concentrations of oxidant (MCPBA more than OCl^-) lead to a reduction in the degree to which the heme absorbance is recovered. Heme oxidation in the presence of a large excess of oxidant, particularly H_2O_2 and peroxoacid, has been shown to be accompanied by porphyrin ring degradation and formation of nonabsorbing or low-absorbing products.[25,31] We suggest the marked increase in k_1 and k_2 that occurs with increasing peroxoacid concentration to be a result of porphyrin ring degradation producing reducing ligands, which then attack the oxidized dfh species and provide an additional pathway for decomposition to free heme.[32,33] At the present time, mechanistic details are unknown; however, the fact that higher regeneration rates are observed following heme oxidation by MCPBA than by OCl^- or ClO_2^- (Table IV) probably reflects a peroxoacid induced degradation of the porphyrin ring system.

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Notes

Contribution from the Department of Chemistry,
University of Florence, Via G. Capponi 7,
50121 Florence, Italy, and
Institute of Agricultural Chemistry,
University of Bologna, 40146 Bologna, Italy

A Comment on the ^1H NMR Spectra of Cobalt(II)-Substituted Superoxide Dismutases with Histidines Deuteriated in the ε1-Position

Lucia Banci,[†] Ivano Bertini,*[,†] Claudio Luchinat,[‡]
and Maria Silvia Viezzoli[†]

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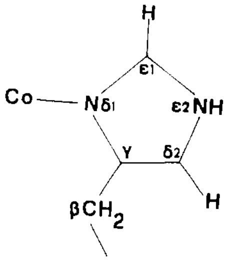
Erythrocyte superoxide dismutase Cu_2Zn_2SOD is a dimeric metalloenzyme containing one copper(II) and one zinc(II) ion bridged by a histidinato residue in each subunit. E_2Co_2SOD and $Cu^I_2Co_2SOD$ are artificial derivatives of the native Cu_2Zn_2SOD[1,2] that permit the investigation through ^1H NMR spectroscopy of the protons of the cobalt domain.[3,4] Cobalt(II) is bound to three histidines and to one aspartate, as shown in the inset of Figure 1; each of the histidines is coordinated through its $N^{\delta1}$ nitrogen:[5]



The ^1H NMR spectra of the bovine E_2Co_2SOD and $Cu^I_2Co_2SOD$ derivatives are shown in Figure 1. In the $Cu^I_2Co_2SOD$ derivative there are six sharp signals downfield; three of these (shaded in Figure 1) disappear when the spectrum is recorded in D_2O. The latter are assigned as the $N^{\epsilon2}$ exchangeable protons of the three coordinated histidines. The other three signals are due to $H^{\delta2}$ of the same histidines that are in a meta-like position with respect to the coordinating nitrogen.[3] Their T_1 and line shape are consistent with signals assigned to meta-like protons of histidines in similar compounds.[6–10] In E_2Co_2SOD one NH signal is missing, probably because it exchanges rapidly on the NMR time scale. One or two broader signals are also present in the spectra, which were tentatively assigned to Hε1 (ortho-like protons) of the above histidines because they are expected to be broader than the meta-like protons.[4,11]

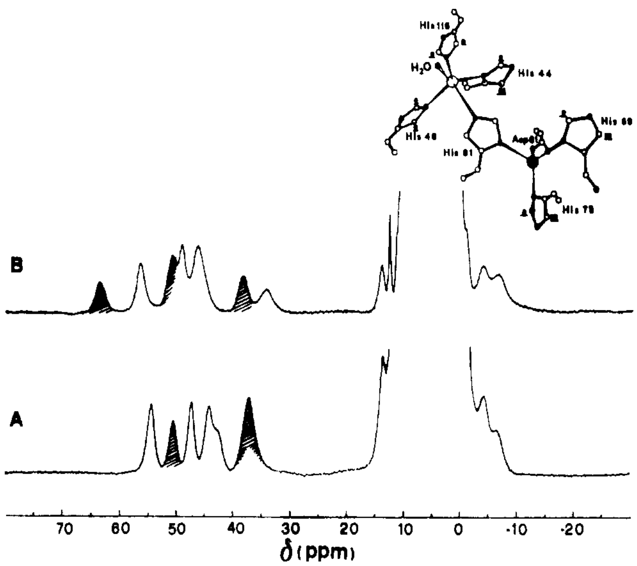


Figure 1. 200-MHz ^1H NMR spectra of bovine E_2Co_2SOD (A) and bovine $Cu^I_2Co_2SOD$ (B). The spectra are recorded in 50 mM acetate buffer, in H_2O, pH 5.5, at 27 °C. The inset shows a scheme of the metal site of SOD.

