Stereoisomerism and Equilibrium Properties of Oxygen-Carrying Cobalt(II) Complexes of Histamine and Its Derivatives: A New Approach to an Old System

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The complex formation equilibria between pH 2 and 11.2 in Co(II)−L−O_2 ternary systems (L = histamine, glycylhistamine, and sarcosylhistamine) have been studied by pH-metric, spectrophotometric, and ^{1}H-NMR spectroscopic methods. In contrast to earlier findings, we detected several protonation states of oxygen-carrying complexes in the pH range 7−11.2. The active species in oxygen uptake is the CoL_2 complex already suggested in the Co(II)−histamine−O_2 system; however, in the case of pseudopeptides, both $CoLH_{-1}$ and CoL_2H_{-1} complexes can take up oxygen. The ^{1}H-NMR study revealed stereoisomerism of oxygenated species in the same protonation states, undergoing slow ligand exchange. In the case of the $Co_2(Hist)_4(OH)(O_2)$ complex, among the 20 possible geometrical isomers, at least six can be distinguished by their imidazole proton signals. The most abundant isomers have axial−equatorial imidazole coordination, presumably on steric grounds. The stability constants determined by pH studies for the above systems proved that {4N}-coordinated species have a greater affinity for oxygen uptake than the {3N}-coordinated ones. They also showed the stabilizing effect of the deprotonated amide nitrogen in the oxygenated complexes of pseudopeptides.

Introduction

The complexation of molecular oxygen by transition metals has generated considerable interest in the last 3 decades. In this respect, a great number of kinetic and pseudoequilibrium studies have been published on the dioxygen binding of cobalt(II) complexes, with a variety of chelating ligands.[1−10] The overwhelming majority of thermodynamic studies concerning oxygen-carrying cobalt(II) complexes in aqueous solution report the formation of only one oxygen-carrying peroxo dimer between pH 6 and 10 (μ-peroxo monobridged or μ-peroxo-μ-hydroxo dibridged complex), depending on the nature of the ligand. The imidazole donor groups in cobalt(II) complexes are reported to enhance the thermodynamic stability as well as to inhibit the autoxidation of the oxygenated species, owing to the strong donation of electron density from the ligand to the metal, which promotes electron transfer from the metal to coordinated oxygen.[4,5] In line with our investigations on the structure and dynamics of coordination spheres about metal ions in solution,[11] we recently studied the coordination properties of histamine-containing peptides[12] as models for the transport of trace metals between tissues and blood, with special attention on their polynuclear complexes. Some of these complexes are known to be very sensitive to oxygen, and our interest turned now to the oxygen-carrying properties of these ligands in the presence of transition metal ions.

Early studies on cobalt(II)−histamine complexes[6−8] established the formation of a 2:1 (Co:O_2) adduct with oxygen in aqueous solution, containing a μ-peroxo bridge between the two metal centers, which slowly degraded in nearly 2 weeks half-life time to yield monomeric Co(III) species.[7] However, the existence of a second μ-hydroxo bridge between the two cobalt ions is disputed. On the basis of proton balance studies, Michaelidis and Martin[7] proposed—while from similar measurements Wilkens *et al*.[6] and Powell and Nancollas[8] rejected—the formation of a μ-hydroxo bridge. In the Co(II)−glycylhistamine−O_2 system also, a single oxygenated species has been reported previously[4] at pH ∼8, having μ-peroxo-μ-hydroxo bridges between the two cobalt centers.

Our experiments tried to solve the above ambiguities: they strongly support the presence of one μ-peroxo-μ-hydroxo dibridged species existing under a variety of stereoisomeric forms and suffering further deprotonations at higher pH. Recently, a number of more or less deprotonated oxygen-carrying species have been determined for some Co(II) complexes of dinucleating, macrocyclic ligands.[9] But except for a recent paper dealing with the Co(II)−histidine−O_2 system,[10] no real equilibrium study is available for amino acid or peptide type molecules. Our aim was to reinvestigate the above Co−L−O_2 systems (where L = histamine (Hist), glycylhistamine (Glyhist), and sarcosylhistamine (Sarhist)), so as to give a complete equilibrium picture between pH 2 and 11.2, together

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(1) McLendon, G.; Martell, A. E. *Coord. Chem. Rev.* **1976**, *19*, 1.
(2) Jones, R. D.; Summerville, D. A.; Basolo, F. *Chem. Rev.* **1979**, *79*, 139.
(3) Niederhoffer, E. C.; Timmons, J. H.; Martell, A. E. *Chem. Rev.* **1984**, *84*, 137.
(4) Harris, W. R.; Martell, A. E. *J. Am. Chem. Soc.* **1977**, *99*, 6746.
(5) Vogt, A.; Kufelnicki, A.; Jezowska-Trzebiatowska, B. *Polyhedron* **1990**, *9*, 2567.
(6) Miller, F.; Simplicio, J.; Wilkens, R. G. *J. Am. Chem. Soc.* **1969**, *91*, 1962.
(7) Michaelidis, M.; Martin, R. B. *J. Am. Chem. Soc.* **1969**, *91*, 4683.
(8) Powell, H. K. J.; Nancollas, G. H. *J. Am. Chem. Soc.* **1972**, *94*, 2664.
(9) (a) Basalotte, M. G.; Martell, A. E. *Inorg. Chem.* **1988**, *27*, 4219. (b) Szpoganicz, B.; Motekaitis, R. J.; Martell, A. E. *Inorg. Chem.* **1990**, *29*, 1467.
(10) Wang, J.; Collange, E.; Aymes, D. J.; Paris, M. R.; Furnaise, R. *Bull. Soc. Chim. Fr.* **1994**, *131*, 37.
(11) Delpuech, J.-J. *Analyst* **1992**, 267, and references therein.
(12) (a) Gajda, T.; Henry, B.; Delpuech, J.-J. *J. Chem. Soc., Dalton Trans.* **1992**, 2313. (b) Gajda, T.; Henry, B.; Delpuech, J.-J. *J. Chem. Soc., Dalton Trans.* **1993,** 1301. (c) Gajda, T.; Henry, B.; Delpuech, J.-J. *J. Chem. Soc., Faraday Trans.* **1994**, *89*, 157. (d) Gajda, T.; Henry, B.; Delpuech, J.-J. *Inorg. Chem.* **1995**, *34*, 2455. (e) Gajda, T.; Henry, B.; Aubry, A.; Delpuech, J.-J. *Inorg. Chem.* **1996**, *35*, 586.

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with a detailed structural study of the complexes formed using ^{1}H-NMR spectroscopy.

Experimental Section

Materials. The histamine dihydrochloride was a Fluka product. Glycyl- and sarcosylhistamine were prepared according to the procedure described previously.[13] Stock solutions of cobalt(II) perchlorate (Fluka products) were standardized complexometrically. pH titrations were performed using Titrisol (Merck) NaOH standard solutions.

Methods. For oxygen uptake measurements, a constant cobalt(II) concentration ($[Co^{2+}] = 3.36 \times 10^{-4}$ mol dm^{-3}) and different metal-to-ligand ratios (between 1:2 and 1:8) have been used. The solutions were saturated with air and then enclosed in the measuring cell. The oxygen consumption was determined by a Radelkis Aquacheck-3 oxygen analyzer, using the amperometric method. The measuring cell and the solution are separated by a polypropylene membrane, which is permeable to oxygen. The oxygen entered into the measuring cell is reduced on the cathode ($E = -650$ mV); this results in a diffusion current directly proportional to the oxygen concentration. The measuring range of the analyzer is 0−400% of the oxygen content in air-saturated water, and the reproducibility is ±1%.

The coordination equilibria were investigated by potentiometric titrations under nitrogen or oxygen atmosphere, maintained by a stream of nitrogen or oxygen, respectively. For the equilibrium measurements, an IBM-compatible, PC-controlled, fully automatic titration set has been used, including a Dosimat 665 (Metrohm) autoburet and an Orion 710 pH meter (precision 0.1 mV). The ionic strength was adjusted to 0.1 mol dm^{-3} with $NaClO_4$, and the cell was thermostated to 298.0 ± 0.1 K. Changes in pH were followed by using an Orion (Catalog No. 81-02) combined glass electrode. For the quantitative evaluation of the data, correlation 1 was used between the experimental electromotive force values (E) and the equilibrium hydrogen ion concentrations ($[H^+]$),

$$E = E_0 + \frac{RT}{F}\ln [H^+] + j_H[H^+] + j_{OH}[H^+]^{-1}K_w \qquad (1)$$

where j_H and j_{OH} are fitting parameters in acidic and alkaline media for the correction of experimental errors, mainly due to the liquid junction potential and the possible alkaline and acidic errors of the glass electrode, and K_w is the autoprotolysis constant of water[14] ($10^{-13.75}$).

The formation constants (β_{pqrs}) for the following generalized reaction were evaluated from the pH titration data with the PSEQUAD computer program:[15]

$$pM + qL + rH + sO_2 \rightleftarrows M_pL_qH_r(O_2)_s$$

In this notation, L is the neutral ligand; consequently, the fully protonated ligands are referred to as LH_2. The metal-promoted further deprotonations of the neutral ligand or, equivalently, the mixed hydroxo complexes formed are denoted as MLH_{-1}, MLH_{-2}.

