

considered outer sphere in nature. Davies<sup>22</sup> has analyzed the variations in rate constants for the latter reactions in terms of outer-sphere precursor formation and the attainment of a species in which the distribution of OH<sup>-</sup> in the inner sphere of cobalt(III) is optimal for hydroxide-mediated electron transfer within the precursor complex. A specific interaction of this type is consistent with the present system.

The slower reaction ( $k_3K_h = 935 \text{ s}^{-1}$ ) for the oxidation of Ni(cyclam) compared with other anionic or neutral substrates (e.g., I<sup>-</sup>,  $k_3K_h = 2.9 \times 10^3 \text{ s}^{-1}$ ) may reflect either the higher redox potential of the Ni(II)L/Ni(III)L couple (0.95 V) or the fact that electrostatic repulsion terms will be greater in the case of cationic reductants. The former argument does not explain the relatively low reaction rates for the Co<sup>2+</sup>, Mn<sup>2+</sup>,

and Fe<sup>2+</sup> reactions.<sup>23</sup> Differences in the rates will be however expected if a specifically oriented precursor is formed prior to the electron transfer. A series of preequilibria<sup>22</sup> describing such a process have been suggested as contributing to the compensating variation in  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$  observed in these reactions. The fact that a metal complex ion is involved rather than the smaller aquo ions will have a bearing on these effects.

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**Registry No.** [Ni<sup>III</sup>L]<sup>2+</sup>, 46365-93-9; Co(III)(aq), 15275-05-5; [NiL(OH<sub>2</sub>)<sub>3</sub>]<sup>3+</sup>, 72360-42-0; [NiL(MeCN)<sub>2</sub>]<sup>3+</sup>, 47099-85-4; CoOH<sup>2+</sup>(aq), 68842-90-0.

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Contribution from the Istituto di Chimica Generale ed Inorganica, Facoltà di Farmacia, University of Florence, 50121 Florence, Italy, and Laboratorio per lo Studio dei Composti di Coordinazione del CNR, Florence, Italy

## pH-Dependent Properties of a CoN<sub>4</sub>(OH<sub>2</sub>) Chromophore: A Spectroscopic Model of Cobalt Carbonic Anhydrase

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The complexes [Co(H<sub>2</sub>O)L](ClO<sub>4</sub>)<sub>2</sub> and [Co(N<sub>3</sub>)L]ClO<sub>4</sub>, where L stands for tris[(3,5-dimethyl-1-pyrazolyl)methyl]amine, have been prepared and characterized. The aqua complex has been found to have two acidic groups in the pH range 5.5-9.5, which have been attributed to the bound water ( $pK_a \approx 9$ ) and to the apical nitrogen ( $pK_a \approx 7.4$ ). The behavior of this compound as well as of its azide derivative has been compared with that of cobalt(II)-substituted carbonic anhydrase; in particular, it is shown that the deprotonation of the coordinated water molecule can by itself cause a dramatic change in the electronic spectra of the cobalt(II) chromophore and a strong reduction of the anion affinity for the solvent coordination site, both puzzling properties of the above enzyme.

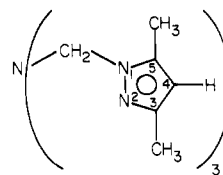
### Introduction

Carbonic anhydrase is a zinc(II) metalloenzyme with a water molecule in the metal coordination sphere, whose catalytic activity depends on an acid-base equilibrium with  $pK_a$  of about 7.<sup>2</sup> The same equilibrium also controls the inhibiting ability of anions like NCO<sup>-</sup>, N<sub>3</sub><sup>-</sup>, halides, etc.<sup>2</sup> The identity of the acidic group involved is still controversial;<sup>3</sup> among various possibilities, the coordinated water molecule has been often regarded as a reasonable candidate.<sup>4</sup>

Cobalt(II) can be successfully substituted to the native metal ion yielding a still active enzyme with similar acid-base properties.<sup>5</sup> The electronic spectra of the latter derivative are strongly pH dependent,<sup>2a</sup> although they are probably sensitive to more than one acid-base equilibrium<sup>6</sup> (actually about four or five deprotonations take place in the enzyme in the pH range 5-9<sup>2b</sup>), the major variation can again be ascribed to a single acidic group with  $pK_a$  of about 7. However, the observed spectral changes cannot be taken as diagnostic of ionization of the coordinated water molecule. Indeed, none of the few known cobalt(II) complexes with a single water molecule bound in water solution<sup>7-11</sup> are suitable to reproduce the spectral features and variations observed in the cobalt enzyme.

