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Complexation of iron(III) by natural organic ligands in the Central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method

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Abstract

A highly sensitive voltammetric technique was developed to examine Fe speciation in seawater. The technique involves adding an Fe(III)-complexing ligand, salicylaldoxime, which competitively equilibrates with inorganic and organic Fe(III) species in ambient seawater. The Fe(III)–salicylaldoxime complex then is measured by adsorptive cathodic stripping voltammetry (ACSV). This new method revealed that 99.97% of the dissolved Fe(III) in central North Pacific surface waters is chelated by natural organic ligands. The total concentration of Fe-binding ligands is approximately 2 nM, a value greatly in excess of ambient dissolved iron concentrations. The titration data can be modeled as consisting of two classes of Fe-binding ligands, a strong ligand class (L_1) with an average surface-water concentration equal to 0.44 nM with a conditional stability constant $K^{cond}_{L_1/Fe'} = 1.2 \times 10^{13}$ M^{-1}, and a weaker ligand class (L_2) with an average concentration equal to 1.5 nM with $K^{cond}_{L_2/Fe'} = 3.0 \times 10^{11}$ M^{-1}. The low concentration of dissolved Fe present in surface waters (~ 0.2 nM), coupled with the excess of strong Fe-chelators, results in extremely low equilibrium concentrations of dissolved inorganic iron, [Fe′] ≈ 0.07 pM. In the deeper waters there is a 2 nM excess of Fe-binding ligands with a stability constant similar to that of the L_2 class of ligands observed in surface waters, resulting in dissolved Fe(III) existing primarily in the chelated form in deep waters as well. The stability constants of the natural ligands are comparable to the model ligands desferal, a siderophore, and the prosthetic heme group, protoporphyrin-IX. The high degree of organic complexation of iron makes it critically important to reevaluate our perceptions of the marine biogeochemistry of iron and the mechanisms by which biota can access this chelated Fe.

1. Introduction

Iron is arguably the most important of all the bioactive trace metals in the oceans (Bruland et al., 1991). This first-row transition metal plays a key role in the biochemistry and physiology of oceanic phytoplankton (Morel et al., 1991; Sunda et al., 1991; Geider and La Roche, 1994; Wells et al., 1995). Concentrations of dissolved Fe in oceanic waters range from approximately 1 nM down to values as low as 20 pM in surface waters of remote, high-nitrate, low-chlorophyl (HNLC) regimes (Gordon et al., 1982; Landing and Bruland, 1987; Martin et al., 1989, 1991; Bruland et al., 1994). These low Fe concentrations are suggested to limit phytoplankton growth and biomass in certain oceanic regions. This hypothesis is supported by evidence that iron enrichment experiments in HNLC waters of the subarctic, equatorial, and sub-antarctic Pacific enhance phytoplankton growth (Martin and Fitzwa-

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ter, 1988; Martin et al., 1990,1991,1994; Price et al., 1994).

The oceanic chemistry of Fe is highly complicated and still not fully understood. Dissolved Fe can exist in two different oxidation states in seawater, Fe(III) and Fe(II). Fe(III) is the thermodynamically stable form in oxygenated waters. There are several processes, however, that reduce Fe(III), leading to measurable steady state concentrations of Fe(II) in surface seawater (Waite and Morel, 1984; Hong and Kester, 1986; O'Sullivan et al., 1991; Johnson et al., 1994; Gledhill and van den Berg, 1995-this volume). In surface seawater at pH 8, inorganic Fe(II) is oxidized back to Fe(III) with a half-life of several minutes (Millero and Sotolongo, 1989).

The inorganic speciation of dissolved Fe(III) and Fe(II) differ considerably. Inorganic species comprising dissolved Fe(III) are dominated by the hydrolysis products, $Fe(OH)_2^+$, $Fe(OH)_3^0$, and $Fe(OH)_4^-$. The free hydrated Fe^{3+} ion is not only an extremely rare species, being only 10^{-10} to 10^{-11} of the summed concentration of the hydrolysis species, it is also the slowest of the inorganic species to react with ligands or surface sites due to its slow water-loss rate constant (Hudson et al., 1992). In marked contrast, inorganic Fe(II) exists primarily as the free Fe^{2+} ion (Millero et al., 1995-this volume), which is much more soluble and kinetically reactive than free Fe^{3+}.

There is a lack of knowledge concerning the degree to which either Fe(III) or Fe(II) is complexed with natural organic ligands in the oceans. During the last decade, however, convincing evidence has accrued demonstrating that a number of other bioactive trace metals are strongly influenced by organic complexation. For example, greater than 99.8% of Cu in oceanic surface waters exists chelated with a low concentration of strong Cu-binding organic ligands (van den Berg, 1984; Moffett and Zika, 1987; Coale and Bruland, 1988; Coale and Bruland, 1990) and greater than 98% of dissolved Zn in oceanic surface waters is complexed with roughly 1 nM of strong and relatively Zn-specific organic ligands (Bruland, 1989; Donat and Bruland, 1990). These findings led us to question whether Fe speciation is also strongly influenced by analogous classes of strong chelators existing at nanomolar concentrations.

In order to determine the chemical speciation of Fe in seawater, it is necessary to develop a highly sensitive technique with minimal sample perturbation. This is a difficult task, since total dissolved Fe can be as low as 20 pM in oceanic surface waters (Martin et al., 1991; Bruland et al., 1994), and the technique must be able to determine concentrations of Fe species which comprise sub-classes of this low total dissolved concentration. In addition, Fe is a ubiquitous contaminant that can easily compromise the analysis at any step from the initial sampling to the final measurement.

We present here details of the development of a new technique which allows determination of Fe(III) speciation in seawater. It is based upon the competitive equilibrium established between natural Fe-chelators in a seawater sample and an added ligand, salicylaldoxime, followed by adsorptive cathodic stripping voltammetry of the Fe(III)–salicylaldoxime complex. The technique can be applied at the ambient seawater pH and requires only the addition of the competing ligand and a pH buffer. When we began developing this technique, an Fe speciation method sensitive enough to determine organic complexation of Fe in seawater was not available, though there was a method for determining **total** dissolved Fe based upon the added ligand 1-nitroso-2-naphthol (1N-2N) and adsorptive cathodic stripping voltammetry (ACSV) (Yokoi and van den Berg, 1992). We chose not to adapt this method for speciation studies because it required substantial additions of H_2O_2 as a catalytic enhancer, sodium dodecyl sulfate (SDS) as a surfactant, and was performed at pH 6.9. We were concerned about how these various sample manipulations would affect speciation studies, particularly given that the technique involved a catalytic enhancement that is mechanistically not well understood. We opted, instead, to develop a new technique which had minimal sample perturbation and could be performed at pH 8. As a test of our new method, we present results of laboratory studies with model ligands as well as field observations on the complexation of Fe(III) with natural organic ligands in central North Pacific seawater.

2. Theory

The analytical approach utilizing competitive ligand equilibration, adsorptive cathodic stripping

voltammetry (CLE/ACSV) to determine metal speciation in seawater was first developed to determine Cu speciation with the added ligand catechol (van den Berg, 1984). This approach has subsequently been used with a variety of other competing ligands to determine the organic speciation of Cu (Donat and van den Berg, 1992; van den Berg and Donat, 1992; Donat et al., 1994), Zn (van den Berg, 1985; Donat and Bruland, 1990), Ni (van den Berg and Nimmo, 1987; Donat et al., 1994) and, most recently, Fe (Gledhill and van den Berg, 1994; van den Berg, 1995-this volume; Wu and Luther, 1995-this volume; Rue and Bruland, this work). We have developed a new, relatively simple and highly-sensitive CLE/ACSV method in order to determine the degree to which organic complexation influences Fe(III) speciation. An outline of the theory underlying this approach for determining dissolved iron speciation is presented below.

In an ambient seawater sample, the mass balance equation for dissolved iron can be expressed as:

$$[Fe_T] = [Fe'] + [FeL_i]$$

where [Fe′] represents the sum of all the **inorganic** species and $[FeL_i]$ represents the **organically** bound fraction, with L_i being classes of natural organic ligand(s). We assume that in our samples, analyzed on board ship a few hours after collection, that [Fe(II)′] is insignificant due to rapid oxidation kinetics. Thus, when we use the term Fe′, it is referring to Fe(III)′. The reaction between Fe′ and one class of Fe-binding organic ligands is:

$$Fe' + L' \rightleftharpoons FeL$$

where L′ is the Fe-binding organic ligand(s) not already bound to Fe(III). The mass action expression representing this equilibrium is:

$$K^{cond}_{L/Fe'} = [FeL]/([Fe'] \cdot [L'])$$

where $K^{cond}_{L/Fe'}$ is the conditional stability constant with respect to inorganic Fe′ under these specific conditions (pH 8.0 seawater). Values for $K^{cond}_{L/Fe'}$ differ from $K^{cond}_{L/Fe^{3+}}$ by the inorganic side reaction coefficient $\alpha_{Fe'} = [Fe']/[Fe^{3+}]$ (e.g. $K^{cond}_{L/Fe^{3+}} = \alpha_{Fe'} \cdot K^{cond}_{L/Fe'}$). Since we do not know the extent of any side reactions of any potential natural Fe-binding ligands, L^{n-}, with H^+, Ca^{2+} or Mg^{2+}, and thus do not have a value for $\alpha_{L'} = [L']/[L^{n-}]$, we are unable to estimate a value for $K'_{L/Fe^{3+}} = [FeL'^{n+3}]/([Fe^{3+}][L'^{n-}])$ (the ionic strength corrected stability constant with respect to concentrations of free Fe^{3+} and free L'^{n-}). To illustrate the importance of these considerations when extrapolating thermodynamic stability constants to ocean water conditions, we can examine desferal (desferrioxamine B or deferoxamine), a well-characterized siderophore. For desferal, the thermodynamic stability constant is $K_{L/Fe^{3+}} = 10^{31.9}$ M^{-1} (Martell and Smith, 1975). The ionic strength corrected stability constant is $K'_{L/Fe^{3+}} = 1029.6$ M^{-1} (with activity coefficient corrections using the mean salt method as in Hudson et al., 1992). The conditional stability constant with respect to free hydrated Fe^{3+} is $K^{cond}_{L/Fe^{3+}} = 10^{26.5}$ M^{-1} (with an $\alpha_{L'} = 10^{3.1}$ due to H^+ and Mg^{2+} side reactions with L^{2-}), while the conditional stability constant with respect to Fe′ is $K^{cond}_{L/Fe'} = 10^{16.5}$ M^{-1} (with an $\alpha_{Fe'} = 10^{10.0}$ (Hudson et al., 1992) due to the side reactions of Fe^{3+} with OH^-). Thus, the thermodynamic stability constant of free Fe^{3+} and L^{2-} at $\mu = 0$ differs by a factor of $10^{15.4}$ from the conditional stability constant of Fe′ with respect to L′ under surface seawater conditions. The conditional stability constant, $K^{cond}_{L/Fe'}$, is the constant that most readily provides insight into the **effective** influence of the Fe-binding organic ligands on Fe speciation, since the ratio of organically complexed Fe(III) to inorganic forms of Fe(III) is given by $[FeL]/[Fe'] = K^{cond}_{L/Fe'} \cdot [L']$.