In the following, charges are omitted, and the coordinated water molecules (necessary to ensure cobalt(II) hexacoordination) are not shown. The constants were calculated as the averages of eight independent titrations. The sample volume was 40 cm^3, and the metal-to-ligand ratio was varied from 1:1.5 to 1:5, with the metal ion concentration between 2×10^{-4} and 1×10^{-3} mol dm^{-3}.

The visible absorption spectra were recorded on a Hewlett-Packard 8452A diode array UV/visible spectrophotometer. ^{1}H-NMR spectra were recorded on a Bruker AM-400 spectrometer with dioxane as internal standard and mostly in aqueous solution (H_2O), using presaturation to eliminate the solvent signal.

Results

Cobalt(II) Complexes in Inert Atmosphere. To determine the equilibrium properties of oxygenated complexes, it is necessary to know the stability of the Co(II) complexes formed under oxygen-free atmosphere. In the case of glycyl- and sarcosylhistamine, these data have been already reported by us,[12d] while for histamine we redetermined them in the course of this work. The formation constants are listed in Table 1. Concerning the main species formed in the Co(II)−histamine system, our results agree well with earlier findings[16] ($\log \beta_{110} = 5.02$ and $\log \beta_{120} = 8.78$). However, the protonated MLH complex, forming in *ca.* 10% near pH 6, is not reported by Eilbeck *et al.*[16]

Table 1. Stability Constants and Derived Data for Complexes Formed in the Systems Co(II)−L−O_2 (as Their Logarithms)[a]

β_{pqrs}[a]	histamine	glycylhistamine	sarcosylhistamine
0110	9.74(1)	8.04	8.31
0120	15.81(1)	14.82	15.08
1110	11.19(4)	10.10	10.17
1100	5.08(1)	3.13	2.84
11−10		−5.18	−5.19
11−20		−15.41	−15.66
1200	8.83(1)	5.65	4.78
12−10		−2.26	−3.07
22−31		−8.00(2)	−9.99(2)
22−41		−17.28(2)	−19.38(2)
24−11	19.17(2)		
24−21	9.37(2)		
24−31	−1.69(3)	−1.50(2)	−3.76(2)
pK^{22-31}_{22-41}		9.28	9.39
pK^{24-11}_{24-21}	9.80		
pK^{24-21}_{24-31}	11.06		
$\log K^1_{O_2}$		2.36	0.39
$\log K^2_{O_2}$	1.51	2.96	2.38

[a] $\beta_{pqrs} = [Co_pL_qH_r(O_2)_s]/[Co]^p[L]^q[H]^r[O_2]^s$, $I = 0.1$ mol dm^{-3} ($NaClO_4$), $T = 298$ K, with estimated errors in parentheses (last digit).

Oxygenated Cobalt(II) Complexes. First, we studied the oxygen uptake of the investigated systems as a function of pH. In air-saturated solutions, the oxygen uptake begins at slightly acidic pH, where only a fraction of the cobalt(II) complexes can be oxygenated. This fraction increases with addition of base and was found to be complete at pH 8−9, depending on the ligand and the [Co]:[L] ratio. At higher metal-to-ligand ratio or at higher concentrations, oxygenation begins at lower pH, *e.g.*, in the case of histamine, the formation of oxygenated species begins at pH ∼6.5 and 7.0 when $[Co] = [L]/2 = 7.5 \times 10^{-3}$ and 1.0×10^{-3} mol dm^{-3}, respectively. The average quantity of consumed oxygen was in good agreement with the expected Co:O_2 = 2:1 adduct. The formation of oxygen-carrying complexes, however, was found to be very slow between pH 7.0 and 8.5. During pH measurements, reaching equilibrium after each new addition of base is of critical importance. Thus, before pH titration, we determined the time needed to reach equilibrium under the conditions used for pH measurements. For this reason, formation of the oxygenated complexes was monitored spectrophotometrically as a function of time. Three series of such spectra in the Co(II)−histamine−O_2 system are shown in Figure 1 at different stages of titration. The most important result of these measurements is that a well-defined equilibrium between Co(histamine)$_x$ and its (their) oxygenated complex(es) is obtained for any base addition. As can be seen, between pH 7.0 and 8.6, *ca.* 40−60 min is needed to reach the equilibrium, while above this pH range, the equilibrium reaction is fairly fast. This fact makes the pH study

(13) Henry, B.; Gajda, T.; Selve, C.; Delpuech, J.-J.; Arnould, J. M. *Amino Acids* **1993**, *5*, 113.
(14) Högfeldt, E. *Stability Constants of Metal−Ion Complexes. Part A: Inorganic Ligands*; Pergamon: New York, 1982; p 32.
(15) Zékány, L.; Nagypál, L. PSEQUAD. In *Computational Methods for theDetermination of Stability Constants*; Legget, D., Ed.; Plenum: New York, 1985.
(16) Eilbeck, W. J.; Holmes, F.; Thomas, T. W. *J. Chem. Soc. A* **1969**, 113.

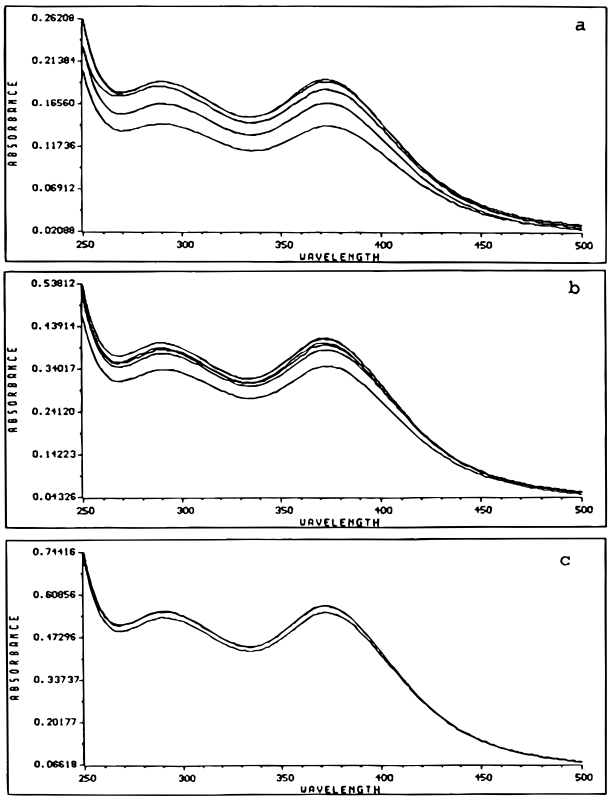

Figure 1. Sets of absorption spectra of the Co(II)−histamine−O_2 system ($[Co^{2+}] = 1.015 \times 10^{-3}$, $[L] = 3.036 \times 10^{-3}$ mol dm^{-3}, air-saturated solution, 0.2 cm thick cell) at different times t after addition of a given amount of base: $t = 4$, 15, 25, 40, and 60 min from the bottom to the top (pH 7.75, 7.52, 7.43, 7.41, and 7.39) (a); $t = 2$, 12, 25, 40, 50, and 70 min (pH 8.00, 7.80, 7.75, 7.74, 7.73, and 7.72) (b); $t = 2$, 15, and 30 min (pH 8.60, 8.54, and 8.53) (c). Sets A, B, and C correspond to increasing quantities of added base, and the pH's simultaneously measured are shown in parentheses.

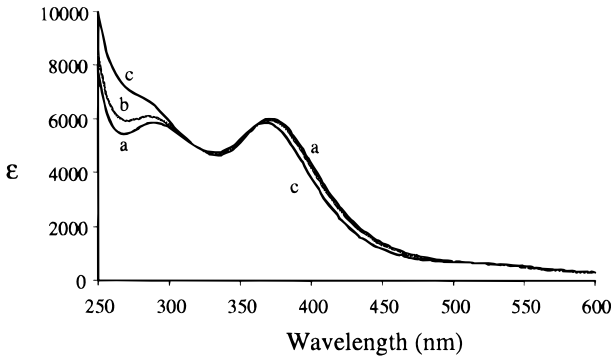

Figure 2. Absorption spectra of the Co(II)−histamine−O_2 systems at pH 8.91 (a), 10.14 (b), and 11.41 (c) (ϵ refers to dimeric complexes).

possible under oxygen atmosphere in order to obtain a detailed equilibrium picture. It is important to note, however, that, between pH 7 and 8.6, a delay of 50 min is needed for reaching the equilibrium between two successive additions of base. In this way, the operating time increased significantly (6−8 h).

At pH 8.91, the UV/vis absorption spectrum (Figure 2a) displays two characteristic maxima, which have been reported to show the presence of nonplanar μ-peroxo-μ-hydroxo dibridged Co(II) dimers.[17] These two bands can be attributed to O_2^{2-} $\pi^*_v \rightarrow$ Co (372 nm, $\epsilon = 6220$) and $\pi_\sigma^* \rightarrow$ Co (288 nm, $\epsilon = 6000$) charge transfer transitions.[17] The oxygenated complexes of pseudopeptide derivatives of histamine show similar spectral features, *i.e.*, a maximum at 362 nm and a shoulder at ∼280 nm, in the case of both glycylhistamine and sarcosylhistamine at pH 8.6. These two bands are present between pH 7 and 11.2. Their intensity increases in the course of time (Figure 1) so as to reach a long-time stable equilibrium at each stage of titration. These facts are in favor of a gradual formation of the μ-peroxo-μ-hydroxo dimer and not of a direct irreversible oxidation to cobalt(III).

