Table I. Rate Constants (k) for Transport of Cs^+ as a Function of the Nigericin:PC Ratio in the Lipid Bilayer[a,b]

$10^3 \times$ nigericin:PC ratio	k(in → out)/s^{-1}
1.071	0.030
2.145	0.055
2.681	0.101
3.217	0.130
4.021	0.160

[a] These data give a first-order rate constant of 46.9 ± 3.3 mol of lipid·(mol of nigericin)$^{-1}$·s^{-1} (correlation coefficient 0.9807). [b] Conditions: 25 mM Cs^+; vesicles prepared from 40 × 10^{-6} mol egg yolk PC at 313 K; spectra run at 303 K on a Bruker AM300 spectrometer at 39.37 MHz. Nigericin:PC ratios vary between 1:934 and 1:249.

(where d = the chemical shift difference between the two sites and $t1 = 1/(2d)$. The first two pulses of this sequence specifically invert the Cs^+(out) magnetization. The variable delay then allows chemical exchange and relaxation to occur. This sequence is preferable to the DANTE sequence[15] when only two signals are involved, as it is over in a much shorter period with less loss of inverted magnetization. The relaxation times at each site were determined separately before the addition of any ionophore and were typically ca. 11.5 s (in) and 3.7 s (out). The Cs(out) relaxation time is ca. 5 times the Li(out) relaxation time observed in our $^7Li^+$ magnetization-transfer experiments[9,11] and allows rates that are slower than those for $^7Li^+$ transport to be measured.

The equations describing the behavior of two exchanging sites in a magnetization-transfer experiment have been derived by Morris and Freeman.[17] These equations were used in a least-squares program that allowed best fit values for the exchange rate to be calculated. Nigericin-mediated exchange rates for ^{133}Cs that we have measured by this technique vary between 0.02 and 0.3 s^{-1}. These values are about 1 order of magnitude less than the values we obtained for $^7Li^+$ transport and are probably the slowest rates yet measured by a dynamic NMR technique.

A typical set of intensity vs time measurements for the Cs^+(in) peak is shown in Figure 1, and a typical set of rate data is given in Table I. As in the case of Na^+ and K^+, the exchange is observed to be first order in nigericin, suggesting that one nigericin molecule transports one cesium ion. Confirmation of this depends upon a study of the dependence of rate on metal ion concentration.

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Investigation of Copper-Zinc Superoxide Dismutase Ser-137 and Ala-137 Mutants

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Ser- and Ala-137 mutants of human copper-zinc superoxide dismutase (Cu,ZnSOD) have been thoroughly characterized in an attempt to understand the subtle effect of the nature of the residue at position 137 on the structure of the copper site and on the activity profile. The results show that the nature of the residue at position 137 determines the presence of water in the active cavity as monitored through water ^1H nuclear magnetic relaxation dispersion. Also, the hyperfine shifts experienced by the protons of His-48 in the Cu_2Co_2 derivative are sensitive to the group at the 137 position. These effects are not detected through electronic and EPR spectroscopies. The activity profiles of Ser-137 and Ala-137 mutants are virtually identical and are very close to that of the Ile-137 mutant at pH < 10. The drop in activity above pH 10 closely parallels that observed in the wild type, at variance with the Ile-137 mutant that shows a marked decrease in activity already below pH 10. The activity profiles definitely show evidence of a pK_a between 6 and 7. The affinity of N_3^- for the Ser-137 and Ala-137 mutants is very similar to that for the wild type. This is again at variance with the Ile-137 derivative that shows an N_3^- affinity twice as large.

Introduction

Copper-zinc superoxide dismutase (SOD, hereafter) is a dimeric Cu,Zn system that is extraordinarily efficient as a catalyst for dismutation of superoxide.[1-8] This is accounted for by both the electrochemical potential of the pair Cu^{2+}/Cu^+, which is intermediate between the potentials of the pairs O_2/O_2^- and O_2^-/O_2^{2-},[8-10] and the residues at the entrance of the catalytic cavity that increase the affinity for the substrate.[8,11-14] Arg-143 (numbering of the human isoenzyme) is close to the copper ion[11-13] (Figure 1) and probably assists the substrate in entering the cavity;[8,11-13,15-18] such a role was proposed on the basis of chemical modification experiments[19,20] and recently demonstrated through site-directed mutagenesis.[21-23] Opposite Arg-143 is a Thr residue (Thr-137) (Figure 1) whose terminal OH is thought to form a

