A Water ^{1}H and ^{17}O N.M.R. Study on PHG-Modified SOD

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Received March 28, 1984

Abstract

The low-activity phenylglyoxal (PHG) modified bovine copper zinc superoxide dismutase (SOD) has been studied through ^{1}H and ^{17}O NMR of solvent water.

Water ^{1}H NMR T_1^{-1} values have been measured at magnetic fields between 4 and 60 MHz. The data for PHG modified SOD provide evidence that water is still semicoordinated in the axial position, though possibly at a greater distance than in native SOD. Water ^{17}O NMR data support this finding.

Introduction

Copper zinc superoxide dismutase (SOD) is a dimeric metalloenzyme, containing one copper(II) and one zinc(II) ion per monomer. The X-ray structure has shown [1] that the zinc(II) ion, whose role is essentially structural, is bound to one oxygen atom from Asp-81 and three nitrogen atoms from His-61, -69 and -78. His-61 is also bound to the catalytic copper(II) ion, whose coordination sphere is completed by three more nitrogen atoms from His-44, -46 and -118, and by a water molecule. The coordination geometry around copper(II) is rather distorted with the four nitrogen atoms forming an averaged square planar coordination polyhedron, completed by the water molecule in the axial position.

Anions are known to bind the copper(II) ion in the native enzyme [2–4] and to act as competitive inhibitors [5]. Phenylglyoxal (PHG) has been shown to quench the enzymatic activity of SOD, and to bind the Arg-141 residue [6], located 600 pm away from the catalytic copper(II) ion. Although PHG does not bind the metal, PHG-modified SOD has a lower affinity for anions compared with the native enzyme [7]. This lowering of affinity could be attributed to electrostatic factors, *i.e.* the positive charge of Arg-141, decreased through interaction with PHG, is no longer able to drive the anions towards the metal ion, or for purely steric reasons, with the PHG molecule bound to Arg-141 blocking the access of inhibitors, anions, substrate and solvent molecules to the catalytic metal ion.

Since the spectroscopic properties of native and PHG-modified SOD are different, we have undertaken a water ^{1}H and ^{17}O NMR study to check whether a water molecule is still present in the coordination sphere of the copper(II) ion in the PHG-modified SOD.

Experimental

Bovine SOD was purchased from Diagnostic Data Inc. (Mountain View, Ca.) and PHG was purchased from Sigma Chemical Company (St. Louis, Mo.); both were used without further purification. The PHG-modified SOD was prepared according to the reported procedure [6]. $H_2{}^{17}O$ (^{17}O content was 18.2%) was purchased from MSD Isotopes (Montreal, Canada) and added to the enzyme solutions to a final ^{17}O content of about 5%.

Copper(II) concentrations in enzyme solutions were checked through electronic spectroscopy ($\epsilon_{680} = 300\,M^{-1}\,cm^{-1}$ per dimeric unit [8]).

^{1}H NMR measurements were performed on an instrument based on a Bruker CXP 100 consol and a Varian DA 60 1.41 T electromagnet; water ^{1}H NMR relaxation measurements were performed at room temperature, between 4 and 60 MHz; the ^{17}O NMR measurements were performed at 10 °C at 8.134 MHz.

Water ^{1}H T_1^{-1} values were measured using the inversion-recovery method. ^{17}O T_2^{-1} values were obtained directly from the half height linewidth of the water ^{17}O signal ($T_2^{-1} = \pi\Delta\nu$), appropriately reduced for the line broadening introduced by exponential weighing of the free induction decay.

Results and Discussion

Water ^{1}H NMR T_1^{-1} data on PHG modified SOD solutions at frequencies between 4 and 60 MHz are reported in Fig. 1, together with the data obtained on native and zinc deprived SOD [9]. A first examination of this figure indicates that the kind of interaction of solvent water with the

0020-1693/84/$3.00

TABLE I. G (pm^{-6}) and τ_c (s) Values Calculated from ^1H NMR Relaxation Measurements for Native, Zn-deprived and PHG-modified SOD.

Enzyme	G	τ_c
Cu_2Zn_2SOD	$2.2 \pm 0.3 \times 10^{-15}$	$2.6 \pm 0.3 \times 10^{-9}$
Cu_2E_2SOD[a]	$2.6 \pm 0.3 \times 10^{-15}$	$3.7 \pm 0.4 \times 10^{-9}$
PHG-modified SOD	$1.6 \pm 0.2 \times 10^{-15}$	$4.4 \pm 0.4 \times 10^{-9}$

[a]Cu_2E_2SOD = Zn-deprived SOD.

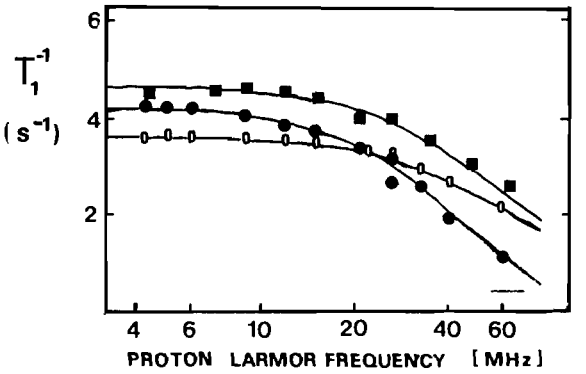

Fig. 1. Room temperature water ^1H NMR T_1^{-1} data as a function of proton Larmor frequency for 8.2×10^{-4} *M* solutions of native (o) [9], Zn-deprived (▪) [9] and PHG-modified (•) SOD. The values for the CN^- and N_3^- adducts of the native protein (——) are also reported.

