P3

Behavior of Copper and Zinc Ions in the Binding Sites of Superoxide Dismutase, Bovine Erythrocytes

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The authors are very much interested in the selective binding of copper and zinc of bovine erythrocytes superoxide dismutase [Cu_2Zn_2SOD; superoxide oxidoreductase, EC 1.15.1.1] in the respective native binding sites without mistake. In order to examine the behavior of metals, we determined the apparent binding constants of copper ions to the apo-SOD, Cu_2E_2SOD by equilibrium dialysis against 2-picolinic acid in various pH solutions [1]. These copper binding constants were found to be pH dependent [2].

Apo-superoxide dismutase, E_2E_2SOD can also bind four zinc ions to two native copper and two native zinc binding sites. Four stepwise apparent binding constants of zinc ions to the apo-SOD were determined similarly, by means of equilibrium dialysis against 2-PA in HEPES buffer of pH 6.25.

In order to investigate the selective binding of copper and zinc ions with apo-SOD, E_2E_2SOD in the respective native binding sites, the authors carried out equilibrium dialysis in the presence of $CuSO_4$ and $ZnSO_4$ in 0.1 *M* HEPES buffer on pH 6.5. They bind selectively to their respective binding sites in the ratio of Cu:Zn = 1:1, recovering SOD activity.

As the geometry of copper ions and zinc ions is not the same, we carried out the determination of the intrinsic apparent binding constants of two zinc ions by the competitive replacement between Cu_2E_2SOD and various concentrations of 2-picolinato-Zn complex, by means of equilibrium dialysis.

The authors have established the HPLC of native-SOD, apo-SOD and metal-replaced-SOD. It is much easier to identify the purity of self-purified and marketing SOD, very rapidly. This HPLC is also helpful in the chemical study of metalloenzyme coordination.

Determination of the apparent binding constants is very useful in the investigation of the active sites of Cu_2Zn_2SOD. The authors recommend HPLC as one of the best tools in the study of bioinorganic chemistry. The mechanism of SOD activity will be discussed.

TABLE I. Apparent Binding Constants of four Cu and four Zn SOD.

	k_1	k_2	k_3	k_4	pH
Cu	13.9	13.4	11.1	10.6	6.25
Zn	10.9	11.1	7.8	6.5	6.25

1 J. Hirose, K. Iwatsuka and Y. Kidani, *Biochem. Biophys. Res. Comm., 98,* 58 (1981).
2 J. Hirose, T. Ohira, H. Hirata and Y. Kidani, *Arch. Biochem. Biophys., 218,* 179 (1982).

P4

Kinetic Characterization of the Active Site Region of Carbonic Anhydrase

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In native carbonic anhydrase the coordinated zinc ion, which is part of the active site region, may be replaced by Co(II), with retention of enzyme activity. The Co(II)-substituted protein has spectral properties which make it more amenable to spectroscopic studies.

The protolytic reactions in the active site region of bovine Co(II)-carbonic anhydrase B have been studied by spectrophotometric titrations employing a new computer-controlled high performance titration system [1] and by temperature jump and electric field jump relaxation spectrometry. Among the fast elementary steps involved, it has been possible to differentiate between the initial protonation/deprotonation reactions, probably occurring at the surface region, an intramolecular proton transfer to the active site region and a rearrangement within the coordination sphere of the heavy metal ion.

The binding constants of the anionic inhibitors sulphate, cyanide, cyanate and thiocyanate to bovine Co(II)-carbonic anhydrase B have been determined employing the new spectrophotometric titration system. The experiments were carried out either by pH titrations at various inhibitor concentrations or by anion titrations at various pH values.

The dynamic properties of the anionic binding site of Co(II) carbonic anhydrase have been characterized on the basis of temperature jump studies. The kinetic results obtained from the investigation of the elementary steps involved in the binding of anionic inhibitors such as cyanide, cyanate and thiocyanate provide information about the mechanism of action of this enzyme. Evidence will be presented that the overall binding of monovalent anions must be characterized by a process comprising at least two steps, one corresponding to the