The pH range required for oxygenation is nearly the same as the one previously observed for the formation of cobalt(II) complexes in the absence of oxygen;[12d] this strongly suggests that these complexes $Co(Hist)_2$ in the case of histamine, $CoLH_{-1}$ and CoL_2H_{-1} in the case of pseudopeptides, see below) play the role of active species in oxygen uptake. In the presence of oxygen, the above parent complexes are immediately transformed into the more stable oxygen-carrying dibridged dimers as soon as they are formed; this shifts the complex formation processes to lower pH. The quantity of oxygenated adducts and their rate of formation increase from pH 7 to 9, presumably because of a simultaneously increasing availability of the active species and hydroxide ion.

On further increasing the pH, the first maximum in the UV/vis spectrum at 372 nm (see above) is shifted to 366 nm, while the other maximum becomes a shoulder at pH 11.2. This pH dependence suggest the existence of at least one more oxygen-carrying species in solution.

The pH titration curves of the three systems studied are shown in Figure 3. Titration curves of 1:2 Co(II):ligand systems up to pH 8−9 show an equivalence point in the presence of oxygen after addition of 4.5 and 5.5 base equivalents per metal ion in the case of histamine and pseudopeptides, respectively. In the case of histamine, the formation of the parent complex, and then of the μ-peroxocobalt(II) dimer, formally requires two neutral ligand molecules per cobalt ion, *i.e.*, four base equivalents to deprotonate successively the imidazolium ring and the ammonio NH_3^+ end of each histamine molecule. The additional 0.5 base equivalent consumption strongly supports the formation of a μ-hydroxo bridge between the two cobalt(II) ions, in addition to the μ-peroxo one, in the oxygen-carrying complex $[Co(Hist)_2(OH)(O_2)Co(Hist)_2]$; it also accounts for the pH shift toward lower values in the titration curve under oxygen atmosphere, as shown by the position of curves b below curves a in Figure 3A.

The explanation is more complex in the case of pseudopeptides, since these ligands may be tridentately coordinated to the metal ion after metal-promoted deprotonation of the amido nitrogen. As far as the parent complexes are concerned, the structure of complexes of the general type MLH_{-1}, ML_2, and ML_2H_{-1}, where M = Co, Ni, or Cu and L = glycylhistidine[18−20] or glycylhistamine,[12b,d] has been thoroughly investigated in the literature. The central metal ion is {3N}-coordinated in species MLH_{-1} by the terminal amino, deprotonated amido, and N^3-imidazolic nitrogens.[12d] The bis-complex ML_2 was shown to exhibit spectral properties[12b,20] revealing {4N} coordination on the metal ion and to deprotonate into ML_2H_{-1} with nearly the same pK as for the deprotonation of an NH_3^+ group in the free ligand LH itself (for both ligands and for all metal ions). This strongly suggested the presence of a free NH_3^+ group in ML_2, which remains uncoordinated after deprotonation into ML_2H_{-1}.

(17) (a) Solomon, E. I.; Tuczek, F.; Root, D. E.; Brown, C. A. *Chem. Rev.* **1994**, *94*, 827. (b) Miskowski, V. M. *Comments Inorg. Chem.* **1987**, *6*, 193.

(18) Sovago, I.; Farkas, E.; Gergely, A. *J. Chem. Soc., Dalton Trans.* **1982,** 59.

(19) Farkas, E.; Sovago, I.; Gergely, A. *J. Chem. Soc., Dalton Trans.* **1983,** 1545.

(20) Pogni, R.; Della Lunga, G.; Basosi, R. *J. Am. Chem. Soc.* **1993,** *115*, 1546.

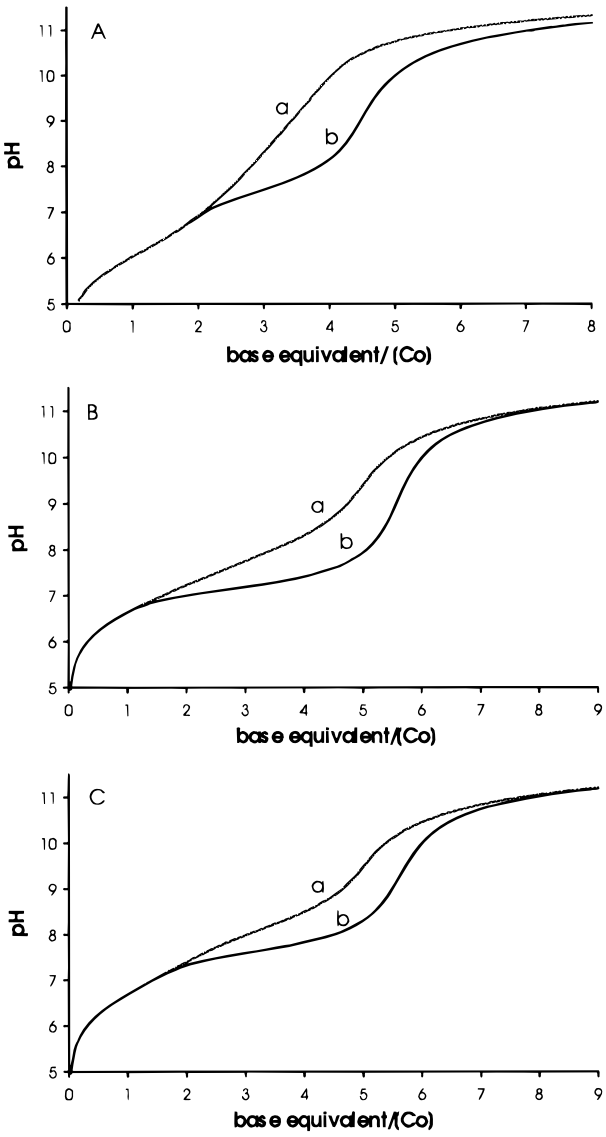

Figure 3. Average base consumption per cobalt(II) ion in the absence of (a) and under (b) oxygen atmosphere in Co(II)−histamine (A), Co(II)−glycylhistamine (B), and Co(II)−sarcosylhistamine (C) systems.

The complex CoL_2H_{-1} was assumed to derive from $CoLH_{-1}$ by the addition of a second ligand molecule, monodentately coordinated through the imidazole ring. In this view, the composition of CoL_2 H_{-1} is better described as $Co(LH_{-1})L$, *i.e.*, the cobalt ion is coordinated by four nitrogens, and the two remaining coordination sites are available to form oxygenated complexes. Under nitrogen atmosphere, five base equivalents per metal ion are needed to reach pH 9.5 at a 1:2 metal-to-ligand ratio. In the presence of oxygen, the formation of the above-mentioned species shifted to lower pH, due to their transformation into oxygenated complexes, while the additional 0.5 base equivalent consumption again supports the predominant presence of the μ-hydroxo-μ-peroxo bridged Co(II) dimer at pH 8−9 (see the discussion below).

For all the oxygenated systems, further deprotonations were observed at higher pH above the equivalence points in Figure 3, presumably on the N^1-pyrrolic nitrogens of coordinated imidazole units (see below).

NMR Spectroscopy. Information on the structures of oxygen-carrying complexes can be obtained by ^{1}H-NMR spectroscopy owing to their diamagnetic nature. In spite of this possibility, very limited data are available about ^{1}H- or ^{13}C-NMR spectra of oxygen-carrying cobalt(II) complexes containing amino acid or peptide type ligands.[21] Figure 4 shows the imidazole region of the ^{1}H-NMR spectrum of the Co(II)−histamine−O_2 system recorded at different pH values and, in each case, after pH equilibration. Under the conditions used, the formation of the diamagnetic, oxygen-carrying complex begins at pH ∼6.6, where its spectrum is detected as a set of weak, sharp lines superimposed on the intense, paramagnetically broadened signals of the Co(II)−histamine complexes (Figure 4a). The sharp lines pattern remains unchanged up to pH ∼9, *i.e.*, the number of lines, their frequencies, and their relative intensities are not modified in this pH range when NMR spectra are recorded at equal time intervals after preparing the oxygenated complex (2 h in Figure 4). The only change is the absolute intensity of the whole set, which is continuously increasing at the expense of lines representing the paramagnetic parent complexes. At pH 8.84 (Figure 4b), the parent Co(II) complexes are no longer detected as well as the free ligand itself. This, again, shows that metal ions and histamine molecules are totally engaged in 1:2 diamagnetic oxygenated complex(es), in line with the presence of CoL_2 units assumed previously by pH measurements.