Recently human SOD has been expressed in *Escherichia coli* (HECSOD)[12] and it has been conceivable to prepare a SOD

[†] University of Florence.

[‡] University of Bologna.

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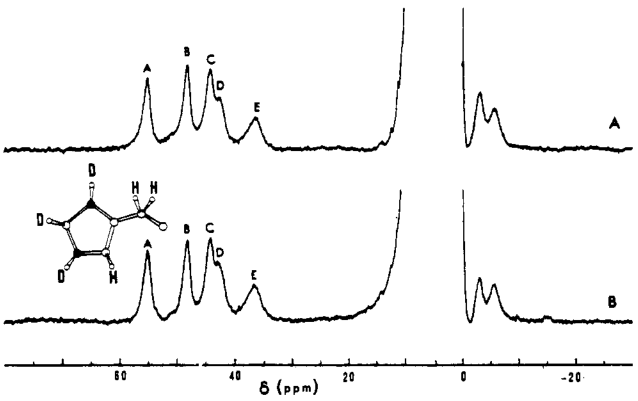

Figure 2. 200-MHz ^{1}H NMR spectra of E_2Co_2HECSOD (A) and E_2Co_2-deuteriated histidine-HECSOD (B). The spectra are recorded at 27 °C, in 50 mM acetate buffer, in D_2O, pH 5.5.

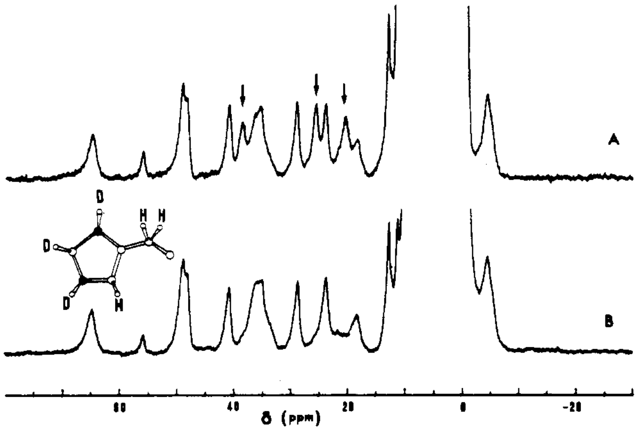

Figure 3. 200-MHz ^{1}H NMR spectra of Cu_2Co_2HECSOD (A) and Cu_2Co_2-deuteriated histidine-HECSOD (B). The arrows in the spectra of Cu_2Co_2HECSOD show the signals that disappear in Cu_2Co_2-deuteriated histidine-HECSOD. The spectra are recorded at 27 °C, in 50 mM acetate buffer, in D_2O, pH 5.5.

derivative with deuteriated histidines in the ϵ1-position in order to check the assignment of the ^{1}H NMR spectra of the above derivatives as well as of the Cu_2Co_2SOD in which the signals of all the residues bound to copper and cobalt are observed. This has been achieved by substituting the regular culture medium used for *E. coli* with a growth medium containing a large amount of histidine deuteriated on the ϵ1-position. In such conditions, even if the *E. coli* strain is not auxotroph for histidine, the produced SOD results to contain deuteriated histidines.