2.1. Competitive ligand equilibration (CLE)

A CLE/ACSV analysis consists of two phases. First, equilibrium is established between a known quantity of a well-characterized added competitive ligand, in this case Fe(III)-binding salicylaldoxime (SA), and the ligands naturally present in the system. The new mass balance created is:

$$[Fe_T] = [Fe'] + [Fe(SA)_2] + [FeL]$$

The reactions describing these competing equilibria are:

$$\begin{array}{ccc} Fe' + L' & \rightleftharpoons & FeL \\ + & & \\ 2SA' & & \\ \downarrow\uparrow & & \\ Fe(SA)_2 & & \end{array}$$

and the mass action expression for complexation of Fe′ by salicylaldoxime is:

$$B^{\text{cond}}_{2\text{SA}/\text{Fe}'} = [\text{Fe(SA)}_2]/([\text{Fe}'] \cdot [\text{SA}']^2)$$

The side reaction coefficient for Fe(SA)_2 with respect to Fe′ is denoted by:

$$\alpha'_{\text{Fe(SA)}_2} = [\text{Fe(SA)}_2]/[\text{Fe}'] = B^{\text{cond}}_{2\text{SA}/\text{Fe}'} \cdot [\text{SA}']^2$$

For this method $[\text{SA}'] = [\text{SA}_\text{T}]$, since $[\text{SA}'] \gg [\text{Fe}_\text{T}]$.

For the initial technique development, a salicylaldoxime concentration was chosen such that $\alpha'_{\text{Fe(SA)}_2}$ was somewhere between 10 and 100, the rationale being that $\alpha'_{\text{Fe(SA)}_2}$ should be at least 10 so that SA would effectively outcompete the inorganic side reactions of $[\text{OH}^-]$ for $[\text{Fe}^{3+}]$. An $\alpha'_{\text{Fe(SA)}_2}$ an order of magnitude greater than $\alpha_{\text{Fe}'}$ is necessary for optimal sensitivity of the technique. On the other hand, for our initial applications we selected an $\alpha'_{\text{Fe(SA)}_2}$ less than 100 in hopes that SA would not overwhelm any potential natural Fe-chelators. The strength and concentration of salicylaldoxime should ideally be adjusted so that the equilibrium reaction above is shifted towards the Fe(SA)_2 species enough to be detectable, yet not have SA completely outcompete the natural Fe-binding chelators (in contrast to total metal determinations), that is, $\alpha'_{\text{Fe(SA)}_2}$ must be less than α'_{FeL}. Since $\alpha'_{\text{Fe(SA)}_2}$ is proportional to $[\text{SA}]^2$, CLE methods allow investigators to change the analytical window of their technique to more fully probe the binding strength of any natural Fe-chelators.

2.2. *Adsorptive cathodic stripping voltammetry (ACSV)*

The second phase of any CLE/ACSV method is the adsorptive cathodic stripping voltammetric (ACSV) determination of the concentration of the metal-added ligand complex formed in the competitive equilibration. This entails adding a ligand that forms a metal complex which readily adsorbs onto a Hg drop and from which the metal can be reduced. The bis-salicylaldoxime Fe(III) complex has a distorted octahedral structure (Podder, 1963; Burger et al., 1965; Egneus, 1972) in which inter-ligand hydrogen bonding causes both salicylaldoxime ligands to be situated in the same x–y plane (with two H_2O or OH^- groups occupying the fifth and sixth coordination positions in the z-axis) (Fig. 1a). The aromatic rings of the two salicylaldoxime ligands thus are oriented in the same plane, allowing the complex to readily adsorb onto the Hg electrode surface and the Fe(III) to be readily accessible for reduction. Moreover, this inter-ligand hydrogen bonding ensures formation of a well-defined 1:2 stoichiometry, which is important for calculating $\alpha'_{\text{Fe(SA)}_2}$ and was one of the key considerations for choosing SA as our added ligand. In addition to forming a well-defined planar

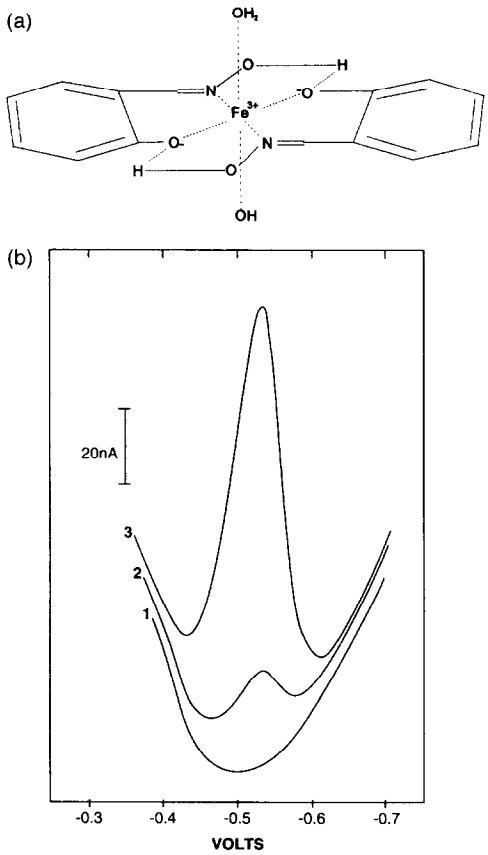

Fig. 1. (a) Structure of the bis-salicylaldoxime Fe(III) complex. Note inter-ligand hydrogen bonding which serves to orient the ligands in the same $x-y$ plane and sterically preclude the attachment of a third salicylaldoxime ligand. The coordination sites on the z-axis are occupied by either H_2O or OH^-. (b) Actual cathodic stripping voltammetric scans using our most sensitive current range setting of 200 nA full scale on the VERTEX V station T4, 20 m sample. The first scan is of a zero Fe addition (ambient $[\text{Fe}_\text{T}] = 0.1$ nM, with no detectable Fe(SA)_2). The second and third scans are Fe additions which yield Fe(SA)_2 signals equivalent to 0.09 and 0.63 nM Fe(SA)_2, respectively.

complex, we chose salicylaldoxime because at concentrations of 10^{-6} to 10^{-4} M its conditional stability constant (see below) results in $\alpha'_{Fe(SA)_2}$ values that were likely to be in a useful range to probe natural Fe-chelators.

Following the adsorption step, the iron reduction current measured from the adsorbed $Fe(SA)_2$ during the cathodic stripping, i_p (Fig. 1b), is directly related to $[Fe(SA)_2]$ in the solution through a proportionality factor S:

$$i_p = S \cdot [Fe(SA)_2]$$

where S is the sensitivity determined from the linear portion of the iron titration curve, occurring after any natural iron-complexing organic ligands have been saturated with iron.

$[Fe']$ is related to the iron reduction peak current, i_p, by the following relationship:

$$[Fe'] = i_p/(S \cdot \alpha'_{Fe(SA)_2})$$

The concentration of FeL can thus be calculated from:

$$[FeL] = [Fe_T] - i_p/S - i_p/(S \cdot \alpha'_{Fe(SA)_2})$$

2.3. Calibration of SA

Due to the lack of adequate literature values, it was first necessary to experimentally determine the stability constant of the $Fe(SA)_2$ complex. We determined $B^{cond}_{2SA/Fe'}$ by establishing a competition between SA and the well-characterized ligand ethylenediaminetetraacetic acid (EDTA). EDTA was allowed to competitively equilibrate with SA for 5 h. This type of calibration was initially described by van den Berg (1985) for zinc with pyrrolidine dithiocarbamate (PDC) as the competing ligand. In the presence of EDTA and SA, the mass balance for Fe becomes:

$$[Fe_T] = [Fe'] + [Fe(SA)_2] + [FeEDTA]$$

where [FeEDTA] includes Fe(III) present as $Fe(OH)EDTA^{2-}$ and $FeEDTA^-$. In a sample containing iron and SA, with no EDTA, the reduction current is at its maximum:

$$i_{p,0} = S \cdot [Fe(SA)_2] = S \cdot ([Fe_T] - [Fe'])$$

When EDTA is present, the iron reduction current is decreased by the amount of iron complexed by the EDTA:

$$i_{p,i} = S \cdot ([Fe_T] - [Fe'] - [FeEDTA])$$

The ratio, X, of the iron reduction current obtained in the presence of EDTA ($i_{p,i}$) to that obtained in its absence, ($i_{p,0}$) is then:

$$X = i_{p,i}/i_{p,0}$$

$$= B^{cond}_{2SA/Fe'} \cdot [SA']^2 \Big/ \left(B^{cond}_{2SA/Fe'} \cdot [SA']^2 + K^{cond}_{EDTA/Fe'} \cdot [EDTA']\right)$$

$[EDTA']$ is the total concentration of EDTA not complexed with iron:

$$[EDTA'] = [EDTA_T] - (1 - X) \cdot [Fe_T]$$

Values for $B^{cond}_{2SA/Fe'}$ can then be calculated from:

$$B^{cond}_{2SA/Fe'} = X \cdot K^{cond}_{EDTA/Fe'} \cdot [EDTA']/([SA']^2 - X \cdot [SA']^2)$$

Values for $B^{cond}_{2SA/Fe'}$ in seawater at pH 8.0 were determined by calibration against EDTA. The overall side reaction of EDTA ($\alpha_{EDTA'} = 10^8$) with respect to the major cations and H^+ was calculated from the stability constants (25°C, $\mu = 0.7$) for the formation of EDTA complexes with Ca^{+2}, Mg^{+2} from Arena et al. (1983) and for H^+, Na^+, and K^+ from Daniele et al. (1985). Stability constants ($\mu = 1.0$) for Fe(III) with respect to EDTA were obtained from Martell and Smith (1982). Conditional stability constants were calculated using the $\alpha_{EDTA'}$ from above and an $\alpha_{Fe'} = 10^{10.0}$ from Hudson et al. (1992).