The main feature of the spectra in Figure 4b is the appearance of multiple signals indicating that several chemically different species are in slow equilibrium on the NMR time scale. Concerning, however, the stoichiometry and the protonation state of complexes, both pH-metric and spectrophotometric results suggest the existence of only one dominant complex at pH ∼9. As already mentioned, the consumption of 0.5, 2.0, and 4.5 mol of dioxygen, histamine, and base per mole of metal ion and the UV/vis evidence for a μ-peroxocobalt(II) dimer require the stoichiometry of the oxygenated complex to be $Co(Hist)_2(O_2)$-$(OH)Co(Hist)_2$. The unchanged UV/vis pattern between pH 7 and 11 and the long-time stability of the oxygenated solution are not in favor of monomeric superoxo complexes as well as of Co(III) complexes. The presence of a monobridged μ-peroxocobalt(II) dimer in equilibrium with the dibridged μ-peroxo-μ-hydroxo complex is also highly unlikely, at least after pH equilibration. The first reason for this is the measured base consumption at pH 9. A second reason is the unchanged NMR pattern from pH 6.6 to 9 mentioned above. The addition of base should shift this equilibrium toward the formation of the dibridged complex (DC) at the expense of the monobridged species (MC). Under these conditions, the NMR pattern should be continuously altered, the intensities of the DC lines increasing at the expense of the MC lines, contrary to experimental results. An objection at this point could be that this equilibrium is fast enough on the NMR time scale to average the signals of both complexes into a single set of lines. This objection can be simply removed since the variable DC:MC molar ratio should result in frequency shifts on increasing the pH, again in contradiction with experimental observations.

Thus, the probable reason for line multiplicity is the inequivalence of imidazole protons, owing to the existence of a great variety of isomers in this complex. In the free ligand, the carbon-bound proton C^2-H in the imidazole ring exchanges easily with deuterium in D_2O. This can help to assign the signals. The spectra of the complex (a and b respectively) with or without deuteration at pH 9 are shown in Figure 5. For both protons C^5-H and C^2-H, 14 lines are visible in the spectrum. It can be seen (Figure 5a) that the C^5-H protons are located between 6.8 and 7.3 ppm; consequently, by difference, the C^2-H protons are dispersed between 5.6 and 8.3 ppm. This fact may suggest the coordination of the N^3 nitrogen in the complex. This

(21) Brown, C. E.; Vidrine, D. W.; Czernuszewicz, R.; Nakamoto, K. *J. Inorg. Biochem.* **1982**, *17*, 247.

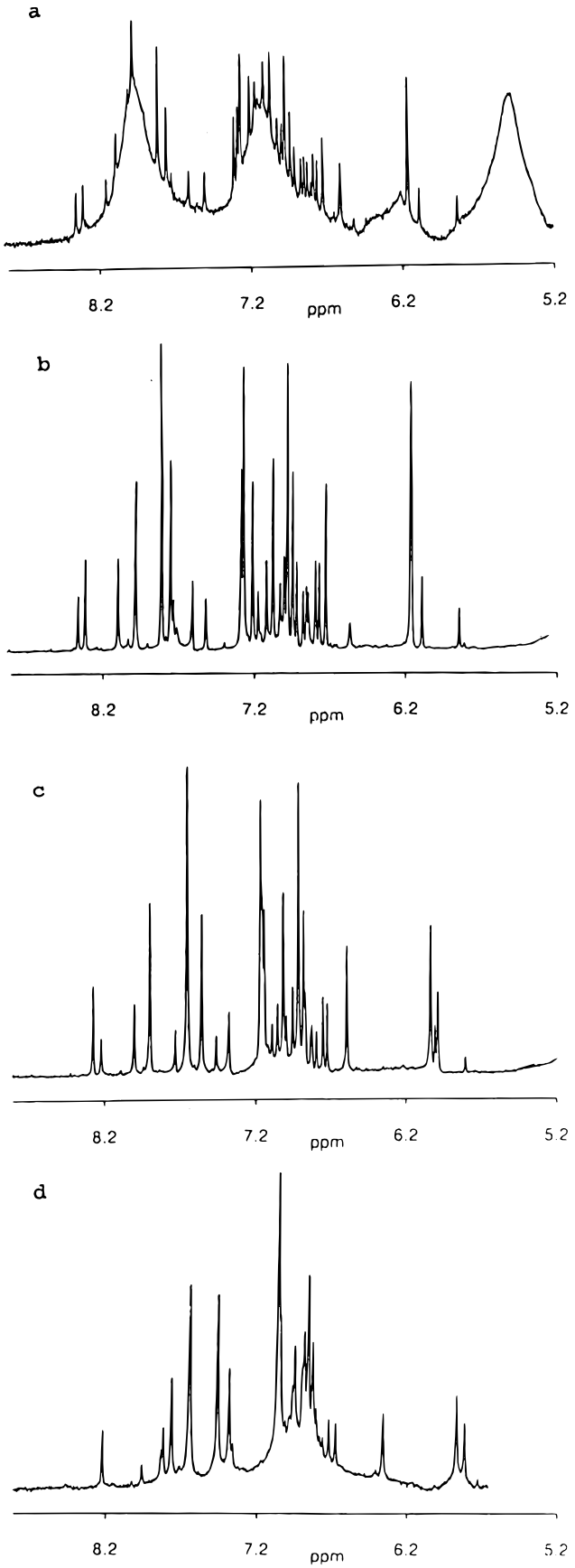

Figure 4. Imidazole region of ^{1}H-NMR spectra of the Co(II)−histamine−O_2 system at pH 6.6 (a), 8.84 (b), 10.42 (c), and 11.4 (d). $[Co^{2+}] = 7.5 \times 10^{-3}$ mol dm^{-3} and $[Co^{2+}]$:[L] = 1:2.

observation was verified using N^3- and N^1-methylhistamine. Only the latter can form an oxygenated complex with Co(II) giving similar spectral features. In this way, the possibility of

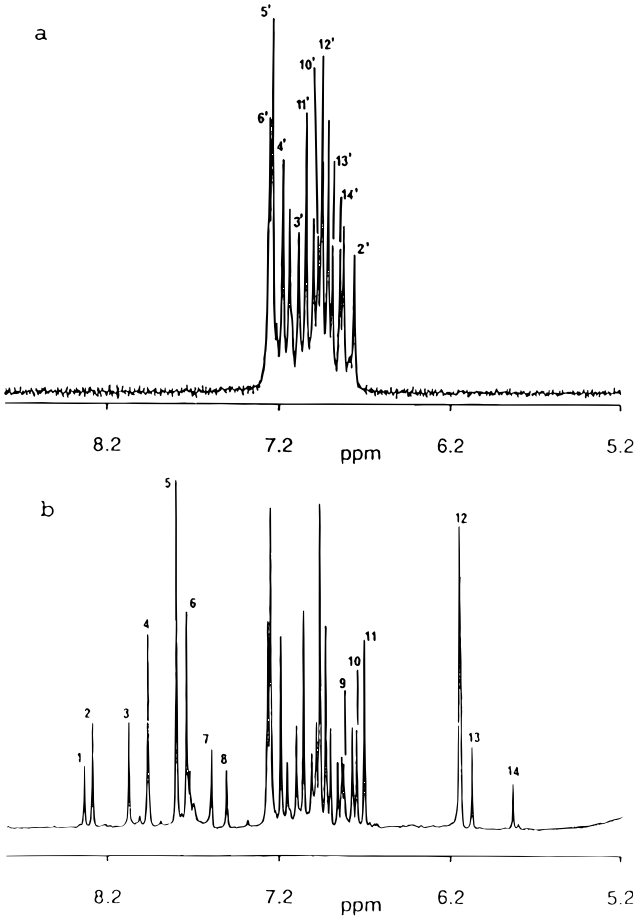

Figure 5. Imidazole region of ^{1}H-NMR spectra of Co(II)−histamine−O_2 systems ($[Co^{2+}] = [L]/2 = 7.5 \times 10^{-3}$ mol dm^{-3} at pH 8.8): deuterated at C^2-H proton of imidazole ring (a) and non-deuterated (b).

different modes of chelation, and consequently of linkage isomers (binding through N^1 or N^3), to account for signal multiplicity can be discarded. The fact that two histamine molecules are attached to each cobalt(II) ion in the oxygenated complex shows that the four sites of coordination left available about each metal ion after the formation of the dibridged dimer are fully occupied by the two histamine ligands. Unsymmetrical bidentate ligands, such as histamine, may still be arranged in different ways about the two metal centers in this complex (see the discussion), giving rise to a variety of *stereoisomers* and, consequently, to line multiplicity.

Finally, the large number of signals (14) observed in NMR spectra brings evidence for the presence of a μ-peroxo-μ-hydroxo dimer $[(Hist)_2Co(O_2)(OH)Co(Hist)_2]$ and not of a μ-peroxo monobridged mixed hydroxo dimer $[(OH)(Hist)_2Co(O_2)(Co(Hist)_2]$, where the OH^- group is coordinated to only one metal ion (completing the Co(II) coordination spheres with a water molecule). The former complex alone can, indeed, give rise to geometrical isomerism owing to its rigid dibridged structure, and this greatly increases the number of nonequivalent imidazole units required to account for line multiplicity (see next section).

Discussion

Stereoisomerism in the Hist/O_2/Co(II) System at pH 9. Little attention has been given to stereoisomerism of Co(II) oxygenated complexes, which may arise from the unsymmetrically dibridged structure. In the case of the μ-peroxo-μ-hydroxobis[bis(ethylenediamine)Co^{III}] complex, two isomers

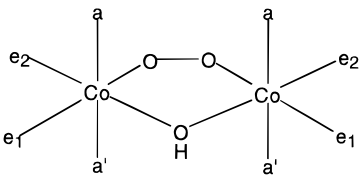

Figure 6. Sites of coordination in the μ-peroxo-μ-hydroxo dibridged cobalt dimer.

