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 $[Cu^{++}]/[protein] \leqslant 0.1$, 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

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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

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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.