CROSS RELAXATION AND SPIN DIFFUSION EFFECTS ON THE PROTON NMR OF BIOPOLYMERS IN H_2O

Solvent saturation and chemical exchange in superoxide dismutase

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1. Introduction

Water–protein interactions have been studied with proton magnetic resonance [1–6]. A population of hydration water is typically observed. This water exchanges with bulk water sufficiently slowly that spin magnetization can be transferred efficiently from surface protein protons to hydration water protons via mutual spin–flips arising from a dipole–dipole interaction. Magnetization can also be transported rapidly within a large molecule by multiple mutual proton spin–flips [7]. In the present study, we examine the effects these magnetization transfers have on high resolution protein-proton spectra which are obtained in H_2O. Other workers have studied the effect of the protein-proton pool on H_2O relaxation resulting from this interaction, whereas we report distortions of the entire macromolecule spectrum when H_2O is saturated. Our results have important implications in several areas including the use of saturation methods of solvent suppression [8,9] and the measurement of solvent exchange rates of exchangeable protons in large biopolymers [10].

2. Experimental

Bovine copper–zinc superoxide dismutase was isolated from liver as in [11]. NMR samples consisted of $\sim 1.0 \times 10^{-3}$ M superoxide dismutase (mol. wt 31 200), 0.05 M phosphate buffer, 1.0×10^{-4} M EDTA and 10% D_2O/90% H_2O. The enzyme was reduced by addition of a minimum amount of solid sodium dithionite.

Proton Fourier transform NMR spectra were obtained at 270 MHz as in [10]. Spectra are reported in ppm from sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). The pulse sequence used in all the experiments reported here consisted of a 0.5 s pre-irradiation pulse of the desired frequency, a 1.0–10.0 ms homogeneity spoil to eliminate any residual transients, a short delay for recovery from the spoil and a 2-1-4 observation pulse [10]. The intensity of the pre-irradiation pulse in the transfer of water saturation experiments was set to be just sufficient to saturate the water resonance, as observed in a separate experiment. The aliphatic region of the protein spectrum was saturated in the transfer of aliphatic proton saturation experiments by rapidly sweeping the pre-irradiation frequency from 0.0–3.5 ppm about 30 times in 0.5 s. We verified that water was not significantly saturated ($< 5\%$) by this procedure in a separate experiment.

3. Results and discussion

A typical transfer of water saturation experiment

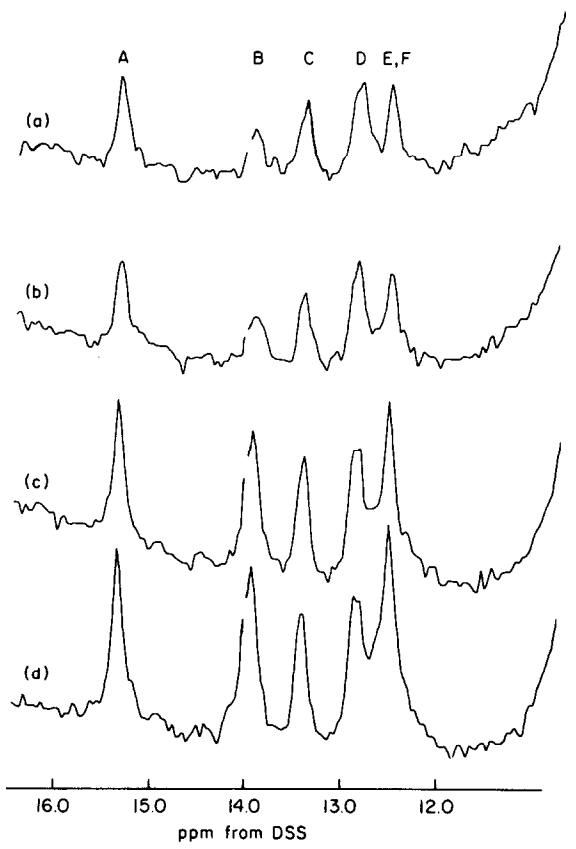

Fig.1. Transfer of saturation experiment between H_2O and the histidine NH resonances of superoxide dismutase, pH 6.1, 23°C. Frequency of pre-irradiation relative to H_2O resonance frequency (a) 0 Hz, (b) +10 Hz, (c) +50 Hz, (d) +200 Hz. Spectrum d is essentially that obtained when no pre-irradiation is used. A single point glitch at 14.06 ppm has been removed by hand.