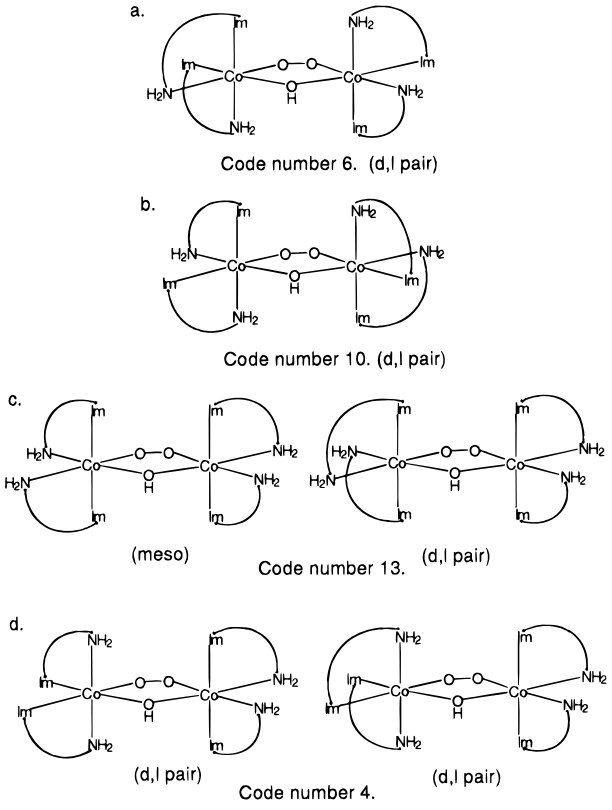

Figure 7. Structure proposed for the major isomers in the Co(II)−histamine−O_2 system at equilibrium between pH 6.6 and 9: [ae_2, $a'e_2$], 56% (a); [ae_1, $a'e_1$], 16% (b); [aa', aa'], 10% (c); and [e_1e_2, aa'], 7% (d).

were detected by X-ray structure determination.[22] In our cases, however, the presence of unsymmetrical bi- or tridentate ligands may further increase the number of isomers. The μ-peroxo-μ-hydroxo bridged cobalt dimer core offers four sites of coordination to histamine on each cobalt ion. Two of them (e_1, e_2) are in and the other two (a, a') are perpendicular to the equatorial plane of the complex (Figure 6). Unsymmetrical bidentate ligands such as histamine may occupy these sites in any of 36 arrangements, if we exclude *cis* diaxial and vicinal coordination to the cobalt dimer core, on steric grounds. To classify these isomers, we may first specify the coordination site of one end of the molecule, *e.g.*, the N^3 nitrogen atom of each of the four imidazole units. Coordination sites are enunciated as a given sequence—*the site code*—of the four letters e_1, e_2, a, and a', two of them for each cobalt center. Thus, the isomer represented in Figure 7a has the site code [ae_2, $a'e_2$]. The enantiomer obtained by interchanging the cobalt centers can be named as [$a'e_2$, ae_2]. In fact, as shown in Table 2, one code name only [ae_2, $a'e_2$] is used to designate this *dl* pair of enantiomers, which are indiscernible by NMR spectroscopy. Thirteen different site codes, numbered from 1 to 13 in Table 2, are possible under the limited conditions mentioned above. Certain site codes may correspond, in fact, to two geometrical isomers, each one with its own spectrum. This occurs whenever the second end of the ligand can occupy either of two coordination sites. Thus, the sequence [*aa'*, *aa'*] (site code 13) represented in Figure 7c can be completed in either of two ways specifying the position of the amino site in each ligand (shown in parentheses): [$a(e_2)$-$a'(e_1)$, $a(e_2)a'(e_1)$] or [$a(e_1)a'(e_2)$, $a(e_2)a'(e_1)$]. Each of these sequences corresponds respectively to an achiral (meso form) or chiral (two enantiomers) isomer. This set of stereoisomers will be characterized by its code number 13 and will be given the name isomer 13 in the following. Isomers displayed in Table 2 have been listed on this basis, together with the site code to which each of them belongs. The last column shows the number (N) of NMR lines expected for each geometrical isomer and their relative intensities (I). It can be seen that up to 20 geometrical isomers and 63 singlets for each proton C^2-H or C^5-H may thus be detected.

Table 2. Classification of the Geometrical Isomers (Chiral or Achiral) of [$Co(Hist)_2(OH)(O_2)Co(Hist)_2$] Dimer According to the Total Number (N_e) of Equatorial N^3 Coordinations and the Site Code, Together with the Number (N) and Relative Intensities (I) of C^2-H, C^5-H Resonances and the Type of Spectral Sets Involved

N_e	code no.	site code	isomers (number and type)	NMR signals N	NMR signals I	spectral sets
4	1	[e_1e_2, e_1e_2]	1 meso[a]	2	2	(e_1e_2)
			1 *dl*[b]	2	2	
3	2	[e_1e_2, ae_2]	2 *dl*	2 × 4	1	(e_1e_2), (ae_2)
	3	[e_1e_2, ae_1]	2 *dl*	2 × 4	1	(e_1e_2), (ae_1)
2	4	[e_1e_2, aa']	2 *dl*	2 × 4	1	(e_1e_2), (aa')
	5	[ae_2, ae_2]	1 meso	2	2	(ae_2*)
	6	[ae_2, $a'e_2$]	1 *dl*	2	2	(ae_2)
	7	[ae_2, ae_1]	1 *dl*	4	1	(ae_2*), (ae_1*)
	8	[ae_2, $a'e_1$]	1 *dl*	4	1	(ae_2), (ae_1)
	9	[ae_1, ae_1]	1 meso	2	2	(ae_1*)
	10	[ae_1, $a'e_1$]	1 *dl*	2	2	(ae_1)
1	11	[ae_2, aa']	2 *dl*	2 × 4	1	(ae_2*), (aa'*)
	12	[ae_1, aa']	2 *dl*	2 × 4	1	(ae_1*), (aa'*)
0	13	[aa',aa']	1 meso	1	4	($aa'aa'$)
			1 *dl*	2	2	

[a] Achiral (meso) isomer. [b] Optically active *dl* isomer pair.

Line Multiplicity of Imidazolic Protons. Fourteen lines only are observed for each imidazole proton in fresh solutions of the complex at pH 9. This may arise from the presence of a limited number of isomers but also from the alternative event of overlapping resonances. In this event, we may assume that chemical shifts of imidazolic protons are determinated by N^3 coordination sites in the complex, *i.e.*, solely by the site code, without regard for the position of the amino ends; this means that we neglect the position of histamine connecting arms in molecular formulas such as those in Figure 7, and that stereoisomers belonging to a given code number have the same spectrum. The importance of N^3 coordination is shown by the fact that C^2-H lines are much more scattered than C^5-H lines, as a result of a closer proximity to the N^3 nitrogen. In this view, the observed bisection of C^2-H lines into two separate subsets (of eight and six resonances, lying on either side of C^5-H resonances, fortunately located just in between with the exception of one line) could be simply assigned to equatorial and axial N^3-coordinated imidazole units (or vice versa), respectively. Another consequence is to enumerate C^2-H resonances not from the set of 20 isomers, but from the set of 13 site codes; this decreases the number of resonances to 38. Further reduction of this number could then arise from the additional assumption that the coordination spheres of each cobalt ion are not influencing chemical shifts of each other due to the distance. This means that we would have to consider the lines arising from the truncated site codes (e_1e_2), (ae_1), (ae_2), (a,a') relative to each cobalt ion considered alone. This condition, however,

(22) Fallab, S.; Zehnder, M.; Thewalt, U. *Helv. Chim. Acta* **1980**, *63*, 1491.