In order to investigate the acid-base properties of the coordinated water molecule, the influence of the water deprotonation on the electronic spectra, and the possibility of substitution of the H<sub>2</sub>O or OH groups by other ligands, we have

prepared and characterized the complexes [Co(H<sub>2</sub>O)L](ClO<sub>4</sub>)<sub>2</sub> and [Co(N<sub>3</sub>)L]ClO<sub>4</sub>, where L is tris[(3,5-dimethyl-1-pyrazolyl)methyl]amine.



This ligand has been chosen for the following reasons: (i) pyrazolyl-containing ligands are known to stabilize low oxidation states<sup>12</sup> and therefore should protect cobalt(II) from

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Table I. Analytical and Some Physical Data for the Complexes

		analytical data				$\Lambda_M^a$ $\text{S cm}^2 \text{ mol}^{-1}$	$\mu_B^{\text{eff}}$ $\mu_B$ (295 K)
		% C	% H	% N	% Co		
$[\text{Co}(\text{H}_2\text{O})\text{L}](\text{ClO}_4)_2$	calcd	35.03	4.73	15.88	9.54	169 ( $\text{CH}_3\text{OH}$ ) 197 ( $\text{H}_2\text{O}$ )	4.25
	found	35.00	4.90	16.40	9.20		
$[\text{Co}(\text{N}_3)\text{L}](\text{ClO}_4)$	calcd	39.90	5.02	25.85			4.30
	found	39.52	5.15	25.40			

<sup>a</sup> Reference values of 2:1 electrolytes in  $10^{-3} \text{ mol dm}^{-3}$   $\text{CH}_3\text{OH}$  solutions give an average  $\Lambda_M = 190 \text{ S cm}^2 \text{ mol}^{-1}$ .<sup>18</sup> Conductivity of ethylenediamine dihydrochloride in  $10^{-3} \text{ mol dm}^{-3}$  water solution is  $240 \text{ S cm}^2 \text{ mol}^{-1}$ .

oxidation even at high pH values; (ii) the tripodal geometry of the ligand and the three methyl groups in the 3-positions should favor five-coordination with a single water molecule; (iii) pyrazoles bear some similarity to imidazoles, which are the coordinating groups from the protein in carbonic anhydrase.<sup>2a</sup>

### Experimental Section

The ligand tris[(3,5-dimethyl-1-pyrazolyl)methyl]amine was synthesized by allowing  $\text{N}(\text{CH}_2\text{Cl})_3$  and potassium 3,5-dimethylpyrazolate to react as already reported.<sup>13</sup>  $[\text{Co}(\text{H}_2\text{O})\text{L}](\text{ClO}_4)_2$  was obtained upon evaporation of an ethanol solution containing  $\text{Co}(\text{H}_2\text{O})_6(\text{ClO}_4)_2$  and the ligand in 10% excess.  $[\text{Co}(\text{N}_3)\text{L}]\text{ClO}_4$  was prepared by adding an equimolar amount of potassium azide to a methanol solution of the aqua complex.

The complexes were recrystallized from ethanol or methanol, filtered off, and washed with petroleum ether. The analytical data are reported in Table I. The presence of water in the  $[\text{Co}(\text{H}_2\text{O})\text{L}](\text{ClO}_4)_2$  complex is confirmed by infrared spectra which show bands in the region  $3200\text{--}3400 \text{ cm}^{-1}$  (OH stretching) and  $1600\text{--}1700 \text{ cm}^{-1}$  (HOH bending).

The conductivity data were obtained with a WTW Model LBR/B conductance bridge. Concentrations of the solutions were approximately  $10^{-3} \text{ mol dm}^{-3}$ . Magnetic measurements were performed with the Faraday method by using the apparatus and the techniques already reported.<sup>14</sup> Infrared spectra were obtained with a Perkin-Elmer Model 283 grating spectrophotometer on samples prepared as Nujol mulls.

Potentiometric measurements were performed with a potentiometer, Orion Model 801, in the pH range 9.2–5.0, with  $10^{-3} \text{ mol dm}^{-3}$  complex solutions.

