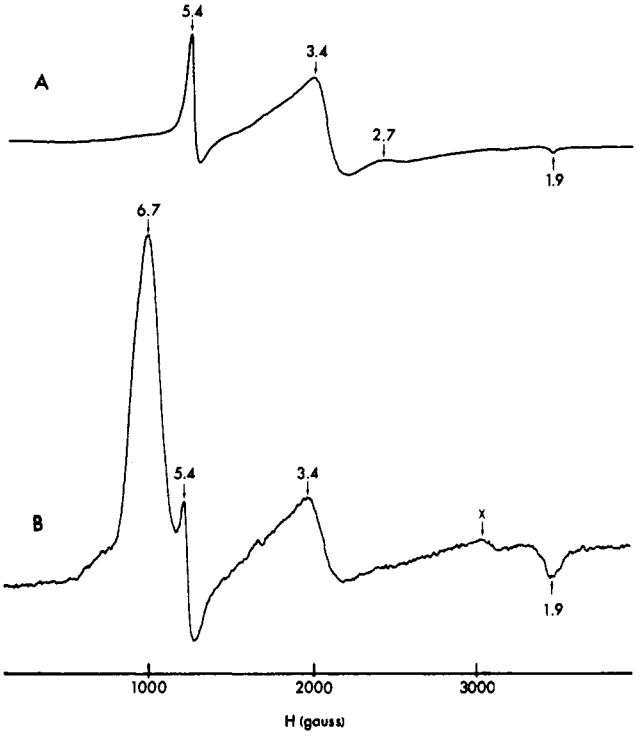

Figure 5. Electron spin resonance spectra of dimeric metalloporphyrin species (1:1 toluene–methylene chloride; 5.8 K): (A) $((TTOP)Fe)_2$; (B) mixture of $((TTOP)Fe)_2$, $((TTOP)Mn)_2$, and $Mn(TTOP)_2Fe$.

leave spin–spin relaxation between metal centers as the major contributor to broadening of the ESR signals at higher temperatures.

Optical spectra of solutions containing both the hetero dimer and homo dimers exhibit no new bands that could be assigned to the hetero dimer. It thus appears that band intensities are only a function of the total quantity of a particular metalloporphyrin in solution.

Discussion

Nuclear magnetic resonance spectroscopy of manganese(III) porphyrins has received only scant attention due to the large line widths evident for the high-spin d^4 ion. Deuterium resonances for coordinated pyridine residues have previously been identified in far downfield positions,[15] but no (phenolato)manganese(III) porphyrin complexes have previously been reported. Thus, it is of interest to compare spin density delocalization patterns for both iron(III) and manganese(III) porphyrins. An upfield-shifted pyrrole proton signal in manganese(III) tetraalkylporphyrins is well rationalized by the $(d_{xy})^1(d_{xz},d_{yz})^2,(d_{z^2})^1(d_{x^2-y^2})^0$ electronic configuration in which the π-symmetry $(d_{xz},d_{yz})^2$ unpaired spin is delocalized to the pyrrole β-carbon atoms. An additional unpaired electron in the strongly σ-interacting $d_{x^2-y^2}$ orbital of high-spin iron(III) porphyrins serves to induce large downfield pyrrole proton chemical shifts. Examination of Figure 4A,D reveals that chemical shift directions for coordinated phenolato ion are the same for both iron(III) and manganese(III) compounds. A singly occupied σ-symmetry d_{z^2} orbital is directed toward the phenolato oxygen atom in each case and must transfer appreciable spin density to the phenyl ring. Alternation in signs of chemical shifts around the phenyl ring is consistent with π-spin polarization of the phenolato aromatic ring. Transfer of manganese(III) spin density in this manner must be relatively less efficient than that of iron(III) as revealed by the approximate 50% attenuation of phenolato signal shifts in the manganese(III) derivatives.

Formation of the hetero Mn–Fe dimer under basic conditions is not surprising, but statistical distribution of dimeric species might have been anticipated. The metal discrimination involved in dimer formation likely reveals both thermodynamic differences in axial ligand bond formation and differences in the affinity of the porphyrins to the basic alumina column. The higher affinity of iron porphyrins for axial ligands and oxophilic character of the iron(III) center suggest that $((TTOP)Fe)_2$ is thermodynamically the most stable species among the three possible dimeric structures. Formation of the homo iron dimer upon addition of 3% MeOH–CH_2Cl_2 to the basic alumina column containing the hetero dimer indicates that all three dimeric species are in equilibrium. As $((TTOP)Fe)_2$ is produced, it is eluted down the column faster than the manganese-containing species and cannot be used to re-form the mixed-metal dimer. Hence, isolation of the pure Fe–Mn dimer by standard chromatographic methods is unlikely.

Generation of the mixed Mn–Fe porphyrin is rather facile, and in principle the $TTOP^{2-}$ ligand might be utilized to generate a large variety of homo and hetero dimetal complexes. Combinations of Cr(III), Mn(III), Fe(III), and Co(III) mixed species should be readily accessible. This general technique for preparation of mixed-metal complexes of defined geometry is thus offered for investigations of electron transfer and spin–spin interactions between metal centers and as a potential source of compounds for multielectron-redox catalysis.

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Cobalt(III) Complexes of L-Histidylglycylglycinate: Preparation, Characterization, and Conformation

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The preparations of three cobalt(III) complexes of L-histidylglycylglycinate are reported. In one complex, $[Co(NH_3)(H_{-2}HisGG)]$, the peptide is coordinated as a quinquedentate chelate through the terminal NH_2, two deprotonated peptide nitrogens, the carboxylate, and the imidazole group. In another, $[Co(NH_3)_2(H_{-2}HisGG)]$, the carboxylate is replaced by a second NH_3. The third complex has properties consistent with the superoxo binuclear complex $NH_4[(H_{-2}HisGG)Co(O_2^-)Co(H_{-2}HisGG)]$. The structures of the first two complexes were derived from the complexes' electronic absorption, circular dichroism, and ^1H and ^{13}C NMR spectra. The coordinated imidazole's NH-1 has acid dissociation constants at 298 K of 10.73 ± 0.04 and 10.69 ± 0.04 for $[Co(NH_3)(H_{-2}HisGG)]$ and $[Co(NH_3)_2(H_{-2}HisGG)]$, respectively. In the diammine complex, the free carboxylate group has a pK_a value of 4.34 ± 0.04 at 298 K.

Introduction

This paper reports the first examples of N-terminal histidine peptides coordinating to a metal via the NH_2, the imidazole moiety, and two peptide-N^- donors. The complexes involve the coordination of L-histidylglycylglycinate to cobalt(III) with and without the terminal CO_2^- bound to the metal (Figure 1).

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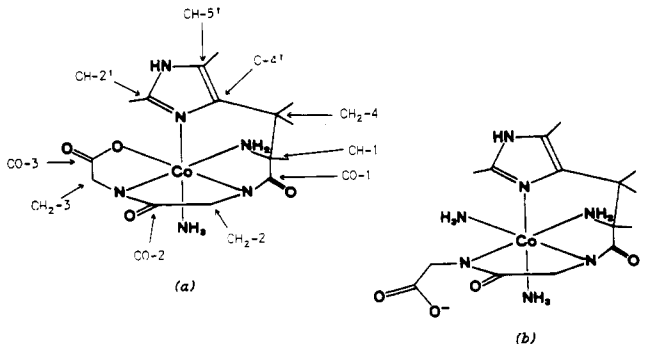

Figure 1. Structure of (a) $[Co(NH_3)(H_{-2}HisGG)]$ and (b) $[Co(NH_3)_2(H_{-2}HisGG)]$.

Histidine-like terdentate chelation via the NH_2, peptide-*O*, and the imidazole moiety has been proposed previously for some divalent metal complexes,[1,2] but the preference for bidentate chelation via the NH_2 and imidazole groups has also been stressed.[2-6] Other forms of chelation have been reported, such as a binuclear complex with NH_2, peptide-N^-, and peptide-O chelation to one metal with the imidazole N-3 binding to the second metal[2,5,7] and, for the above tripeptide, NH_2, two peptide-N^- donors, and carboxylate.[7]

In this paper the syntheses of $[Co(NH_3)(H_{-2}HisGG)]$ and $[Co(NH_3)_2(H_{-2}HisGG)]$ are reported and the structures of the complexes are characterized by various spectroscopic techniques. The conformations of the chelate ring formed by NH_2 and imidazole coordination have been determined by an analysis of the ^1H NMR spectra. The acid dissociation constants for NH-1 in the coordinated imidazole have been determined for the two complexes, and the constant for the free carboxylate has been determined for the diammine complex.