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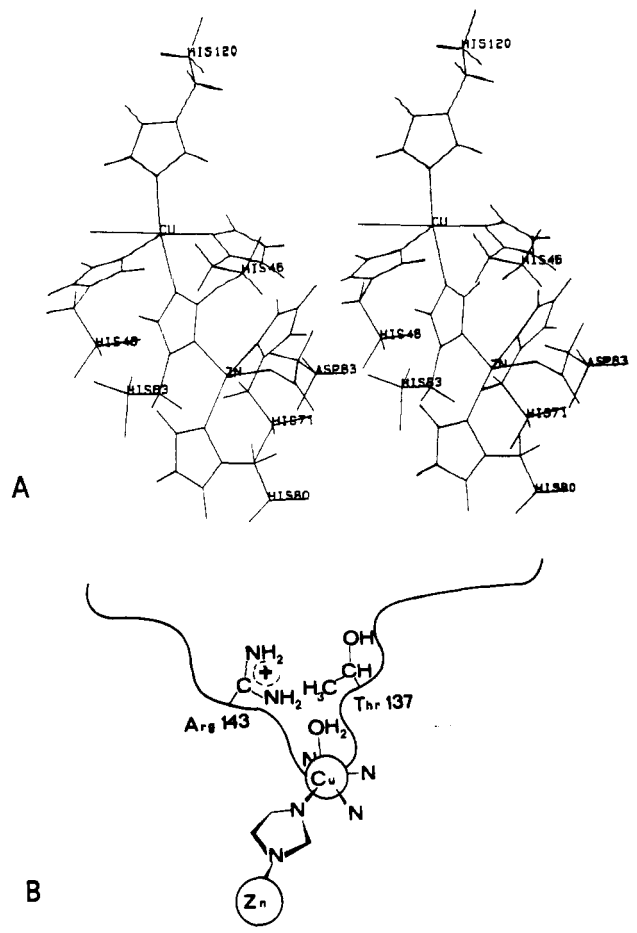

Figure 1. Schematic drawing of the superoxide dismutase cavity (A) and of the copper site (B).

hydrogen bond either with a glutamic acid (Glu-133)[13] or with a water molecule that is weakly coordinated to Cu^{2+}.[11]

Through site-directed mutagenesis, Thr-137 has been changed into Ile-137.[24] Despite the hydrophobic nature and the large steric hindrance of the substituent residue, the activity in the range of pH 5–9 is about 75% of that of the wild type. Water ^1H NMRD (nuclear magnetic relaxation dispersion) measurements have shown that there are no exchangeable protons that sense the paramagnetic copper center;[25] this has been interpreted as due to the absence of the water molecule, which in the wild type is weakly coordinated to copper(II). In agreement with the NMRD

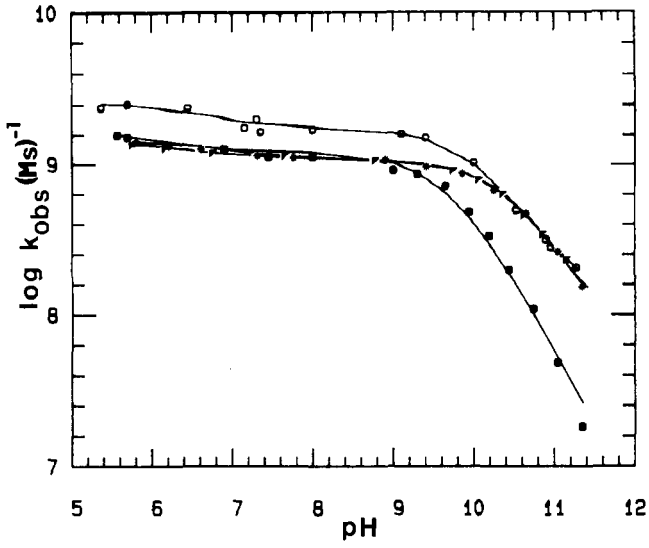

Figure 2. pH dependence of specific activity of WT (○), Ser-137 (✱), Ala-137 (▼), and Ile-137 (●). The lines represent best fit curves calculated by taking into consideration two different pK_a's. All the solutions are in 0.01 M formate and 0.05 M phosphate buffer.

results, the electronic and EPR spectra and the ^1H NMR spectrum of the Cu_2Co_2SOD mutant show a more tetragonal structure with a plane defined by Cu^{2+} and four His nitrogens.[24,26] Furthermore, the Ile-137 mutant has the pK_a of the high-pH activity profile lower than that of the wild type.[24] The groups determining this pK_a are not yet understood.

We felt that it would have been meaningful to further investigate the presence of the water molecule inside the active cavity as related to the nature of the 137 residue and to follow the activity and its pH dependence for different mutants in position 137, as well as the variations in the coordination geometry around the metal ions. With this in mind, we have prepared and characterized the mutants Ala-137 and Ser-137: the former is hydrophobic like Ile-137 but less bulky; the latter is hydrophilic like Thr-137 but also less bulky. The new systems have been investigated through kinetic studies, electronic and EPR spectroscopies, water ^1H NMRD, and ^1H NMR spectroscopy of the Cu_2Co_2 derivative. In the last case, the interaction with anions was also studied.

Experimental Section

Preparation of the Mutants. The mutant human SOD genes containing the Thr-137 to Ser-137, Ala-137, and Ile-137 changes were made by using cassette mutagenesis as previously described.[27] Yeast expression plasmids containing the mutant human genes were constructed and transformed into yeast strain 2150-2-3.[28] The mutant SOD proteins obtained from these modified strains were fractionated to >95% purity, as previously reported.[21,28,29] All mutants are also modified in two positions distant from the active site: Cys-6 and Cys-111 are substituted by Ala and Ser (AS), respectively. These modifications increase the thermostability of the enzyme, facilitating the use of a heat step during the purification.[29] As the active cavity structure and the enzymatic properties are not affected by this modification, hereafter we refer to the AS mutant as wild type (WT).