The electronic spectra were recorded on a Cary 17D spectrophotometer. The spectra in the near-infrared region were recorded in  $\text{D}_2\text{O}$  or deuteriomethanol solutions. The binding of  $\text{N}_3^-$  to the cobalt complex in the pH range 6–9.5 was followed from the spectral variations in the visible region.

The 80-MHz  $^1\text{H}$  NMR spectra of the complexes in  $\text{D}_2\text{O}$  or  $\text{CD}_3\text{OD}$  were recorded on a Varian CFT 20 spectrometer operating at  $20^\circ\text{C}$ . Pulses of  $90^\circ$  were employed with repetition times of 0.05–0.1 s. The short relaxation times of the protons of paramagnetic complexes permitted enhancement of the ligand signals relative to the signals of the solvent protons, which are almost saturated under these conditions. Chemical shifts were measured from external tetramethylsilane.  $T_1$  measurements were performed by using the inversion recovery method; water proton relaxation measurements were performed as previously described.<sup>15</sup> The reported pH values in  $\text{D}_2\text{O}$  solutions are uncorrected, and they correspond to pD  $-0.4$ .<sup>16</sup>

The aqua complex was tested as catalyzer of the  $\text{CO}_2$  hydration reaction following the method of Roughton and Booth.<sup>17</sup> No increase in the hydration rate is observed up to  $10^{-3} \text{ mol dm}^{-3}$  complex concentration at pH 8.8.

### Results and Discussion

The complex  $[\text{Co}(\text{H}_2\text{O})\text{L}](\text{ClO}_4)_2$  is high-spin: its reflectance spectrum shows absorption at  $6.8$ ,  $13.6$ , and  $19.2 \text{ cm}^{-1} \times 10^{-3}$  (Figure 1). The analogies with other  $[\text{CoXL}]^+$  complexes (X = halides, pseudohalides) previously reported<sup>13</sup> lead us to assign the complex a five-coordinate structure. The

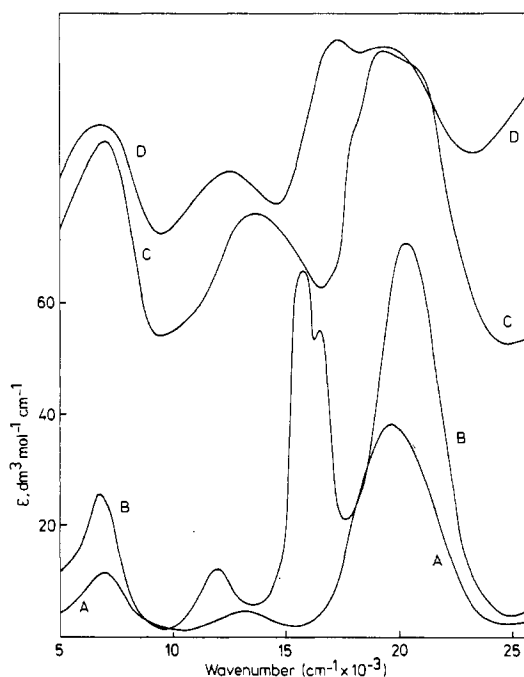


Figure 1. Electronic spectra of the  $[\text{Co}(\text{H}_2\text{O})\text{L}](\text{ClO}_4)_2$  complex in water solution at pH 7.5 (A) and 9.6 (B) and as a solid (C) and electronic spectrum of solid  $[\text{Co}(\text{N}_3)\text{L}]\text{ClO}_4$  (D).