2.4. Calculation of $[L_T]$ and $K^{cond}_{L/Fe'}$

The conditional stability constants, $K^{cond}_{L/Fe'}$, and concentrations, $[L_T]$, of the natural Fe-chelators were calculated from the ACSV titration data by Scatchard and Langmuir transformation methods applied to the titration data (van den Berg and Kramer, 1979; Ružić, 1982; van den Berg, 1982). For our system, the Scatchard transformation involves plotting the ratio [FeL]/[Fe'] against [FeL] and, if the data can be interpreted as a 1:1 complex between Fe(III) and ligands with closely similar stability constants, a

straight line will result whose y-intercept is $K^{cond}_{L/Fe'} \cdot [L_T]$ and whose x-intercept yields $[L_T]$. The Langmuir linearization (also referred to as a van den Berg/Ružić linearization) involves plotting the ratio [Fe']/[FeL] against [Fe'] for the titration data. If the data can be interpreted as above, a straight line will result whose y-intercept yields $1/(K^{cond}_{L/Fe'} \cdot [L_T])$ and whose slope yields $1/[L_T]$. From these linearizations, estimates of $K^{cond}_{L/Fe'}$ and $[L_T]$ can be obtained. If two classes of Fe-binding ligands exist, for example, one class composed of a smaller concentration of stronger Fe-chelators and a second class composed of a larger concentration of weaker chelators, then the transformations yield nonlinear plots. For such a case of two ligands, a Scatchard transformation will have two linear regions that can be used to resolve the concentrations of both L_1 and L_2, as well as their respective conditional stability constants, $K^{cond}_{L_1/Fe'}$ and $K^{cond}_{L_2/Fe'}$ (Mantoura and Riley, 1975; Ružić, 1982). The presence of two or more ligand classes with differing conditional stability constants can be recognized in a Langmuir transformation by curvature at lower [Fe']. The reciprical of the slope of the linear region at higher [Fe'] provides a good estimate of the total ligand concentration. It is more difficult, however, to calculate individual K's and L's when multiple ligands are present from Langmuir transformations (Ružić, 1982). It should be noted that even if we can determine the concentrations and conditional stability constants of two classes of Fe-binding ligands, these values are still model derived parameters that probably represent weighted averages of mixtures of many ligands. This characterization nevertheless can still provide us with a good estimate of the degree of organic complexation and Fe speciation. With these derived values of $K^{cond}_{L/Fe'}$, $[L_T]$'s, and the initial concentration of $[Fe_T]$, the ambient concentrations of [L']'s, [FeL]'s, and [Fe'] can then be calculated.

3. Materials and methods

3.1. Instrumentation

The CLE/ACSV system consists of a Princeton Applied Research (PAR) 303A static mercury drop electrode connected to a PAR 174A voltammetric analyzer (modified to increase the pulse frequency to 8 s^{-1} and to decrease the delay time prior to the current sampling period during a pulse from 40 to 13 ms), whose output is plotted on an X–Y recorder (Houston Instruments) (Donat and Bruland, 1990). The working electrode was a "large" mercury drop (2.8 mm^2), the reference electrode was Ag/saturated AgCl, saturated KCl, and the counter electrode was a platinum wire.

During CLE/ACSV analyses, all samples were contained in FEP-Teflon voltammetric cell cups, and stirred with a PTFE-Teflon-coated stirring bar driven by a PAR magnetic stirrer (model 305). All sample manipulations were performed within a positive-pressure clean-sampling van or in a class-100 clean-air work area at room temperature.

3.2. CLE/ACSV reagents

A 0.11 M stock solution of salicylaldoxime (Janssen Chimica) was prepared every other week in optima grade methanol (Fisher). A sub-standard (0.011 M) was prepared in Milli-Q water. A 1.3 M stock buffer solution of HEPES (N-2-hydroxyethylpiperazine-N'-3-propanesulfonic acid; Aldrich) was prepared in 1 M QNH_4OH (Q refers to reagents that are cleaned by sub-boiling quartz distillation): an addition of 50 μl of this HEPES solution to 10 ml of seawater buffered the pH to 8.0. Iron standard solutions were prepared by dilution of 1000 ppm atomic absorption standards (Fisher) with Milli-Q, and acidified to pH 3 with sub-boiling quartz-distilled hydrochloric acid (QHCl). For determination of $B^{cond}_{2SA/Fe'}$, a 0.04 M stock solution of Na_2EDTA (Fisher) was prepared at pH $\sim$ 8.5 and diluted with Milli-Q water. In experiments with model ligands, a 3.3 mM stock solution and subsequent dilutions of desferal (butanediamide,N'-[5-[[4-[[5-(acetylhydroxyamino)-pentyl]amino]-1,4-dioxobutyl]hydroxyamino]pentyl]-N-(5-aminopentyl)-N-hydroxy, monomethanesulfonate, commonly known as desferrioxamine B mesylate or deferoxamine, provided by CIBA GEIGY, [138-14-7]) were prepared in Milli-Q water every other day and a 19 mM stock solution of protoporphyrin-IX (Aldrich) was prepared in Milli-Q water. For pH optimization experiments, 1.0 M solutions of PIPES (pH 6.8) (piperazine-N,N'-bis(2-ethanesulfonic acid); Aldrich) and HEPES (pH 8.5) (N-(2-hydroxy-

ethyl)piperazine-N'-2-ethanesulfonic acid; Aldrich) were each prepared in 1M QNH_4OH.

Trace metals and metal chelating organic ligands were removed from seawater according to the procedure described by Donat and Bruland (1988). In this report, such seawater is referred to as "UVSW".

3.3. Sample collection and handling

This study was performed on fresh seawater samples obtained from a station in the North Pacific Central Gyre, station ALOHA (22°45'N, 158°W) during January 1994 and on a frozen sample from VERTEX V station T4 at the eastern edge of the North Pacific Central Gyre (33.3°N, 139.1°W) collected July 1984. Seawater was collected in Teflon-coated 30 l GO-Flo sampling bottles (General Oceanics) mounted on a Kevlar hydrowire, and tripped with a Teflon messenger (Bruland et al., 1979; Bruland, 1980). Upon recovery, the GO-Flo bottles were pressurized with filtered nitrogen gas and the seawater transferred via Teflon tubing through 142 mm diameter, 0.2 μm pore size polycarbonate membrane filters (Nuclepore) mounted in Teflon filter sandwiches (Millipore), and into 1 or 2 l FEP-Teflon bottles for speciation work and 1 l polyethylene bottles for total dissolved trace metal analyses by organic extraction. Subsamples for speciation determinations at station ALOHA were stored in the dark at lab temperature and analyzed within several hours of collection (range of 1 to 10 h); subsamples for total dissolved metal determinations were acidified to pH < 2 with QHCl and returned to our shore-based laboratory for analysis. The sample from VERTEX V station T4 was filtered through a 0.3 μm Nuclepore filter and collected in a 2 l FEP-Teflon bottle and frozen immediately. The sample was thawed just prior to analysis.

3.4. Total dissolved iron determinations

Total dissolved iron was concentrated from the acidified samples in our lab using an ammonium 1-pyrrolidine-dithiocarbamate (APDC)/diethylammonium diethyldithiocarbamate (DDDC) chelation/solvent extraction technique, described in detail by Bruland et al. (1979,1985) and by Landing and Bruland (1987). Fe in the extracts was determined by graphite furnace atomic absorption spectrometry using a Perkin-Elmer 5000 spectrophotometer.

3.5. CLE / ACSV Fe speciation determinations

Determination of Fe speciation in seawater samples by CLE/ACSV followed the method outlined below.

Subsamples (10 ml) of seawater were pipetted into a series of up to 15 FEP-Teflon voltammetric cell cups and 6.5 mM HEPPS buffer was added. Iron was added to all but two cups, yielding concentration additions from 0 to 5 nM. The Fe-equilibration cell cups were placed in individual polyethylene containers fitted with airtight lids, and the added Fe was allowed to equilibrate with the natural ligands for 1 h at laboratory temperature. After this equilibration period, the aliquots were transferred to a second series of ligand-equilibration cell cups which had been previously conditioned with the seawater sample containing the salicylaldoxime. At this point, 27.5 μM salicylaldoxime was added to the first aliquot of the Fe titration, left to equilibrate for at least 10 min and then deaerated for 4 min using oxygen-free, water saturated, nitrogen gas.

The $Fe(SA)_2$ complexes from the sample were subsequently adsorbed onto a fresh mercury drop at an applied potential of -0.05 V for 10 min while the sample was stirred at 700 rpm. After the 10 min adsorption period, the stirrer was stopped and 15 s later the potential was scanned in the differential pulse mode from -0.05 to -0.7 V at 20 mV s^{-1} (pulse amplitude 25 mV, pulse rate 8 s^{-1}) and the cathodic stripping current from adsorbed $Fe(SA)_2$ recorded. Cell cups were rinsed with only Milli-Q between analyses and the same cup was consistently used for each added Fe concentration.

3.6. CLE / ACSV determinations of $B^{cond}_{2SA/Fe'}$

To determine $B^{cond}_{2SA/Fe'}$, subsamples (10 ml) of UVSW containing 6.5 mM HEPPS (pH 8.0), 27.5 μM salicylaldoxime, and 10 nM Fe were pipetted into FEP-Teflon cell cups. EDTA was added to one set of cups yielding concentrations ranging from 1.2×10^{-8} to 5×10^{-5} M in 5 increments. All aliquots were allowed to equilibrate for at least 5 h before the

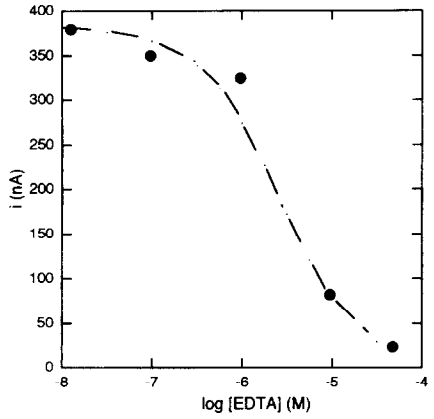

Fig. 2. CLE/ACSV Fe reduction peak current obtained in UVSW as a function of EDTA concentration. An Fe concentration of 10 nM and an adsorption time of 4 min were used. Filled circles (●) represent actual titration points and the curved line represents the best fit of the data using a $B^{cond}_{2SA/Fe'} = 9.6 \times 10^{10}$ M^{-2}.

iron reduction currents were determined in stirred samples (700 rpm) using 4 min adsorption periods at −0.05 V.

The effect of additions of EDTA on the iron reduction current is shown in Fig. 2. The curve in this figure represents the best fit of the data to an average $B^{cond}_{2SA/Fe'}$. We obtained a $B^{cond}_{2SA/Fe'} = 9.6 \times 10^{10}$ M^{-2}, using an $\alpha_{Fe'} = 10^{10.0}$. At an [SA'] = 27.5 μM, this results in an $\alpha'_{Fe(SA)_2} = 73$.

4. Results

4.1. Method development

The development of this CLE/ACSV technique consisted of a series of experiments designed to optimize critical analytical parameters. The results of these experiments are presented below.

Effect of varying [SA]

The dependence of the iron peak reduction current on the salicylaldoxime concentration is shown in Fig. 3A. The iron reduction current increased with SA concentrations up to 20 μM, and began to significantly decrease at concentrations beyond 40 μM. A salicylaldoxime concentration of 27.5 μM was used for all analyses reported here.