Experimental Section

Materials. L-Histidylglycylglycine was purchased from Bachem and used without further purification. Cobalt(III) "peroxo dimer", $[(NH_3)_5Co(O_2)Co(NH_3)_5](NO_3)_4 \cdot 2H_2O$ was prepared by the method of Davies and co-workers.[8] All other reagents were of AnalaR grade. The pK_a values of NH-1 of the coordinated imidazole were determined at 298 K by titration with standard CO_2-free sodium hydroxide by using a TPS 1852 digital pH meter (±0.01 pH unit) and a thermostated cell with a combination-glass electrode and a nitrogen bubbler.

Preparation of Cobalt(III) Complexes of L-Histidylglycylglycine. The "peroxo dimer" (1.0 g, 0.017 mol) was added with constant stirring to a solution of L-histidylglycylglycine[9] (0.95 g, 0.0034 mol) in dilute aqueous ammonia (10 mL) at pH 9 kept below 5 °C. Stirring was continued for 3 h before the solution was transferred to a refrigerator for approximately 14 h. The solution was filtered; the addition of acetone to the filtrate yielded an oil, which was triturated with acetone to give an orange-brown solid that was filtered off under vacuum, washed with acetone, and air-dried.

The crude product was redissolved in water and chromatographed on a BioGel P2 column (50-100 mesh, 3 × 80 cm) with dilute aqueous ammonia (pH 9) as eluant. A pink fraction remained adhered to the column. Two orange fractions separated and, after collection, were freeze-dried.

Fraction 1, which eluted first, was redissolved in water and chromatographed on a Sephadex CM-C25 column (Na form, 2 × 50 cm) with water as eluant. A pink band adhered to the top of the column while the orange band was eluted and freeze-dried after the pH was raised to 9. As the ^1H and ^{13}C NMR spectra of the product showed very broadened peaks, the compound (in water) was first chromatographed on Biorad Chelex 100 (50–100 mesh, Na form, 1 × 30 cm) to remove any cobalt(II) impurities and then on Sephadex DEAE-A25 (Cl form, 2.5 × 25 cm) with water as eluant. A small amount of pink compound eluted with the solvent front while the orange material adhered to the top of the column. This was eluted off with dilute hydrochloric acid (pH 2). The fraction was collected, its pH was raised to 9 with ammonia, and the solution was freeze-dried. The product was finally chromatographed on Fractogel TSK HW-40(F) (2 × 40 cm) with water as eluant. Only one homogeneous band was observed. This was collected, dried over phosphorus pentoxide until a wet solid was obtained, and then dried over anhydrous copper sulfate for 2 days to yield 60 mg of product whose analyses and properties were consistent with the superoxide dimer $NH_4[(H_{-2}HisGG)Co(O_2^-)Co(H_{-2}HisGG)] \cdot 4H_2O$. Anal. Calcd for $C_{20}H_{36}Co_2N_{11}O_{14}$: C, 31.1; H, 4.7; N, 19.9. Found: C, 31.2; H, 5.6; N, 20.1.

Fraction 2 (eluted second off the P-2 column) was redissolved in water and chromatographed on Sephadex CM-C25 (Na form, 2 × 50 cm) with water as eluant. Two orange fractions separated. The first that eluted, fraction 2-1, comprising approximately 70% of the total fraction had an absorption maximum at 456 nm. The second band, fraction 2-2, had an absorption maximum at 460 nm. The pH of both fractions was raised to 9 with ammonia before freeze-drying. Each fraction was rechromatographed as above to ensure purity. Fraction 2-1 (50 mg) has an analysis and spectroscopic properties consistent with the formula $[Co(NH_3)(H_{-2}HisGG)] \cdot 3.5H_2O$ in which the peptide is coordinated as a quinquedentate chelate via NH_2, two peptide-N^- donors, CO_2^-, and the imidazole. Anal. Calcd for $C_{10}H_{22}CoN_6O_{7.5}$: C, 29.6; H, 5.5; N, 20.7. Found: C, 29.6; H, 5.3; N, 20.6. Fraction 2-2 (20 mg) has an analysis and spectroscopic properties consistent with the formula $[Co(NH_3)_2(H_{-2}HisGG)] \cdot 3H_2O$ in which the peptide is coordinated as a quadridentate chelate via NH_2, two peptide-N^- donors, and the imidazole. Anal. Calcd for $C_{10}H_{24}CoN_7O_7$: C, 29.1; H, 5.9; N, 23.7. Found: C, 29.3; H, 5.9; N, 23.8. The reaction was repeated at pH 9.5–10 and in liquid ammonia to give a higher yield of the diammine but a lower yield of the monoammine complex.

Table I. ^1H Chemical Shifts[a] of L-His-Gly-Gly, $[Co(NH_3)(H_{-2}HisGG)]$, and $[Co(NH_3)_2(H_{-2}HisGG)]$

	chem shift					
pH	CH-1	CH_2-2	CH_2-3	CH_2-4	CH-2′	CH-5′
			Free Peptide			
9.5	3.71	3.91, 3.93	3.77	2.94	7.67	6.96
6.6	4.19	3.98, 4.00	3.78	3.20	7.95	7.14
4.3	4.39	4.01, 4.11	3.80, 3.82	3.43, 3.45	8.66	7.45
1.0	4.41	4.05, 4.12	4.06	3.45, 3.46	8.71	7.48
			$[Co(NH_3)(H_{-2}HisGG)]$[b]			
7.0	4.13	3.62, 3.72	4.31, 4.40	3.27, 3.36	7.30	7.00
			$[Co(NH_3)_2(H_{-2}HisGG)]$[c]			
7.0	4.00	3.64, 3.82	3.46, 4.40	3.19, 3.31	7.45	7.01

[a] In ppm from DSS. [b] NH_2: 5.77, 5.49. NH_3: 2.65. [c] NH_2: 5.29, 4.95. NH_3: 3.24, 3.46.

Spectroscopic Studies. A Cary 17 spectrophotometer and a Jobin Yvon Mark III dichrograph were used to measure the UV–visible and circular dichroism (CD) spectra, respectively. The ^{13}C and ^1H NMR spectra were recorded on a Bruker CXP300 instrument. Spectra were recorded with the compounds dissolved in D_2O with sodium 3-(trimethylsilyl)propane-1-sulfonate and dioxane (δ 67.4) as internal references for the ^1H and ^{13}C NMR spectra, respectively. The pH values of the solutions for the NMR studies were adjusted by addition of either concentrated perchloric acid or sodium deuteroxide solution. The electron spin resonance measurements were made with a Bruker ER200D spectrometer by using DPPH, $g = 2.0036$ as a g marker.

Results

UV–Visible and Circular Dichroism Spectra. The UV–visible and CD spectra of $[Co(NH_3)(H_{-2}HisGG)]$ are shown in Figure 2, which also contains the spectra[10] of $[Co(NH_3)_2(H_{-2}PGG)]$ and $[Co(NH_3)_2(H_{-2}AGG)]$ for comparison, where P and A refer to L-phenylalanyl and L-alanyl.

(1) Yokoyama, A.; Aiba, H.; Tanaka, H. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 112.
(2) Boggess, R. K.; Martin, R. B. *J. Inorg. Nucl. Chem.* **1975**, *37*, 1097.
(3) Agarwal, R. P.; Perrin, D. D. *J. Chem. Soc., Dalton Trans.* **1975**, 268.
(4) Agarwal, R. P.; Perrin, D. D. *J. Chem. Soc., Dalton Trans.* **1975**, 1045.
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(8) Davies, R.; Mori, M.; Sykes, A. G.; Weil, J. A. *Inorg. Synth.* **1971**, *12*, 197.
(9) Abbreviations: HHisGG is the zwitterion, HisGG the anion, and H_{-2}HisGG the ligand with both peptide groups deprotonated.
(10) Evans, E. J.; Hawkins, C. J.; Rodgers, J.; Snow, M. R. *Inorg. Chem.* **1983**, *22*, 34.