paramagnetic center is essentially similar in all three cases. Water proton T_1^{-1} enhancement values, T_{1p}^{-1}, depend on the external magnetic field according to the Solomon equation [10]

$$\frac{T_{1p}^{-1}}{f} = T_{1M}^{-1} = \frac{2}{15}\frac{\gamma_I^2 g^2 \beta^2 S(S+1)}{r^6}\left(\frac{7\tau_c}{1+\omega_s^2\tau_c^2} + \frac{3\tau_c}{1+\omega_I^2\tau_c^2}\right)$$

where f is the molar fraction of bound water, r is the metal-proton distance, ω_I and ω_s are the nuclear and electron Larmor frequencies, respectively, and τ_c is a correlation time which is determined by the electronic relaxation time when the rotational time is slow, as happens for the present system. All the other symbols have the usual meaning. The equation holds when electronic relaxation follows an exponential law, g is isotropic, and the hyperfine coupling with the metal nucleus is zero. It has been recently shown in our laboratory [11] that the experimental curves of T_{1M}^{-1} *versus* magnetic field in copper(II) systems approach the behavior predicted by the Solomon equation at magnetic fields higher than 1 MHz, *i.e.* when the $\hat{I}\cdot\underset{\approx}{A}\cdot\hat{S}$ term of the Hamiltonian is smaller than the Zeeman term.

The above equation can be rewritten in the following form

$$T_{1p}^{-1} = \frac{[E]}{111} K \cdot G \frac{3\tau_c}{1+\omega_I^2\tau_c^2}$$

where K is a product of physical constants, $G = \Sigma_{i=1}^{n} 1/r_i^6$ takes into account the interaction of all the exchanging protons at a distance r_i from the metal, [E] is the concentration of the paramagnetic species, and 111 is the molarity of water protons. Typical G values for a coordinated water molecule are around 4×10^{-15} pm^{-6}. The G and τ_c parameters for native, Zn-deprived and PHG-modified SOD solutions are shown in Table I. These figures show that the order of magnitude of the electronic relaxation time is the same in all systems, although there is an increase on passing from native to Zn-deprived to PHG modified SOD, and that in all cases water interacts with the copper center, though at a greater distance than the usual. The G values give estimates of the Cu–O distances of 230, 220 and 250 pm for the native, Zn-deprived and PHG-modified SOD, respectively.

Addition of N_3^- and CN^- to the PHG-modified SOD solution causes a reduction in the water proton T_1^{-1} value (Fig. 1) whose limit is the same as that for the native enzyme [4], but a higher anion concentration is required to obtain it; this is again consistent with previous reports from spectrophotometric titrations [7]. Owing to the very similar EPR [7], water proton T_1^{-1} and electronic absorption behaviours [7] for the N_3^- and CN^- adducts of native, Zn-deprived and PHG modified SOD, the resulting chromophores are the same in all cases, whatever the N_3^- or CN^- mode of approaching the metal ion.

The investigation of fluoride is made difficult by its low affinity. ^{19}F T_2^{-1} measurements on solutions of PHG-modified SOD, containing increasing amounts of F^-, indicate that the latter ion interacts with the paramagnetic center, since a line broadening is observed with respect to analogous solutions of the reduced enzyme, but that the affinity constant is much lower than $1\ M^{-1}$.

Water ^{17}O NMR data for native and PHG-modified SOD solutions are reported in Table II. It appears that the paramagnetic contribution to the linewidth for the PHG-modified SOD is lower than for the

TABLE II. T_{2p}^{-1} Values of Water ^{17}O Nuclei Measured at 10 °C for Native and PHG-modified SOD.[a]

Enzyme	pH	T_{2p}^{-1} (s^{-1})
Cu_2Zn_2SOD	8.7	160 ± 20
PHG-modified SOD	8.7	65 ± 20

[a]Protein concentration 1.33×10^{-3} *M* in 0.156 *M* $NaHCO_3$.

native enzyme. The increase in the τ_c value calculated from water ^1H NMR relaxation data for PHG-modified SOD with respect to the native enzyme would cause an increase in the T_{2p}^{-1} value of water ^{17}O nuclei in the former enzyme which is not experimentally recorded. The relaxation mechanism for water ^{17}O nuclei bound to a paramagnetic center is contact in origin and it is a function of the electronic relaxation time of the metal ion. A change in T_{2p}^{-1} value of the ^{17}O nucleus for the former derivative is accounted for by a lower value of the hyperfine coupling constant. Such Cu–O lengthening is consistent with a more axial geometry of the copper chromophore of the PHG-modified SOD, as has been shown by EPR measurements [7].

The permanence of a coordinated water molecule in PHG-modified SOD, and the analogy between the latter derivative and the native SOD as far as the cordination of anions is concerned, indicate that the decrease in activity is not due to a significant change in the coordination sphere, and therefore the hypothesis that PHG affects the properties of SOD by modifying the positive charge distribution in the cavity is more creditable.

Acknowledgement

Thanks are expressed to Dr. J. S. Valentine for providing the first sample and stimulating us to perform these measurements. Two of the authors also acknowledge the support of the U.S. Army, through its European Research Office (contract No. DAJA 37-81-C-0754).

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