seen that the higher stability of $CuNTA^-$ is entirely an entropy and not an enthalpy effect. The more exothermic ΔH for $CuHY^-$ is the result of the coordination of an additional N atom to the metal ion enabling the special structural requirements to be met for the formation of relatively strain-free chelate rings.[27] In the NTA^{3-} ion, the charged carboxyl groups are more restricted than those in HY^{3-}, and the resulting localization of charge will lead to a more effective orientation of water molecules. Upon complex formation, the release of these water molecules will account for some of the observed $\Delta S(CuNTA^-) - \Delta S(CuHY^-)$ difference. Another factor contributing to this entropy difference of 16 cal deg^{-1} $mole^{-1}$ will be the greater loss of librational entropy of the larger HY^{3-} ion as compared with NTA^{3-} when they coordinate with the Cu^{2+} ion.

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Oxygenation and Oxidation of Cobalt(II) Chelates of Amines, Amino Acids, and Dipeptides[1]

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Abstract: In the absence of oxygen, glycylglycine undergoes a cobalt(II) ion promoted amide hydrogen ionization near pH 10. For the 2:1 complex the two amide hydrogen ionizations occur in a cooperative manner, yielding a light blue solution with a magnetic susceptibility of 4.1 BM. These results and the absorption spectrum of the light blue solution suggest an equilibrium between high and low spin states in an octahedral complex. Admission of oxygen to pink or blue solutions of octahedral cobalt(II) complexes rapidly yields yellow or, at higher concentrations, brown solutions of binuclear oxygenated complexes. Depending upon the ligands these complexes decompose at a variety of rates to yield red mononuclear cobalt(III) chelates. Proton balance studies conducted for the oxygenation and oxidation reactions indicate that the binuclear oxygenated complexes of ethylenediamine, histamine, and glycinamide contain hydroxo as well as oxygen bridges. With sufficient ligand, no hydroxo bridges appear with the other ligands studied including diaminopropionic acid, histidine, histidinamide, and histidylglycine. A minimum of three nitrogen donors seems necessary for formation of oxygenated complexes which are obligatory intermediates in the oxidation of cobalt(II) complexes by molecular oxygen. The product cobalt(III) chelates appear to be derived from the binuclear oxygenated complexes by filling the coordination position vacated by the departing peroxo group with an additional chelating group if available, otherwise by a hydroxo group. Absorption spectra and circular dichroism results are reported for all three kinds of complexes studied.

Since the discovery of reversible oxygenation of high-spin, octahedral bis(histidinato)cobalt(II) to yield a brown diamagnetic, binuclear oxygenated complex,[2] subsequent extensions to other ligands have been confusing owing to misidentification of the red complexes of monomeric cobalt(III), obtained from the over-all oxidation reactions, as oxygenated intermediates. Especially in the cases of amino acids other than histidine, and dipeptides where concentrations of oxygenated intermediates do not build up except at high pH, reported results require reinterpretation. Elemental analysis,[3] structures,[4] magnetic susceptibilities,[5] absorption spectra,[3,4,6,7] slow rates of appearance,[4,7] charge on complex,[8] and pmr spectra,[8] all attributed to the oxygenated complexes of dipeptides, should be reassigned to red cobalt(III) chelates. Oxygenation of the bis(histidine) complex of cobalt(II) to give the brown, binuclear, oxygenated complex is so rapid that a stopped-flow method was employed to measure the rates.[9] The formation of H_2O_2 was indicated polarographically in oxidation of the glycylglycine chelate.[10]

This research attempted to define more precisely for a variety of ligands the requirements and conditions for oxygenation and oxidation of cobalt(II) chelates. Since these reactions are often studied at high pH, it is necessary to investigate first the structures of cobalt(II) chelates, especially dipeptides, in alkaline solutions in the absence of oxygen in order to learn the characteristics of complexes undergoing oxygenation. Thus the first parts of the Results and Discussion sections are taken up with a study of cobalt(II) chelates. Extensive spectrophotometric and proton balance studies are then reported and discussed for the oxygenation and oxidation reactions.

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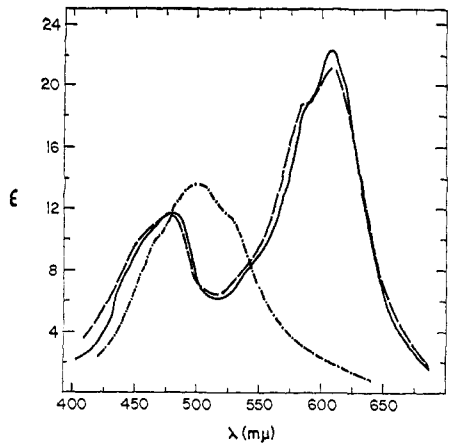

Figure 1. Extinction coefficient per mole of cobalt(II) ion in oxygen-free solutions containing a 20-fold excess of dipeptide ligands. Dotted and dashed curve represents glycylglycine at pH 9.0, solid curve glycylglycine at pH 11.5, and dashed curve glycyl-L-alanine at pH 11.5.