Table 3. Spectral Sets and Characteristic C^2−H Lines (for Numbering See Figure 6) Assigned to the Geometrical Isomers of the $[Co(Hist)_2(OH)(O_2)Co(Hist)_2]$ Dimer, Indicated by Their Code Number (see Table 2) and Their Relative Proportions (%) in the Course of Time *t*

	no. of characteristic lines			assigned isomer proportions (%)				
spectral sets	axial	equatorial	code no.	$t = 0$	$t = 0.5$ h	$t = 2$ h	$t = 6$ h	$t = 70$ h
ae_2	5	12	6	27	30	34	48	56
ae_1	4	11	10	15	16	16	20	16
aa'*	{2, 3}		11 + 12	22	22	21	8	10
aa'	7		4	7	8	12	8	7
$aa'aa'$	6		13	10	11	11	11	10
ae_1* + ae_2*	{1, 8}	{9, 14}	(5 + 7 + 9) + (11 + 12)	32 {10[a], 22}	26 {4[a], 22}	22 {1[a], 21}	8 {0[a], 8}	10 {0[a], 10}
e_1e_2		{13, 10}	1 + 2 + 3 + 4	16 {9[b], 7}	17 {9[b], 7}	20 {8[b], 12}	13 {5[b], 8}	7 {0[b], 7}

[a] Value obtained by combining data from spectral sets aa'* and ae_1* + ae_2*. [b] Value obtained by combining data from spectral sets aa' and e_1e_2.

proves to be too severe, reducing the number of lines to only seven. A looser condition allows some interaction between the two coordination spheres only when the two imidazole units are in vicinal *cis* diaxial positions (*aa* or $a'a'$), still on steric grounds. Thus, we still consider that (e_1e_2) lines will appear at the same frequency in the isomers described by the codes 1 [e_1e_2, e_1e_2], 2 [e_1e_2, ae_2], 3 [e_1e_2, ae_1], and 4 [e_1e_2, aa']. This set of lines will be called a spectral set (e_1e_2) in the following. The presence of one spectral set (ae_2) still holds in the isomers [ae_2, $a'e_2$] due to molecular symmetry. On the contrary, (ae_2) lines will separate into two spectral sets denoted as ae_2 and ae_2* for site codes 2, 6, and 8 (no diaxial interaction) and 5, 7, and 11 (one diaxial interaction), respectively. Similar splittings are observed for (ae_1) and (aa') lines with spectral sets ae_1, ae_1*, and aa', aa'*. The case of the tetraaxial isomer stands apart (two diaxial interactions), and its spectrum (one line) is designated by the site code itself in the following ($aa'aa'$). Each spectral set is found in one or several site codes, as shown in Tables 2 and 3. Spectral sets have the following composition: e_1e_2, a couple of equatorial lines of equal intensity; ae_1, ae_2, ae_1*, and ae_2*, one equatorial and one axial signal, of equal intensity; aa' and $aa'aa'$, a single axial line; aa'*, a couple of axial lines of equal intensity. This means that, under these assumptions, the whole spectrum may contain up to 14 lines; this is the number actually observed by ^{1}H NMR. Moreover, the prediction of eight axial and six equatorial resonances strongly suggests that each of these subsets of lines should be assigned to the low- and high-field parts, respectively, of the C^2-H spectrum (see above).

Line Assignment. In a first step, lines should be assigned to the eight spectral sets described above. The spectral sets thus obtained should then be assigned to the 13 possible isomers defined by their code number. Line assignment and the subsequent identification of isomers, in fact, should be performed in parallel, and many ambiguities are possible in the whole procedure. In view of this, the consideration of intensities is of fundamental importance to connect lines to each other, since resonances from the same spectral set on the one hand, and those from different spectral sets of the same isomer on the other hand, should have equal intensities. In fact, equally intense lines can also arise from different isomers fortuitously present in equal amounts. Fortunately, equilibria in this mixture of stereoisomers are very slow. The proportions of stereoisomers are consequently variable in the course of time, and this may help us to recognize sets of lines with similar time behavior, so as to assign each of them to the same isomer.

The 14 C^2-H lines (Figure 8), numbered 1−14 along increasing fields (see Figure 5), obtained at different recording times, show the presence of (i) two intense lines (5 and 12), (ii) three medium-sized lines (4, 6, and 11) slowly decreasing with time, and (iii) nine weaker lines, with contrasting behaviors: a group of four lines (1, 8, 9, and 14) simultaneously decreasing to nearly zero, two groups of two lines of equal intensity (2, 3 and 10, 13), and one single line (7) slowly

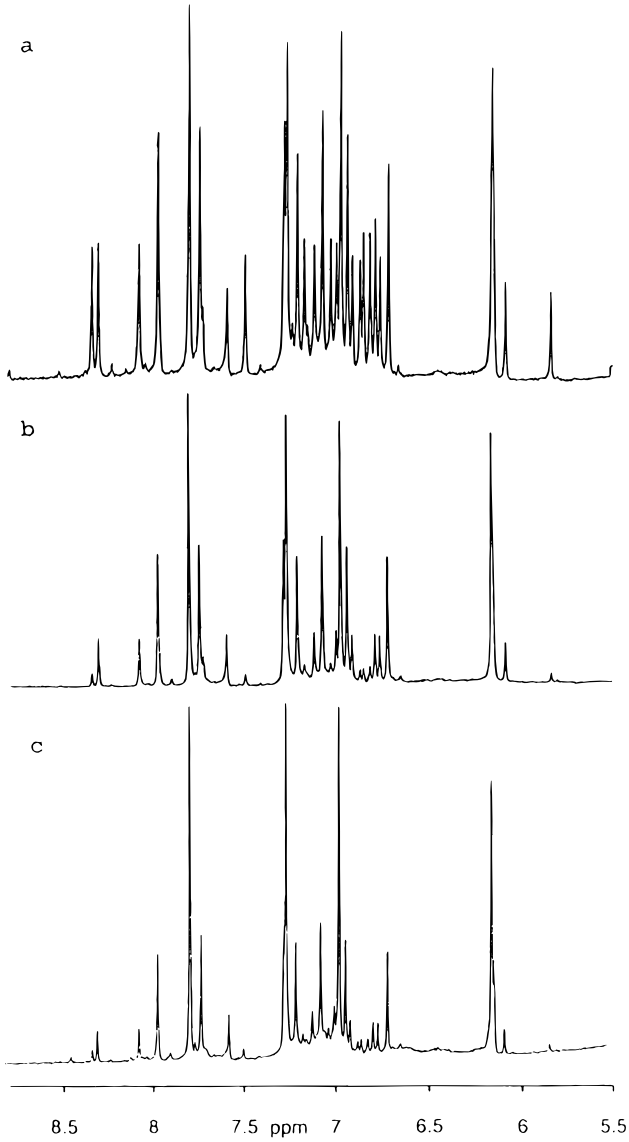

Figure 8. Imidazole region of ^{1}H-NMR spectra of Co(II)−histamine−O_2 system as a function of time: freshly prepared solution (a) and the solution 6 h (b) and (c) 40 h after ($[Co^{2+}] = [L]/2 = 7.5 \times 10^{-3}$ mol dm^{-3} at pH 9).

decreasing with time. C^5-H lines are much more imbricated (Figure 5), and so counting 14 proton lines is less straightforward. A salient feature in the C^5-H spectrum is the presence of two intense lines, clearly associated with C^2-H lines 5 and 12. This correlation can be shown by 2D COSY NMR, since there is a weak (undetected) scalar coupling ($J \approx 1$ Hz) between protons C^2-H and C^5-H from the same imidazole units: cross-correlation peaks are thus found between the two upfield (C^2-H and C^5-H) intense lines on the one hand and the two downfield intense lines on the other hand (the correlated C^5-H protons are denoted in Figure 5 by the corresponding primed numbers). There is, unfortunately, no NMR procedure to correlate protons belonging to different imidazole units as a help in assigning NMR lines to the various isomers.

Information on the nature of the isomers can, however, be obtained if we admit the above bisection proposed for equatorial and axial lines on the basis of spectral sets and coded site sequences. Detailed line assignments are reported in the Appendix. Remaining ambiguities were removed by assigning the most intense lines to isomers assumed to be energetically favored on qualitative consideration of the steric strain induced by coordinated imidazole rings, depending on the distance between them (see the Appendix). The identification of stereoisomers may then be summarized as follows. Under the working hypotheses mentioned above, solutions at equilibrium may contain up to six isomers. The proportions of these isomers can be approximately computed from the intensities of appropriate lines and from the number of equivalent nuclei in each of them. Isomers 6 and 10, having an axial−equatorial N^3 coordination on each cobalt (Figure 7a,b) and no vicinal diaxial interaction [ae_2, $a'e_2$] and [ae_1, $a'e_1$], are the most abundant, 72% at equilibrium, with 56 and 16% of the first (lines 5 and 12) and second (lines 4 and 11) isomers, respectively. Then, there are two sets of isomers with the same abundance of about 10% (Figure 7c): the mixture of (indiscernible) triaxial isomers 11 and 12 [ae_2, aa'] and [ae_1, aa'] (lines 1, 2, 3, 8, 9, and 14) and the tetraxial isomer 13 [aa', aa'] (line 6). The isomers 1, 2, 3, and 4, with two geminal diequatorial imidazole units [e_1e_2, e_1e_2], [e_1e_2, ae_2], [e_1e_2, ae_1], and [e_1e_2, aa'], are energetically unfavored, with an overall percentage of 7% and a total predominance of the fourth isomer [e_1e_2, aa'], which logically contains the minimum number of equatorial imidazoles (Figure 7d).

In freshly prepared solutions, the less stable isomers are present to a large extent at the expense of the thermodynamically stable ones. The isomers having either a vicinal diaxial interaction (coded 5, 7, and 9) or a geminal diequatorial N^3 coordination (coded 1, 2, and 3) were not detected at equilibrium. However, at the beginning, these isomers exist to an extent of 10 and 9%, respectively. Among the isomers which still exist at equilibrium, the proportion of isomers 11 and 12 having a vicinal diaxial interaction is logically decreased from 22 in fresh solutions to 10% at equilibrium, while the overall proportions of the thermodynamically favored isomers 6 and 10 are simultaneously increased from 42 to 72%, and those of isomers 4 and 13 are unchanged (7 and 10%). The initial mixture of isomers is clearly out of equilibrium. Kinetic products formed after oxygen uptake are converted into thermodynamically stable isomers (Table 3) in about 1 day. During this interconversion, the pH of the solution and its absorption spectra remain unaltered; this shows that the decomposition of the μ-peroxo-μ-hydroxo bridges and the subsequent formation of monomeric Co(III) complexes can be neglected during this time. This also shows that some caution should be exercised in the handling of these solutions and in the interpretation of experimental data, depending on the time of measurements.