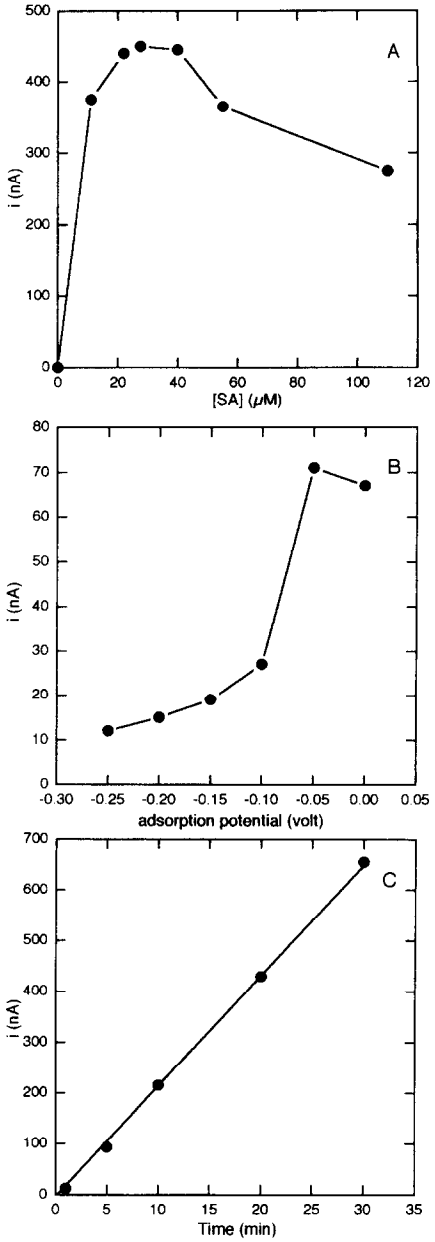

Fig. 3. (A) ACSV Fe reduction peak current obtained in UVSW as a function of salicylaldoxime concentration using an Fe concentration of 10 nM and an adsorption time of 4 min. (B) ACSV Fe reduction peak current obtained in UVSW as a function of deposition potential using an Fe concentration of 2 nM and an adsorption time of 4 min. (C) ACSV Fe reduction peak current obtained in UVSW as a function of adsorption time using an Fe concentration of 2.5 nM.

Effect of varying the deposition potential

The dependence of the iron peak reduction current on the deposition potential is shown in Fig. 3B. Deposition potentials between −0.25 and 0.00 volts were tested. A potential of −0.05 V yielded the greatest iron response and was used for all further work.

Effect of mercury in the cell cup

In early experiments we noticed a slight decrease in the iron response as a function of increasing drops of mercury in the bottom of the preconditioned cup (1–2% per drop). This occurred at both higher (2.5 nM) and lower (< 0.5 nM) concentrations of Fe. Subsequent analyses were performed on separate 10 ml aliquots of the experimental solution to ensure that the only Hg drop in contact with the solution was the one specifically extruded for the adsorption/stripping cycle.

Effect of varying adsorption time

The dependence of the iron peak reduction current on adsorption time in UVSW is shown in Fig. 3C. At an iron concentration of 2.5 nM, the iron reduction current was a linear function of adsorption time up to 30 min.

Linearity of response

The iron peak reduction current obtained as a function of iron concentration in UVSW was linear up to Fe concentrations of approximately 5 nM, with a sensitivity of 16 $nA \cdot nM^{-1} \cdot min^{-1}$. Beyond 5 nM, the iron response leveled off, probably due to precipitation of Fe′ during the 1 h equilibration period prior to adding the SA. If the SA is added before the iron, the response is linear with the same slope of 16 $nA \cdot nM^{-1} \cdot min^{-1}$ to at least 20 nM total Fe. For these titrations of UVSW the y-intercept was zero, which suggests that there was no significant adsorption on the cell cup walls which would lead to an apparent Fe-binding ligand concentration in the UVSW, i.e. the Fe blank, as well as any Fe-chelator blank, were both undetectable in UVSW. For titrations of samples containing dissolved Fe-chelators, once the ligands are titrated, the response to increasing iron additions is linear to the point at which the excess [Fe′] in the initial equilibration with the natural Fe-chelators approaches 5 nM.

Interferences

An additional requirement for CLE/ACSV methods is that none of the natural Fe-binding organic ligands form adsorbing electroactive complexes with Fe. We checked this by performing Fe titrations of seawater samples in the absence of SA. No ASCV signal was observed at Fe additions up to 5 nM, an Fe concentration that would have fully titrated any Fe-chelators.

Experiments were also conducted to investigate whether Fe(II) is being measured in addition to Fe(III) by our technique. 5 nM Fe(II) additions (from a 0.5 μM Fe(II) standard containing 0.045 M $NH_2OH \cdot HCL$) were made to UVSW containing buffer and 27.5 μM salicylaldoxime which resulted in no CSV signal. Either Fe(II) does not appreciably complex with SA at a concentration of 27.5 μM, or the complex, if formed, does not adsorb (if it did form an adsorbable complex with this concentration of SA, at our deposition potential the adsorbed Fe(II) would have been oxidized to Fe(III) and would have appeared as a current during the CSV step). Since field collected seawater samples sat in the dark at room temperature for a few hours prior to analysis, presumably any Fe(II) that might have been initially present in the samples would have been oxidized to Fe(III) during this time.

Salicylaldoxime has also been used to determine copper complexation under similar conditions (Campos and van den Berg, 1994), but Cu does not interfere with Fe determinations since the Cu peak and the Fe peak are separated by 150 mV and the Fe peak is thus fully resolvable.

Effect of equilibration time

Using a competitive ligand equilibrium approach requires consideration of the kinetics of two complexation reactions; that of the added Fe with the natural ligands, and that of the system with the added salicylaldoxime. Rather than adding the competing ligand and the Fe simultaneously, thereby requiring the equilibrium to be established between the added Fe, a high concentration of competing ligand (27.5 μM) and a low concentration of natural Fe-binding ligand(s) (1–2 nM); we took the approach used by van den Berg (1984) of initially adding just the metal to the natural sample (Fig. 4, point *A*) and letting it first equilibrate with the low concentration of natural

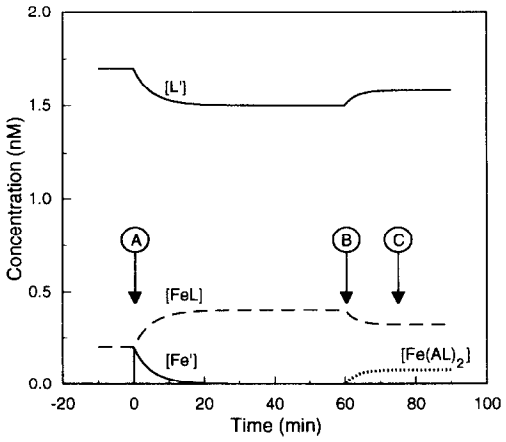

Fig. 4. Modeled complexation kinetics for a titration in a seawater sample. Initial conditions include $[L_T] = 1.9$ nM, $[Fe_T] = 0.2$ nM, and $K^{cond}_{L'/Fe'} = 5 \times 10^{11}$ M^{-1}. Ambient values prior to addition of Fe at point *A* are $[L'] = 1.7$ nM, $[FeL] = 0.2$ nM, and $[Fe'] = 0.02$ pM. At time zero (point *A*), a 0.2 nM Fe addition was made and equilibrated with a rate constant of 1.8×10^6 $M^{-1}s^{-1}$ (the rate which we experimentally observed and is consistent with Hudson et al., 1992). Upon reaching equilibrium, this lowered the [L'] by 0.2 nM, increased the [FeL] by 0.2 nM, and increased [Fe'] to 0.5 pM. After a 1 h equilibrium period, the competing ligand, 27.5 μM salicylaldoxime was added (point *B*). The system comes to a new equilibrium (point *C*, based on experimental results) where an electroactive, detectable fraction of Fe now exists as the $Fe(SA)_2$ complex with the added ligand salicylaldoxime. The ACSV analysis begins at point *C*.

Fe-binding ligand for 1 h. Experiments were conducted on natural samples to determine the equilibration time necessary for the added Fe′ to come to equilibrium with the natural organic ligands. It was determined that under these conditions the added Fe′ comes to equilibrium with the natural Fe-binding ligands within 40 min. This equilibration time is consistent with a forward rate constant of 2×10^6 $M^{-1}s^{-1}$, a value similar to that predicted based upon the rate-limiting step of water loss from the inner coordination sphere of the labile ferric hydroxy species in pH 8 seawater (Hudson et al., 1992).

After 1 h (Fig. 4, point *B*), 27.5 μM of SA was added and an additional 14 min equilibration permitted Fe′ and FeL to come to equilibrium with the high concentration of SA (Fig. 4, point *C*). Experiments were conducted to determine the time necessary for the added SA to come to equilibrium with the added Fe′ and the natural ligands. It appeared to rapidly equilibrate within 10 min with no further observed difference between equilibration times of 10 min and 3 h. This equilibration rate between the added ligand and the natural ligands seems fast if one were to consider a simple disjunctive mechanism (Hering and Morel, 1988) whereby the FeL independently dissociates and then the competing ligand binds to the Fe′. Instead, an adjunctive mechanism, with the added SA assisting in the dissociation of the natural Fe chelate by forming a tertiary complex, likely hastens the formation of $Fe(SA)_2$. Such adjunctive mechanisms are common for Fe-siderophores (Crumbliss, 1991; Birus et al., 1993) and are known to markedly hasten ligand exchange reactions. The large concentration of SA, particularly relative to [L'] ($[SA']/[L'] \geq 10{,}000$), ensures that the second equilibration step with the competing ligand is rapid.

Table 1
Depth distibutions of Fe-chelator concentrations and their conditional stability constants as calculated from the Scatchard transformations from station ALOHA in the Central North Pacific

Sample depth (m)	$[Fe_T]$ (nmol/kg)	$[L_{1T}]$ (nM)	$K^{cond}_{L_1/Fe'}$ (M^{-1})	$[L_{2T}]$ (nM)	$K^{cond}_{L_2/Fe'}$ (M^{-1})
20	0.24	0.37	1.5×10^{13}	1.5	3.8×10^{11}
35	0.27	0.53	1.1×10^{13}	1.4	3.2×10^{11}
70	0.27	0.43	1.6×10^{13}	1.5	2.9×10^{11}
110	0.17	0.45	0.5×10^{13}	1.5	2.0×10^{11}
160	0.09	0.5	1.1×10^{13}	1.3	3.8×10^{11}
300	0.27	0.6	1.2×10^{13}	1.5	3.3×10^{11}
500	0.67	–	–	2.8	5.8×10^{11}
1000	0.72	–	–	2.4	3.1×10^{11}
2000	0.77	–	–	2.5	3.0×10^{11}

4.2. Field studies

Seawater samples for iron speciation determinations were collected from depths of 20 to 2000 m at a station in the North Pacific central gyre (ALOHA, 22°45′N, 158°W) during January 1994. The surface mixed-layer during this time of the year extended down to approximately 100 m. There was a strong subsurface fluorescence maximum just below the mixed layer centered around 110 m, which decreased with depth to background levels by a depth of 160 m. The total dissolved iron concentrations in these samples are presented in Table 1.