The ^{1}H NMR spectra of D_2O solutions of the deuteriated and native E_2Co_2SOD do not differ at all (Figure 2). Likewise, D_2O solutions of deuteriated and native $Cu^{I}_2Co_2$SOD display identical spectra (data not shown). On the other hand, in the case of Cu_2Co_2SOD all the protons assigned to $H^{\epsilon 1}$ protons of the histidines in the copper domain disappear (Figure 3), thus confirming that the assignment of such signals was correct and that we are indeed dealing with a fully deuteriated protein. We conclude that the spectra of native E_2Co_2SOD and $Cu^{I}_2Co_2$SOD do not show the ortho-like protons because they are too broadened by the hyperfine coupling with the paramagnetic center. This is a meaningful result: the ortho-like protons of histidines bound to pseudotetrahedral cobalt(II) may be broadened beyond detection, presumably by Curie relaxation, already at 200 MHz.[6,13,14] The alternative assignment is that the two broad signals are due to geminal protons of the coordinated aspartate. Asp-81 has been already suspected as originating one hyperfine-shifted signal in the downfield region for the $Cu^{I}_2Ni_2$SOD, Ag_2Ni_2SOD, and Cu_2Ni_2SOD derivatives.[15] If the D and E signals in Figure 2

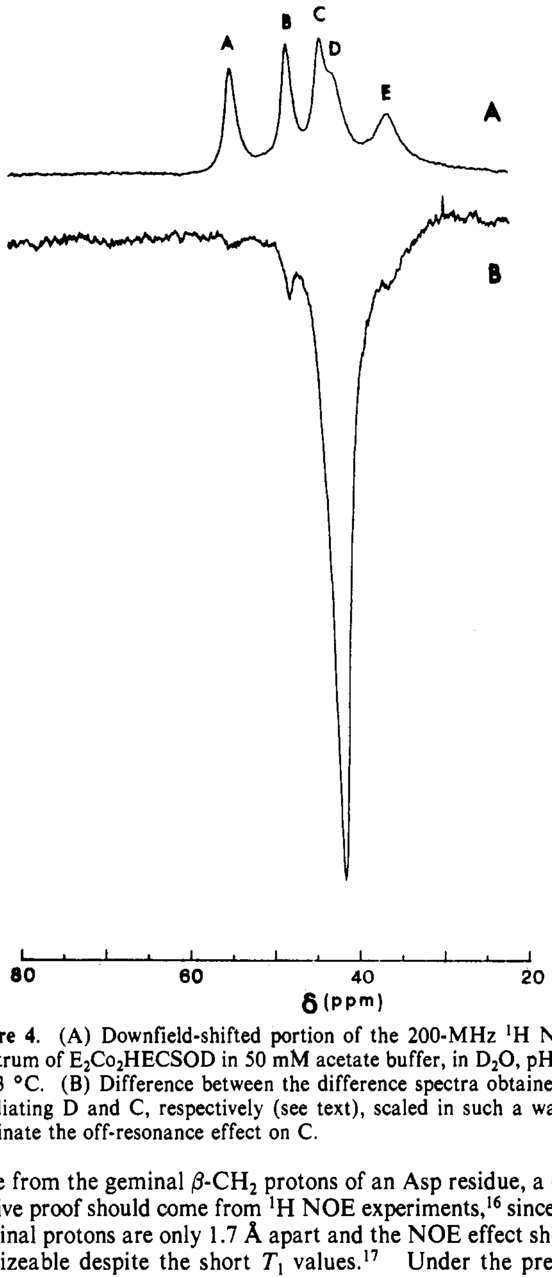

Figure 4. (A) Downfield-shifted portion of the 200-MHz ^{1}H NMR spectrum of E_2Co_2HECSOD in 50 mM acetate buffer, in D_2O, pH 5.5, at 33 °C. (B) Difference between the difference spectra obtained by irradiating D and C, respectively (see text), scaled in such a way to eliminate the off-resonance effect on C.

arise from the geminal β-CH_2 protons of an Asp residue, a conclusive proof should come from ^{1}H NOE experiments,[16] since the geminal protons are only 1.7 Å apart and the NOE effect should be sizeable despite the short T_1 values.[17] Under the present conditions (steady-state NOE) NOE on signal j when i is irradiated is given by

$$\eta_{ij} = \sigma_{ij}/\rho_j$$

where ρ_j is the nonselective T_1^{-1} of signal j and σ_{ij} is proportional to r_{ij}^{-6} (r_{ij} is the interproton distance) times the rotational correlation time τ_r (slow-motion limit).[18] Since we want to ascertain whether signal E gives any NOE in response to irradiation of signal D, we must make sure to avoid even slight off-resonance effects, which are negative as the expected NOE's. The safest way of performing the experiment is by using as a reference spectrum one that is irradiated at the right side of signal E, in a position symmetrical with respect to the irradiated signal D. In such a way off-resonance effects cancel in the subtraction. Therefore,