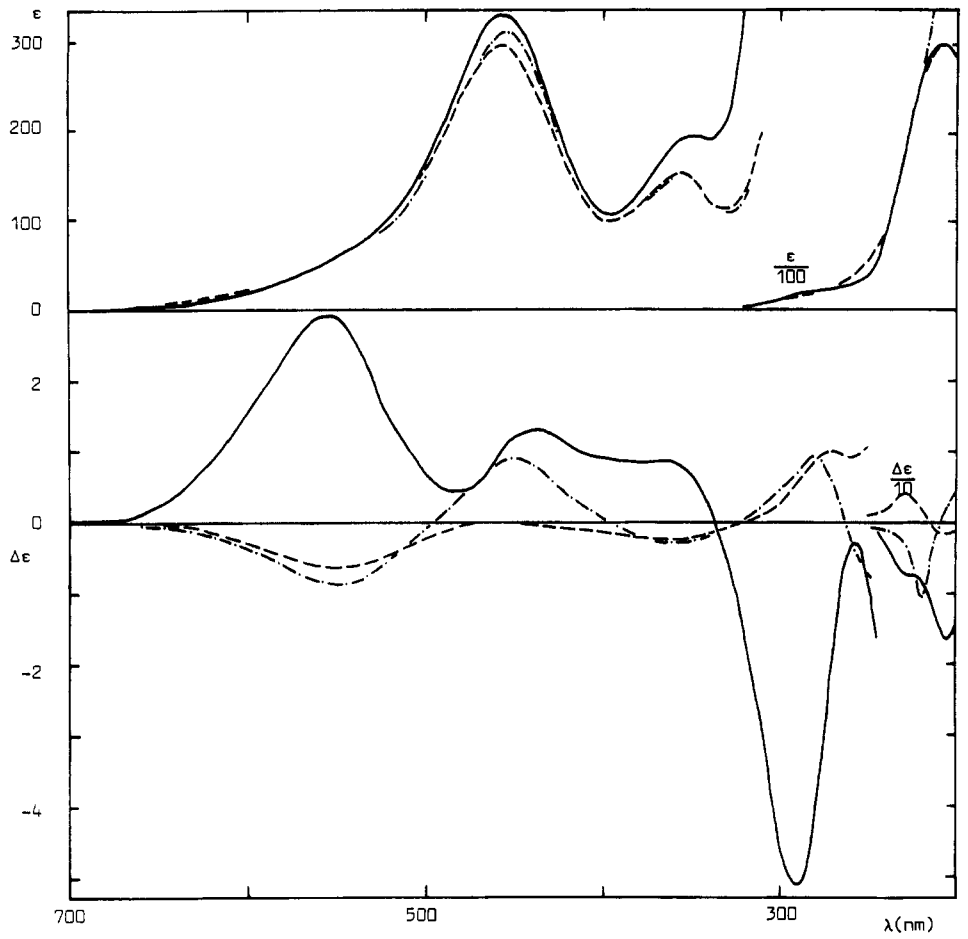

Figure 2. Absorption spectra (upper) and circular dichroism spectra (lower) of aqueous solutions of $[Co(NH_3)(H_{-2}HisGG)]$ (–), $[Co(NH_3)_2(H_{-2}AGG)]$ (- - -), and $[Co(NH_3)_2(H_{-2}PGG)]$ (–·–).

Table II. Proton–Proton Coupling Constants[a] for L-Histidylglycylglycine, $[Co(NH_3)(H_{-2}HisGG)]$, and $[Co(NH_3)_2(H_{-2}HisGG)]$

pH	$^3J_{CH\text{-}1,CH_2\text{-}4}$	$^2J_{CH_2\text{-}2}$	$^2J_{CH_2\text{-}3}$	$^2J_{CH_2\text{-}4}$	$^4J_{CH\text{-}4,CH\text{-}5'}$	$^4J_{CH\text{-}2',CH\text{-}5'}$
			Free Peptide			
9.5	6.6	−17.0				1.1
6.6	6.6	−17.1				1.1
4.3	5.9, 6.7[b]	−16.8	−17.3	−15.8		1.2
1.0	6.5, 6.3[b]	−16.9		−16.2		1.4
			$[Co(NH_3)(H_{-2}HisGG)]$[c]			
7.0	4.9, 2.3[b]	−17.8	−19.8	−17.4	1.0, 0.6[b]	0.9
			$[Co(NH_3)_2(H_{-2}HisGG)]$[d]			
7.0	2.4, 4.3[b]	−18.3	−17.3	−17.3	0.2, 0.7[b]	0.5

[a] In Hz. [b] J value for the more shielded CH_2-4 proton. [c] $^2J_{NH_2} = -10.7$; $^3J_{NH,CH\text{-}1} = 5.1$ (higher shielded NH); $^5J_{CH\text{-}1,CH_2\text{-}2}$ and $^5J_{CH_2\text{-}2,CH_2\text{-}3} < 1.0$. [d] Coupling observed between NH_2 and CH_2-4 sufficient to broaden resonances markedly; $^5J_{CH\text{-}1,CH_2\text{-}2} < 1.0$.

The spectra for $[Co(NH_3)_2(H_{-2}HisGG)]$ are given in Figure 3 and compared with spectra[11] for $[Co(NH_3)_3(H_{-2}PGG)]$ and $[Co(NH_3)_3(H_{-2}AGG)]$.

^{1}H NMR Spectra. Chemical shift and coupling constant data for the free peptide, $[Co(NH_3)(H_{-2}HisGG)]$, and $[Co(NH_3)_2(H_{-2}HisGG)]$ are presented in Tables I and II. The spectra before and after N–H deuteration are given in Figures 4 and 5 for the two complexes. Assignments were made on the basis of chemical shift changes for the free peptide with pH, by proton decoupling experiments, and by reference to previous assignments for histidine, peptides, and peptide complexes.[12-14] The vicinal coupling constants for the CH-1 and CH_2-4 protons have been analyzed in terms of the rotamers about the CC bond (Figure 6) for the free peptide at different pH values and for the above two complexes by the method of Feeney,[15] and the results are given in Table III. Yokoyama and co-workers have reported the acid dissociation constants for the CO_2H, the imidazole, and the NH_3^+ groups of L-His-Gly-Gly to be 3.17, 5.52, and 7.62, respectively. At pH 9.5 the peptide is in the anionic form, at pH 6.6 the peptide is in the zwitterionic form with the NH_2 group largely protonated, at pH 4.3 the peptide has both the NH_2 and the imidazole group

Table III. Side-Group Rotamer Populations Based on the Method of Feeney[a] for L-Histidylglycylglycine, $[Co(NH_3)(H_{-2}HisGG)]$, and $[Co(NH_3)_2(H_{-2}HisGG)]$

pH	J_{AX}, Hz	J_{BX}, Hz	n_I	n_{II}	n_{III}
		Free Peptide			
9.5	6.6	6.6	0.4	0.4	0.2
6.6	6.6	6.6	0.4	0.4	0.2
4.3	6.7	5.9	0.3	0.4	0.3
	5.9	6.7	0.4	0.3	0.3
1.0	6.3	6.5	0.4	0.3	0.3
	6.5	6.3	0.3	0.4	0.3
		$[Co(NH_3)(H_{-2}HisGG)]$			
7.0	2.3	4.9	0.0	−0.1	1.1
		$[Co(NH_3)_2(H_{-2}HisGG)]$			
7.0	2.4	4.3	−0.1	0.0	1.1

[a] Reference 15.

(11) Enright, M. P. Honours Thesis, University of Queensland, 1982.
(12) Evans, E. J.; Grice, J. E.; Hawkins, C. J.; Heard, M. R. *Inorg. Chem.* **1980**, *19*, 3496.
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(15) Feeney, J. *J. Magn. Reson.* **1976**, *21*, 473.

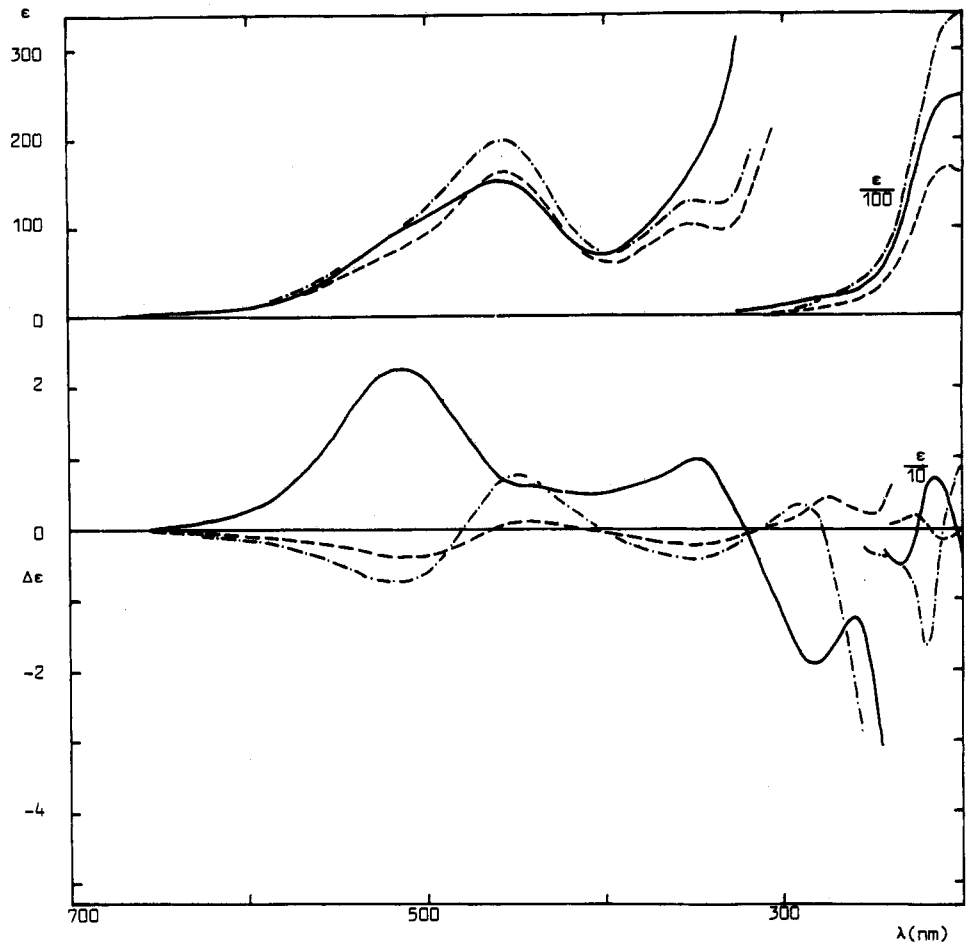

Figure 3. Absorption spectra (upper) and circular dichroism spectra (lower) of aqueous solutions of $[Co(NH_3)_2(H_{-2}HisGG)]$ (–), $[Co(NH_3)_3(H_{-2}AGG)]$ (- - -), and $[Co(NH_3)_3(H_{-2}PGG)]$ (–·–).