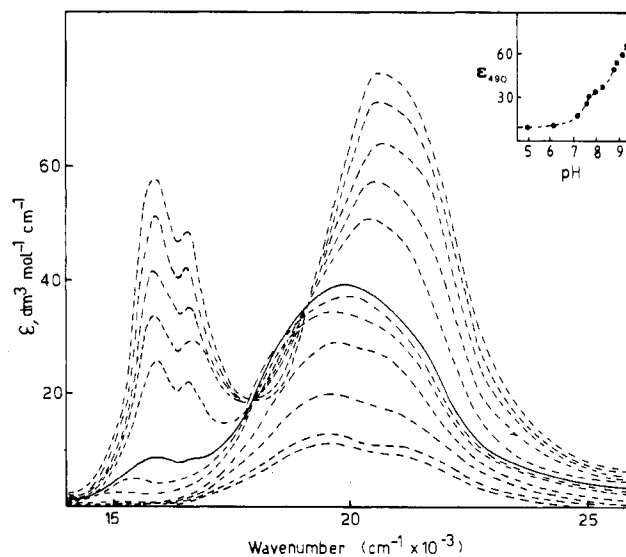


Figure 2. pH dependence of the electronic spectra of the  $[\text{Co}(\text{H}_2\text{O})\text{L}](\text{ClO}_4)_2$  complex in water: from the top, pH 9.55, 9.3, 9.15, 8.9, 8.7, 8.3, 7.9, 7.7, 7.6, 7.2, 6.2, 5.0. The pH of the solid line (8.3) is that resulting from dissolving the complex in water at  $10^{-3} \text{ mol dm}^{-3}$  concentration. The insert shows the variation of  $\epsilon_{490}$  with pH.

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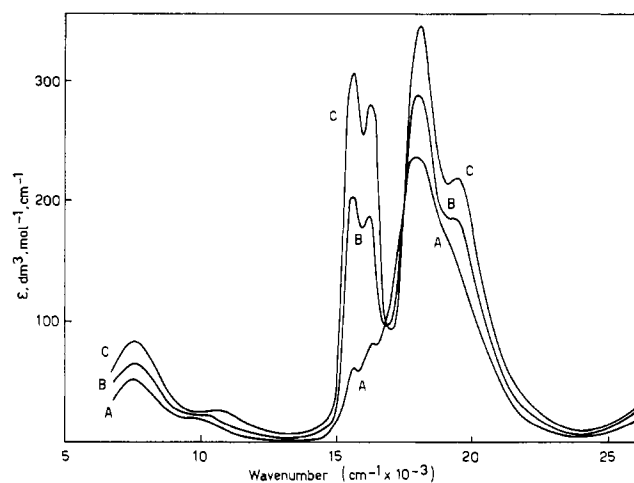
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complex is soluble in water, in methanol, and in most polar organic solvents. Conductivity measurements in water and methanol provided values indicative of 2:1 electrolyte<sup>18</sup> (Table



**Figure 3.** Electronic spectra of the isoenzyme B of cobalt(II) human carbonic anhydrase at pH 6.1 (A), 7.8 (B), and 9.5 (C).

I), in agreement with the proposed structure. In water solution at about pH 7.5 and in methanol solution the electronic spectra are very similar to that recorded on the solid sample (Figure 1). The spectrum obtained in water solution is dramatically pH dependent, as shown in Figure 2. The analysis of the spectral data indicates the presence of two acid-base equilibria (Figure 2, insert): upon acidification of the aqua complex (full-line spectrum) the color of the solution fades, indicating that the apical nitrogen is protonated and six-coordinated polyaqu complexes are formed;<sup>19</sup> upon increase of pH the spectrum of a new species appears, indicating the presence of a further single acid-base equilibrium, as shown by the presence of two isobestic points, whose  $pK_a$  is estimated to be  $8.9 \pm 0.2$ .<sup>20</sup> This equilibrium should be ascribed to the deprotonation of the bound water molecule to yield a  $[\text{Co}(\text{OH})\text{L}]^+$  chromophore; the electronic spectrum of the hydroxo complex (Figure 1), although markedly different from that of the conjugated acid, is still consistent with five-coordination.

The electronic spectra of both the low- and the high-pH forms of cobalt carbonic anhydrase are reported in Figure 3: a striking similarity is apparent with the spectra of the present  $[\text{Co}(\text{H}_2\text{O})\text{L}]^{2+}$  and  $[\text{Co}(\text{OH})\text{L}]^+$  chromophores. The coordination geometry of both forms of carbonic anhydrase is generally believed to be pseudotetrahedral, with a  $\text{N}_3\text{O}$  donor set;<sup>1a</sup> the presence of a fifth nitrogen donor in the case of the model complex results in an increase in energy of the highest F-F transition and in a decrease of the overall molar absorbance. Nevertheless, the deprotonation of the water molecule in the model complex successfully mimics the spectral variations of the cobalt enzyme with pH, making less speculative the occurrence of the same process in the enzyme.