Results of Scatchard transformations of the titrations performed on each of the samples are presented in Table 1. The iron titration data and transformations for the 20 and 70 m samples are presented as examples in Table 2 and Fig. 5A–F. The titration data for the other sample depths may be obtained from the authors. The sensitivity, S, for the linear region of the higher Fe additions was 16.5 nA · $nM^{-1} \cdot min^{-1}$, a value identical to what was observed on aliquots of UVSW under similar conditions. Since the slopes for the UVSW and the seawater sample (once the ligands had been titrated) were equivalent, we conclude that there does not appear to be additional classes of weaker ligands (i.e. with $K^{cond}_{L/Fe'} > 10^9$ M^{-1}) at higher concentrations (> 5 nM) present in the samples. The similarity between the sensitivity in UVSW and samples from the central North Pacific also suggests that there is no significant interference by natural surfactants present in the sample.

The Scatchard and Langmuir transformations of the titration data of the representative 20 and 70 m samples (Fig. 5B,C,E,F) could not be modeled by a single class of Fe-chelators. Instead, the Scatchard plots could be readily interpreted as resulting from two classes of Fe-binding ligands. For the 20 and 70 m samples, we obtained values of 0.37 and 0.43 nM for the concentration of L_{1T}, the stronger of the two Fe-binding ligands. The conditional stability constant of this ligand class was determined to be $K^{cond}_{L_1/Fe'} = 1.5 \times 10^{13}$ M^{-1} and 1.6×10^{13} M^{-1}, respectively. The weaker class of Fe-binding ligands, L_2, had $[L_{2T}] = 1.5$ and 1.6 nM, with conditional stability constants of $K^{cond}_{L_2/Fe'} = 3.8 \times 10^{11}$ M^{-1} and 2.9×10^{11} M^{-1} for the 20 and 70 m depths, respectively.

Table 2
Iron titration data and Scatchard transformations for the 20 and 70 m samples from station ALOHA in the Central North Pacific. Current (nA) values represent the mean of duplicate or triplicate analysis

$[Fe_{Total}]$ (nM)	$[Fe_{added}]$ (nM)	Current (nA)	$[Fe(SA)_2]$ (nM)	[Fe′] (nM)	[FeL] (nM)	[FeL]/[Fe′]	[Fe′]/[FeL]
ALOHA 20 m sample							
0.24	0.0	1.0	0.00592	0.00008	0.234	2802.36	0.0004
0.29	0.05	2.0	0.01183	0.00017	0.278	1664.35	0.0006
0.39	0.1	5.0	0.02958	0.00042	0.360	861.76	0.0012
0.59	0.2	7.5	0.04437	0.00063	0.545	869.75	0.0011
0.69	0.4	13.8	0.0828	0.0012	0.606	526.35	0.0019
0.89	0.6	19.4	0.1164	0.0016	0.772	476.95	0.0021
1.29	1.0	40.0	0.2387	0.0033	1.048	313.72	0.0032
2.29	2.0	119	0.7111	0.0099	1.569	157.92	0.0063
5.29	5.0	585	3.496	0.049	1.745	35.72	0.0280
ALOHA 70 m sample							
0.27	0.0	1.0	0.0059	0.0001	0.264	3161.73	0.0003
0.37	0.1	2.5	0.0148	0.0002	0.355	1700.29	0.0006
0.47	0.2	8.0	0.0473	0.0007	0.422	631.17	0.0016
0.67	0.4	11.0	0.0661	0.0009	0.603	657.03	0.0015
0.87	0.6	15.0	0.0897	0.0013	0.779	622.18	0.0016
1.27	1.0	40.0	0.2387	0.0033	1.028	307.73	0.0032
2.27	2.0	98.0	0.5858	0.0082	1.676	204.87	0.0049
3.27	3.0	285	1.703	0.024	1.543	64.84	0.0154
5.27	5.0	567	3.389	0.047	1.834	38.74	0.0258

The Langmuir plots also indicate the presence of two or more ligand classes and provide an estimate of the total Fe-binding ligand concentration that agrees with the sum of $[L_{1T}]$ and $[L_{2T}]$ from the Scatchard plots.

The concentrations of Fe-chelators, $[L_{1T}]$ and $[L_{2T}]$, from throughout the water column at station ALOHA are shown in Fig. 6A, where total ligand concentrations as well as $[Fe_T]$ for each depth are

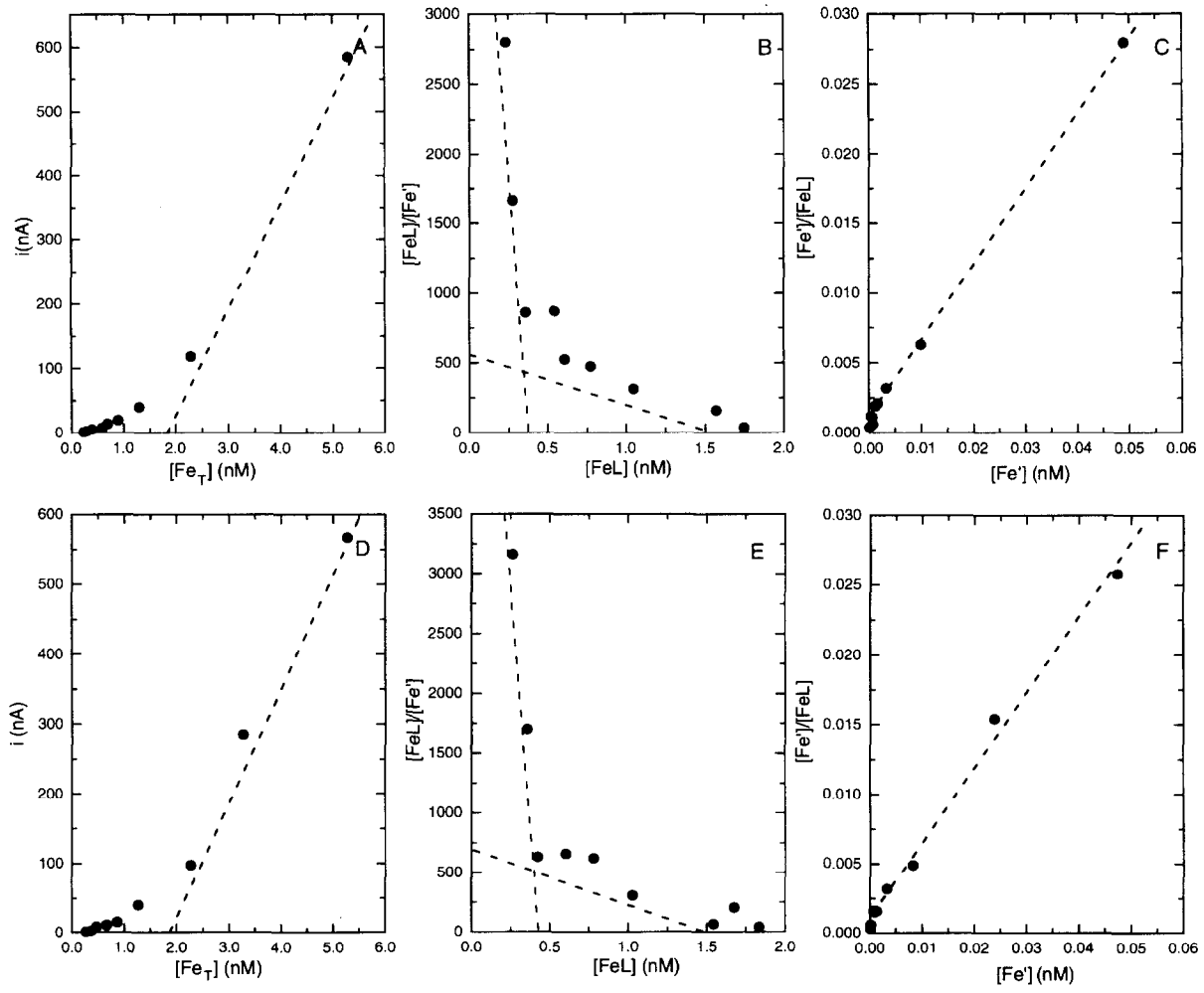

Fig. 5. (A) CLE/ACSV Fe titration of a typical surface water sample from 20 m depth, station ALOHA. The dashed line has a slope equivalent to that for which the ligand has been fully titrated by the added Fe (16.5 nA · nM^{-1} · min^{-1}). It intersects the x-axis at the $[L_T] = 1.83$ nM (calculated from the Langmuir transformation). (B) Scatchard transformation of the 20 m sample. The two dashed linear components of the curve are the resolved contributions from the stronger ligand class and the weaker ligand class; their x-axis intercepts and slopes yield $[L_1] = 0.37$ nM with a $K^{cond}_{L_1/Fe'} = 1.5 \times 10^{13}$ M^{-1} and $[L_2] = 1.5$ nM with a $K^{cond}_{L_2/Fe'} = 3.8 \times 10^{11}$ M^{-1}. (C) Langmuir transformation of the 20 m sample. The dotted line represents the best fit through the last four points of the titration; its slope corresponds to $1/[L_T]$. (D) CLE/ACSV Fe titration of a typical surface water sample from 70 m depth, station ALOHA. The dashed line has a slope equivalent to that for which the ligand has been fully titrated by the added Fe (16.5 nA · nM^{-1} · min^{-1}). It intersects the x-axis at the $[L_T] = 1.88$ nM (calculated from the Langmuir transformation). (E) Scatchard transformation of the 20 m sample. The two dashed linear components of the curve are the resolved contributions from the stronger ligand class and the weaker ligand class; their x-axis intercepts and slopes yield $[L_1] = 0.43$ nM with a $K^{cond}_{L_1/Fe'} = 1.6 \times 10^{13}$ M^{-1} and $[L_2] = 1.6$ nM with a $K^{cond}_{L_2/Fe'} = 2.9 \times 10^{11}$ M^{-1}. F) Langmuir transformation of the 70 m sample. The dotted line represents the best fit through the last four points of the titration; its slope corresponds to $1/[L_T]$.