Activity Measurements. Activities of the mutants as a function of pH were measured through pulse radiolysis experiments, using the 2-MeV Van der Graaf accelerator at Brookhaven National Laboratory, as previously reported.[24]

Metal Substitution. The apoproteins were obtained through extensive dialysis against 10 mM EDTA, in 50 mM acetate buffer at pH 3.8.[1] The

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Table I. Best Fit Parameters of Activity Data for 137-Mutants and Wild Type[a,b]

	log k_{obs1}	pK_{a1}	log k_{obs2}	pK_{a2}	log k_{obs3}
WT	8.8 ± 0.3	7.1 ± 1.4	9.2 ± 0.1	10.7 ± 0.1	6.9 ± 1.3
Ser-137	8.6 ± 0.1	6.9 ± 0.7	9.0 ± 0.1	10.5 ± 0.1	7.3 ± 0.7
Ala-137	8.6 ± 0.4	6.3 ± 1.3	9.0 ± 0.1	10.4 ± 0.1	7.6 ± 0.3
Ile-137	≅9[c]	≅6[c]	9.1 ± 0.1	9.7 ± 0.2	

[a] The best fit parameters are obtained by assuming two pK_a's. [b] Deviations are expressed as $\pm 3\sigma$. [c] Ile-137 data have been fitted by assuming two pK_a's consistently with the data of the other mutants, but the data show large indetermination on the first k_{obs} and pK_a; furthermore, it is not possible to determine k_{obs3} (alkaline pH limit).

chelating agent was removed through dialysis against 100 mM NaCl in the same buffer and then against acetate buffer alone, gradually increasing the pH from 3.8 to 5.5. Protein concentrations were measured by the Coomassie method.[30] The Cu_2Co_2 derivatives were obtained by adding first Co^{2+} and then Cu^{2+}, following the metal incorporation spectrophotometrically.[31] Cobalt uptake, in the 2:1 cobalt-to-protein ratio, is instantaneous for all the mutants, while complete copper uptake was achieved within 12 h, as confirmed by the characteristic electronic spectrum[32] and the ^{1}H NMR spectrum.[33]

Physical Measurements. The absorption spectra were obtained with a Cary 17D spectrophotometer. CD spectra were recorded on a Jasco J 500C spectrophotometer, by using one cm path length cells. The differential dichroic absorption, $\Delta\epsilon$, has units of M^{-1} cm^{-1}.

^{1}H NMR spectra were recorded at 200 MHz by using a Bruker MSL 200 instrument with the modified DEFT pulse sequence[34,35] in order to suppress H_2O and bulk protein signals.

EPR spectra at room temperature were recorded on a Bruker ER200 operating at 9.8 GHz (X-band).

^{1}H NMRD experiments were performed by using the field cycling relaxometer home-built at the IBM laboratories, Yorktown Heights, NY, as previously described.[36,37]

Results

Kinetic Measurements. The activity of the mutants as a function of pH is reported in Figure 2, together with that of the wild-type enzyme. Both the wild type and the mutants show an inflection in the activity profile at acidic pH, between pH 5.7 and 6.9, with a decrease in activity of about 40%. This detail of the activity profile of the wild type had already been detected.[24] Then, there is a second drop of activity at alkaline pH. Best fit parameters of the four profiles are reported in Table I. The data show a decrease in k_{obs} with a pK_a of about 6–7 and a major decrease with a pK_a of about 11. At variance with the Ile-137 mutant, for which the pK_a was found to be lower than that of the WT,[24] the new mutants have the same pK_a or even slightly higher. It should be noted that, with the exception of the previously investigated Ile-137 mutant, a third value of k_{obs} is required for the high-pH limit. Such a value is very small. It is reported in Table I as a best fit parameter only to make the evaluation of pK_{a2} more reliable.

Electronic, CD, EPR, and ^{1}H NMRD Measurements. The electronic spectra of the Cu,ZnSOD mutants in position 137 are reported in Figure 3, together with that of the wild type. The absorption maximum in the d–d region of the spectra is shifted toward higher wavenumber on going from the wild type and the Ser-137 mutant to the Ala-137 and the Ile-137 mutants. The absorption is centered around 14 700 cm^{-1} in the wild type and Ser-137, at 15 000 cm^{-1} in Ala-137, and at 16 000 cm^{-1} in Ile-137. The CD spectrum of the Ala-137 mutant also shows a smaller blue shift with respect to the wild type and SOD-Ser-137 than that observed in SOD-Ile-137 (Figure 4).