Table IV. ^{13}C Chemical Shifts[a] of L-Histidylglycylglycine, $[Co(NH_3)(H_{-2}HisGG)]$, and $[Co(NH_3)_2(H_{-2}HisGG)]$

	chem shift									
pH	CH-1	CO-1	CH_2-2	CO-2	CH_2-3	CO-3	CH_2-4	CH-2′	C-4′	CH-5′
					Free Peptide					
9.5	55.70	178.14	43.41	172.00	44.12	177.21	32.65	137.02	133.86	118.36
6.6	54.53	171.68	43.41	172.90	44.15	177.24	29.99	136.81	131.49	118.12
4.3	53.18	169.78	43.24	171.53	44.17	177.09	27.21	135.67	126.72	119.50
1.0	53.13	169.55	43.18	172.18	42.13	174.11	26.97	135.56	126.43	119.64
					$[Co(NH_3)(H_{-2}HisGG)]$					
7.0	56.90	179.72	51.49	179.11	48.88	186.80	27.33	136.78	133.13	116.69
					$[Co(NH_3)_2(H_{-2}HisGG)]$					
7.0	56.96	178.82	50.02	181.89	49.67	179.40	27.09	137.75	133.10	116.89

[a] In ppm from Me_4Si.

Table V. ^{13}C NMR Chemical Shift Differences[a] on Protonation and Chelation

	chem shift diff									
reacn	CH-1	CO-1	CH_2-2	CO-2	CH_2-3	CO-3	CH_2-4	CH-5′	C-4′	CH-2′
$NH_2 \rightarrow NH_3^+$ [b]	−1.17	−6.46	0.00	+0.90	+0.03	+0.03	−2.66	−0.24	−1.37	−0.21
$Im \rightarrow ImH^+$ [c]	−1.35	−1.90	−0.17	−1.37	+0.02	−0.15	−2.78	+1.38	−4.77	−1.14
$CO_2^- \rightarrow CO_2H$ [d]	−0.05	−0.23	−0.06	+0.65	−2.04	−2.98	−0.24	+0.14	−0.29	−0.11
$L^- \rightarrow Co(NH_3)L$	+1.20	+1.58	+8.08	+7.11	+4.76	+9.59	−5.32	−1.67	−0.73	−0.24
$L^- \rightarrow Co(NH_3)_2L$	+1.26	+0.68	+6.61	+9.89	+5.55	+2.19	−5.56	−1.47	−0.76	+0.73

[a] In ppm. [b] δ(pH 6.6) − δ(pH 9.5). [c] δ(pH 4.3) − δ(pH 6.6). [d] δ(pH 1.0) − δ(pH 4.3).

protonated, and at pH 1.0 all three groups are protonated.

^{13}C NMR Spectra. Chemical shift data for the free peptide in its various ionic forms and for the two complexes are given in Table IV. The ^{13}C chemical shifts for L-Ala-Gly-Gly, L-Phe-Gly-Gly, and L-Tyr-Gly-Gly in their various ionic forms have been analyzed in terms of the changes in chemical shifts on protonation at the NH_2 terminus and the carboxylate group.[12] On the basis of that work and the assignments of Christl and Roberts,[16] assignments for the free peptide were made for the peptide backbone, CH-1, CH_2-2, CH_2-3, CO-1, CO-2, and CO-3. The imidazole ring carbons of histidine have been assigned by Reynolds and

(16) Christl, M.; Roberts, J. D. *J. Am. Chem. Soc.* **1972**, *94*, 4565.

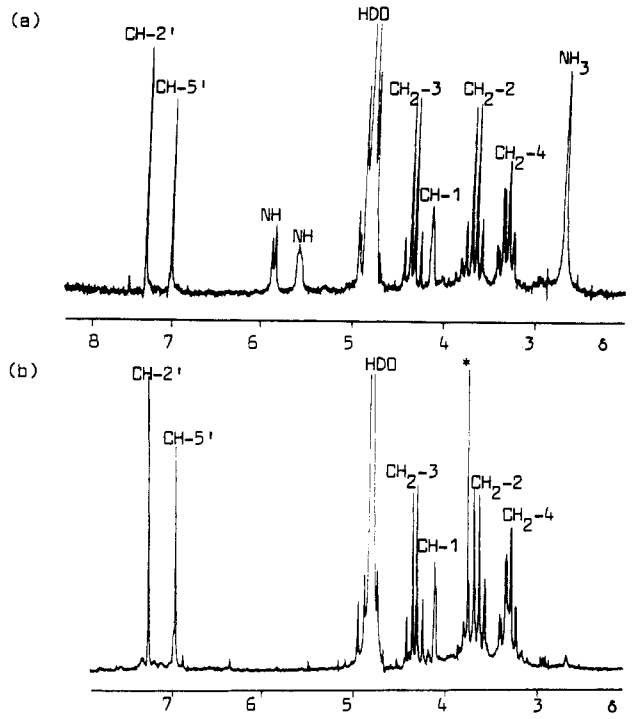

Figure 4. 300-MHz ^1H NMR spectrum of $[Co(NH_3)(H_{-2}HisGG)]$ in D_2O (a) before and (b) after ^2H exchange. An asterisk denotes dioxane in the latter spectrum as ^{13}C reference.

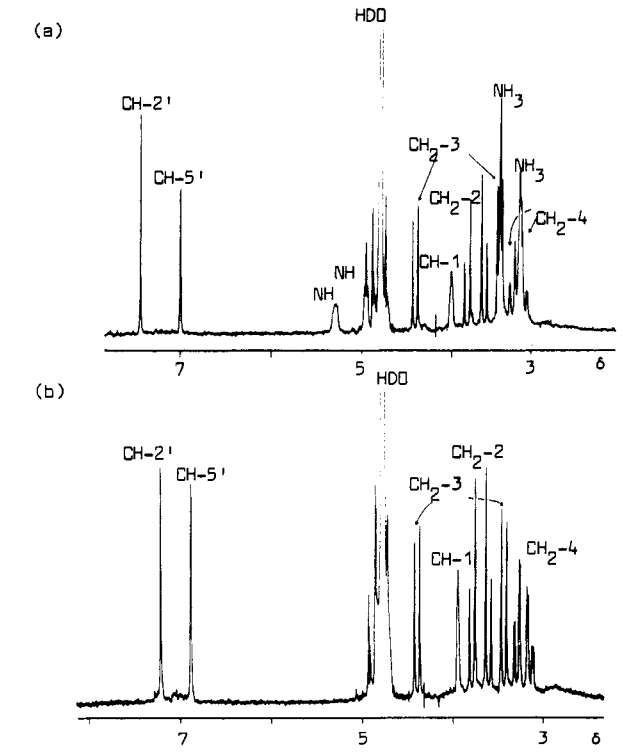

Figure 5. 300-MHz ^1H NMR spectrum of $[Co(NH_3)_2(H_{-2}HisGG)]$ in D_2O (a) before, and (b) after ^2H exchange. In the latter, addition of base to promote deuteration has resulted in partial deprotonation of the imidazole ring and associated shifts to lower shielding for the CH-2′ and CH-5′ resonances.

co-workers,[17] and their assignments are used here for L-His-Gly-Gly. Protonation of the imidazole ring shifts C-4′ 4.77 ppm to higher shielding on lowering of the pH from 6.6 to 4.3. A similar shift of 4.8 ppm has been reported by Reynolds for the

(17) Reynolds, W. F.; Peat, I. R., Freedman, M. H.; Lyeria, J. R. *J. Am. Chem. Soc.* **1973**, *95*, 328.

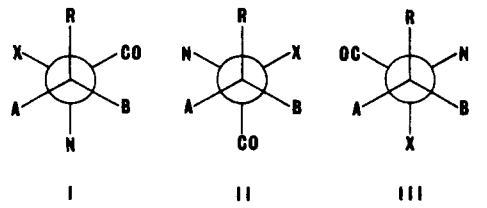

Figure 6. Side-group rotamers where A, B, and X are protons.

amino acid histidine.[17] The other ring carbons, CH-5′ and CH-2′ shift by much smaller amounts for both L-His-Gly-Gly and L-histidine: L-His-Gly-Gly, +1.38 and −1.14; L-histidine, +0.7 and −2.4, respectively. A summary of the shifts of the ^{13}C resonances on protonation of L-His-Gly-Gly is given in Table V.