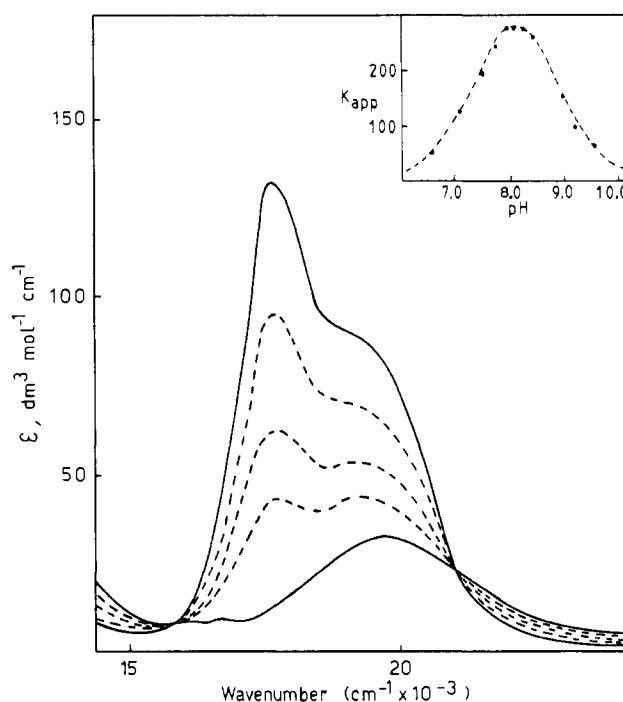
Of course this finding does not allow any further inference on the catalytic mechanism besides providing some support to those mechanisms which involve  $\text{H}_2\text{O}$  and  $\text{OH}^-$  along the process.<sup>4</sup>

Another property of the enzyme to be accounted for is the decrease in affinity of anions for the metal site with increasing

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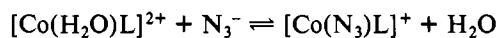
(19) The protonation rate is very low (about 1 h to reach constant pH), and the  $pK_a$  is estimated to be  $7.4 \pm 0.2$ . Tertiary amine nitrogens have  $pK_a$  values of around 10, which are lowered upon coordination to a metal ion, depending on the stability of the complex. On the other hand, the pyrazole nitrogens are expected to have  $pK_a$  values of around 2.

(20) The uncertainty of the latter value is due to precipitation of hydroxo species at high pH values: above pH 9.6 the amount of complex in solution becomes too low to perform any measurement. Potentiometric titrations yielded  $pK_a = 9.1 \pm 0.1$  for the above equilibrium; the whole titration in the pH range 9.2–5.0 required 1.7 mol of protons/mol of complex.



**Figure 4.** Electronic spectra of  $10^{-3} \text{ mol dm}^{-3} [\text{Co}(\text{H}_2\text{O})\text{L}](\text{ClO}_4)_2$  with increasing amounts of  $\text{NaN}_3$ : from the bottom, 0,  $1.5 \times 10^{-3}$ ,  $3.0 \times 10^{-3}$ ,  $9.0 \times 10^{-3}$ ,  $0.2 \text{ mol dm}^{-3}$ . The insert shows the pH dependence of the apparent affinity constant of  $\text{N}_3^-$  for the  $[\text{Co}(\text{H}_2\text{O})\text{L}]^{2+}$  complex.

pH. When  $\text{N}_3^-$  is added to water solutions of the  $[\text{Co}(\text{H}_2\text{O})\text{L}]^{2+}$  complex, a new spectrum is obtained similar to that of the  $[\text{Co}(\text{N}_3)\text{L}]\text{ClO}_4$  complex (Figure 1), which has been isolated in the solid state (see Experimental Section). When the spectra are recorded at increasing amounts of  $\text{N}_3^-$  (Figure 4), two isobestic points appear, and the affinity constant for the equilibrium



