

distance and charge considerations lead us to conclude that this density is due to a third Zn^{2+} (no other metals have been detected in native PLC), which is liganded to the amino and carboxyl groups of Trp 1 to form a five-membered chelate ring similar to that observed in many Zn-amino acid complexes (for example, with aspartic²⁰ and glutamic²¹ acids). The third Zn^{2+} is also coordinated to His 14 and Asp 122.

The resulting constellation of metal ions resembles that in alkaline phosphatase from *E. coli*¹⁴; one of a family of enzymes that have an absolute requirement for Zn^{2+} but can bind other metals with retention of activity²². The Zn-Zn distances in PLC are similar to those reported for Cd-substituted alkaline phosphatase¹⁴. Interestingly, the synthesis of both PLC and alkaline phosphatase are P_i -repressed in *B. cereus* (P. M. Guddal, K. Schulstad, T. Johansen and C.L., unpublished observations) and together represent a P_i retrieval system for the bacterium. The similarity in the active sites in the two enzymes may therefore be more than coincidental (alkaline phosphatase hydrolyses the product from PLC).

Figure 3a shows the environment of the metal ions. Asp 122 forms a carboxylate bridge between Zn1 and Zn3 (Zn-Zn distance 3.3 Å). Two such bridges occur in myohemerythrin (Fe-Fe, 3.23 Å) (ref. 23) and are probably formed by Asp 51 in AP (ref. 14) and between the metal ions in DNA polymerase I (ref. 16). A molecule of water or OH^- forms a second bridge between Zn1 and Zn3; again this is similar to the oxide bridge in myohaemerythrin. The coordination of Zn2 resembles that in carboxypeptidase²⁴, except that only one of the carboxyl oxygens from Glu 146 is bonded to the metal ion. Metal coordination is completed by two further water molecules so that all three metal ions are approximately trigonal bipyramidal. Histidines 14 and 118 are approximately parallel and are oriented in a manner which resembles charge transfer stacking with a ring-ring distance of 3.7 Å.

Atomic absorption analyses of the metal content of PLC (refs 12, 19 and 25) have consistently indicated two metal ions per mol of PLC and the same is true of a solution state EXAFS study²⁶. The metal stoichiometry, however, was calculated from atomic absorption analysis using a relative molecular mass (M_r) of 23,000 obtained from calibrated gel filtration²⁷. Recalculation using the true M_r of 28,520 or 28,585, in the case of three Zn ions³, gives 2.3 Zn ions per mol PLC, indicating that the third metal site is at least partially occupied even in solution. Furthermore, both the analyses and the EXAFS study were carried out on PLC after an ion-exchange^{12,13,19,26} or multi-stage dialysis treatment²⁵ to remove unbound metal ions, a treatment which would also remove loosely bound metal ions. By contrast, crystallization was performed in the presence of $\sim 10 \mu M Zn^{2+}$. □

Note added in proof: PLC has extensive homology with the first two-thirds of the recently published sequence of the alpha-toxin (phospholipase C) of *Clostridium perfringens*³⁸⁻⁴⁰.

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ERRATA

Arachidonic acid metabolites as intracellular modulators of the G protein-gated cardiac K^+ channel

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FIGURES 2 and 3 of this letter were inadvertently transposed during the editing process.

Geochemical implications of the formation of the Moon by a single giant impact

H. E. Newsom & S. R. Taylor

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THE cover for the 2 March issue of *Nature* (shown right), illustrating the Review Article by Newsom and Taylor, was not properly credited. The computation to provide the image was done at Sandia National Laboratories, Albuquerque by Marlan Kipp and H. J. Melosh. Calculations were performed using the new 3-D code CTH on a Cray I XMP with a solid-state hard disk. The full computation required some 60 hours of computational time.