is shown in fig.1 for the 6 observed histidine NH protons of bovine Cu, Zn superoxide dismutase [12]. We label these A–F, where F is the broad peak under resonance E. When the long selective pre-irradiation pulse is applied at the water frequency (fig.1a), a substantial amount of water saturation is transferred to the histidine NH resonances. Expressed as % intensity reduction upon saturation of water, the values are: A, 60%; B, 94%; C, 63%; D, 48%; E, F, 81%. As the pre-irradiation pulse is moved away from the water frequency (fig.1b–d) the NH intensities increase in proportion to the water resonance intensity.

The transfer of saturation from water could occur by several mechanisms, including chemical exchange with water, direct solvent cross-relaxation and protein-proton mediated solvent cross-relaxation. Measurements of line widths and longitudinal relaxation rates on peaks B and F at several pH values indicate chemical exchange is the dominant mechanism of saturation transfer for these protons. However, the chemical exchange mechanism can be ruled out for peaks A and C by real time deuterium exchange experiments [13] where an exchange rate of less than 0.1×10^{-4} s^{-1} is observed. Resonances D and E show pH-independent longitudinal relaxation rates and linewidths below pH 10 and also are probably not exchanging rapidly enough for transfer of saturation by chemical exchange to be important. Thus, for at least some resonances, substantial saturation transfer occurs with virtually no contribution from chemical exchange.

In the direct solvent cross-relaxation mechanism of saturation transfer, magnetization is transferred to the NH protons by a single mutual spin–flip of the water–protein interface. This is a negative solvent–solute nuclear Overhauser effect (NOE) [14]. The extremely slow chemical exchange of protons A and C is strong evidence against solvent contact and a direct process of saturation transfer for these protons. The protein-proton mediated mechanism of solvent cross-relaxation is able to explain transfer of water saturation to slowly exchanging, buried protein protons. In this mechanism water proton saturation is transferred to a protein proton at the water protein interface by a mutual spin–flip and then, by a series of several rapid mutual spin–flips within the protein, to the histidine NH protons. The first step could also occur by chemical exchange of a labile proton on the protein surface.

The plausibility of the protein-proton mediated mechanism can be established by showing that:

1. Cross-relaxation within the protein is rapid relative to spin-lattice relaxation.
2. The rate of magnetization transfer from water to the protein is comparable to the average longitudinal relaxation rate of the protein protons.

The results in fig.2 demonstrate that the first requirement is met. Spectrum 2a shows the aliphatic and histidine NH resonances. Spectrum 2b shows these regions after application of a pre-irradiation pulse which selectively saturated ~70% of the aliphatic

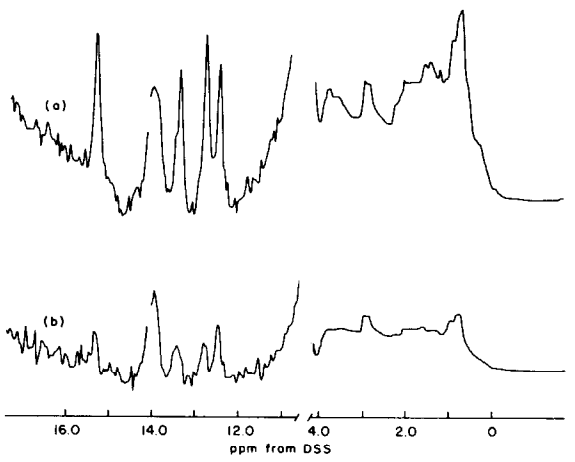

Fig.2. Transfer of saturation experiment between the aliphatic region and the histidine NH protons of superoxide dismutase, pH 7.55, 20°C. (a) Aliphatic and histidine NH regions with no pre-irradiation. (b) Aliphatic and histidine NH regions after application of a pre-irradiation pulse which saturates about 70% of the resonances from 0.0–3.5 ppm as described in the text. The two regions were recorded at different gains. A single point glitch at 14.06 ppm has been removed by hand.