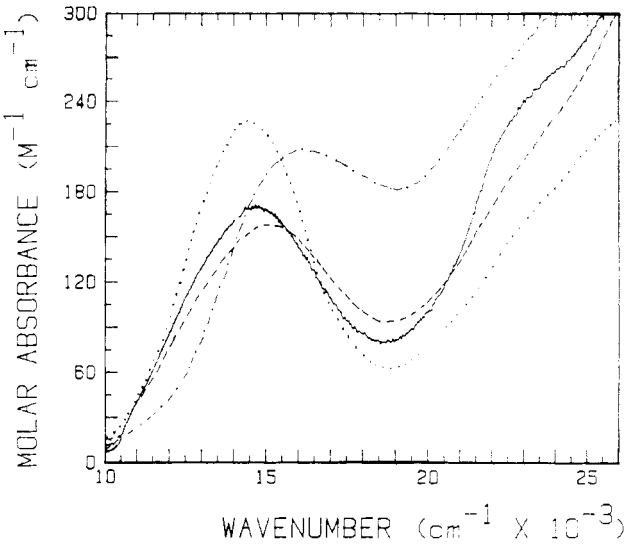

Figure 3. Electronic spectra of WT (···), Ser-137 (—), Ala-137 (--), and Ile-137 (-·-), at pH 7.5 in 50 mM Hepes. Molar absorbance is calculated per mole of copper. The protein concentrations are about 0.5 mM.

The EPR spectra (X-band, 9.8 GHz), recorded at room temperature, of SOD-Ala-137 and SOD-Ser-137 are rhombic, both with $g_\parallel = 2.26$, $g_\perp(av) = 2.07$, and $A_\parallel = 140 \times 10^{-4}$ cm^{-1}. They resemble the spectrum of the wild type and that of the AS mutant[22] but differ from that of Ile-137, which is essentially axial with a larger $A_\parallel(162 \times 10^{-4}$ $cm^{-1})$.[38]

The ^{1}H NMRD data are shown in Figure 5. On the ordinate scale, we report the water ^{1}H T_1^{-1} values, with the contribution of bulk solvent and the diamagnetic contribution subtracted, normalized to 1 mM copper concentration. The height of the profiles around 10 MHz is proportional to the correlation time τ_c and to the number and distance of the exchangeable protons sensing the paramagnetic metal ion.[8,36,39] The general shape of the profile indicates that τ_c does not vary much from one derivative to the other. From the height of the profile at 10 MHz, it appears that the Ser-137 mutant has either more water in the cavity or a better coordinated water molecule with respect to the WT. Then the Ala-137 derivative follows, which has a profile similar to that of the WT. The above data can be fitted by using an equation for the paramagnetic contribution to nuclear relaxation derived by including the hyperfine coupling of the copper nucleus.[36,39] If it is assumed that only one water molecule interacts with copper, and only the distance between oxygen and copper is allowed to vary together with the correlation time τ_c, the fitting provides values of $\tau_c = 1.5 \times 10^{-9}$ and 2.1×10^{-9} s, with a Cu–H distance of 3.7 and 3.2 Å for Ala-137 and Ser-137, respectively, which compare with the value of $\tau_c = 2.2 \times 10^{-9}$ s and a Cu–H distance of 3.5 Å for the wild type[25] and $\tau_c = 3.8 \times 10^{-9}$ s and a Cu–H distance of 4.7 Å for Ile-137.[25] The best fit curves are reported in Figure 5. The average, over the experimental points, of the square error is 5.3×10^{-3} mM^{-2} s^{-2} for the wild type, 2.3×10^{-2} mM^{-2} s^{-2} for Ser-137, 6.6×10^{-2} mM^{-2} s^{-2} for Ala-137, and 5.3×10^{-3} mM^{-2} s^{-2} for Ile-137. It should be noted that if τ_c increases, the same ^{1}H water T_1^{-1} value is reproduced by a larger Cu–H distance. However, at these small T_1^{-1} values, second-sphere and outer-sphere contributions may be significant, and the above distances should be considered just as an indication of the overall amount of solvent protons sensing the paramagnetic copper(II) center.

