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Detection of Copper at 10^{-10} *M* Level by its Recombination with Copper-free Superoxide Dismutase

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Bovine copper zinc superoxide dismutase (BSOD) is a metalloprotein characterized by a very high catalytic activity, $k \simeq 1.8 \times 10^9\ M^{-1}\ s^{-1}$ [1], and produces a large effect on the ^{19}F nuclear magnetic relaxation rates, due to the cupric ions in the native site of the holoenzyme [2].

These properties have been utilized to measure very low concentrations of the enzyme by polarographic [3] and pulsed NMR techniques [4] which can measure BSOD concentrations as low as 10^{-11} *M* and 10^{-8} *M* respectively.

In connection with these highly sensitive methods the reconstitution of native site of the enzyme, starting from Cu^{2+} ions and the copper-free enzyme, offers the possibility of measuring very low copper concentrations.

The preparation of the copper-free enzyme and the binding of copper ions to copper-free enzyme has been extensively studied [5–7]. On this basis and from a new series of experiments in which the enzyme activity has been determined by the polarographic method, a practical linear increase of the kinetic rate constant value of the reaction between superoxide dismutase and O_2^- with the $[Cu^{2+}]/[$Cu-free enzyme$]$ ratio has been observed for values of the ratio in the range 0–1.

The slopes of these plots, which represent the specific activity of the reconstituted copper sites in the above reported conditions, have been found to be independent of the protein concentration in the range 10^{-6}–10^{-10} *M*. In all these experiments the restoration of the catalytic properties is completed after an hour since Cu^{2+} addition to the copper-free protein.

The relaxation rates (T_{1p}^{-1}, T_{2p}^{-1}) of $^{19}F^-$ solutions show a linear dependence on $[Cu^{2+}]/[$Cu-free enzyme$]$ ratio in the range 0–2. By increasing this ratio over 2 no sensibile changes in the ^{19}F nuclear relaxation rates have been observed (see Fig. 1). On these basis and because of the very high affinity of the cupric ion to its native site the binding of Cu^{2+} ions to the copper-free enzyme has been easily measured in a variety of experimental conditions, such as pH (7–10), temperature (10–35 °C), ionic strength (0.02–0.2 *M*). A good agreement between experimentally measured and effective Cu^{2+} concentrations has been obtained in these experiments. The sensitivities were 10^{-10} *M* for the polarographic method and 2×10^{-8} *M* for the $^{19}F^-$ NMR method respectively, with a standard deviation of 10–15% in both cases. All the solutions had been previously purified by treatment with Chelex 100 (BioRad) to eliminate the metal ion traces present in the reagents. Al^{3+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Cr^{3+}, Pb^{2+}, Fe^{3+} at concentrations tenfold higher than that of the Cu^{2+}

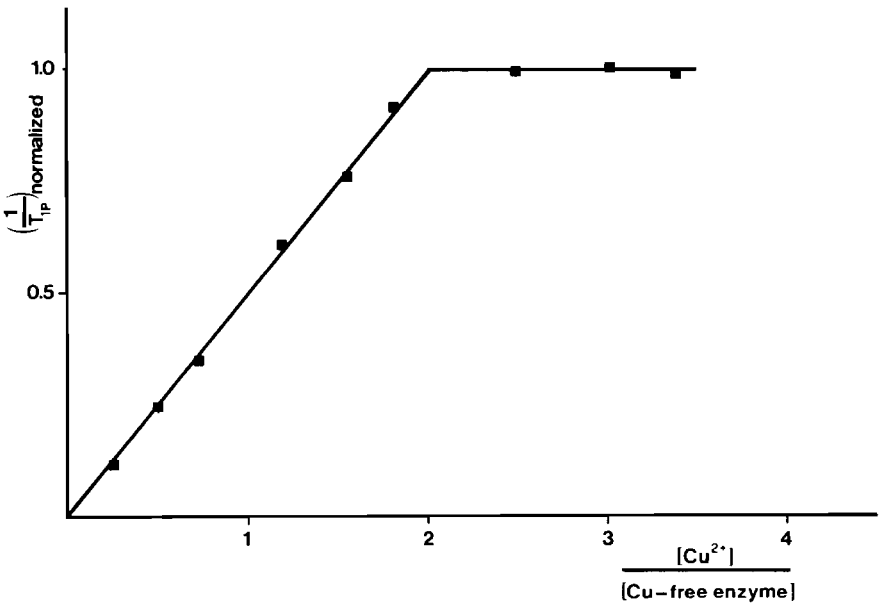

Fig. 1. $^{19}F^-$ relaxation rate as a function of the $[Cu^{2+}]/[$Cu-free enzyme$]$ ratio.

do not interfere both with the recombination process and with activity or relaxivity measurements.

Different batches of copper-free enzyme or its storage for months at −20 °C lead to slight changes in the calibration curves.

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