Coordination of the above alanyl, phenylalanyl, and tyrosyl tripeptide anions as quadridentates to cobalt(III) causes the backbone ^{13}C resonances to shift to lower shielding: CH-1, +2.7 to +4.5 ppm; CO-1, +0.6 to +1.5 ppm; CH_2-2, +8.4 to +8.5 ppm; CO-2, +6.8 to +6.9 ppm; CH_2-3, +4.6 ppm; CO-3, +9.7 to +9.8 ppm.[12] The assignments in Table IV for $[Co(NH_3)(H_{-2}HisGG)]$ give similar shifts on chelation of the peptide (Table V).

The carbonyl resonances at δ 179.72 and 179.11 are assigned to CO-1 and CO-2, respectively, as this assignment gives a closer agreement with the shifts observed for the other peptides on coordination than the reverse assignment. However, the small separation of the two resonances prevented an unambiguous assignment. The assignments of CH_2-4 and CH-5′ are unambiguous. Off-resonance decoupling was used to distinguish the resonances for C-4′ and CH-2′ at δ 133.13 and 136.78, respectively.

The proposed structure for $[Co(NH_3)_2(H_{-2}HisGG)]$ (Figure 1) has coordination via the NH_2 and two peptide-N^- donors. The ^{13}C resonances for triammine complexes with the same coordination for the peptides Gly-Gly-Gly, L-Ala-Gly-Gly, and L-Phe-Gly-Gly have been assigned, and the effect of this type of coordination by the peptide anions on the chemical shifts has been determined.[11] This data provided the basis for the assignments in Table IV. The assignments for CH_2-3 and CO-3 in the uncoordinated C-terminus group were confirmed by observing the change of the chemical shifts with a change of pH from 7 to 1 for these four complexes.

Acid Dissociation Constants. The pK_a values determined for the pyrrole nitrogen (NH-1) in $[Co(NH_3)(H_{-2}HisGG)]$ and $[Co(NH_3)_2(H_{-2}HisGG)]$ at 298K are 10.73 ± 0.04 and 10.69 ± 0.04, respectively. Potentiometric titration of $[Co(NH_3)_2(H_{-2}HisGG)]$ with acid at 298 K gave a pK_a value of 4.34 ± 0.04 for the free carboxylate group.

Electron Spin Resonance. Fraction 1 from the reaction of L-His-Gly-Gly with "peroxo dimer" in the solid at 77 K has a single isotropic ESR signal with g = 2.01 with some incipient fine structure. Frozen aqueous solutions of the complex gave a similar spectrum. When the solution was bubbled with nitrogen for 30 min and the spectrum remeasured, the intensity of the signal at g = 2.01 was considerably reduced and a new signal at g = 4.29 appeared. Bubbling the solution with air for several hours did not restore the g = 2.01 signal, but the g = 4.29 signal disappeared.

Discussion

The close similarity of the absorption spectrum of $[Co(NH_3)(H_{-2}HisGG)]$ (Figure 2) to the spectra[10] reported for diamminecobalt(III) complexes of various tripeptides strongly suggests that the coordination modes are similar. These latter complexes have the tripeptide bound as a quadridentate with the terminal amino group, two deprotonated peptide nitrogens, and the carboxylate group coordinated. Microanalysis data are consistent with only one ammonia group in the former complex. The similarity in the absorption spectra is explained by the coordination of the side-chain imidazole trans to the ammonia. This would not greatly affect the position and splitting of the $^1A_{1g} \rightarrow {}^1T_{1g}$ band as imidazole and ammonia have similar values for the spectrochemical parameter Δ. For example, the bis(imidazole) complex $[Co(ImH)_2(H_{-2}GGG)]$ in water has an absorption maximum at 452 nm compared to 456 nm for the related di-

ammine complex.[18] On the other hand, the maximum of the $^1A_{1g} \rightarrow {}^1T_{1g}$ absorption band for $[Co(OH_2)_2(H_{-2}GGG)]$ absorbs at 438 nm,[18] ruling out water as the potential sixth donor group.

A comparison of the UV–visible absorption spectra of $[Co(NH_3)_5(ImH)]^{3+}$ and $[Co(NH_3)_6]^{3+}$ reveals that for the imidazole complex the charge-transfer band extends to much higher wavelength, almost obscuring the $^1A_{1g} \rightarrow {}^1T_{2g}$ band at approximately 330 nm.[19] In the spectrum of $[Co(NH_3)(H_{-2}HisGG)]$ the charge-transfer bands extend to higher wavelengths than for the diammine peptide complexes and the $^1A_{1g} \rightarrow {}^1T_{2g}$ band at 350 nm is partially obscured, consistent with the coordination of the imidazole group. The very intense band at 207 nm (ϵ 2.97 × 10^4) has a shoulder at approximately 270 nm that could result from this coordination as it is not discernible in the spectra of the diammine complexes.

The CD spectrum of $[Co(NH_3)(H_{-2}HisGG)]$ has a large positive Cotton effect at 554 nm and a smaller positive band at 438 nm both arising from the $^1A_{1g} \rightarrow {}^1T_{1g}$ (O_h) transition. The spectrum also has a large negative band ($\Delta\epsilon$ −5.1) at 290 nm in the vicinity of the absorption shoulder attributed above to a charge-transfer transition involving the imidazole. The CD spectrum shows two other bands at 229 ($\Delta\epsilon$ −7.6) and 205 nm ($\Delta\epsilon$ −16.7). Comparison of this spectrum with those of the diamminecobalt(III) complexes of the N-substituted tripeptides shown in Figure 2 shows marked differences. The CD bands at approximately 290 and 554 nm for the histidyl complex are opposite in sign to those at similar positions for the other *N*-peptides and larger in size, while the band at 438 nm for the histidyl complex has the same positive sign as the corresponding bands for the L-phenylalanyl peptide complexes but is shifted 12 nm to lower wavelength. The total rotational strength for the d–d transitions for the L-histidyl complex is positive while for the diamminecobalt(III) complexes of the N-terminal tripeptides with the L configuration it is negative.

The rotational strength induced in the d–d transitions is derived from the vicincal effect of the asymmetric center and from any chiral puckering of the chelate ring system. Obviously coordination of the side-chain imidazole would exert a strong influence on the CD spectrum through both these mechanisms and would appear to be the explanation for the greatly increased rotational strength and change in sign of both the charge-transfer and d–d transitions.

Crystal structure studies of $[Co(NH_3)_2(H_{-2}AGG)]$ have revealed marked nonplanarity in the N-terminal chelate ring with the methyl group occupying an equatorial orientation and the chelate ring possessing the δ conformation.[10] From Dreiding models it can be seen that coordination of the side-chain imidazole in $[Co(NH_3)(H_{-2}HisGG)]$ will increase the degree of puckering in the N-terminal chelate ring and possibly in the middle and C-terminal chelate rings as well. It enforces the λ conformation in the N-terminal chelate ring. Proton NMR studies of the complex, which will be discussed later, have shown that the electrons from the peptide groupings are delocalized around the chelate rings. Twisting of this delocalized system as a result of imidazole coordination would lead to the introduction of rotational strength in the peptide transitions and also in the d–d transitions via the charge-transfer transitions.

The preference for conformations of opposite chirality for the alanyl and histidyl chelates and the associated enantiomeric twisting of the "delocalized" backbone would enforce rotational strength of opposite sign in some d–d and charge-transfer transitions. The Cotton effects for these two complexes do have opposite signs for the low-energy component of the T_{1g} band that transforms as a rotation about the NH_2–N^- axis,[10] for the T_{2g} band, and for the charge-transfer bands in the 270–290- and 230-nm regions. For the phenylalanyl peptide complex the phenyl group sits above the peptide ring in a position similar to that of the imidazole group for at least 50% of the time without the enforcement of the λ conformation for the N-terminus chelate ring.[12] In agreement with the above interpretation this complex has the same signs as the alanyl peptide complex except in the 220–230-nm region.[10]

The similarity in shape and position of the $^1A_{1g} \rightarrow {}^1T_{1g}$ band for $[Co(NH_3)_2(H_{-2}HisGG)]$ and the triammine peptide complexes (Figure 3) is striking and suggests the same mode of coordination, terminal NH_2 and two deprotonated peptide nitrogens with the terminal carboxylate group free and three amine ligands bound meridionally. However, the $^1A_{1g} \rightarrow {}^1T_{2g}$ band observed at approximately 350 nm for the triammine complexes is obscured in the spectrum of the histidyl complex by the charge-transfer band at approximately 280 nm. This charge-transfer band, which is not discernible in the spectra of the triammine complexes, is probably associated with the coordination of the side-chain imidazole, as discussed above for the monoammine complex.