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an NOE experiment was performed by irradiating at signal D and at its symmetrical position with respect to E, alternately, and subtracting the FID's. In addition, a similar experiment was performed by irradiating at signal C and at its symmetrical position with respect to E. The resulting difference spectra were subtracted, in order to rule out any possible contribution of C to the NOE on E. The result of such subtraction is shown in Figure 4. Signal E is clearly apparent as a negative shoulder of the irradiated signal D and therefore demonstrates the presence of sizable NOE. Off-resonance effects are apparent on signal B and, to a lesser extent, on signal A, for which complete cancellation has not occurred. The intensity of the NOE observed on signal E is consistent with D and E being the geminal β-CH_2 protons of Asp-81.

The assignment of β-CH_2 protons of aspartic and γ-CH_2 protons of glutamic residues has always been a problem in the NMR spectroscopy of paramagnetic proteins, and very few documented examples are available. Some significant work had been done by Goff and Que in dinuclear iron complexes.[19]

We have now put the assignment of the protons of the cobalt domain in cobalt-substituted SOD's on a firmer basis through deuteriation and ^1H NOE experiments: the ortho-like protons of histidines may relax much faster than the meta-like protons in tetrahedral cobalt(II) systems, and the CH_2 protons of Glu and Asp are quite broad and experience shifts similar to those of meta-like protons of histidines.

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Contribution from the Dipartimento di Chimica
e Chimica Industriale, Università di Pisa,
via Risorgimento 35, I-56100 Pisa, Italy

Cobalt Carbonyl Chemistry in Wet Ethereal Solutions: $[Co_6(CO)_{15}]^{2-}$ and $Co_6(CO)_{16}$ Cluster Formation or Stepwise Water Gas Shift Reaction

Tiziana Funaioli, Paolo Biagini, and Giuseppe Fachinetti*

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The reduction of water by CO to form CO_2 and H_2 (water gas shift reaction (WGSR)) is an important process catalyzed in homogeneous phase by metal carbonyls.[1] Therefore, the chemistry of metal carbonyls in water or in water-containing solutions is a subject of large interest: several group 6 or group 8 metal carbonyls, precursors for WGSR, were investigated in detail in their reactions with water or with hydroxide ion.[2] From this point of view, $Co_2(CO)_8$ (**1**), promoting the WGSR under drastic conditions,[3] has been somewhat neglected. It is known that water, in which **1** is not soluble, slowly disproportionates **1**;[4] from the resulting pink solution, especially when concentrated, some $HCo(CO)_4$ and $Co(OH)_2$ can be formed. Subsequently, we found that the disproportionation–synproportionation equilibrium (1) is established at room temperature in tetrahydrofuran (THF) solutions of **1** in the presence of small amounts of water ($H_2O/\mathbf{1}$ molar ratio <10).[5]

$$\tfrac{3}{2}Co_2(CO)_8 \xrightleftharpoons{\text{THF, } H_2O} [Co(H_2O)_x(THF)_y]^{2+} + 2[Co(CO)_4]^- + 4CO \quad (1)$$

These findings indicate that the chemistry of **1** is strongly affected by water concentration. In order to better define these effects and the possibilities offered by **1** as a catalyst for the WGSR, we carried out the present study, where the electron transfers occurring in wet ethereal solutions of **1** are investigated under various $H_2O/\mathbf{1}$ molar ratios and CO pressures.

