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Biochemistry

A cadmium enzyme from a marine diatom

The ocean biota contains a vast reservoir of genomic diversity¹. Here we present the sequence and preliminary characterization of a protein that is a cadmium-containing carbonic anhydrase from the marine diatom *Thalassiosira weissflogii*. The existence of a cadmium enzyme in marine phytoplankton may indicate that there is a unique selection pressure for metalloenzymes in the marine environment², and our discovery provides a long-awaited explanation for the nutrient-like behaviour of cadmium in the oceans³.

In marine diatoms, the metals cadmium, cobalt and zinc can functionally substitute for one another to maintain optimal growth rates. This effect is at least partly due to metal replacement in the metal-binding site of the enzyme carbonic anhydrase⁴, which is involved in the acquisition of inorganic carbon for photosynthesis. In addition to a zinc carbonic anhydrase that can substitute cobalt in its active site *in vivo*⁵, *T. weissflogii* has a putative cadmium carbonic anhydrase that is also involved in acquiring inorganic carbon⁶.

We purified a protein, CDCA1, from this organism that has carbonic anhydrase activity and contains cadmium (Fig. 1a, b; Genbank accession number AY772014). Determination of its sequence was complicated by the presence of a triple repeat (see supplementary information): the three amino-acid sequences are virtually identical (about 85% identity), but there is more variation in their encoding DNA (about 78% identity). Contrary to an earlier estimate⁶, we have determined the relative molecular mass of CDCA1 as about 69K (for methods, see supplementary information). CDCA1 has probably not been sequenced before, as there are no hits in the NCBI database. It is significantly different from any of the known major classes of carbonic anhydrases^{7,8}, and therefore represents the first member of a new class of carbonic anhydrases, the ζ class.

The genome of *T. pseudonana* contains a single sequence that is highly homologous to

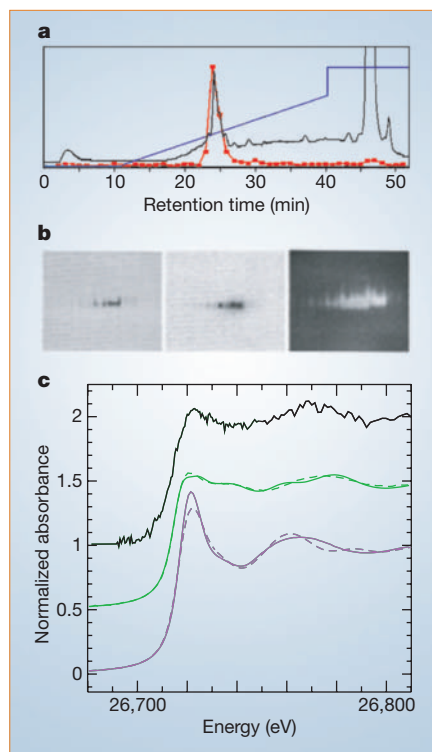


Figure 1 Characterization of the carbonic anhydrase CDCA1 from a marine diatom. **a**, High-pressure liquid chromatography (HPLC) of the CDCA1 enzyme, showing co-elution of purified CDCA1 (black trace, absorbance at 280 nm) and of ¹⁰⁹Cd-radiolabelled enzyme (red, c.p.m., 330 c.p.m at peak) at 24 min. Blue line, NaCl gradient. **b**, Non-denaturing gel electrophoresis of HPLC fractions containing the main peak of protein and radiolabel shows an intense Coomassie-blue-stained protein band (left) that co-migrates with the ¹⁰⁹Cd radiolabel (centre) and carbonic anhydrase activity (right). **c**, Cadmium K-edge X-ray absorption spectra from purified CDCA1 (top) compared with two tetrahedral, thiolate-coordinated species, [Cd(SPh)₄](Me₃N)₂ (green solid line), cadmium phytochelatin (green broken line), and with two octahedral species [Cd(H₂O)₆]²⁺ (purple solid line) and [Cd(midazole)₆](NO₃)₂ (purple broken line). The similarity of the CDCA1 spectrum to that of the tetrahedral species suggests that the metal site has tetrahedral symmetry and involves cysteinyl ligands to the metal. (For methods, see supplementary information.)

the three repeats in *T. weissflogii*, corresponding to a protein of 25.5K (see supplementary information). The presence of expressed-tag sequences shows that this putative cadmium-containing carbonic anhydrase is expressed, indicating that only a single repeat of CDCA1 may be necessary for activity. The amino-terminal sequence of the *T. pseudonana* enzyme (not available for CDCA1) has only 15 amino acids preceding the homologous sequence from *T. weissflogii*.

X-ray absorption near-edge spectroscopy of the purified protein confirms the presence of the cadmium-binding site (Fig. 1c). Comparison with standards⁹ indicates that the site probably has a roughly tetrahedral geometry and that the cadmium ion is bound by two or more thiolates — as in the zinc-containing β class of carbonic anhydrases in higher plants, which have two cysteines and a histidine at the metal-binding site¹⁰. The spectra are also consistent with an

active site containing an activated water molecule, as in other carbonic anhydrases.

Gene-expression analysis shows that there is an increase in the abundance of *cdca1* transcripts within 1 hour of increasing the concentration of cadmium or of decreasing the partial pressure of carbon dioxide in the medium (results not shown). Expression of CDCA1 may therefore be controlled in part by the availability of cadmium and carbon dioxide in sea water.

High-throughput sequencing of a sea-water sample has revealed that the marine environment may contain unique genes¹. We have identified and partially characterized one such gene — for a carbonic anhydrase from a marine diatom that, to our knowledge, is the first native enzyme so far discovered to contain cadmium. Because of the extraordinarily low concentrations of many essential trace metals in sea water, it is likely that there are other metalloenzymes in marine organisms that use unusual metals for activity and contribute to trace-metal geochemical cycling in the oceans.

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Corrigendum

Animal behaviour: Elephants are capable of vocal learning

J. H. Poole, P. L. Tyack, A. S. Stoeger-Horwath, S. Watwood *Nature* **434**, 455–456 doi:10.1038/434455a (2005)

The African elephant Calimero spent 18 years with two Asian elephants at Bioparco in Rome, Italy, not the Basel Zoo, Switzerland. He was later transferred to Basel, where the recordings described in this Brief Communication were made.