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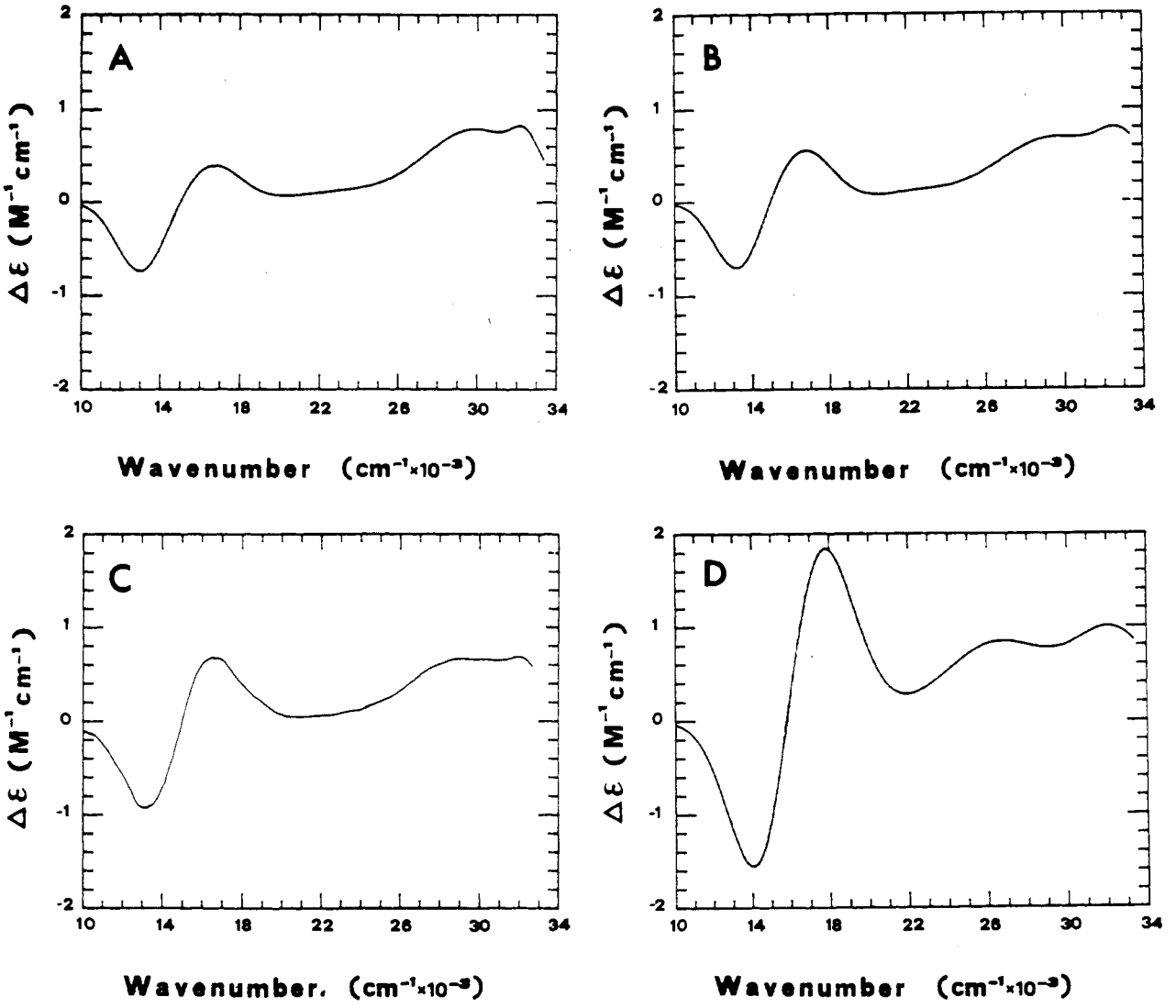

Figure 4. CD spectra of WT (A), Ser-137 (B), Ala-137 (C), and Ile-137 (D), at pH 7.5 in 50 mM Hepes. $\Delta\epsilon$ is expressed per mole of copper. The protein concentrations are about 0.5 mM.

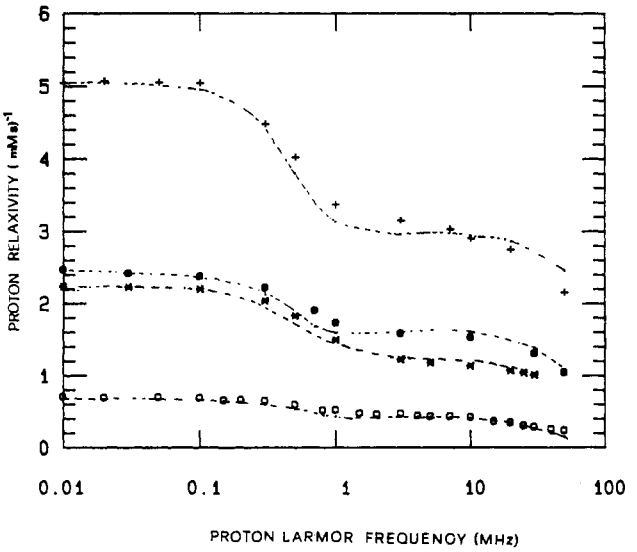

Figure 5. Water ^{1}H NMRD profiles at 298 K, pH 5.5, of solutions of WTSOD (●), Ser-137-SOD (+), Ala-137-SOD (✱), and Ile-137-SOD (○). Dotted lines represent best fitting curves calculated as discussed in the text.

The Cu_2Co_2 Derivatives. The derivatives with cobalt at the site of zinc are suitable for ^{1}H NMR spectroscopy and, at least in the case of the WT, display the same catalytic efficiency as the native derivative.[40-42] The high-spin cobalt(II) ion has very short electronic relaxation times, thus providing sharp, well-resolved ^{1}H NMR lines for protons of residues directly bound to the metal ion. On the contrary, the copper(II) ion has long electronic relaxation times, giving rise to broad, undetectable lines.[36] Magnetic coupling,[43,44] through the histidinato bridge, provides copper with the efficient electronic relaxation mechanisms of cobalt,[45,46] thus yielding NMR spectra with sharp lines for protons of residues bound to both copper and cobalt. The spectra are reported in Figure 6. Through H/D exchange, the His NH signals have been assigned. Other signals have been assigned by comparison with the fully assigned spectrum of the WT derivative[47-49] and by comparing the shift pattern upon addition of