Comparison of the CD spectrum of $[Co(NH_3)_2(H_{-2}HisGG)]$ with the CD spectra of the cobalt(III) triammine complexes of L-Ala-Gly-Gly and L-Phe-Gly-Gly (Figure 3) reveals differences similar to the differences noted above for $[Co(NH_3)(H_{-2}HisGG)]$ and the cobalt(III) diammine complexes. Nearly all bands in the spectra of the diammine L-His-Gly-Gly complex show large increases in rotational strength and most are opposite in sign to the corresponding bands for the triammine complexes. As observed for the monoammine L-His-Gly-Gly complex, the charge-transfer band at 284 nm is negative while the triammine L-Ala-Gly-Gly and L-Phe-Gly-Gly both have positive bands with lower rotational strength in this region. As for the monoammine L-His-Gly-Gly complex, the total rotational strength of the d–d bands of $[Co(NH_3)_2(H_{-2}HisGG)]$ is positive while for the triammine complexes it is negative. The differences are once again ascribed to the coordination of the imidazole side-group in a position apical to the peptide coordination (Figure 1).

The ^1H NMR spectra of the free tripeptide in its anionic, zwitterionic, and cationic forms show the same trends on protonation that have been observed for other tripeptides.[12] Protonation at the terminal NH_2 group causes the largest shift in the aliphatic resonances at the triplet that appears at δ 4.19 for the zwitterionic form of the free peptide. This resonance integrates for one proton and is assigned as CH-1. A shift of 0.26 ppm to lower shielding is also seen for the CH_2-4 doublet ($^3J_{CH\text{-}1,CH_2\text{-}4}$ = 6.6 Hz) which is centered at 2.94 ppm in the anion. Of the aliphatic peaks these two resonances also show the largest shifts on protonation of the imidazole group, after which the CH_2-4 protons become nondegenerate ($\Delta\delta_{AB}$ = 0.02 at pH 4.3). The resonance for CH-1 is not resolved completely into four lines at the lower pH values, but the middle peak of the triplet is broadened. An AB pattern is observed for CH_2-2 at all four pH values, and the chemical shifts of these two protons do not move markedly on protonation of any of the ionizable groups. Protonation of the carboxylate group causes a significant shift only for CH_2-3, which is observed as an AB pattern (δ 3.80, 3.82) at pH 4.3 and as a singlet, δ 4.06, at pH 1.0. The imidazole ring protons, CH-2′ and CH-5′, move 0.71 and 0.31 ppm to lower shielding, respectively, on protonation of the imidazole N-3. The corresponding shifts for CH-2′ and CH-5′ for Gly-Gly-L-His are +0.71 and +0.28 ppm, respectively.[20]

For all forms of the peptide, coupling is observed between the protons attached to C-5′ and C-2′, with ^4J values between 1.1 and 1.4 Hz being observed. The proton attached to C-5′ also couples to the CH_2-4 protons, resulting in a complicated multiplet for this proton. Although this coupling is too small to be measured accurately, it is of the order of 0.5 Hz and is sufficient to broaden the CH_2-4 resonance. At the pH values 4.3 and 1.0 where the CH_2-4 protons are nondegenerate, the chemical shift difference is too small to allow determination of which proton is the more strongly coupled.

The vicinal coupling constants for the CH-1 and CH_2-4 protons have been analyzed in terms of the rotamers about the CC bond for the free peptide in its various ionic forms by using the method of Feeney.[15] The results in Table III show that, irrespective of the assignment of the A and B CH_2-4 protons, the rotamer

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(20) Hawkins, C. J.; Martin, J. *Inorg. Chem.* **1983**, *22*, 3879.

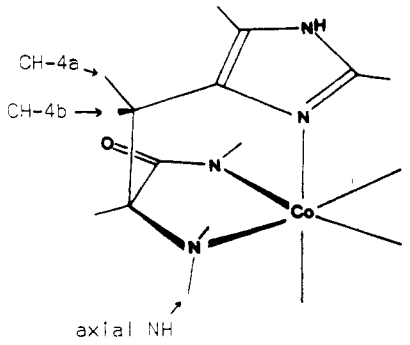

Figure 7. "W" relationship between CH-4a and the axial NH.

preferences are distributed fairly evenly between the three rotamers, especially at the lower pH values.

The ^{1}H NMR spectra of $[Co(NH_3)(H_{-2}HisGG)]$ before and after deuteration are shown in Figure 4. The broad resonance integrating for three protons at δ 2.65 disappears after deuteration and is consequently assigned to the NH_3 group. The doublet observed at δ 5.77 and the multiplet at δ 5.49 also disappear on deuteration of the complex and are assigned as the terminal NH_2 resonances. The observation of an NH resonance as a doublet is unusual as both NH groups are generally coupled to the CH-1 proton as well as to each other. For example in $[Co(NH_3)_2(H_{-2}AGG)]$ the two NH resonances have vicinal coupling constants $^3J_{NH,CH\text{-}1}$ = 9.9 and 6.5 Hz as well as a geminal coupling constant $^2J_{NH_2}$ = −10.0 Hz. However, in the present case only a geminal coupling constant of −10.7 Hz is observed for the NH resonance at lower shielding.

For $[Co(NH_3)_2(H_{-2}AGG)]$, the marked nonplanarity in the N-terminal chelate ring[10] will give rise to dihedral angles consistent with the above NH_2CH vicinal coupling constants. For $[Co(NH_3)(H_{-2}HisGG)]$ the $^3J_{NH,CH\text{-}1}$ value is 5.1 Hz for the higher shielded NH and is very small for the NH resonance to lower shielding. This is consistent with a structure where the N-terminal chelate ring is puckered markedly in the opposite direction to the puckering observed for $[Co(NH_3)_2(H_{-2}AGG)$, which is necessary if the imidazole ring is coordinated in the apical position.

The resonance at δ 5.49 (axial NH) is further complicated by a small coupling to the higher shielded CH_2-4 proton at δ 3.36. Although this is too small to be measured accurately (~0.5 Hz), it is sufficient to broaden both resonances, and a noticeable sharpening in the resonance for the CH_2-4 proton to higher shielding was observed on deuteration or on decoupling at the higher shielded NH resonance. Long-range couplings (0.4–2 Hz) across four bonds in saturated systems are well documented and generally confined to a planar zigzag or W configuration.[21] Thus the resonance of the CH_2-4 proton at higher shielding may be assigned as the proton that is "W" to the axial NH and in later discussion will be referred to as CH-4a (Figure 7).

Both CH_2-2 and CH_2-3 are observed as AB patterns, with CH_2-3 showing large shifts of 0.54 and 0.63 ppm to lower shielding on coordination. Although CH_2-2 gives rise to an AB pattern for the L-Ala-Gly-Gly, L-Phe-Gly-Gly, and L-Tyr-Gly-Gly diammine complexes, the resonance for CH_2-3 in these complexes is a singlet.[12] For $[Co(NH_3)(H_{-2}HisGG)]$, $\Delta\delta_{AB}$ is 0.09 ppm for CH_2-3, a difference that could possibly be due to the proximity of a bound imidazole side chain.

In the spectrum of the undeuterated complex, the resonance for CH-1 appears as a broad unresolved peak at δ 4.13 due to coupling with the axial NH. Upon deuteration of the complex and removal of this coupling, a partially resolved multiplet is observed for CH-1. This may be further resolved by decoupling at CH_2-2, which also markedly sharpens the resonance for CH_2-3. ^5J coupling (~1 Hz) between the CH_2-2 protons and the protons on CH-1 and CH_2-3 has been observed previously[12] and is indicative of the electronic delocalization around the peptide backbone in the complex. Although it is sufficient to broaden the CH-1, CH_2-2, and CH_2-3 resonances of $[Co(NH_3)(H_{-2}HisGG)]$, the ^5J coupling around the chelate rings appears to be less than 1 Hz and could not be resolved.

Coupling between the protons attached to C-2′ and C-5′ was observed for the free peptide and is also present in the complex ($^4J_{CH\text{-}2',CH\text{-}5'}$ = 0.9 Hz). Small couplings are also observed between the proton attached to C-5′ and the CH_2-4 protons of the complex. The CH_2-4 proton that is "W" to the axial NH, CH-4a, couples to CH-5′ with J = 0.6 Hz while CH-4b couples to CH-5′ with J = 1.0 Hz. Allylic couplings across one double and three single bonds are negative in sign with values of 0 to −3 Hz, are zero at θ = 0 and 180°, and are at a maximum (largest negative value) at θ = 90°.[21,22] In between these values theory predicts that the function should show a $\cos^2\theta$ dependence. This suggests that the CH-4a proton is tilted closer to the plane of the imidazole ring than CH-4b.