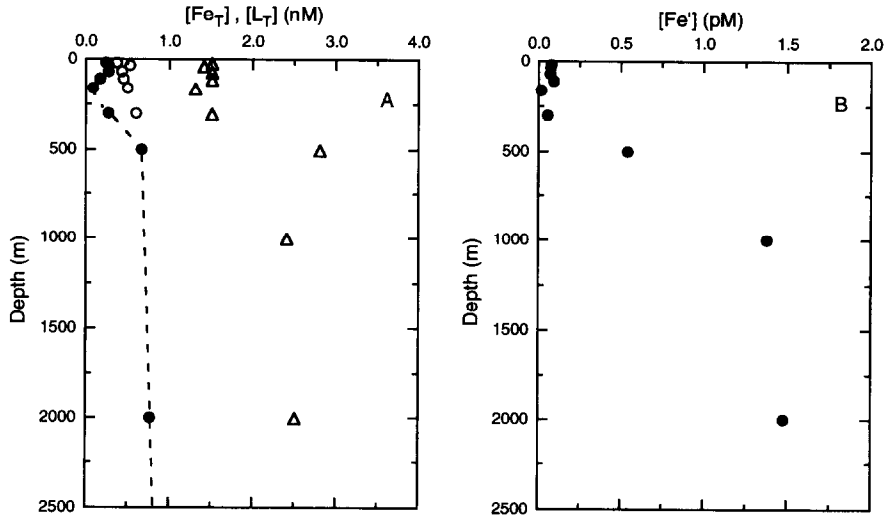

Fig. 6. (A) Depth profiles of total dissolved Fe, [Fe$_T$] (●), and Fe-chelator concentrations, [L$_{1T}$] (○) and [L$_{2T}$] (△), at station ALOHA in the central North Pacific. (B) Depth profile of corresponding equilibrium inorganic Fe concentrations, [Fe′].

plotted on the same scale. The average [Fe$_T$] from the surface waters (20–110 m) at station ALOHA was determined to 0.24 ± 0.05 nM. The average [L$_{1T}$] = 0.44 ± 0.06 nM with a $K^{cond}_{L_1/Fe'} = 1.2 \pm 0.5 \times 10^{13}$ M^{-1}. The average [L$_{2T}$] = 1.5 ± 0.1 nM with a $K^{cond}_{L_2/Fe'} = 3.0 \pm 0.8 \times 10^{11}$ M^{-1}. These average concentrations and stability constants, together with the ambient dissolved Fe concentration, [Fe$_T$], can then be used to calculate the ambient Fe speciation. At equilibrium, the 0.24 nM of dissolved Fe is speciated as follows: 0.207 nM exists as FeL$_1$, 0.033 nM as FeL$_2$, and only 0.00007 nM (0.07 pM) exists as Fe′. An average of 99.97% of the Fe is organically complexed with 86.2% associated with the stronger L$_1$ ligand, and 13.8% associated with the weaker L$_2$ class of ligands. Only 0.03% of the Fe exists as inorganic hydrolysis species, Fe′. A summary of the average ambient surface water iron speciation from station ALOHA are presented in Table 3. Fig. 6B presents the depth distribution of equilibrium [Fe′].

We also analyzed a surface sample from a summer-time station at the eastern edge of the North Pacific central gyre (VERTEX V station T4) that had been filtered, quickly frozen at sea, and then stored frozen in a Teflon bottle until just prior to analysis. Samples stored in this manner have been reported to have metal-binding ligand concentrations and respective stability constants similar to those of fresh samples analyzed on board ship (Gledhill and van den Berg, 1994; van den Berg, 1995-this volume). The results from a Scatchard transformation of the titration data are presented in Table 3. We again observed two classes of Fe-binding ligands in excess of the dissolved Fe; the the concentration of the strong

Table 3
Average ambient surface water (20–110 m) iron speciation from station ALOHA in the Central North Pacific and VERTEX V station T4 at the eastern margin of the North Pacific central gyre. The precision values represent one standard deviation of the four surface water samples (20–110 m)

Sample	[Fe$_T$] (nmol/kg)	[L$_{1T}$] (nM)	$K^{cond}_{L_1/Fe'}$ (M^{-1})	[L$_{2T}$] (nM)	$K^{cond}_{L_2/Fe'}$ (M^{-1})	[Fe′] (pM)	[FeL]/[Fe$_T$]
ALOHA	0.24 ± 0.05	0.44 ± 0.6	$1.2 \pm 0.5 \times 10^{13}$	1.5 ± 0.06	$3.0 \pm 0.8 \times 10^{11}$	0.07	0.9997
T4	0.1	1.0	0.8×10^{13}	1.5	3.0×10^{11}	0.01	0.9999

L_1 class of Fe-chelator was 1 nM. At equilibrium, only 0.01 pM of Fe(III) would exist as Fe′. In this case, 99.99% of the dissolved Fe would exist as either FeL_1 (94.1%) or FeL_2 (5.9%) at equilibrium.

5. Discussion

5.1. Surface waters

In the surface waters at two sites in the central North Pacific, the concentration of the iron-binding ligands exceeds the concentration of the total dissolved Fe by 1.5 to 2.5 nM. These ligands can be modeled as being composed of stronger (L_1) and weaker (L_2) classes of Fe-binding organic ligands, with the concentration of both ligand classes exceeding the ambient dissolved Fe concentration. Using the average of the results obtained in the surface waters at station ALOHA, we estimate that 0.21 nM of the 0.44 nM L_1 is chelated to the ambient dissolved Fe ($[FeL_1] = 0.21$ nM), leaving 0.23 nM of excess L'_1. Only 0.03 nM of the 1.5 nM of L_2 is chelated with Fe ($[FeL_2] = 0.03$ nM), leaving essentially all of L_2 as excess chelator available to bind Fe entering surface waters ($[L'_2] = 1.5$ nM). The side reaction coefficients of these excess concentrations of L′ are $\alpha'_{FeL_1} = 2800$ and $\alpha'_{FeL_2} = 450$, yielding an overall $\alpha'_{FeL} = 3300$. This means that only 0.03% of the dissolved Fe will exist as Fe′, vs. 99.97% as FeL. Thus, the equilibrium concentration of inorganic Fe′ in the surface waters of station ALOHA is estimated to be only 0.07 pM. The equilibrium concentration of Fe′ in the surface waters of the VERTEX V station T4 site is only 0.01 pM. A concentration of Fe′ of 10^{-14} M is equivalent to a concentration of free Fe^{3+} ions of 1×10^{-24} M, less than 1 hydrated Fe^{3+} ion per liter!

It should be noted that these are the first samples on which we have applied our new method. Although the results unequivocally point to the dominance of Fe speciation by organic complexation, our interpretation of these data suggesting two discrete classes of Fe-chelators is somewhat model dependent. For this initial study we set up a competitive equilibrium with salicylaldoxime in which $\alpha'_{Fe(SA)_2} = 73$, a value which in hindsight turns out to be relatively low compared to the α'_{FeL} observed for the natural Fe-binding ligands of approximately 3300. As a result, the characterization of the L_1 ligand class is based upon relatively small analytical signals. In future studies we plan to both increase the adsorption time (to enhance the sensitivity) and to perform some titrations with a higher concentration of SA (thus increasing $\alpha'_{Fe(SA)_2}$ in order to better compete with stronger Fe-chelators), thereby allowing us to verify and better characterize the stronger classes of Fe-chelators.

Our average conditional stability constant of the strong Fe-binding ligand of $K^{cond}_{L_1/Fe'} = 1.2 \times 10^{13}$ M^{-1} is equivalent to a $K^{cond}_{L_1/Fe^{3+}} = 1.2 \times 10^{23}$ M^{-1}. The conditional stability constant with respect to Fe′ depends on our assumption about the inorganic side reaction coefficient, $\alpha_{Fe'} = 10^{10.0}$. Recent estimates of $\alpha_{Fe'}$ at pH 8 range from $10^{10.0}$ (Hudson et al., 1992) to $10^{11.5}$ (Byrne et al., 1988). If for example $\alpha_{Fe'}$ is assumed to be $10^{11.0}$, then the estimate for $K^{cond}_{L_1/Fe'}$ becomes an order of magnitude lower, $K^{cond}_{L_1/Fe'} = 1.2 \times 10^{12}$ M^{-1}. In this case, 99.7% of the Fe(III) would be complexed and 0.3% would exist as Fe′ (vs. 99.97% and 0.03% as reported above). Thus, even if our choice of $\alpha_{Fe'}$ is off by more than an order of magnitude, our calculations still suggest that the ambient dissolved Fe(III) is more than 99% organically complexed.

Other determinations of iron speciation in seawater are from van den Berg and coworkers using a CLE/ACSV method with 1-nitroso-2-napthol (1N-2N) as the competing ligand. Gledhill and van den Berg (1994), working at pH 6.9, found a class of Fe-binding ligands in North Sea coastal waters having conditional stability constants with respect to Fe^{3+}, $K^{cond}_{L/Fe^{3+}}$, falling within the range of $10^{19.8}$–$10^{22.5}$ M^{-1}. Van den Berg (1995-this volume) obtained values from the Mediterranean Sea using a CLE-ACSV method with 1N-2N at pH 8.0. He determined the concentration of Fe-binding ligands to range from 4 to 13 nM (dependent upon the analytical window used) and an average $K^{cond}_{L/Fe^{3+}} = 10^{21.8}$ M^{-1}. Van den Berg chose a value for $\alpha_{Fe'}$ of $10^{11.4}$, which results in a value of $K^{cond}_{L/Fe'} - 10^{10.4}$ M^{-1}. His value for $K^{cond}_{L/Fe^{3+}} = 10^{21.8}$ M^{-1} is close to that which we observed for our weaker L_2 class of ligands. Van den Berg estimated that iron is more than 99% complexed by this class of natural organic ligands throughout the water column in the Mediter-

ranean Sea. Thus, there is some indication that organic complexation of Fe by this class of ligands may be a ubiquitous feature in the oceans.

In the Mediterranean Sea studies (van den Berg, 1995-this volume), titrations at four different 1N-2N concentrations were performed with an $\alpha'_{Fe(NN)_3}$ varying from 0.4 to 320. He observed that the ligand concentration determined from the titration data decreased from 13 to 5.7 nM, and the conditional stability constant ($K^{cond}_{L/Fe^{3+}}$) increased from $10^{19.4}$ to $10^{22.5}$ M^{-1}, as the $\alpha'_{Fe(NN)_3}$ was increased from 0.4 to 320. This observation prompted van den Berg to suggest that "there are perhaps several Fe complexing ligands in the seawater". He did not, however, observe the presence of a low concentration of stronger Fe-binding ligands such as the L_1 class which we detected in the surface waters of the central North Pacific. While low concentrations of L_1-type ligands may have been present, they would likely have been masked by the relatively high concentration of dissolved Fe (3 nM) present in the surface waters of the Mediterranean Sea. The concentration of dissolved Fe in the surface waters of the central North Pacific is over an order-of-magnitude lower than that found in the Mediterranean Sea studies.

Wu and Luther (1995-this volume) used a slight modification of the method of Gledhill and van den Berg (1994) to investigate potential Fe(III)-organic complexation in surface waters of the Northwest Atlantic Ocean. These studies were carried out at pH 6.9 with just 1N-2N (13.6 μM) and PIPES buffer. They observed that dissolved Fe at the slope and shelf water stations was largely present as 1N-2N nonlabile Fe species which they attributed to either stable Fe-organic complexes, inorganic crystalline Fe hydrolysis products, and/or small Fe colloids. Wu and Luther reported evidence that Fe-binding organic ligands from shelf and slope environments have different kinetics for complexing added Fe and of equilibration with added 1N-2N. Using a kinetic analysis on one of their stations, they determined a value of $K^{cond}_{L/Fe^{3+}} = 10^{20.6}$ M^{-1}, a value within the range of that observed by Gledhill and van den Berg (1994) for North Sea samples.