protons from 0.0–3.5 ppm and $< 5\%$ of the water protons. The histidine NH resonances, A, C, D, E are nearly 70% saturated in spectrum b, indicating that magnetization transfer to the NH protons from aliphatic protons by cross-relaxation is rapid relative to the intrinsic spin-lattice relaxation rate of the NH protons. Substantial saturation is also transferred to the aromatic and amide regions of the spectrum. Resonance B shows only a small effect because it is exchanging rapidly with water, which acts as a pool of non-saturated spins. For the conditions of this experiment, proton F is exchanging with water very rapidly and its resonance is broadened beyond detection.

We can also show that substantial saturation is transferred from water to all protein protons (fig.3). Spectrum 3a shows the normal proton spectrum, while spectrum 3b was obtained while water was nearly completely saturated. As shown by the difference, 3c, between 3a and 3b, the majority of resonances saturate to some degree. The intensity loss in fig.3b, relative to fig.3a, is 35–40% across the spectrum. This indicates that the rate of magnetization transfer from water to the protein is roughly equal to the average longitudinal relaxation rate of all protein protons, estimated to be 1.4 s^{-1} from the average relaxation rate in D_2O solutions [13]. We conclude that the protein-proton mediated mechanism of solvent cross-relaxation is a plausible explanation for the transfer of saturation results presented in fig.1. The limiting step in the mechanism is magnetization transfer from water to the protein.

Our results have implications in several areas. Previous investigations have used longitudinal relaxation rates and transfer of saturation data to determine chemical exchange rates of protons in small molecules [15]. In these cases, solvent cross-relaxation results in limited enhancement ($\leqslant 50\%$) of resonances (positive NOEs). This limit on the possible enhancement makes estimates of the chemical exchange rates of protons possible even where longitudinal relaxation is not completely dominated by chemical exchange. In large molecules cross-relaxation will result in intensity decreases in a transfer of saturation experiment (negative NOEs) [7]. Direct or protein-proton mediated solvent cross-relaxation and chemical exchange are of the same form making it difficult to distinguish between them. The results presented here show that protein-proton mediated solvent cross-relaxation is likely to interfere with attempts to measure exchange kinetics by pulsed NMR methods when the exchange rate is slower than the rate of cross-relaxation with neighboring spins. This rate is estimated to be 4 s^{-1} for a histidine NH proton interacting with a single C2-H proton on the same ring [7], assuming a rotational correlation time of 1.8×10^{-8} s. The apparent longitudinal relaxation rate of histidine NH protons A, C, D, E, measured by selective saturation-recovery, is about 4-times this rate (17.0 ± 3.0 s^{-1}). These values predict a substantial negative NOE between the NH and C2-H protons of each residue. We have observed such NOEs [13]. Considering the C4-H and other nearby protons with which the NH protons can interact, it is probable the apparent longitudinal relaxation rate is dominated by cross-relaxation from neighboring protons.

Our observations also show that saturation methods of solvent suppression [8,9] may result in serious distortion of intensities of non-exchangeable, as well as exchangeable peaks. The effects are expected to be more serious the larger the protein [7], because

magnetization transfer is proportional to the rotational correlation time and competing relaxation processes are inversely proportional to it. The distortion would not be serious when the method is used to eliminate the residual HDO signal in predominantly deuterated solvent. Results similar to those reported here have also been observed by others in our laboratory studying hemoglobin (mol. wt 68 000) and transfer RNA (mol. wt 25 000) [16].