Regardless of the assignments of the CH_2-4 protons, rotamer III (Figure 6), which is the rotamer necessary for side-chain imidazole coordination, is found to be exclusively populated. With the side chain in this orientation, CH-4a may be unambiguously assigned as the A proton by virtue of its "W" coupling to the axial NH proton. Thus J_{AX} is 2.3 Hz, and a unique solution, n_{III} = 1.1, is obtained. The exclusive orientation of the side chain in this position is by no means proof of imidazole coordination, but it does lend strong support to the argument for this proposal.

In the ^{1}H NMR spectra of $[Co(NH_3)_2(H_{-2}HisGG)]$ (Figure 5), the two broad peaks at δ 3.24 and 3.46 disappear on deuteration of the complex and so are assigned as NH_3 resonances. Integration by weighing gave an area approximately equivalent to three protons for each peak. The absence of a third peak attributable to an NH_3 group under conditions where deuteration is very slow lends strong support to the proposal that this "triammine-like" complex does in fact contain two bound NH_3's and a bound imidazole side chain. Enright reports chemical shifts of δ 3.44, 3.43, and 3.32 for the NH_3 bound in the plane of the chelate rings in the triammine cobalt(III) complexes of Gly-Gly-Gly, L-Ala-Gly-Gly, and L-Phe-Gly-Gly, respectively.[11] Thus, the resonance at δ 3.46 in the spectrum of $[Co(NH_3)_2(H_{-2}HisGG)]$ may be similarly assigned as the "equatorial" NH_3. The ammine groups bound trans to each other in the apical positions of the triammine complexes both resonate at δ 2.7–2.8 except for the L-Phe-Gly-Gly complex where interaction with the phenyl group moves one of the apical NH_3 resonances to δ 1.98.[11] The remaining ammine resonance of $[Co(NH_3)_2(H_{-2}HisGG)]$ at δ 3.24 must be due to an NH_3 bound in an apical position, and its less shielded chemical shift compared to those of the apical NH_3's of the triammine complexes is probably a result of being trans to an imidazole group instead of an ammonia. In the monoammine L-His-Gly-Gly complex, the resonance for the apical NH_3 group was shifted 0.2 ppm to lower shielding compared to those to the diammine complexes. Compared to the equivalent resonances for the triammine complexes a shift of 0.4 ppm to lower shielding is observed here for the apical NH_3 of $[Co(NH_3)_2(H_{-2}HisGG)]$.

The NH resonances at δ 5.29 and 4.95 (d, ^2J ~ −10 Hz) are both observed at higher shielding than the NH resonances of the monoammine L-His-Gly-Gly complex, the largest shift occurring for the "doublet" resonance, which moves from δ 5.77 to 4.95. The triammine complexes reported by Enright also showed large shifts in the NH resonances compared to those for the corresponding diammine complexes.[11,12] Obviously, whether the fourth coordination position in the chelate ring plane is occupied by the carboxylate group or an ammine group will have a strong influence on the NH_2 group because of its close proximity.

The NH resonance at δ 4.95 is complicated by the superposition of a HDO spike, but if, in fact, it is only a doublet and shows no coupling to CH-1, it suggests a similar situation as described for $[Co(NH_3)(H_{-2}HisGG)]$ with CH-1 pulled up out of the plane of the chelate rings by coordination of the imidazole ring in the apical position. The other NH shows a coupling of about 5 Hz to CH-1,

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(22) Sternhill, S. *Q. Rev., Chem. Soc.* **1969**, *23*, 236.

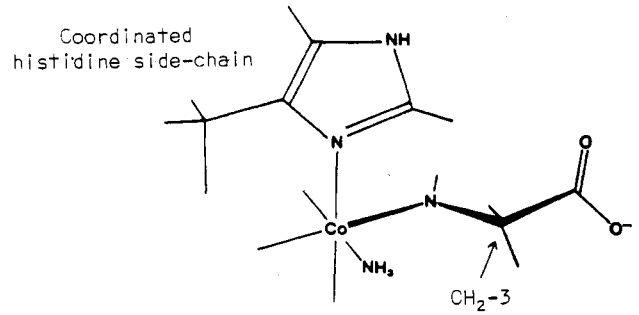

Figure 8. Probable orientation of CH_2-3 protons in $[Co(NH_3)_2(H_{-2}HisGG)]$.

similar to that observed in the monoammine complex, strongly suggesting that the NH–CH dihedral angles are similar for both complexes.

As well as coupling to CH-1 and to the equatorial NH ($^2J \sim -10$ Hz), the axial NH shows a small coupling to the lower shielded CH_2-4 proton at δ 3.31. Although this coupling could not be resolved, it is sufficient to broaden markedly both resonances and a noticeable sharpening in the resonance for the CH_2-4 proton to lower shielding was observed on deuteration or on decoupling at the lower shielded NH resonance. This ^4J coupling was also observed for the monoammine complex and allows assignment of the CH_2-4 resonance to lower shielding as the proton that is "W" to the axial NH, and thus designated as CH-4a. The observation of ^4J coupling to the NH resonance at δ 5.29 confirms its assignment as the axial NH because "W" coupling between the equatorial NH and either of the CH_2-4 protons is not possible.

In the monoammine complex, CH-4a is the higher shielded of the two CH_2-4 protons, δ 3.27, and couples to CH-1 with $^3J_{CH\text{-}1,CH\text{-}4a} = 2.3$ Hz. In $[Co(NH_3)_2(H_{-2}HisGG)]$, the chemical shifts of the CH_2-4 protons are reversed, and it is the proton to lower shielding, δ 3.31, that is now CH-4a. The observation that it is the lower shielded CH_2-4 proton that shows a $^3J_{CH\text{-}1,CH\text{-}4a}$ coupling of 2.4 Hz confirms this assignment. A very small change in the chemical shift of this proton of 0.04 ppm to lower shielding is observed for the L-His-Gly-Gly diammine complex compared to the monoammine complex. In contrast, the chemical shift of the other CH_2-4 proton, CH-4b, moves from δ 3.36 in the monoammine complex to δ 3.19 in the diammine complex. This much larger difference is a consequence of the different shielding effects of a Co–O(carboxylate) bond and a Co–N(ammonia) bond and the closer proximity of CH-4b to this coordination position (Figure 7).

In the spectrum of the complex before deuteration, the resonance for CH-1 is a broad unresolved peak. Even when the coupling from the axial NH is removed by deuteration, it remains unresolved. Resolution into four lines was achieved by irradiation at the resonance frequency of the CH_2-2 proton to lower shielding, thus removing the small ^5J coupling around the chelate rings. Unexpectedly the CH-1 proton appears to be more strongly coupled to the lower shielded CH_2-2 proton with $^5J_{CH\text{-}1,CH\text{-}2} \sim 0.8$ Hz. Coupling to the other CH_2-2 proton appeared to be much smaller. As the carboxylate group is not coordinated in this complex, ^5J coupling between CH_2-2 and CH_2-3 is not expected and is not observed.

The resonances for both CH_2-2 and CH_2-3 appear as AB patterns. One of the most interesting features of the spectrum is the very large difference in chemical shift between the protons attached to C-3: $\Delta\delta_{AB} = 0.94$ ppm. In the cobalt(III) triammine complexes of L-Ala-Gly-Gly and L-Phe-Gly-Gly, $\Delta\delta_{AB}$ for CH_2-3 has values of 0.10 and 0.04 ppm, respectively.[11] The large difference observed for $[Co(NH_3)_2(H_{-2}HisGG)]$ is most easily explained by a structure where the imidazole group is coordinated, bringing it in close proximity to CH_2-3. The carboxylate group is not coordinated and in its favored orientation will almost certainly be directed away from the imidazole ring, and the CH_2-3 protons will then point toward the ring (Figure 8). Inspection of Dreiding models reveals that, regardless of their exact orientation, both protons will be deshielded by the aromatic group. As this effect falls off rapidly with distance, the proton closer to the ring will be the more strongly deshielded. If one considers a structure where the CH_2-3 protons are orientated as in Figure 8, a rough estimate of the size of the long-range anisotropic effect of an aromatic ring may be gained by utilizing the data of Johnson and Bovey for a benzene ring.[23] If a benzene ring occupied the same position in the complex as the imidazole ring, a chemical shift difference of about 0.5 ppm would be induced in the CH_2-3 protons. As will be discussed below, the ^{13}C chemical shift of CH_2-3 is deshielded more than the corresponding resonances for the triammine complexes, also consistent with an apically bound and thus very close imidazole group.