Fe enrichment experiments (Martin et al., 1989) have been used to investigate whether phytoplankton are limited by low Fe concentrations in remote HNLC regimes. Our work makes it clear that it is important to know the ambient conditions of the system which control Fe speciation (e.g. not just $[Fe_T]$, but also $[L_T]$'s and their respective $K^{cond}_{L/Fe'}$, and therefore [Fe']) in order to properly interpret the chemical speciation as a function of added Fe. For example, nanomolar additions of Fe do not yield nanomolar concentrations of Fe' (Fig. 7). Given the above ambient conditions at station ALOHA, the [Fe'] is a nonlinear function of $[Fe_T]$. An Fe addition of 1 nM

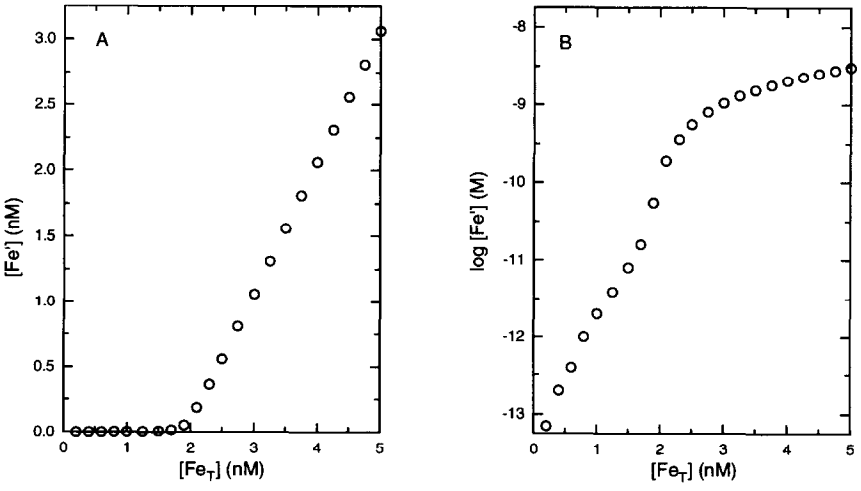

Fig. 7. Effect of hypothetical Fe additions on the chemical speciation of dissolved Fe. Initial values are the average ambient conditions for surface waters at station ALOHA: $[L_{1T}] = 0.44$ nM with $K^{cond}_{L_1/Fe'} = 1.2 \times 10^{13}$ M^{-1} and $[L_{2T}] = 1.5$ nM with $K^{cond}_{L_2/Fe'} = 3.0 \times 10^{11}$ M^{-1}.

increases the [Fe′] from 0.07 to 2 pM, with > 99.7% of the added Fe ending up in the form of [FeL], not [Fe′]. It is not until the added Fe completely titrates the total Fe-binding organic ligands (0.44 nM of L_1 and 1.5 nM of L_2) that we begin to see the added [Fe_T] appear principally as [Fe′]. It is as yet unknown whether excess concentrations of such strong Fe-chelators exist in low-Fe HNLC regimes.

Coincidently, Fe additions in shipboard grow out experiments generally range from 1 to 10 nM (Buma et al., 1991; Martin et al., 1991). If Fe complexation in these waters resembles that at station ALOHA,

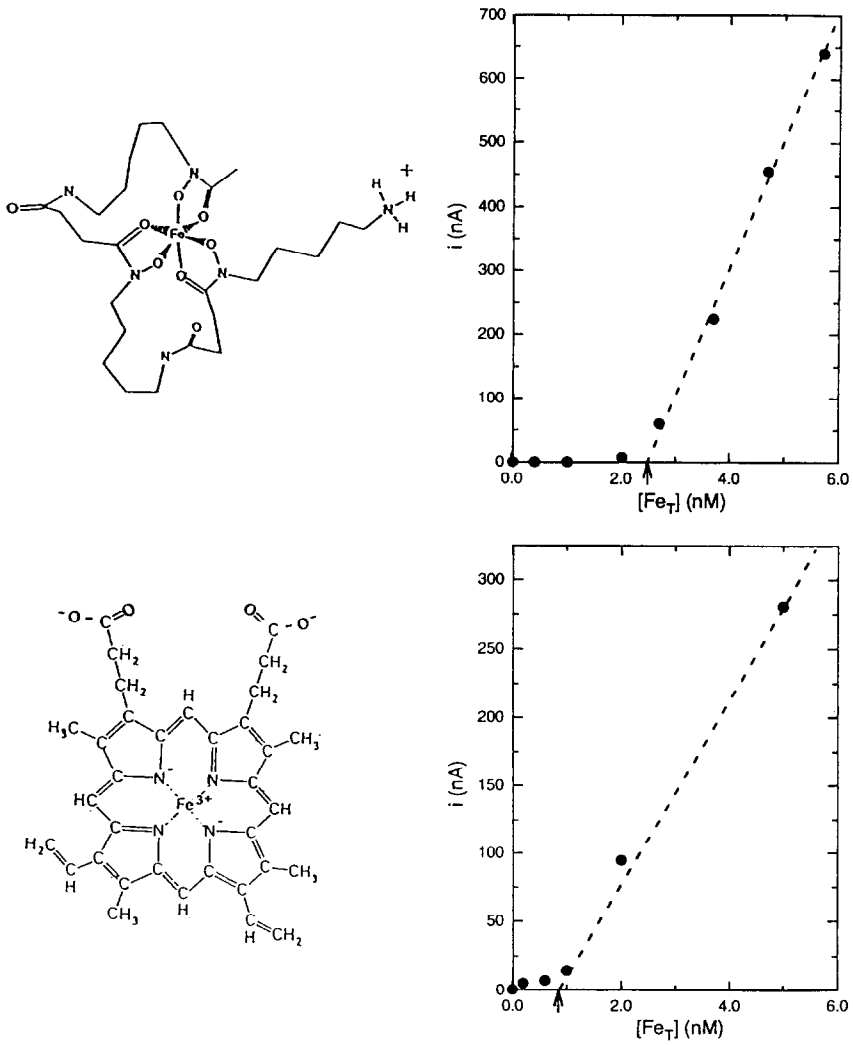

Fig. 8. (top, left) Structure of the Fe-Desferal® chelate (from Crumbliss, 1991). (right) CLE/ACSV Fe titration of 2.5 nM Desferal® added to UVSW (adsorption time was 15 min). The dashed line has a slope equivalent to that for which the Desferal® has been fully titrated by the added Fe. It intersects the x-axis at the [L_T] = 2.5 nM, as calculated using the slope of all the points from the linear Langmuir transformation. (bottom, left) Structure of the Fe-protoporphyrin-IX chelate. (right) CLE/ACSV Fe titration of 0.09 nM protoporphyrin-IX added to UVSW (adsorption time was 15 min). The dashed line has a slope equivalent to that for which the protoporphyrin-IX has been fully titrated by the added Fe. It intersects the x-axis at the [L_T] = 0.9 nM, as calculated using the slope of all the points from the linear Langmuir transformation.

additions at the low end of this range would be less than the excess Fe-binding ligand. Additions at the high end, where the Fe addition would exceed the Fe-chelator concentration, would lead to a substantially different chemical speciation of Fe. Fe additions on the order of 1 nM or less would be "buffered" by the excess Fe-chelator and maintained in solution as FeL. In contrast, Fe additions near 10 nM would have titrated the Fe-chelator concentration and presumably be partially precipitated as colloidal hydrous ferric oxide.

Dissolved inorganic Fe′ is currently thought of as the chemically relevant form of Fe for phytoplankton uptake (Hudson and Morel, 1990). The ambient equilibrium [Fe′] at station ALOHA, however, is on the order of 0.07 pM and at VERTEX V station T4 it is only 0.01 pM. These low Fe′ values are well below diffusion limited concentrations for adequate iron uptake by oceanic nanoplankton (> 2.0 μm in size) (Hudson and Morel, 1993). Moreover, values obtained here also appear to be below the diffusion limit for even the smaller picoplankton, strongly suggesting that some portion of the organically-bound iron must become biologically accessible for phytoplankton growth to be possible. These results raise a series of intriguing questions: what are the sources of these Fe-binding ligands, how accessible is this chelated Fe to phytoplankton and heterotrophic bacteria, and what are the mechanisms by which the biota can access this chelated Fe to fulfill their Fe requirements?

5.2. Model ligand studies

It is possible that these organic ligands could enhance Fe uptake by some phytoplankton species if they are part of a siderophore uptake system. Microorganisms isolated from coastal and oceanic habitats have been shown to produce siderophores in iron deficient culture media (Trick et al., 1983, Trick, 1989; Reid et al., 1993; Haygood et al., 1993). In no cases, however, have soluble Fe–siderophore complexes been identified in seawater samples, although little effort has been directed toward their detection. We investigated the complexation of Fe with the well-characterized siderophore, desferal, produced from a terrestrial fungus, as a model for the natural Fe-chelators found in the oceans. The Fe-desferal complex is shown in Fig. 8 (top, left). Results of an Fe titration of 2.5 nM desferal added to UVSW are presented in Fig. 8 (top, right). Our titration data resulted in a measured $[L_T]$ of 2.5 nM and a value for $K^{cond}_{L/Fe'}$ of $> 10^{13}$ M^{-1}. The value calculated from constants found in the literature is $K^{cond}_{L/Fe'} = 10^{16.5}$ M^{-1}. This experiment provides a good check on the ability of our method to accurately determine $[L_T]$, and demonstrates that the conditional stability constants are of the right order, although in this case the siderophore's stability constant was too high to accurately determine with the relatively low $\alpha'_{Fe(SA)_2}$ we used. This experiment also demonstrates that Fe–siderophore complexes such as Fe-desferal are not elecrochemically active with respect to our ACSV method.

We cannot exclude the possibility that there is yet another subclass of natural Fe-chelators present in surface seawater in addition to L_1 and L_2. If this ligand class had an even stronger conditional stability constant (such as desferal) and existed at 0.1 or 0.2 nM concentrations, we would not have been able to quantify it. In the Fe titrations, we generally did not detect measurable $[Fe(SA)_2]$ until after approximately 0.1 nM of Fe was added (our detection limit for $[Fe(SA)_2]$ was approximately 10 pM). Thus, there could be 0.1 or 0.2 nM of stronger Fe-binding ligands present in surface seawater in addition to the ligands we report. As noted above, our future plans with longer deposition times and with increased concentrations of SA will help us better understand the range of Fe-chelator affinities in seawater, and thus, characterize any classes of even stronger Fe-binding ligands that may exist.

