BINDING OF WATER TO "TYPES I AND II" Cu^{2+} IN PROTEINS

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Summary

Water proton spin-lattice relaxation times have been measured at 30MHz between 280 - 333 K in aqueous solutions of proteins containing Type I Cu^{2+} ions (azurin and umecyanin) and Type II Cu^{2+} ions (benzylamine oxidase and superoxide dismutase). These measurements show that Type II Cu^{2+} is accessible to exchangeable water molecules but Type I is not. This behaviour is consistent with the EPR and optical properties of these ions and their likely biochemical functions.

Copper is present in proteins as either diamagnetic Cu^{+} or paramagnetic Cu^{2+} ions. The electron paramagnetic resonance (EPR) and optical properties of the Cu^{2+} ions suggest they occur in two distinct kinds of "site" which have been designated Types I and II (1). Type II Cu^{2+} exhibits similar properties to small Cu^{2+} complexes, while those of Type I Cu^{2+} are unique; the latter has a strong optical absorption band at approximately 600 nm and an anomalously low value (<300 MHz as compared with values >420 MHz in most small complexes and Type II proteins) for the hyperfine coupling constant A_{11}. Proton spin-lattice relaxation measurements in aqueous solutions of plastocyanin (2) (Type I) and superoxide dismutase (3) (Type II) suggest that the Cu^{2+} in these sites may also differ in their ability to bind water molecules: Type II ions are accessible to exchangeable water molecules whereas Type I ions are not. Herein, we show by similar measurements that the same situation prevails in other Type I (azurin and umecyanin) and Type II (benzylamine oxidase) proteins.

Methods

Azurin was isolated from cells of the bacterium Pseudomonas fluorescens by the procedure described by Sutherland and Wilkinson (4). The isolated

protein was homogeneous as monitored by both OD_{625nm}/OD_{280nm} ratio (4) and EPR (5) methods.

Umecyanin was prepared from horseradish peelings using the procedure described by Paul and Stigbrand (6); the fraction from Sephadex G50 chromatography was comparable in purity to that obtained by these workers as monitored by OD_{610nm}/OD_{280nm} ratio, gel electrophoresis, and EPR methods (6,7).

Benzylamine oxidase, purified from pig plasma (8), was homogeneous as judged by gel electrophoresis and analytical ultracentrifugation. The purified preparation contained less than 0.2% ceruloplasmin according to the assay of Curzon (9).

Superoxide dismutase was purified from ox blood according to the method of McCord and Fridovich (10). The protein was homogeneous as monitored by gel electrophoresis and analytical ultracentrifugation. The EPR spectrum indicated identical Cu^{2+} ions.

Samples of azurin, umecyanin, benzylamine oxidase and superoxide dismutase were made up in potassium phosphate buffer (0.05 M, pH 7); protein concentrations were in the range 0.1 - 0.7 mM. These samples were deoxygenated with argon gas.

Results and discussion

Proton spin-lattice relaxation time T_1 measurements (11), were made at 30 MHz on the bulk water in these samples in the temperature range 280-333 K using the $90^\circ - \tau - 90^\circ$ pulse method; the results, normalized to a protein concentration of 0.6 mM, are summarized in figure 1.

The values obtained in the azurin and umecyanin samples are similar to those measured in the buffer solution indicating the Type I Cu^{2+} ion in these proteins has little effect on T_1; the small difference may be attributed to protein-water interaction. In contrast, there is a marked effect on T_1 in the benzylamine oxidase and superoxide dismutase samples suggesting the Type II Cu^{2+} ion is accessible to exchangeable water molecules.

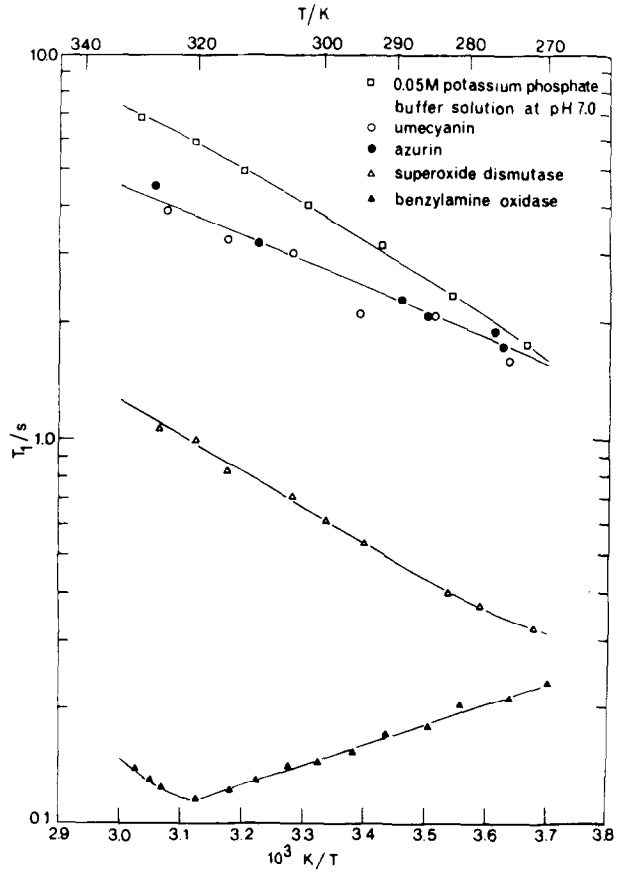

Fig. 1

^{1}H spin-lattice relaxation times T_1 measured at 30 MHz in aqueous copper protein solutions plotted as a function of reciprocal temperature. Protein concentrations in the samples investigated were in the range 0.1 - 0.7 mM; the T_1 values are normalised to 0.6 mM in each case.

These and previous results (2,3) suggest Type II and I Cu^{2+} ions may be differentiated according to whether or not they affect the bulk water proton relaxation rate. Interestingly, Malmström et al (12) have shown that fungal laccase contains two Cu^{2+} ions and two diamagnetic copper ions. One of the Cu^{2+} ions is of Type I, the other of Type II; only the latter has any effect on T_1.

The accessibility of Type II but not Type I Cu^{2+} ions to exchangeable water molecules is consistent with their EPR and optical properties and reflects important differences in their chemical environment and biochemical

function. The role of Type I Cu^{2+} is most likely that of a "redox shuttle" between reducing and oxidising substrates. On the other hand, the role of Type II Cu^{2+} is relatively obscure but its accessibility to exchangeable water suggests the site might function in binding substrates or inhibitors. Significantly, figure 1 shows that there is a substantial difference in the T_1 measurements in the benzylamine oxidase and superoxide dismutase samples suggesting there might be marked differences in both the structure and function of the Type II Cu^{2+} sites in these proteins. A more detailed interpretation of these measurements and its relation to the biochemical activity of these proteins will be the subject of a future publication.

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