The coupling between CH-1 and CH_2-4 is consistent with rotamer III (Figure 6), the conformation necessary for imidazole coordination (Table III). Both CH_2-4 protons couple to CH-5′ with both couplings somewhat smaller than those found in the monoammine complex. The coupling between CH-2′ and CH-5′ is also reduced, with $^4J_{CH\text{-}2',CH\text{-}5'} = 0.5$ Hz compared to 0.9 Hz for the monoammine complex. The chemical shift of CH-5′, δ 7.01, is almost identical with that observed for CH-5′ for $[Co(NH_3)(H_{-2}HisGG)]$, δ 7.00. A larger difference is observed in the chemical shift of CH-2′: diammine, δ 7.45; monoammine, δ 7.30. This is easily explained by structures with the imidazole side chain coordinated because CH-2′ then projects out toward the third amino acid residue. Whether the carboxylate group is bound or whether this coordination position is occupied by ammonia will affect the chemical shift of the CH-2′ proton. In structures where the imidazole group is not coordinated, CH-2′ is much further away from this part of the molecule, and reasons for the difference in its chemical shift between the two complexes are not as readily apparent.

The fact that the peptide backbone ^{13}C resonances for $[Co(NH_3)(H_{-2}HisGG)]$ have chemical shifts similar to those for the corresponding resonances for the diamminecobalt(III) tripeptide complexes, which have coordination via a terminal NH_2, two deprotonated peptide nitrogens, and the terminal carboxylate group, supports the proposed structure in Figure 1.

The side-chain CH_2-4 resonance in the ^{13}C spectrum shows a relatively large shift of 5.32 ppm to higher shielding on complexation. In comparison, shifts of −2.87 and −3.01 ppm are found for the diamminecobalt(III) complexes of L-Phe-Gly-Gly and L-Tyr-Gly-Gly, respectively.[12] This difference could result from the coordination of the imidazole group. The ^{13}C resonances for the imidazole ring in $[Co(NH_3)(H_{-2}HisGG)]$ do not show major changes in chemical shifts relative to those of the free peptide anion. Large changes in these chemical shifts were not observed on coordination of the imidazole group in $[Co(NH_3)_2(H_{-2}GGHis)]$ where it is bound in the plane of the chelate rings.[20] Similarly the ^{13}C resonances for *N*-methylimidazole do not show large shifts on coordination in $[Co(N\text{-}MeIm)_2(H_{-2}GGG)]$ (C-2, −0.07 ppm; C-4, −1.23 ppm; C-5, +1.71 ppm).[18] The shifts observed for the imidazole ring carbons of L-His-Gly-Gly on complexation are of the same order and thus are not inconsistent with a coordinated imidazole group.

A similar comparison of the ^{13}C spectrum of $[Co(NH_3)_2(H_{-2}HisGG)]$ with the spectra of triamminecobalt(III) complexes of tripeptides where coordination of the peptide is via the terminal NH_2 and two deprotonated nitrogens supports the structure of the histidyl peptide complex in Figure 1. The resonance for CH_2-3 at δ 49.67 is 0.8–0.9 ppm to lower shielding than the corresponding resonances for the triammine complexes. As stated above, the difference may arise from the close proximity of the imidazole ring when it is bound in the apical position (Figure 8). As for the monoamminecobalt(III) complex of L-His-Gly-Gly, large changes in the chemical shifts of the imidazole ring carbons are not observed on complexation of the peptide. The CH-2′ resonance is 0.97 ppm to lower shielding than the corresponding resonance in the monoammine complex whereas in contrast the chemical shifts for CH-4′ and CH-5′ are individually very similar for the

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two complexes. Examination of Dreiding models reveals that on imidazole coordination CH-2′ projects out toward the C-terminus peptide residue and its chemical shift would be influenced by whether the carboxylate is bound or free. The CH-1 and CH_2-4 resonances for the two histidyl peptide complexes have very similar chemical shifts as expected for the structures given in Figure 1.

The acid dissociation constant for the pyrrole hydrogen, NH-1, of imidazole has been reported to be in the range 14.2–14.5.[24] The acidity of the pyrrole hydrogen increases upon complexation of a metal ion at N-3. Cobalt(II) lowers the pK_a from that of unbound ligand by about 2 logarithmic units, copper(II) by 1 more logarithmic unit, and palladium(II) by almost another logarithmic unit.[24] Coordination to cobalt(III) lowers the pK_a of imidazole and its derivatives by almost 4 logarithmic units, and values fall in the range 10.0–10.8.[25-28] The pK_a values determined for the pyrrole hydrogens of $[Co(NH_3)(H_{-2}HisGG)]$ and $[Co(NH_3)_2(H_{-2}HisGG)]$ at 298 K, 10.73 ± 0.04 and 10.69 ± 0.04, respectively, provide strong evidence that the imidazole side chain is coordinated in both L-His-Gly-Gly complexes. If it was unbound, pK_a values of approximately 5.5 and 14 would have been expected for $NH\text{-}3^+$ and NH-1, respectively.[1,2]

Potentiometric titration of $[Co(NH_3)_2(H_{-2}HisGG)]$ with acid at 298 K gave a pK_a value of 4.34 ± 0.04 for the free carboxylate group. For the free tripeptide, a value of 3.17 is reported for the pK_a of the carboxylate group.[1] As the charge on the free tripeptide changes from 1+ to 2+ on protonation of the carboxylate group while the charge on the complex changes from 0 to 1+, the decreased acidity of the carboxylate group of the complex is to be expected. Enright determined the pK_a^D of the carboxylate group of $[Co(NH_3)_3(H_{-2}GGG)]$, which also has zero charge, by ^1H NMR titration and reports a value of 4.15.[11] The difference between this value and the value observed for $[Co(NH_3)_2(H_{-2}HisGG)]$ is of a magnitude similar to the difference in the pK_a values for the protonation and deuteration of the free carboxylate group of $[Co(NH_3)_2(H_{-2}GGHis)]$ (0.24 logarithmic unit).[20]

The microanalysis of fraction 1 from the reaction of L-His-Gly-Gly with the "peroxo dimer" is consistent with a superoxide-bridged binuclear cobalt(III) complex with the peptide. It has an ESR spectrum consistent with this. The $g = 2.01$ signal is consistent with a free radical such as the superoxide group. Values for mononuclear cobalt(III) superoxide complexes have been reported in the range 2.013–2.036.[29-31] The low value of the cobalt hyperfine coupling constant for the superoxide resonance is consistent with formulation as a cobalt(III) complex.[32,33] The majority of cobalt(III) superoxide complexes are stable only in nonaqueous solvents at low temperature. Similar complexes with other non-histidyl tripeptides have not been isolated. Thus the observation of such a species under the present conditions is extremely unusual. However, a similar complex has been isolated with Gly-L-His-Gly.[19] They are unstable as would be expected for such superoxide species, being decomposed by dinitrogen to yield a cobalt(II) complex. Attempts at detailed characterization were not successful. For example, ^1H and ^{13}C spectra of the complexes showed extremely broad resonances.

Coordination of imidazole in one of the apical positions of a cobalt(III) complex of a quadridentate ligand is known to stabilize coordination of a superoxide in the other apical position.[33,34] The same reason is possible for the stabilization of the superoxide complexes of the histidyl peptides reported here.

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Registry No. $NH_4[(H_{-2}HisGG)Co(O_2^-)Co(H_{-2}HisGG)]$, 101054-50-6; $[Co(NH_3)(H_{-2}HisGG)]$, 101054-51-7; $[Co(NH_3)_2(H_{-2}HisGG)]$, 101054-52-8; $[(NH_3)_5Co(O_2)Co(NH_3)_5](NO_3)_4$, 16632-71-6.

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Reactions of Molybdenum(V) Tetraphenylporphyrins with Superoxide. Mechanism of the Reactions and the Characterization of an Isolated Dioxygen Complex[1]

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The mechanism of the reactions of $Mo^VO(TPP)X$ (TPP = 5,10,15,20-tetraphenylporphyrin; X = Br, Cl, NCS) with superoxide ion O_2^- in aprotic solvents under anaerobic conditions has been stoichiometrically elucidated. The complex $Mo^VO(TPP)X$ is reduced by O_2^- to $Mo^{IV}O(TPP)$ in dichloromethane containing 1% (v/v) dimethyl sulfoxide at 25 °C via an intermediate, complex **1**. Complex **1** is stable in solution at −80 °C but is converted into $Mo^{IV}O(TPP)$ at room temperature. Complex **1**, a new dioxygen complex of molybdenum tetraphenylporphyrin, was isolated. The chemical formula of complex **1** is ascertained to be [18-crown-6-K][$Mo^VO(TPP)(O_2^{2-})$] where the dioxygen binds side-on with the electronic configuration of peroxide. The structure and oxidation state of the molybdenum–dioxygen unit in the dioxygen complex are maintained in aprotic media over the temperature range between −80 and −20 °C.

Introduction

Dioxygen complexes of metalloporphyrins have been of great interest in relation to the elucidation of the mechanism of transport and storage of dioxygen by hemoglobin and myoglobin,[2] of activation of dioxygen by cytochrome P-450,[3] and of four-electron reduction of dioxygen by cytochrome oxidase[4] in vivo. Dioxygen adducts formed by the reactions of molecular dioxygen with iron(II),[5-11] cobalt(II),[12-14] chromium(II),[15] ruthenium(II),[16]

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