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Table II. Chemical Shifts (ppm) of the ^1H NMR Signals of the Wild Type and of the 137 Mutant Cu_2Co_2SOD Derivatives at 200 MHz, 300 K

signal	WT	Ser-137	Ala-137	Ile-137	assignment
A	66.3	67.8	65.4	64.7	His-63 Hδ2
B	56.9	56.8	56.9	57.6	His-120 Hδ1
C	50.0	50.9	48.6	48.8	His-46 Hε2
D	49.6	49.8	50.2	50.2	His-71 Hδ2
E	49.2	49.8	48.6	47.7	His-80 Hδ2
F	46.8	47.0	47.2	47.7	His-80 Hε2 (His-71 Hε2)
G	41.4	40.8	40.5	47.7	His-46 Hδ2
H	39.1	37.9	38.7	43.8	His-120 Hε1
I	36.9	36.0	36.3	36.5	Asp-83 Hβ1 (Asp-83 Hβ2)
J′	36.0	36.0	36.3	36.5	Asp-83 Hβ2 (Asp-83 Hβ1)
J	35.6	34.5	35.5	36.5	His-71 Hε2 (His-80 Hε2)
K	35.0	34.5	36.3	38.5	His-48 Hδ1
L	28.8	28.3	29.9	37.9	His-48 Hδ2
M	25.8	26.7	25.5	20.2	His-46 Hε1
N	24.1	23.5	24.5	23.8	His-120 Hδ2
O	19.8	20.3	21.4	22.4	His-48 Hε1
P	18.9	19.4	18.5	16.7	His-46 Hβ1
Q	−5.7	−4.8	−5.3	−5.3	His-46 Hβ2 (−)
R	−5.7	−4.8	−5.3	−7.3	− (His-46 Hβ2)

increasing amounts of N_3^- to the various derivatives. The shift values, together with their assignment for the three mutants and that of the WT, are reported in Table II. The spectra indicate the overall close similarity in the structure of the active site for all the mutants and the WT enzyme. However, analysis of the shifts of signals K, L, and O, which are assigned to His-48,[49] is instructive. His-48 in the WT is bound to copper in a distorted way (N(His-46)–Cu–N(His-48) = 130°)[11] and experiences a large variation in shift upon anion binding. Compared to the spectrum of the wild type, there is almost no variation (see Table II) for the Ser-137 mutant whereas there is a larger downfield hyperfine shift (of about 2 ppm on the average) for the Ala-137 mutant; in the case of the Ile-137 mutant,[24] these signals undergo an even larger variation in the hyperfine shifts toward the downfield region.

In addition, other small variations in the shift values for the other signals are observed, indicating other small rearrangements in the active site. The signals from protons of the cobalt domain seem to be less affected by residue substitution at position 137. This is consistent with the close similarity of the ^1H NMR spectra of all the E_2Co_2 derivatives (not shown).

Azide binds the copper ion in SOD, and the exchange between bound and free azide is fast on the NMR time scale.[8,43,50] From the pattern of the shifts of the signals in the presence of increasing amounts of N_3^-, the affinity constants for the two new mutants have been estimated: 90 ± 7 (3σ) M^{-1} for Ser-137 and 110 ± 8 M^{-1} for Ala-137. These values compare with those of 94 ± 5 M^{-1} for the native enzyme and 138 ± 2 M^{-1} for the Ile-137 derivative under the same conditions.[24] The final adducts for all the mutants are similar to each other. In particular, differences in the shift values between the mutants and the WT are smaller for the adducts with azide with respect to the samples without azide, indicating that anion binding, even if the noninhibited derivatives show some structural variation, induces similar ligand arrangement.

Discussion

Ser-137 and Ala-137 SOD mutants appear quite similar to the native enzyme from the point of view of electronic and EPR spectroscopies on one side and of activity profiles on the other. If the water ^1H T_1^{-1} profiles and the affinity constant of azide are considered, then some subtle differences start to appear. The nature of the residue at the 137 position determines the presence of water within the active cavity. Indeed, the content of water sensing copper(II) in the active cavity decreases in the order Ser-137 > Thr-137 ≃ Ala-137 > Ile-137. Possibly this trend is related to the overall bulkiness and hydrophobicity of the 137 residue: the bulk Ile group occupies a large part of the cavity (Figure 1) and provides large hydrophobic contacts. This may account for the difference with the Ala derivative that has smaller size. The Thr derivative probably stabilizes the weakly coordinated water either by a direct hydrogen bond or by participating in a hydrogen-bonding network. Serine, due to its smaller hindrance, possibly can orient in such a way to further stabilize the water molecule and/or to allow for further water to be present in the cavity. The presence of this water molecule practically does not affect the electronic energy levels of the copper(II) ion.[26] In the absence of water, if the CuN_4 chromophore were planar, the unpaired electron would be expected to be in the xy plane and in the $x^2 - y^2$ orbital. Consequently, the shifts of the protons of His-48, which in such a case lies in the xy plane, would be the largest, i.e. of the same size as those of the other histidines. However, His-48 tends to be removed from the CuN_3 plane constituted by the nitrogens of His-46, His-63, and His-120, and thus the protons of His-48 exhibit smaller hyperfine shifts compared to those of the protons of the other histidines. In this way, there is space for a water molecule to approach the copper ion. Indeed, the order of the four derivative is qualitatively the same when the ^1H NMR shifts of signals K, L, and O of His-48 and the relative height of the water ^1H NMRD profiles are considered.

