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.

References

1 B. Reinhammar, 'Advances in Inorganic Biochemistry', Eickhorn/Marzilli, eds., 1979, pp. 91–118 and references therein.
2 L. Morpurgo, M. T. Graziani, A. Desideri and G. Rotilio, *Biochem. J.*, in press (1980).
3 R. S. Himmelwright, N. C. Eickman and E. I. Solomon, *J. Am. Chem. Soc.*, *101*, 1576 (1979).
4 A. Desideri, L. Morpurgo, G. Rotilio and B. Mondovì, *FEBS Lett.*, *98*, 339 (1979), and references therein.
5 B. Mondovì, M. T. Graziani, W. B. Mims, R. Oltzik and J. Peisach, *Biochemistry*, *16*, 4198 (1977).
6 L. Morpurgo, M. T. Graziani, A. Finazzi Agrò, G. Rotilio and B. Mondovì, *Biochem. J.*, in press (1980).
7 L. Avigliano, A. Desideri, S. Urbanelli, B. Mondovì and A. Marchesini, *FEBS Lett.*, *100*, 318 (1979).

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.

References

1 R. G. Shulman, Y. Yafet, P. Eisenberger and W. E. Blumberg, *Proc. Natl. Acad. Sci., USA*, *73*, 1384 (1976).
2 G. Rotilio, L. Calabrese, B. Mondovì and W. E. Blumberg, *J. Biol. Chem.*, *249*, 3157 (1974).
3 L. Calabrese, D. Cocco and A. Desideri, *FEBS Letters*, *106*, 142 (1979).

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.