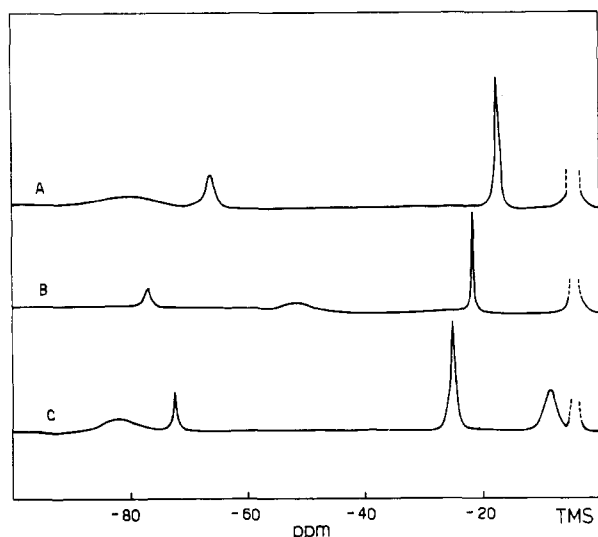
could be determined through a computer treatment of the spectrophotometric data. The apparent affinity constant  $K_{\text{app}}$  at pH 8.1 is  $280 \pm 20 \text{ dm}^3 \text{ mol}^{-1}$ . Such analysis indicates also that only 1:1 adducts are formed. Although the spectrum of the  $\text{N}_3^-$  adduct is not pH dependent, the affinity constants are, as shown in Figure 4 (insert). The dotted line is calculated from the relationship

$$K_{\text{app}} = \frac{K}{\left(1 + \frac{[\text{H}^+]}{K_{a1}}\right) \left(1 + \frac{K_{a2}}{[\text{H}^+]}\right)}$$

with the assumption of  $K = 420$ , negligible affinity for the high pH form,  $pK_{a1} = 7.5$ , and  $pK_{a2} = 8.8$ .

The above data indicate that  $\text{N}_3^-$  reacts only with the acidic form, although the possibility of a small affinity for the high-pH form cannot be ruled out. Again, the data confirm that competition between anions and hydroxide ions can be a reasonable hypothesis also in the enzyme. Despite the many similarities between the model complex and the cobalt enzyme from the chemical point of view, the former derivative does not show any catalytic activity with respect to  $\text{CO}_2$  hydration (see Experimental Section). Obviously, the catalytic efficiency of an enzyme depends on the presence of many concomitant structural requirements, of which the metal chromophore is only a component.

The  $^1\text{H}$  NMR spectra of the  $[\text{Co}(\text{H}_2\text{O})\text{L}](\text{ClO}_4)_2$  complex in  $\text{D}_2\text{O}$  at pH 7.8 and 9.6 and the spectrum obtained in



**Figure 5.**  $^1\text{H}$  NMR spectra of  $[\text{Co}(\text{H}_2\text{O})\text{L}](\text{ClO}_4)_2$  in  $\text{D}_2\text{O}$  at the uncorrected pH values of 9.6 (A) and 7.8 (B) (for the determination of pH see the Experimental Section) and in the presence of  $10^{-1}$  mol  $\text{dm}^{-3}$   $\text{N}_3^-$  (C).

presence of  $10^{-1}$  mol  $\text{dm}^{-3}$   $\text{NaN}_3$  are reported in Figure 5. The latter spectrum consists of four signals of intensities 2:1:3:3, which are assigned to the  $\text{CH}_2$  group, to the C4 proton, and to the 5- $\text{CH}_3$  and the 3- $\text{CH}_3$  groups, respectively.<sup>21</sup> In the

former spectra three signals of relative intensities 1:2:3 are observed; the 3- $\text{CH}_3$  signal is apparently underlying the intense peak of HDO protons. The large isotropic shift differences observed in the signals of the aqua complex at the two pH values, particularly evident in the  $\text{CH}_2$  protons, point out that a considerable conformational change accompanies the deprotonation of the metal-bound water molecule. The NMR spectrum at high pH is somewhat similar to that of the azide derivative. The occurrence of some stereochemical rearrangement upon water deprotonation is also consistent with the variation of the electronic spectra, since switching from  $\text{H}_2\text{O}$  to  $\text{OH}^-$  is not expected to cause any major change in the energy levels, the two groups being close in both the spectrochemical and nephelauxetic series.<sup>22</sup>

Longitudinal relaxation rates of the  $^1\text{H}$  signal of water in cobalt enzyme solutions have been found to be pH independent.<sup>15</sup> Actually, a decrease would be expected on passing from  $\text{H}_2\text{O}$  to  $\text{OH}^-$  owing to the decrease in the number of exchangeable protons interacting with the paramagnetic center, unless the metal-proton distance sensibly decreases in the latter case. In the present aqua complex, the  $T_1$  values of water protons decrease from 3.5 to 2.0 s when the paramagnetic complex is dissolved in water at  $1.0 \times 10^{-3}$  mol  $\text{dm}^{-3}$  concentration. The measured effect is indeed substantially pH independent, although too small to be safely compared with the enzyme results.