The sensitivity of the technique is very high, the maximum source of error being the indetermination of protein and metal ion concentration. The errors on the relaxivity values are mainly due to the scattering of the various points with respect to the calculated curves.

It is worth noting that the presence of water at the various degrees here reported is not related to the activity of the protein.

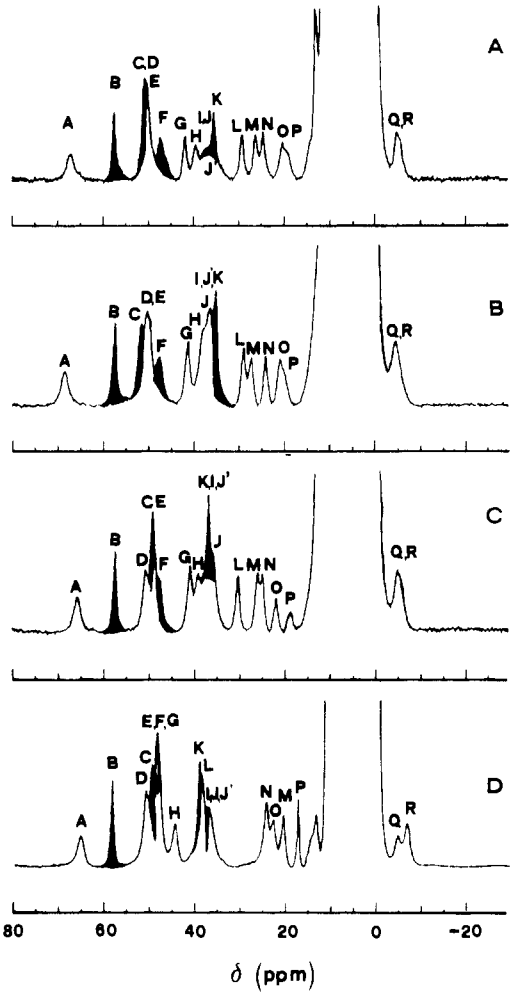

Figure 6. 300-MHz ^1H NMR spectra of (A) Cu_2Co_2WT, (B) Cu_2Co_2Ser-137, (C) Cu_2Co_2Ala-137, and (D) Cu_2Co_2Ile-137. The spectra are recorded at 200 MHz, 300 K. The samples are in 50 mM Hepes, pH 7.5. Filled signals disappear in D_2O.

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This suggests that the electron transfer between superoxide and copper is not mediated by a water molecule near the copper ion. The activity profiles of the WT and of the three mutants at the 137 position are virtually identical, except for a small decrease in the high-pH pK_a of the profile of Ile-137. The first conclusion would be that the kinetic parameters are not much dependent on the nature of the residue at position 137, provided that the WT is the most active derivative. The affinity of N_3^- increases slightly from Ser-137 to WT and Ala-137. Then it is almost 50% larger for the Ile-137 derivative. The azide affinity follows the reverse order of the water ^1H T_1^{-1} values. It has been independently proposed that the affinity of O_2^- (and therefore K_M) changes in the same direction as N_3^- does in a series of mutants at position 143.[22] Here, the overall changes are much smaller and not easily related to N_3^- affinity. In the previous series of mutants, the changes were proposed to originate mainly from electrostatic reasons, the mutated residue being a positively charged arginine. Here, the factors governing activity and anion affinity may not be the same, and in many cases they induce smaller effects.

The variation in the high-pH pK_a of the activity profile in the case of Ile-137 is likely due not to structural effects but only to change in hydrophobicity of the cavity. The small inflection at about pH 6–7 is also due to groups yet undefined; interestingly, it is present also in the mutants without Glu-132 and Glu-133, which are at the opening of the cavity.[13] It may be due to a different group far from the active cavity. Therefore the pH dependence of the activity around neutrality is quite complex.

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Registry No. Ser, 56-45-1; Ala, 56-41-7; Ile, 73-32-5; Thr, 72-19-5; Cu, 7440-50-8; N_3, 14343-69-2; superoxide dismutase, 9054-89-1.