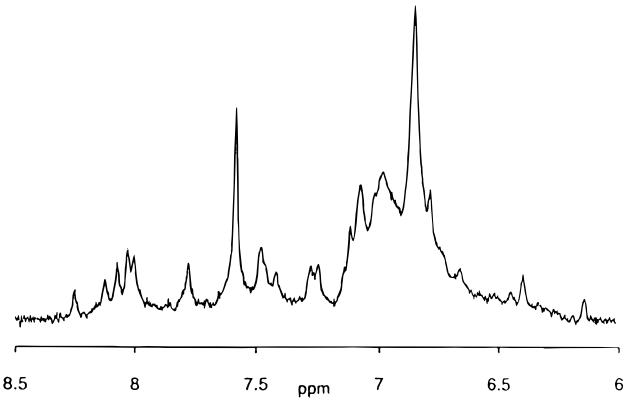

Figure 9. Imidazole region of ^{1}H-NMR spectrum of the Co(II)−glycylhistamine−O_2 system ($[Co^{2+}] = 2[L]/3 = 1.0 \times 10^{-2}$ mol dm^{-3} at pH 8.2).

Oxygenated Cobalt Complexes of Histamine at Higher pH. Coming back to the pH dependence of ^{1}H-NMR spectra, the chemical shifts of signals are identical between pH 6.6 and 9, with increasing signal intensity, due to the sole presence of one oxygen-carrying complex as defined by its protonation state and its stoichiometry ($Co_2(Hist)_4(OH)(O_2)$). At higher pH, however, the signals are gradually shifted upfield, and the character of spectra also changes at pH > 11, indicating the formation of further oxygen-carrying complex(es). However, NMR site ligand exchange between the oxygenated complexes is fast on the NMR time scale (since only a gradual shift was observed as a function of pH without the appearance of new signals), suggesting differences in protonation state and fast proton exchanges rather than structural changes between the successively formed complexes. Similar behavior can be concluded from ^{1}H-NMR spectra of oxygenated complexes with glycyl- and sarcosylhistamine, although the signals appearing in this case (Figure 9) are broad (∼10−20 Hz), probably on kinetic grounds.

Taking into account all the potentiometric and spectroscopic results cited in this study, the best fit of experimental pH curves was obtained by considering the presence of three different protonation states of oxygenated complexes in all systems, as listed in Table 1. The different isomers, belonging to the same protonation states, are indistinguishable by pH study. The species' distribution curves are shown in Figure 10.

In agreement with earlier suggestions, only the bis-Co^{II}-(histamine)$_2$ complex can take up oxygen. In accordance with the high stability of oxygenated complexes, the species CoL_2 is transformed almost quantitatively into the ternary complex Co(II)−histamine−O_2 in the presence of oxygen, leaving a very small concentration of CoL_2 in equilibrium. As mentioned above, the [$Co(Hist)_2(OH)(O_2)Co(Hist)_2$] species containing μ-hydroxo-μ-peroxo bridges form first between pH 7 and 10 after equilibration of the system. The only pseudoequilibrium study reported previously for the Co(II)−histamine−O_2 system[6] considered no μ-hydroxo bridge, thus the results are not comparable. The pK values of the two further deprotonations of the above complex are 9.80 and 11.06, denoted as pK_{24-21}^{24-11} and pK_{24-31}^{24-21}, respectively, in Table 1. In the recently reported Co(II)−histidine−O_2 system[10] (in the absence of the μ-hydroxo bridge, and $I = 0.5$ mol dm^{-3}), the corresponding values are reported to be 10.4 and 11.1, respectively (without assignment of deprotonation). Since the coordination sphere of cobalt is completely filled, these deprotonations cannot involve coordinated water molecules, but rather N^1 (pyrrolic) nitrogens of

bound imidazole rings. However, in oxygen-free solution, the deprotonation of N^1 (pyrrolic) nitrogen, when the metal ion is already coordinated at N^3 nitrogen, takes place at much higher pH (pK ~13 for histamine and ~12.5 for histidine[23]). This fundamental difference is most easily explained by considering the effect of electron transfer from cobalt to dioxygen, *i.e.*, a triply positive formal charge on metal ions in oxygen-carrying complexes, in line with earlier conclusions from different spectroscopic techniques.[17] This statement can be confirmed by the fact that, in the $Co^{III}(NH_3)_2(LH_{-2})$ complex (where L = glycylglycylhistidine), the pK of pyrrolic nitrogen deprotonation[24] is reported to be 9.81, a value close to our findings.

Oxygenated Complexes of Pseudopeptides. In the case of pseudodipeptides, the parent complexes $CoLH_{-1}$ and CoL_2H_{-1} (formed under nitrogen atmosphere, see above) are formed before oxygen ligation. $[Co(LH_{-1})(OH)(O_2)Co(LH_{-1})]$ is the first oxygenated complex to appear in the equilibrated system. This complex may suffer a single deprotonation at higher pH, having pK values of 9.28 and 9.39 for glycyl- and sarcosylhistamine, respectively. However, besides the already-reported[4] $CoLH_{-1}$ species, the CoL_2H_{-1} complex seems to be also able to take up oxygen between pH 7 and 11, forming the $[Co(L_2H_{-1})(OH)(O_2)Co(L_2H_{-1})]$ adduct. Recently, in the Co(II)−glycyhistidine−O_2 system,[25] the presence of a monobridged species $[Co(LH_{-1})(O_2)Co(LH_{-1})]$ was also deduced from pseudoequilibrium studies. The formation of analogous species in our systems did not improve the quality of the fits, presumably because of negligible concentrations.

The best-fit values of formation constants determined and the oxygenation constants calculated for equilibria 2 and 3 are listed in Table 1.

$$2Co(LH_{-1}) + H_2O + O_2 \rightleftarrows [Co(LH_{-1})(OH)(O_2)Co(LH_{-1})] + H, \quad K^1_{O_2} \quad (2)$$

$$\log K^1_{O_2} = \log \beta_{22-31} - 2 \log \beta_{11-10}$$

$$2Co(L_2H_x) + H_2O + O_2 \rightleftarrows Co(L_2H_x)(OH)(O_2)Co(L_2H_x) + H, \quad K^2_{O_2} \quad (3)$$

$$\log K^2_{O_2} = \log \beta_{24(2x-1)1} - 2 \log \beta_{12x0}$$

where $x = 0$ and -1 for histamine and pseudopeptides, respectively.

The log $K^1_{O_2}$ value for glycylhistamine (log $K^1_{O_2}$ = 2.36) is much higher than that reported earlier by Harris and Martell[4] (log $K^1_{O_2}$ = 0.4) but is, however, close to that recently found for the Co(II)−glycylhistidine−O_2 system, where log $K^1_{O_2}$ is reported to lie between 1.9 and 2.3.[25] The difference in stability of the glycyl- and sarcosylhistamine complexes reflects the somewhat lower coordination ability of the latter ligand, due to the steric effect of the *N*-methyl group.[12b,d]

If we assume that the parent cobalt(II) complexes retain their structure in the oxygenated adducts, the metal ion in the first oxygenated complex $[Co(LH_{-1})(OH)(O_2)Co(LH_{-1})]$ is {3N}-coordinated to the ligand. Two of the three remaining sites on each cobalt(II) ion are engaged in the μ-peroxo and μ-hydroxo bridges. The coordination number of cobalt(II) is completed to six by solvating water molecules, as in the parent non-oxygenated complexes themselves. This complex may suffer a single deprotonation at higher pH, having the pK values pK^{22-31}_{22-41} = 9.28 and 9.39 for glycyl- and sarcosylhistamine, respectively (Table 1). Along the same line of reasoning, the second oxygenated complex appears to derive from the first one by attaching a a second ligand molecule on each cobalt(II) ion in the place of the solvated water molecule mentioned above. In view of this, the second ligand molecule is assumed to be monodentately coordinated through the imidazole ring, just as in the parent complex CoL_2H_{-1}. This {4N}-coordinated species seems to have a greater affinity for oxygen uptake than the {3N}-coordinated one, *i.e.*, log $K^2_{O_2}$ > log $K^1_{O_2}$, in line with the higher electron transfer (from metal to oxygen) ability expected for the {4N}-coordinated metal ion.