Experimental Section

Materials and procedures were used as described recently.[6]

$Co_7(CO)_{15}\cdot 2H_2O\cdot 6THF$ (2). A 6.2-g amount (18.13 mmol) of **1** and 0.35 mL (19.44 mmol) of H_2O were dissolved in 600 mL of THF. The resulting dark brown solution was refluxed at 40 °C under a constant 440-mmHg pressure. Pure CO was evolved during the reaction, and after 10 h 6.0 g (89%) of **2** was obtained as black crystals. Anal. Calcd for $Co_7(CO)_{15}\cdot 2H_2O\cdot 6THF$: Co, 31.74; CO, 32.28; THF, 33.20; H_2O, 2.77. Found: Co, 31.27; CO, 32.12; THF, 32.80; H_2O, 2.85. Selected IR bands (Nujol mull): 3413 (m, br), 2045 (m), 1984 (vs), 1956 (vs), 1781 (s), 1756 (m), 1732 (m), 1722 (s), 1697 (m), 1653 (s) cm^{-1}.

Carbonylation of 2 in Toluene. A suspension of 1.45 g (1.11 mmol) of **2** in 100 mL of toluene was vigorously stirred with a magnetic bar in a 500-mL closed vessel filled with CO at atmospheric pressure. After 12 h at 20 °C a dark brown solution and a black solid, $Co_6(CO)_{16}$ (76%), were obtained. Anal. Calcd for $Co_6(CO)_{16}$: Co, 44.14; CO, 55.86. Found: Co, 43.60; CO, 55.10. $\nu(CO)$ (Nujol mull): 2113 (w), 2061 (vs), 2057 (sh), 2026 (w), 2020 (w), 2018 (w), 1772 (vs) cm^{-1}.

$Co_4(OH)_7Co(CO)_4$ (3). A pink solution obtained by dissolving 3.16 g (9.2 mmol) of **1** and 2.5 mL (139 mmol) of H_2O in 300 mL of Et_2O was refluxed under atmospheric pressure. A $CO/H_2 = 2.8$ mixture of constant composition was evolved during 3 h while **3** separated out as a gray solid (96%). Anal. Calcd for $Co_5(OH)_7(CO)_4$: Co, 56.08; OH, 22.62; CO, 21.29. Found: Co, 57.03; OH, 21.63; CO, 20.84. IR (Nujol mull; cm^{-1}): $\nu(OH)$ 3665 (m), 3640 (m), 3594 (m); $\nu(CO)$ 2040 (m), 1980 (s), 1920 (vs).

Carbonylation of 3. A 120-mL rocking steel autoclave containing 0.96 g (1.83 mmol) of **3** suspended in 35 mL fo 0.5% H_2O/THF was pressurized with 80 atm of CO and warmed at 100 °C. Analysis of gases after 12 h revealed the presence of CO and CO_2 only. CO_2 was quantitatively determined by slowly bubbling the reaction gases into 50 mL of 0.106 M $Ba(OH)_2$ solution. The excess of $Ba(OH)_2$ was potentiometrically titrated against 0.1 N HCl. The end point was reached after the addition of 36 mL of HCl. The IR spectrum of the liquid phase showed absorptions due to **1** ($\nu(CO)$ 2070 (vs), 2040 (vs), 1846 (s) cm^{-1}) and to $[Co(CO)_4]^-$ ($\nu(CO)$ 1887 cm^{-1}). Quantitative determination of formed H_2O (3.0 mmol) was performed by IR comparison with standard H_2O/THF solutions. Before analysis, samples were treated with an excess of 2,2′-bipyridine.

Results and Discussion

Depending on the $H_2O/\mathbf{1}$ molar ratio, different reactions occur on CO removal from wet THF solutions of **1**. As mentioned above, in the case of $H_2O/\mathbf{1} = 1$ (molar ratio) the P_{CO}-dependent equilibrium (1) is operating at room temperature in a 0.03 M solution of **1** in THF. By reflux under reduced pressure at 40 °C, CO is lost from this solution while a black crystalline solid analyzing as $Co_7(CO)_{15}\cdot 2H_2O\cdot 6THF$ (**2**) separates out in almost quantitative yields. While some disorder in the crystals prevented a X-ray determination of the structure, the nature of **2** can be inferred from its IR spectrum in CH_3OH, where **2** dissolves with a dark green color. In the CO stretching region the characteristic pattern of absorptions of the anionic cluster $[Co_6(CO)_{15}]^{2-}$ is observed[7] and the spectrum is superimposable on that of an authentic sample of $Cs_2Co_6(CO)_{15}$ in CH_3OH at the same concentration (Figure 1a). It is considered therefore that, in the

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