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O35

X-Ray Absorption Spectroscopy of 3-Fe Clusters in Fe–S Proteins

R. A. SCOTT, S. A. KAZMI

School of Chemical Sciences, University of Illinois, Urbana, Ill. 61801., U.S.A.

H. BEINERT, M. H. EMPTAGE

Institute for Enzyme Research, University of Wisconsin, Madison, Wis. 53706, U.S.A.

J. E. HAHN, K. O. HODGSON

Department of Chemistry, Stanford University, Stanford, Calif. 94305, U.S.A.

C. D. STOUT

Department of Crystallography, University of Pittsburgh, Pittsburgh, Pa. 15260, U.S.A.

and A. J. THOMSON

School of Chemical Sciences, University of East Anglia, Norwich, NR4 7TJ, U.K.

Fe EXAFS and edge spectroscopy have been used to characterize the 3-Fe clusters in aconitase and *Azotobacter vinelandii* ferredoxin I (*Av* Fd I). Fe EXAFS of a frozen solution of oxidized (unactivated) beef heart aconitase indicates a 'compact' cluster structure with Fe–Fe distances of *ca.* 2.7 Å. In combination with independent iron and acid-labile sulfur determinations, these results allow us to propose a structure for the aconitase 3-Fe cluster with a stoichiometry of [3Fe-4S].

Single crystal polarized X-ray absorption spectroscopy was performed on *Av* Fd I crystals with the goal of distinguishing Fe–Fe scattering contributions from the 4- and 3-Fe clusters. Crystallographic results on this protein suggest an 'extended' structure for the 3-Fe cluster with *ca.* 4.1 Å Fe–Fe distances. Polarized spectra were recorded with the X-ray polarization vector both normal to and parallel to the average plane of the irons in the 3-Fe clusters. The Fourier transforms were compared and found to be identical in the region in which a 4.1 Å scattering peak would be expected. Thus, the long (*ca.* 4.1 Å) Fe–Fe distance cannot be detected in *Av* Fd I crystals at room temperature. Low-temperature solution work is planned to look for evidence of this interaction.

O36

A Water Proton and Anion Affinity Investigation of Zinc(II) Deprived Superoxide Dismutase

I. BERTINI, C. LUCHINAT, R. MONNANNI and A. SCOZZAFAVA

Dipartimento di Chimica, Università di Firenze, Florence, Italy

E. BORGHI

ISSEC CNR, via Guerrazzi 39, Florence, Italy

In native superoxide dismutase, copper(II) is bound to four histidine nitrogens in a puckered square planar arrangement, the angles N–Cu–N for the two couples of trans nitrogen atoms being 160° and 230°, respectively. One of these nitrogens belongs to an histidinate residue bridging the zinc ion [1]. The EPR spectrum shows a large rhombic component [2]. Anions like CN^-, N_3^-, NCS^-, NCO^- substitute in our opinion for a histidine nitrogen [3, 4].

Zinc deprived SOD shows an axially symmetrical EPR spectrum [5] indicating that the removal of the bridging requirement leads to a less distorted chromophore. Water proton NMRD of solutions containing SOD have indicated the presence of a coordinated axial water molecule [6]. The same measurements performed on the zinc deprived derivative (Fig. 1) have shown that water is still present, although loosely bound as in the native SOD.

The affinity constants of the anions N_3^- and NCS^- at pH 6.0 for zinc deprived SOD (160, 26 M^{-1}) closely compare with those for the native enzyme (90,

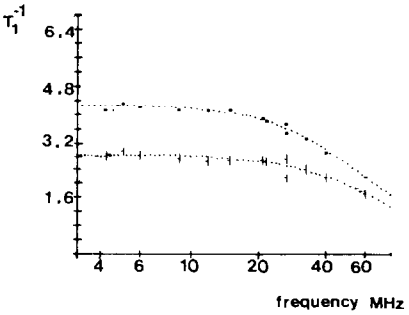

Fig. 1. Magnetic field dependence of the proton relaxation rate of aqueous solutions of native (■) and zinc deprived (+) SOD. The samples were 5.6×10^{-4} M protein, pH 6.0.

120 M^{-1}). These data indicate that anions do not substitute for the bridging histidine in native SOD.

The coordination geometry of the anion adducts is similar for the two enzyme derivatives and the major effect of zinc removal is probably that of providing a more tetragonal chromophore.