In HNLC regimes, a substantial fraction of the total Fe in surface waters is associated with microorganisms as particulate Fe and is recycled on time scales of days (Hutchins et al., 1993). One major biological reservoir is the Fe-porphyrin moiety in cytochromes Fe-chelates which might be released during grazing (Hutchins and Bruland, 1994) or cell lysis. Biomolecules such as porphyrins might be a source of the excess Fe-chelators we observe in solution. However, the original Fe would have to be first removed from the complex during cell-lysis or grazing, or outcompeted by other stronger Fe-chelators in solution. As a model of this type of ligand, we investigated the complexation of Fe with proto-

porphyrin-IX, the cofactor of heme-containing compounds. The structure of the Fe-protoporphyrin-IX compound is shown in Fig. 8 (bottom, left). The results of an Fe titration of 0.90 nM protoporphyrin-IX added to UVSW are presented in Fig. 8 (bottom, right) and yielded a value for $[L_T] = 0.92$ nM, in excellent agreement with the amount added. We determined a $K^{cond}_{L,Fe'} = 10^{12.0}$ M^{-1}, but were unable to find literature values in order to calculate a conditional stability constant for Fe-protoporphyrin-IX in seawater to compare with our determined value. The measured conditional stability constant for Fe-protoporphyrin-IX is similar, however, to the conditional stability constants determined for the naturally occurring Fe-chelators we observe in the water column.

Reid et al. (1993) isolated and characterized a siderophore, alterobactin A, from a marine heterotrophic bacteria from Barbados. They determined a value for $K^{cond}_{L/Fe^{3+}}$ by competition with EDTA in a 0.1 M buffer solution to be $10^{20.7}$ M^{-1}. Using an estimate for the side-reaction coefficient $\alpha_{L'}$ of 10^{28} to 10^{32} (the ratio of the sum of all protonated forms of L (e.g. HL^{5-}, H_2L^{4-}, H_3L^{3-}, etc.) to that of the free L^{6-} species), they calculated the thermodynamic stability constant between free L^{6-} and Fe^{3+} to be $K_{L/Fe^{3+}} = 10^{49-53}$ M^{-1}. Alterobactin A has such a large side reaction coefficient because of the extremely high pK_a of the hydroxyl protons of the two B-hydroxyaspartic acid functional groups. If we assume that any Ca^{2+} or Mg^{2+} side reactions are minor compared to the high side reaction with protons, and that any ionic strength effects due to the difference between the 0.1 M buffer solution and that of seawater will be minor, then a conditional stability constant of alterobactin A with respect to Fe′ in seawater can be estimated to be $K^{cond}_{L/Fe'} = 10^{10.7}$ M^{-1}.

This value is on the low side relative to the conditional stability constants we determined for the L_1 and L_2 ligands in seawater. On the basis of the high value for the proton independent, thermodynamic stability constant of 10^{49-53} M^{-1}, Reid et al. (1993) made the claim that alterobactin A has an "extraordinary" affinity constant and that the Fe-alterobactin A stability constant is "... not exceeded by any other known siderophore,...". Once side-reactions have been taken into account, however, its conditional stability constant in seawater is relatively weak in comparison to the ligands we observe. Nanomolar concentrations of alterobactin A would be relatively ineffective at competing with the Fe-chelators already present. Desferal, on the other hand, has a thermodynamic stability constant of $10^{31.9}$ M^{-1}, a value roughly 20 orders-of-magnitude lower than that for alterobactin A. Desferal, however, has a relatively small side-reaction coefficient ($\alpha_{L'} = 10^{3.1}$) leading to a conditional stability constant in seawater with respect to Fe′ of $K^{cond}_{L/Fe'} = 10^{16.5}$ M^{-1}, a value roughly 6 orders-of-magnitude greater than that estimated for alterobactin A. Desferal would be much more effective than either alterobactin A or our observed L_1 and L_2 ligands at chelating Fe(III) in seawater.

It is important to stress that the thermodynamic stability constants (the normal mode for tabulating Fe-ligand stability constants) may not be a good indicator of the effective binding strength of organic ligands in seawater, because of the markedly different side reactions with H^+, Mg^{2+}, and Ca^{2+}. The conditional stability constant with respect to Fe′ is a more pragmatic approach which allows one to readily estimate the effective strength of an Fe-binding ligand in seawater. In a similar vein, Crumbliss (1991) utilized a pM value ($pM = -\log[Fe^{3+}]$) calulated from the observed H^+-dependent formation constant for a fixed set of physiologically relevant experimental conditions (pH 7.4, $[L_T] = 10$ μM, $[Fe_T] = 1$ μM) as an empirical approach to assess the effective strength of various siderophores.

These results with model ligands suggest, though do not prove, that perhaps some fraction of the organic ligands we observe in the Central North Pacific may be siderophores or other biomolecules such as porphyrins, and that these ligands could originate from phytoplankton and/or heterotrophic bacteria. The conditional stability constants of Fe′ with these model ligands such as alterobactin A, protoporphyrin-IX, and desferal extend from the low to the high end of those we report here for the Fe-binding ligands we find in surface seawater.

5.3. Deep waters

In the intermediate and deep waters (depths of 500 to 2000 m), the Scatchard and Langmuir transformations of the Fe titrations were linear and did not indicate a significant presence of the stronger L_1

class of Fe-binding ligands. Instead, we interpreted the data as having only the L_2 ligand class. Average values for intermediate and deep waters were $[Fe_T] = 0.72 \pm 0.05$ nM, $[L_{2T}] = 2.6 \pm 0.2$ nM, and $K^{cond}_{L/Fe'} = 4.0 \pm 1.6 \times 10^{11}$ M^{-1}. Using these values, 99.9% of the dissolved Fe would exist chelated with L_2, and only 0.13% or 0.9 pM would exist as Fe'. A possible reason that we did not detect the occurance of any of the strong L_1 ligand in the deep waters may be due to the concentration of Fe_T being in excess of L_{1T}; it may be present, but since it is completely titrated by the ambient dissolved Fe concentration, we may be unable to detect L_1. We observed the presence of the strong class of L_1 ligands within the surface 300 m (Fig. 6A) where the concentration of L_1 was in excess of $[Fe_T]$. In intermediate and deep waters, the second, weaker class of ligands, L_2, was still present, however, and dominated the speciation of dissolved Fe.

It should be noted that we performed the Fe titrations of these samples on board ship at 1 atm pressure, a temperature of approximately 25°C, and a pH of 8.0. These conditions are markedly different than those at a depth of 1000m (e.g. 100 atm, 5°C, and pH 7.6). Byrne et al. (1988) estimated that there would be a marked difference in $\alpha_{Fe'}$ between surface waters (25°C and pH 8.0) and 1000 m waters (5°C and pH 7.6) due to these temperature and pH differences. Their estimate of $\alpha_{Fe'}$ was lower by 2.7 orders of magnitude in the cold, more acidic, deep waters than in warm pH 8 surface waters. As yet, we do not know the temperature, pressure, or pH dependency of the conditional stability constants of the natural Fe-chelators. If acidic functional groups such as catechols and/or hydroxamic acids (characteristic of siderophores; Crumbliss, 1991) are important components of the natural Fe-chelators, then there could be a significant pH effect, with the α'_{FeL} decreasing in the more acidic deep waters. However, the corresponding decrease in the inorganic side reaction coefficient, $\alpha_{Fe'}$, may counteract and thus minimize any decrease in $K^{cond}_{L/Fe'}$ due to pH. The standard enthalpy changes for Fe(III) complexing with catechols and hydroxamates is relatively small, so that there may be little temperature effect upon complexation. At present, not enough is known about the natural Fe-chelators to permit confident extrapolation about pH, temperature and pressure effects.

The observation of an excess of relatively strong Fe-chelators in deep waters contrasts with results obtained for Zn and Cu in this same region. Anodic stripping voltammetry measurements reported by Bruland (1989) indicate that 98.7% of the zinc in waters shallower than 200 m is chelated with 1.2 nM of a relatively strong Zn-specific organic ligand or ligand class. But in deep waters, the speciation of Zn was dominated by inorganic speciation. Likewise, Coale and Bruland (1988, 1990) reported that 99.7% of total dissolved Cu in waters shallower than 200 m from this region exists as strong Cu-chelates. The stronger ($L_1 \approx 1.8$ nM) of two Cu-complexing organic ligands dominates Cu speciation in these surface waters; its concentration exceeding the surface dissolved Cu concentrations. Like Zn, the combination of increasing Cu concentrations and decreasing Cu chelator concentrations with depth results in organic complexation being much less important for Cu in deep waters.

In the case of Fe, the L_2 chelator concentration remains in excess of the total dissolved Fe, and therefore Fe is significantly chelated ($> 99\%$) throughout the water column. The relatively uniform concentration of the L_2 class of Fe-chelators, along with its relatively uniform conditional stability constant, raises interesting questions. Such a conservative distribution normally implies that the L_2 class of Fe-chelators would be relatively inert with a long residence time relative to physical mixing processes. It may be, however, that production and destruction rates are closely balanced and that it is much more reactive than the profile would imply. With the limited amount of data available at this time, the stronger Fe-binding ligand, L_1, also has a relatively uniform distribution within the surface 300 m. Such constant concentrations of the Fe-chelators might be expected if they were primarily microbially produced siderophores with regulated concentrations.

6. Conclusions

We present a highly-sensitive CLE/ACSV method to characterize the extent to which the speciation of dissolved Fe in seawater is influenced by complexation with organic chelators. We applied this new method for the first time to a profile at station

ALOHA in the central North Pacific. An excess of ~0.2 nM of strong chelators (L'_1) and 1.5 nM of weaker chelators (L'_2) above ambient dissolved Fe exist within the surface waters of the Central North Pacific. The average conditional stability constants of these ligands are $K^{cond}_{L_1/Fe'} = 1.2 \times 10^{13}$ M^{-1} ($K^{cond}_{L_1/Fe^{3+}} = 1.2 \times 10^{23}$ M^{-1}) and $K^{cond}_{L_2/Fe'} = 3.0 \times 10^{11}$ M^{-1} ($K^{cond}_{L_2/Fe^{3+}} = 3.0 \times 10^{21}$ M^{-1}). We estimate that 99.97% of the dissolved Fe(III) in these surface waters is chelated with these ligands, which serve to buffer the equilibrium concentration of [Fe']. The L_2 class of chelators is also present in the deep waters, with 2 nM excess ligand. Characterizing the nature of these chelators which control the chemical speciation of dissolved Fe is essential in order to understand the factors regulating the biological availability and biogeochemical cycling of iron in seawater.

The existence of an excess of strong iron-binding ligands in the oceans alters the fundamental basis of our understanding of oceanic Fe speciation and biological availability. These results lead to numerous unanswered questions:

(1) What are the biological roles of the Fe-binding ligands?
(2) What organisms and/or what processes are producing them?
(3) By what mechanisms and at what rates do the chelated forms of Fe become biologically available?
(4) To what degree do these Fe-chelators buffer the dissolved Fe in a less particle-reactive form, thereby increasing the residence time of iron in seawater, particularly surface seawater?

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