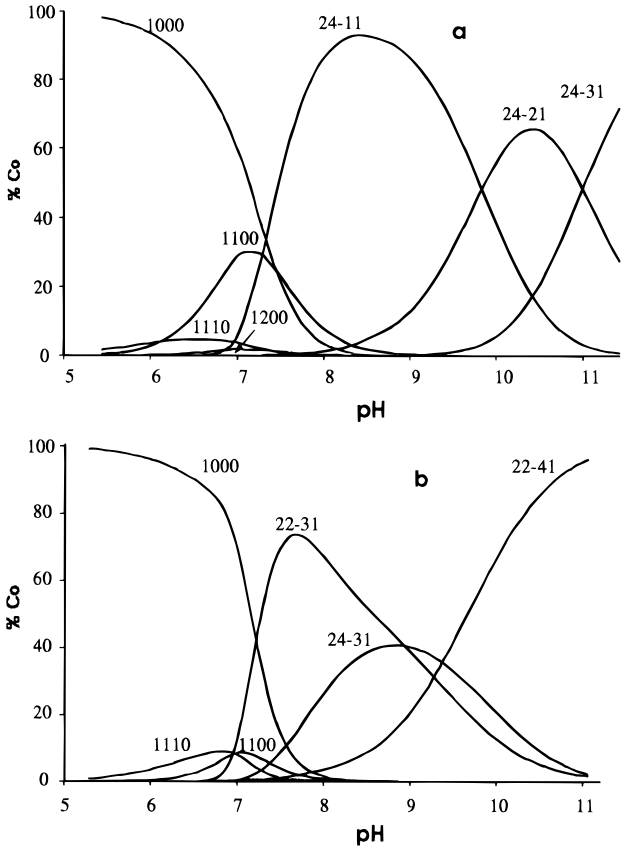

Figure 10. Species distribution in the Co(II)−histamine−O_2 (a) and Co(II)−glycylhistamine−O_2 (b) systems. I = 0.1 mol dm^{-3}, T = 298 K, $[Co^{2+}] = 1.0 \times 10^{-3}$ mol dm^{-3}, and $[L] = 3 \times 10^{-3}$ mol dm^{-3}.

Oxygen uptake appears to be easier in pseudopeptide than in histamine complexes, *i.e.*, log $K^2_{O_2}$ = 1.51 and 2.96 for histamine and glycylhistamine, respectively. This can be tentatively assigned again to a higher electron transfer effect in pseudopeptide oxygenated complexes due to the coordination of the deprotonated amide nitrogen, presumably because of its higher basicity and/or negative charge.

Conclusion

The structure and stability of oxygenated species have been studied in Co(II)−L−O_2 ternary systems (where L = histamine, glycylhistamine, and sarcosylhistamine). Three different protonation states of oxygenated species were evidenced in all systems between pH 7 and 11.2. In the Co(II)−histamine−O_2 system, the oxygen uptake can be attributed to the parent complex CoL_2; however, in the case of pseudopeptides, both $CoLH_{-1}$ and CoL_2H_{-1} complexes seem to participate in the oxygen uptake. The stability constants determined by pH study for the above systems revealed that {4N}-coordinated species

(23) Sundberg, R. J.; Martin, R. B. *Chem. Rev.* **1974**, *74*, 471.
(24) Hawkins, C. J.; Martin, J. *Inorg. Chem.* **1983**, *22*, 3879.
(25) Kufelnicki, A.; Swiatek, M. *Polish J. Chem.* **1993**, *67*, 1345.

have a greater affinity for oxygen uptake than the {3N}-coordinated ones. They also showed the stabilizing effect of the deprotonated amide nitrogen in the oxygenated complexes of pseudopeptides.

In every protonation state, several geometrical isomers, indistinguishable by pH study, have been detected by ^{1}H-NMR spectroscopy. Fourteen singlets are effectively observed for each imidazolic proton C^2-H or C^5-H in the case of histamine. This line multiplicity can be attributed to the presence of a great number (up to 20) geometrical isomers, six of which can be distinguished in the case of the $Co_2(Hist)_4(OH)(O_2)$ complex between pH 7 and 9. This shows, in turn, the absence of fast dynamic processes which would bring magnetic equivalence between coordination sites within the oxygenated complex. This is the first direct evidence of the presence in solution of a μ-hydroxo bridge in addition to the peroxo bridge in the cobalt dimer, since the creation of an asymmetrically dibridged dimer core involves the possibility for a large number of stereoisomers from the combination of nonequivalent axial and equatorial coordination sites. Under reasonable working hypotheses, it is then possible to account for the number of lines observed in NMR spectra and to propose structures for these isomers. Line intensities then permit one to give the proportions of these stereoisomers in the course of time and at equilibrium. Isomers with neighboring {^{3}N}-coordinated imidazole units, *e.g.*, in a vicinal *cis* diaxial or in a geminal diequatorial arrangement, are present only in minor quantities, presumably on steric grounds. These isomers suffer progressive deprotonations on their pyrrolic N^1 nitrogens from pH ~8, and each of them is in fast equilibrium with its N^1-deprotonated analogues on the NMR time scale. This means that C^2-H and C^5-H singlets are progressively shifted (upfield) in the course of deprotonation, without modification of line multiplicity and of the NMR pattern analyzed above, at least in the first stages of deprotonation (pH 8−9) when the induced chemical shift variations are sufficiently weak to prevent line crossing phenomena. In this view, the p*K*'s measured for the abstraction of one and two N^1-H protons refer to an average over the mixture of stereoisomers.

Although reversible, these systems are characterized by slow kinetic processes toward equilibrium. This is used first to obtain definite states of protonation of oxygen-carrying complexes, as determined by pH measurements, with a reaction time of about 1 h. A second evolution, more subtle since it was undetected until now by potentiometric studies, involves a great variety of stereoisomers, depending on the coordination modes of the ligand. This evolution, which occurs at constant pH, lasts 1 day before completion.

The results obtained with these slowly reversible systems emphasize the necessity for additional structural information besides the chemical speciation obtained from equilibrium studies. They also help by providing presumptive evidence for the structural variety of species formed in solution, even if they are not detectable due to fast mutual exchange.

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Appendix

^{1}H-NMR Line Assignment of $Co_2(Hist)_4(OH)(O_2)$ Complexes. The two intense C^2-H lines, one of which (5) is axial and the other one (12) equatorial, can be assigned only to any of the four spectral sets ae_2, ae_1, ae_2*, or ae_1*, and consequently to one of the four isomers 6 [ae_2, $a'e_2$], 5 [ae_2, ae_2], 9 [ae_1, ae_1], or 10 [ae_1, $a'e_1$]. This result shows that complexes with geminal axial−equatorial imidazole coordination are more stable than those with geminal diequatorial (isomers 1, 2, 3, and 4) or vicinal *cis* diaxial coordination (isomers 7, 11, 12, and 13), presumably due to the steric strain brought by neighboring imidazole units. For the same reasons, the *trans* vicinal diaxial coordination is more stable than the *cis* vicinal diaxial coordination. As a consequence, lines 5 and 12 should be preferably assigned to either isomer 6 or 10. Moreover, *trans* vicinal diequatorial coordination is energetically favored in an e_2,e_2 rather than in an e_1,e_1 arrangement, still on the basis of the distance between equatorially coordinated imidazole rings. On this basis, lines 5 and 12 are tentatively assigned to isomer 6, coded as [ae_2, $a'e_2$]. The free energy difference between isomers 6 and 10, however, should be relatively small since, in both cases, imidazole units are relatively far from each other. This suggests that isomer 10 should be assigned to two medium-size resonances, one of which is the equatorial line 11, and the other one either of the two axial lines 4 or 6, preferably line 4 due to the slight difference between intensities of lines 11 and 6. The supernumerary line 6 would be an axial singlet to be assigned to either spectral set aa' or $aa'aa'$. Finally, we cannot exclude that some quantity of isomer 8 [ae_2, $a'e_1$] may contribute to somewhat increasing by equal amounts the intensities of the two above couples of lines 5, 12 and 4, 11.

If we examine now the set of weaker lines, their time behavior shows that axial lines 2 and 3 on the one hand, and equatorial lines 10 and 13 on the other hand, should be associated. This can occur only if the first couple of lines is assigned to the spectral set aa'* and the second one to e_1e_2. Line 7 cannot be simply related to any other in the spectrum and should, consequently, be assigned to either spectral set aa' or $aa'aa'$. This was also the case for line 6. The ambiguity can be removed by noting that aa' is associated with a single isomer, 4 [e_1e_2, aa']. This means that lines 13 and 10 (e_1e_2) should each have an intensity greater than or equal to half that of the singlet representing aa', depending on whether isomer 4 is accompanied with isomers 1, 2, and 3 or not. This condition can be fulfilled only if line 7 is assigned to aa', and consequently line 6 to $aa'aa'$. Lines 7 and 6 are relatively intense in spite of unfavorable steric factors (one geminal diequatorial or two vicinal diaxial interactions in isomers 4 and 13, respectively), because each resonance represents two or four, respectively, equivalent nuclei. Finally, the last quartet of lines 1, 8, 9, and 14 should belong to spectral sets ae_1* and ae_2* and, consequently, to the five isomers 5, 7, 9, 11, and 12. Their intensities are not significantly different, precluding the distinction between the two doublets ae_1* and ae_2*. For solutions at equilibrium, it can be observed that the intensity of lines 1 and 8 is approximately half that of lines 2 and 3, representing the spectral set aa' in isomers 11 and 12. This points to the absence of isomers 5, 7, and 9 and the sole presence of isomers 11 and 12 in nearly equal amounts.

Supporting Information Available: Figures representing the 20 possible geometrical isomers of the $Co_2(Hist)_4(OH)(O_2)$ complex (4 pages). Ordering information is given on any current masthead page.

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