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O37

Diphenoloxidase from Human Erythrocytes

ANNA SISINI and GIAN GAVINO PINNA

Istituto di Chimica Biologica, Facoltà di Medicina, via Muroni 23, Sassari, Italy

MARIA LUISA GANADU and CATERINA SELLITO

Istituto di Chimica Generale, Facoltà di Scienze MFN, via Vienna 2, Sassari, Italy

GIANNI CALARESU

Istituto Zooprofilattico Sperimentale della Sardegna, via Duca Abruzzi 8, Sassari, Italy

ANDREA SCOZZAFAVA

Istituto di Chimica Generale ed Inorganica, Facoltà di Farmacia, via G. Capponi 7, Florence, Italy

The oxidation of *o*-diphenols such as epinephrine and dihydroxyphenylalanine (DOPA) has been observed in human platelets [1], red blood cells both in the cytosol and membranes [2, 3] as well as in most animal tissues [4].

The diphenoloxidase (DPO) from red blood cells (1,2-benzenediol:oxygen oxidoreductase, EC 1.10.3.1) has been recently purified [5], but as yet insufficiently characterized; it is a protein of 150,000 molecular weight arranged in two identical subunits [5]. It oxidizes L-epinephrine and L-DOPA to their respective quinones adrenoquinone and dopaquinone, which in turn give rise spontaneously to adrenochrome (ϵ_{mol}, 485 nm = 4.35 X 10^3) and dopachrome (ϵ_{mol}, 475 nm = 3.72 X 10^3). The latter compound is known as a precursor of melanins.

This DPO may play a role in the *in vivo* oxidation of catecholamines [6], but also in some redox reactions of red blood cells involving reduced glutathione.

We have purified the diphenoloxidase according to Tuil and Demos [5] with minor modifications. Polyacrylamide gel electrophoresis (PAGE) (Fig. 1) of the pure enzyme under both native and denaturing conditions shows virtually only one band in both gels stained for proteins; when gels with native enzyme are stained for activity (incubation with L-DOPA), a black spot of melanin appears at the same position of the protein band. This indicates that the enzyme separated on PAGE is a DPO. Our pure preparation is active on L-epinephrine, L- and D-DOPA, while L-tyrosine is not accepted as substrate.

We have also checked the effect of some cations on catalytic activity. Results reported in Table I show that Cu^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+} and Fe^{3+} all en-

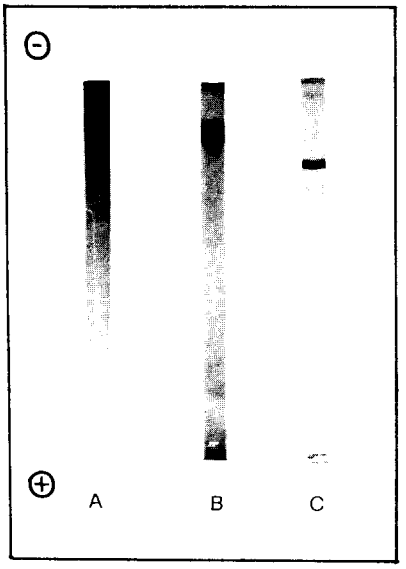

Fig. 1. Polyacrylamide gel electrophoresis of DPO. Protein 20 μg; gel A: native enzyme stained with L-DOPA 2 X 10^{-3} *M* at 25 °C; gel B: native enzyme stained for proteins with Coomassie G-250; gel C: sodium dodecylsulphate (SDS) denatured enzyme stained with Coomassie R-250.

TABLE I. Effect of Some Cations on the Oxidation of L-epinephrine by Diphenoloxidase from Human Erythrocytes. The reaction mixture contained 0.375 *M* Tris-HCl, pH 9.55, L-epinephrine 2 X 10^{-3} *M*, 40 μg enzyme protein and cations 5 X 10^{-7} *M*. Reaction rates were recorded at 485 nm for 2 minutes at 30 °C.

Cation added	Specific activity (nmol adrenochrome per min per mg protein)	% Activation
None	84	–
Mn^{2+}	126	50
Fe^{3+}	164	95
Co^{2+}	122	45
Ni^{2+}	170	102
Cu^{2+}	108	28
Zn^{2+}	116	40