reduction and of reoxidation of the reduced enzyme are affected [6]. Removal of the Type 2 Cu has no effect on the spectroscopic properties of the Type 1 Cu, below pH 7.0, but decreases the enzyme absorbance at 330 nm, usually assigned to the Type 3 Cu, and in the 650–800 nm region. The latter decrease amounts to 250–300 M^{-1} cm^{-1}, a rather high value for a 'normal' type of Cu(II) [6]. Similar results were obtained on removal of the Type 2 Cu from ascorbic acid oxidase [7]. The redox potential of the remaining copper ions and the stability of the tree laccase were also affected by removal of the Type 2 Cu.

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Correlation of K-Absorption Edge of Cobalt Derivatives of Some Metalloproteins

ALESSANDRO DESIDERI

Dipartimento di Fisica, Università della Calabria, Arcavacata, Italy

FABIO COMIN

Laboratori Nazionali Frascati, Gruppo Puls Frascati, Italy

LILIA CALABRESE, DINA COCCO, LAURA MORPURGO, BRUNO MONDOVÌ and GIUSEPPE ROTILIO

Centro di Biologia Molecolare del C.N.R., Rome, Italy

The recent availability of a stable and intense X-ray source using synchroton radiation from high energy electron storage rings has allowed meaningful measurements to be made on dilute systems.

In this report we present measurements, carried out at the ADONE Synchroton (Frascati), of the K-edge absorption spectrum of cobalt derivatives of some metalloproteins.

A K-edge absorption spectrum of a transition metal ion is usually characterized by a first weak absorption assigned to the 1s→3d transition, a second absorption present either as a shoulder or a peak about 9–10 eV higher in energy assigned to the 1s→4s transition, and an intense absorption still higher in energy assigned to the 1s→4p transition.

The K-edge of the cobalt derivative of superoxide dismutase where the cobalt is substituted for the native zinc shows all of these three features. The striking property of this spectrum is the quite intensity of the first peak due to the 1s→3d transition. It has been already shown by Shulman *et al.* [1] that departure from configurations that have inversion symmetry such as octahedral or square planar toward tetrahedral symmetry produces such an effect. Therefore these measurements confirm the previous suggestion obtained by EPR and optical data [2], that the cobalt substituted for the native zinc retains the distorted tetrahedral structure.

A correlation between the K-edge spectrum of this derivative, of that where the cobalt is substituted for the copper in the native superoxide dismutase [3] and of several cobalt derivatives of metalloproteins of known structure such as stellacyanin and carbonic anhydrase will be discussed.

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The Binding of Copper Ions to Copper-Free Bovine Superoxide Dismutase. Applied Aspects

R. STEVANATO, P. VIGLINO and A. RIGO

Institute of Physical Chemistry, University of Venice, Italy

D. COCCO and L. CALABRESE

Institute of Biological Chemistry, University of Rome, Italy

The catalytic activity, $k = 3 \times 10^9$ M^{-1} sec^{-1} [1], close to the diffusion limit and the very high relaxivity of $^{19}F^-$ nucleus, $R_F \sim 10^7$ M^{-1} sec^{-1} [2], due to the copper in the native site of Cu–Zn superoxide dismutase, make possible the measurements of very low concentrations of these enzymes.

In connection with the high sensitivity in the enzyme detection the reconstitution of the native site from Cu^{++} and the copper-free enzyme besides, to offer a good insight into the interaction copper–protein [3–5] presents some interesting applied aspects.

The measurement of copper concentrations much lower than ppb is a good example of these applications. For this purpose the polarographic [6] and the pulsed NMR techniques [2] appear very suitable since they can sense superoxide concentrations of the order of 10^{-11} and 10^{-8} *M* respectively.

Using these techniques, and owing to the very high affinity of the copper ion for its native site, the binding of Cu^{++} to the copper-free enzyme has been easily followed in a variety of experimental conditions. In particular the addition of known amounts of Cu^{++} to the copper depleted superoxide dismutase, at $[Cu^{++}]/[\text{protein}] \leqslant 0.1$, causes a linear and reproducible increase of both the activity and the relaxivity after few minutes incubation.

The method results to be highly specific for the Cu^{++} and allows, as measured by the polarographic activity and by ^{19}F relaxivity, to detect the ion with sensitivity of the order of 10^{-10} and 2×10^{-8} *M* respectively.

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Interaction of Halides with Cu–Zn Superoxide Dismutases

E. ARGESE, P. VIGLINO and A. RIGO

Institute of Physical Chemistry, University of Venice, Italy

Halides (X^-) bind at the copper ion present in the active site of Cu–Zn superoxide dismutases [1–3], and therefore the measurement of the $Cu{-}X^-$ stability constants (K_X) of superoxide dismutases isolated from different sources can offer a good insight into the structure of the active site of these enzymes. In particular K_X values were measured for the resting and working enzyme in parallel sets of experiments carried out in the presence of different concentrations of X^- by NMR measurement of the proton relaxivity of aqueous solutions of superoxide dismutase (resting conditions) and by polarographic determination of the enzyme activity (working conditions). While small halide effects on relaxivity have been detected, stability constants as high as 30 M^{-1} have been calculated for the $Cu{-}X^-$ complexes by the polarographic method.

According the K_X values obtained for the different types of Cu–Zn superoxide dismutases the halides may be arranged in the following series according to their increasing binding capacity: $F^- < Cl^- \lesssim Br^- < I^-$. However irregularities in this pattern have been observed which indicate that the stereochemical and conformational features of the active site are important as much as the chemical features.

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Nuclear Relaxation of Water Protons as a Probe of the Copper Environment in Rhus Vernicifera Laccase

E. F. ORSEGA, A. RIGO, P. VIGLINO

Institute of Physical Chemistry, University of Venice, Italy

L. MORPURGO, M. T. GRAZIANI and G. ROTILIO

Institute of Biological Chemistry, University of Rome, Italy

The presence of different types of copper in Rhus Vernicifera Laccase offers the possibility of obtaining information on the copper environments through the interactions of these paramagnetic centers with the water molecules. The nuclear relaxation rate, R_H, of the water protons has been used as the parameter of these interactions.

The R_H values of aqueous solutions of *holo-* and of type 2 copper depleted laccase samples have shown a little dependence on H^+ concentration, in the pH range 3.5–8, and suggest that the type 2 copper is responsible for a considerable fraction of the proton relaxation. Furthermore it has been observed that the ethylenediaminetetraacetate (EDTA) and the corresponding monoamide derivative with 4-amino-2,2,6,6-tetramethylpiperidinoxyl (EDTA-SL) nicely titrate a fraction of the enzyme relaxivity at pH 4.6. The R_H decrease due to the EDTA-SL addition is paralleled by a decrease of the EPR signal of the $>N{-}O^{\bullet}$ group and by a modification of the Cu^{++} EPR spectra which indicate the presence in the laccase sample of a fraction of Cu^{++} ions which are at least partially chelated by EDTA.