**Registry No.**  $[\text{Co}(\text{H}_2\text{O})\text{L}](\text{ClO}_4)_2$ , 76773-04-1;  $[\text{Co}(\text{N}_3)\text{L}](\text{ClO}_4)$ , 76773-06-3; isoenzyme B, 9001-03-0; Co, 7440-48-4.

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## Electrochemical Study of a Trinuclear Copper(II) System: The $\text{Cu}^{\text{II}}_2\text{Cu}^{\text{III}}-\text{Cu}^{\text{II}}$ Couple and Its Inhibition by Proton

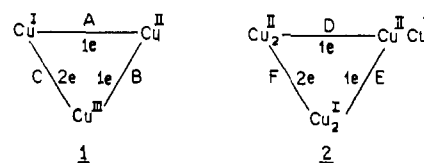
D. DATTA, P. K. MASCHARAK, and A. CHAKRAVORTY\*

Received October 10, 1980

An electrochemical study of a group of spin-doublet ( $S = 1/2$ ) trinuclear copper(II) complexes,  $[\text{Cu}_3\text{O}_p(\text{OH})_{1-p}(\text{ligand})_3](\text{ClO}_4)_{2-p}$  [ $0 \leq p \leq 1$ ; ligand =  $\text{RNC}(\text{R}')\text{C}(\text{R}')\text{NO}^-$ ;  $\text{R} = \text{Et}, n\text{-Pr}, n\text{-Bu}, \text{Ph}$ ;  $\text{R}' = \text{Me}, \text{Ph}$ ], is reported. In general, the complexes as isolated contain both  $\text{Cu}_3\text{O}$  and  $\text{Cu}_3\text{OH}$  cores held by three peripheral oximate bridges of the ligand. Cyclic voltammetry (both dc and ac) and constant potential coulometry establish that only the  $\text{Cu}_3\text{O}$  core displays the novel one-electron-transfer process  $\text{Cu}^{\text{III}}\text{Cu}^{\text{II}}_2 + e^- \rightleftharpoons \text{Cu}^{\text{II}}_3$  with  $E^{\circ}_{298}$  in the range 0.3–0.4 V vs. SCE in several solvents at a platinum working electrode. The rare  $\text{Cu}(\text{III})-\text{Cu}(\text{II})$  mixed-valence species thus discovered have an intense electronic absorption band at  $\sim 830$  nm. The heterogeneous rate constants of electron transfer are reported. Base ( $\text{NEt}_3$ ) converts  $\text{Cu}_3\text{OH}$  to a  $\text{Cu}_3\text{O}$  species in an equilibrium deprotonation reaction. On the other hand an acid ( $\text{HClO}_4$ ) brings about the reverse conversion,  $\text{Cu}_3\text{O} \rightarrow \text{Cu}_3\text{OH}$ . The influence of these reactions on the electrochemical response is analyzed. The identification of a second couple  $\text{Cu}^{\text{II}}_3 + e^- \rightleftharpoons \text{Cu}^{\text{II}}_2\text{Cu}^{\text{I}}$  ( $E^{\circ}_{298} \sim -0.4$  V vs. SCE) at a mercury working electrode in which only the  $\text{Cu}_3\text{OH}$  core can participate is briefly reported. The symbiotic relationship between electron transfer and proton transfer is noted.

The function of copper oxidases and oxygenases is dependent on the variable valency of the metal ion.<sup>1-3</sup> This has stimulated much current interest in electron-transfer studies of model mononuclear and dinuclear copper species having O, N, and S donor ligands. Electrochemical techniques have been effectively used in such studies. The electron-transfer steps thus far identified are A,<sup>4-8</sup> B,<sup>9-12</sup> C,<sup>13</sup> D,<sup>4,14-16</sup> E,<sup>15,16</sup> and F<sup>17</sup>

as schematically summarized in 1 and 2. Though unknown



at present, given proper ligand environment, a similar triangle

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