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

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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 $\geq N–O^{\bullet}$ 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.

The Cu^{++} EDTA titrated is usually less than 10% of the total copper in freshly prepared enzyme. This amount increases steadily with the aging of the laccase solutions both at room temperature and at −30 °C, and this phenomenon seems to be correlated with the increase of the ratio of the optical densities at 330 and 614 nm and with the decrease of the enzyme activity in the oxidation of 1,4-hydroquinone.

In the light of these observations the relaxivity measurements of freshly prepared *holo* and type 2 copper depleted laccase samples have been carried out in the presence of EDTA. In these conditions the molar relaxivity values of the type 2 copper and of the type 1 plus type 3 copper are about 2200 and 2400 M^{-1} sec^{-1} respectively. Since the relaxivity of the Cu^{++} EDTA titrated is of the order of 4000 M^{-1} sec^{-1} the increase of the relaxivity of the laccase solution with the aging, which may be due to the irreversible modification of the native enzyme in a less active form, appears to be related to a change of the environment of some copper ions which leads to a much more efficacious interaction of the paramagnetic center with the water molecules. In conclusion the measurements of the relaxivity of the laccase solutions with and without EDTA appears a very sensitive probe of the state of the copper ions in the enzyme.

Calorimetric Studies of Nucleic Acids Bases in Aqueous and Nonaqueous Solvent

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The heats of solution of cytosine and cytosine monohydrate in water and dimethyl sulfoxide at infinite dilution has been measured calorimetrically in the temperature range 25–40 °C. Enthalpies and heat capacities for the transfer process $H_2O \rightarrow$ DMSO were calculated. Combining these results with calorimetric measurements previously obtained on other nucleic acid bases showed that ΔH_{trans} for cytosine and cytosine monohydrate were more negative than purine but less than adenine, uracil, and thymine. ΔCp_{trans} was found to be positive while ΔCp_{trans} for all other nucleic acid bases were negative. This behavior indicates that cytosine and cytosine monohydrate interaction with DMSO is stronger than it is with water.

The Mn(II) Relaxation Probe in Dynamical Studies on Biomodel Systems in Water Solution

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A combined EPR and NMR analysis on biomodel systems containing manganous ion is presented. The analysis is carried out in terms of the electron spin relaxation theory of Mn(II) in aqueous solution [1, 2]. The spin-Hamiltonian is described by:

$$\mathcal{H} = \mathcal{H}_0 + AS.I + \mathcal{H}_1(\Omega) + \mathcal{H}_{rf}$$

where:

$$\mathcal{H}_1(\Omega) = \sum_{p} \mathcal{H}_1^p(\Omega) = \sum_{pq} (-1)^p F^{2p} D_{q-p}^{2}{}^{2}(\Omega) A^{2q}$$

is modulated by rotational Brownian motion.

The dipole–dipole (DD) direct interaction and the contact Fermi interaction for the nuclear relaxation are described by:

$$\mathcal{H}^{DD}(t) = \gamma_I \gamma_S \hbar r_{IS}^{-3}(t) \{ IS(t) - 3[I \cdot \hat{r}_{IS}(t)]\,[S(t)\hat{r}_{IS}(t)] \}$$

and:

$$\mathcal{H}^{FC}(t) = AI.S(t)$$

and are modulated by several mechanisms.

A novel derivation of the Solomon–Bloembergen–Morgan equations including effects deriving from the ligand field splitting and the spin density delocalization, allows a critical revision of the correlation times in the investigated systems [3].

EPR results, including the frequency dependence and preliminary data performed by longitudinal detection, are discussed [2, 4, 5]. The Electron and Nuclear spin relaxation model is applied to the metal–lipid interaction and to some ternary model systems.

Metal–lipid interactions. T_{1p}^{-1} and T_{2p}^{-1} measurements and frequency dependent EPR spectra have been performed on:

(1) the integral system in which the Mn(II) ions interact with external surface of large 'onion like' structures;

(2) the sonicated system in which the Mn(II) ions interact with both the internal and the external surfaces of simple bilayer versicles;

(3) the sonicated system, as in (2), in which the external Mn(II) ions are mostly replaced by ionic exchange with diamagnetic ions, and only the interaction with the external surface of the vesicles is possible. The relevance of through-water ion–lipid interactions is demonstrated.

Ternary model systems. Some ternary model systems mimicking the metal ion bridging in protein–