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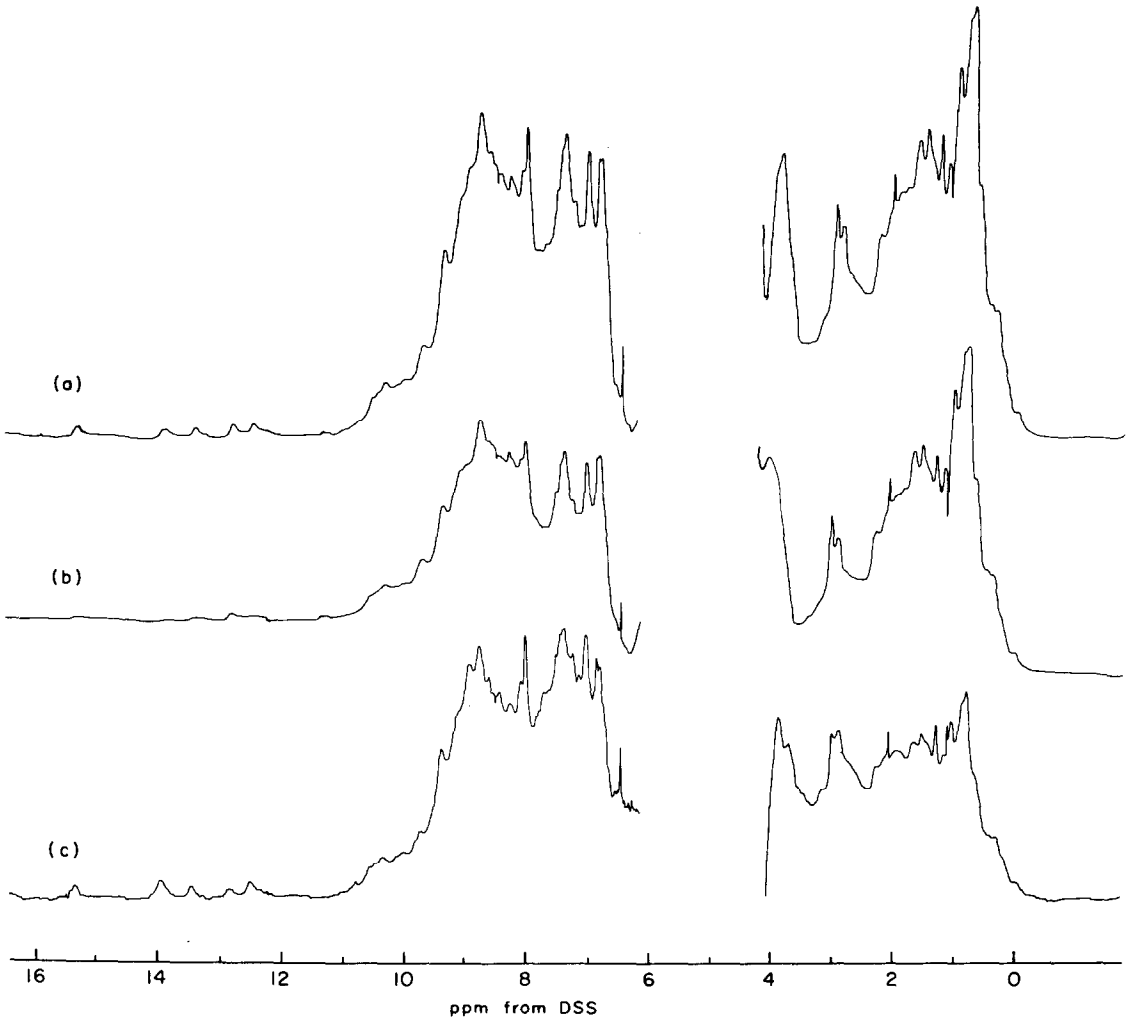

Fig.3. Transfer of saturation experiment between H_2O and the entire spectrum of superoxide dismutase, pH 6.95, 20°C. Spectra a and b were obtained under identical conditions except that for spectrum a, the pre-irradiation pulse frequency was 400 Hz downfield of the H_2O resonance while in spectrum b pre-irradiation was at the H_2O frequency. An independent experiment showed that H_2O was about 95% saturated under the conditions of spectrum b. Spectrum c is spectrum a minus spectrum b, recorded at twice the gain of either a or b.

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