

03-Crystallography of Biological Macromolecules

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transient oxidation states of the binuclear iron center in MMO. The residues lining the proposed oxygen binding site in MMO are significantly smaller in MMO than in R2 allowing binding of both molecular oxygen and methane at this site. This binding site is lined by residues Cys151, Thr213, Ile217 and Ile(Val)239.

This binding site in R2 can be involved in other reactions than producing the radical species of the protein as shown by the mutation of Phe208 to Tyr. This Tyr is transformed into a catechol in the oxygen reaction and a blue protein with ferric dopa interaction is created. The structure of this mutant has been determined at 2.5 Å resolution. The coordination geometry is changed significantly and the dopa 208 and Glu238 both become bidental.

PS-03.04.26 THE ROLE OF THE METAL AND IMPORTANT ACTIVE SITE RESIDUES IN ENZYMATIC CATALYSIS OF ZINC PROTEINASES. By H. Feinberg, H. M. Greenblatt, O. Almog, A. Spungin, D. Ben-Meir, S. Blumberg and G. Shoham*, Department of Inorganic Chemistry and The Laboratory of Structural Chemistry and Biology, The Hebrew University of Jerusalem, Jerusalem 91904, Israel, and Sackler Institute of Molecular Medicine, Tel Aviv University, Tel Aviv 60078, Israel.

Several series of crystallographic studies, combined with enzyme kinetics, surface interactions and electrostatic potential analysis, have been performed in order to determine the yet unknown general mode of catalysis of zinc proteinases. For the detailed structural analysis we chose the one-zinc carboxy-exo-proteinase bovine carboxypeptidase A (CPA) and the two-zinc amino-exo-proteinase *Streptomyces griseus* aminopeptidase (SGAP). In attempts to fully understand the role of the zinc ions in these and other zinc proteinases, and in zinc enzymes in general, several metal derivatives have been prepared and their kinetic behavior studied. These metal-substituted enzymes show very interesting pattern of activities towards various peptide and ester substrates. The catalytic activity is shown to change dramatically with the particular metal substituted and with the specific substrate chosen. Although some crystallographic studies of metal substituted zinc proteinase derivatives have been carried out in the past, no satisfactory explanation has been given to the large kinetic deviations observed. Moreover, the specific role of the active site zinc ion(s) in both the binding of the substrate and the actual enzymatic hydrolysis has never been fully clarified for this family of metallo proteinases.

We have prepared apo-CPA (zinc removed) in which no trace of zinc remains, and have used the resulting enzyme for the preparation of a series of metallo-CPA derivatives. These derivatives were crystallized and their crystal structures were determined at high resolution. In addition to the detailed structures of the metallo-derivatives themselves, we have also analyzed the structures of their complexes with a number of inhibitors and reaction coordinate analogues.

The purpose of these studies was to clarify the role of the active site metal in enzymatic catalysis and especially to ascertain whether the observed changes in the activity of metallo-derivatives of CPA (or zinc proteinases in general) are due to differences in the way in which the metal is bound to the enzyme, due to local changes in the conformation of the active site, or whether different metals affect the binding of substrates in various ways.

Similar studies were also carried out on the apo-enzyme, in order to determine the effect of the zinc ion on the so called "Michaelis-Menten" complex, formed before any reaction takes place. We will present the high resolution and refined structures of native-, apo-, and metallo-enzymes described above, both alone and in complex with various effectors, and discuss the relevance of these data to the role of zinc in biological catalytic activity. Results will be presented of electrostatic potential calculations which are based on the structures determined. These results indicate that in addition to the roles of substrate binding and transition state charge stabilization, the active site metal plays also an important role in long range attraction of the substrate into the active site. Surface interactions in the structures of the enzyme-effector complexes analyzed will be considered for the future rational design of inhibitors and second generation analogues.

Preliminary crystallographic data will be presented for the calcium activated aminopeptidase SGAP. In addition to its importance for the understanding of zinc proteinases this enzyme represents a new class of low molecular mass aminopeptidases of various potential biotechnological applications.

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Magnetostuctural behaviour of the superexchange coupled Cu-dimers

C. Erasmus, W. Haase

*Institut für Physikalische Chemie, TH Darmstadt
Petersenstraße 20, D-6100 Darmstadt, Germany*

Some bioinorganic molecules are formed by exchange coupled Cu-dimers in the active site. The kind of diamagnetic ligands between the paramagnetic centres can vary.

The contribution is summing up the related magnetostuctural results even over extended bridges (~ 11 Å), in general different ligands, e.g. hydrogen bridges.

Theoretical calculations of the exchange coupling parameters are presented for long distance bridges, oximato groups and hemo-cyanin model compounds.

Some new crystal structures on Cu-oligomers are presented and discussed.