Contribution from the Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803-1804

Intramolecular Binding of Nitrogen Bases to a Cofacial Binuclear Copper(II) Complex†

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Coordination of pyrazines and pyridines to the cofacial binuclear complex $Cu_2(NBA)_2$ ($NBAH_2$ = 3,3′-[2,7-naphthalenediylbis(methylene)]bis(2,4-pentanedione)) has been studied by structural and spectroscopic methods. Pyrazines bind more strongly than the corresponding pyridines, suggesting intramolecular, or endo, coordination, which X-ray analysis of the adduct with 2,5-dimethylpyrazine (2,5-Me_2pyz) confirms: $Cu_2(NBA)_2(\mu$-2,5-Me_2pyz)·4CH_2Cl_2, monoclinic, space group $C2/m$ (No. 12); a = 22.941 (6), b = 22.432 (4), c = 11.677 (2) Å; β = 97.32 (2)°; V = 5960 (4) Å^3; Z = 4; R = 0.096, R_w = 0.093 for 372 parameters and 3358 reflections with $I > 1\sigma(I)$. The structure contains two independent $Cu_2(NBA)_2(\mu$-2,5-Me_2pyz) molecules (Cu···Cu = 7.596 (2), 7.559 (2) Å), each with a disordered 2,5-Me_2pyz moiety and overall $2/m$ (C_{2h}) symmetry. Equilibrium constants for binding of substituted pyrazines to $Cu_2(NBA)_2$ range from ca. 0.2 M^{-1} (2,3-diethylpyrazine) to 93 M^{-1} (2-aminopyrazine); binding constants for comparably substituted pyridines are significantly smaller in all cases. Hydrogen bonding between the NH_2 group of 2-aminopyrazine and the O atoms of the $Cu_2(NBA)_2$ host is probably responsible for its unusually large binding constant.

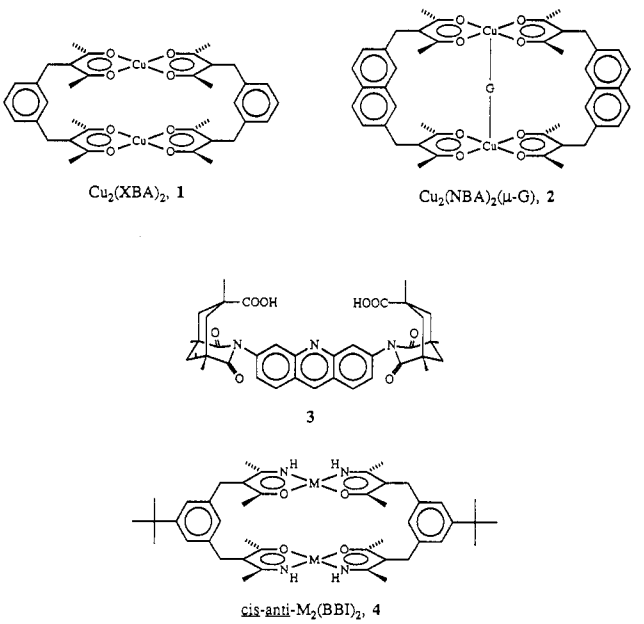
Introduction

Since our initial report of the cofacial binuclear bis(β-diketone) complex $Cu_2(XBA)_2$ (**1**; see Chart I),[1] we have been exploring the use of these and related complexes to bind guest molecules. We showed that the larger complex $Cu_2(NBA)_2$ binds guest molecules G (see **2**) such as Dabco (1,4-diazabicyclo[2.2.2]octane) in an intramolecular fashion.[2] The resulting host–guest complex is similar to those produced by several flexible binucleating macrocycles,[3] but our rigid 2,7-naphthalenediylbis(methylene) bridging groups provide a cavity of well-defined size and shape. We also examined pyrazine, whose lower basicity[4] and larger N···N distance[5] are likely to make intramolecular complexation less favorable.

Pyrazines and Dabco have been used to join mononuclear species to produce binuclear complexes of a variety of metals.[6] One-,[6b,7] two-[8] and three-dimensional[9] polymeric complexes have also been prepared from these diamines. The recognition of pyrazine by organic systems such as the rigid chelating diacid **3** has been studied by both experimental[10] and theoretical[11] methods.

We now report the demonstration by X-ray analysis that pyrazines bind intramolecularly to the discrete binuclear complex $Cu_2(NBA)_2$. Coordination of substituted pyrazines to $Cu_2(NBA)_2$ is generally weaker than that of the parent compound. However, aminopyrazine binds much more strongly, probably because it can hydrogen-bond to the O atoms of the coordinated bis(β-diketone) ligands.

Chart I



$Cu_2(XBA)_2$, **1**

$Cu_2(NBA)_2(\mu$-G), **2**

3

cis-anti-$M_2(BBI)_2$, **4**

† Ligand abbreviations: acacH = 2,4-pentanedione; $XBAH_2$ = *m*-xylylenebis(acetylacetone) (3,3′-[1,3-phenylenebis(methylene)]bis(2,4-pentanedione)); $NBAH_2$ = 2,7-naphthalenediylbis(methylene)bis(acetylacetone) (3,3′-[2,7-naphthalenediylbis(methylene)]bis(2,4-pentanedione)); $BBIH_2$ = 5-*tert*-butyl-*m*-xylylenebis(acetylacetone imine) (3,3′-[5-(1,1-dimethylethyl)-1,3-phenylenebis(methylene)]bis(4-amino-3-penten-2-one)).

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