Experimental Section

The equivalent weight of all high quality ligands obtained from commercial sources was checked by titrations with standard base. Only the middle cut of a distillate collected at 206–207° from technical diethylenetriamine was employed. As described previously[11] titration and proton balance studies were conducted on a radiometer TTTl pH stat with recorder, and reaction vessel thermostated at 25.0°. Purified tank nitrogen was passed through vanadous chloride scrubbers to remove any traces of oxygen.[12] Ionic strength was controlled at 0.5 *M* with KCl.

Oxygen uptake was measured in a Warburg apparatus. Spectra were taken on a Cary Model 11 recording spectrophotometer and circular dichroism on a Durrum-Jasco ORD-UV-5 recording spectropolarimeter with a circular dichroism attachment. Magnetic susceptibilities were determined by an nmr method on a Perkin-Elmer R-20 instrument.[13] The technique was improved by abandoning the use of capillaries and comparing chemical shifts of *t*-butyl alcohol in a pair of solutions with and without cobalt(II) nitrate, all other concentrations being identical. Formation constants were evaluated by the projection strip method.[14]

Results

Titration curves were conducted in an O_2-free environment to pH 11 for solutions containing 2:1 molar ratios of ligands and cobalt(II) ions. These curves exhibited equivalence points near pH 9–10 and at lower pH values than titration curves conducted in the absence of cobalt(II) ion. Acid ionization constants from nitrogen-bound hydrogens and formation constants calculated for three ligands are presented in Table I. Titration of 3:1 L-histidine to cobalt(II) solution yields two equivalence points, the first near pH 7.5 corresponding to the titration of ammonium groups on two chelated ligands and the second with an apparent pK_a = 9.1 approximating that of free histidine. Thus the tendency for the third equivalent of histidine to bind is weak and in the absence of large amounts of excess histidine each bound histidine serves as a tridentate ligand. Additional support for this view is provided by the appearance of $Co(OH)_2$ which precipitates before the expected end points in titrations of 2:1 mixtures of either of the bidentate ligands ethylenediamine (en) or histamine.

Table I. Acid Ionization and Formation Constants for Cobalt(II) Complexes at 25° and 0.5 *M* Ionic Strength

	pK_2	pK_3	Log K_1	Log K_2
L-Histidine	6.10	9.20	6.45	5.05
L-Histidinamide	5.85	7.78	4.53	3.68
L-2,3-Diamino-propionic acid	6.70	9.50	6.2	5.0

In order to inhibit precipitation, titrations were performed on solutions containing a 20:1 molar ratio of dipeptides to cobalt(II) ions. With glycylglycine 1.1 equiv of base was consumed per mole of ligand to reach the equivalence point near pH 10.5. Since glycylsarcosine yields a precipitate at 1.0 equiv near pH 10 in an identical experiment, the additional two ionizations for glycylglycine may be ascribed to cobalt(II) ion promoted ionization of amide hydrogens from two ligands per metal ion.[15] Precipitation also occurs with a 20-fold excess of glycinamide·HCl and glycylglycinamide·HCl but near pH 11 and after the addition of 1.2 equiv of base per mole of the latter ligand, suggesting that additional ionizations have occurred in this last case also.

Light pink colors, typical of high-spin octahedrally coordinated cobalt(II) complexes, are obtained in all cases in oxygen-free systems at pH 9–10 where the titration curves indicate that the complexes are fully formed. Visible spectra show shoulders on an absorption maximum near 500 mμ with ε 10–24. Circular dichroism and visible absorption spectra for 2:1 complexes are tabulated for the first four ligands in Table II. A solution containing 5×10^{-3} *M* cobalt(II) ion and three times as much L-alanine at pH 9.2 gave two minima in CD at 484 and 520 mμ with $\Delta\epsilon$ −0.07 and −0.09, respectively.

Table II. Cobalt(II) Chelates. Circular Dichroism and Absorption Spectra

Ligand	pH	Circular dichroism mμ	Δε	Absorption mμ	ε[a]
L-Histidine	10.0			440	S
		487	+0.50	485	18
		533 S	+0.25	535	S
L-Histidinamide	10.0			440	S
		484	+0.32	485	20
		510	+0.24		
L-Histidylglycine	9.0			440	S
		483	+0.27	485	18
		510	+0.20		
L-2,3-Diamino-propionic acid	8.3			440	S
		481	+0.18	485	10
		522 S	+0.10	530	S
Glycyl-L-alanine	11.5			450	S
		480	−0.09	480	12
		533	−0.06	535	S
		588	−0.22	585	S
		610	−0.22	607	22

[a] S signifies shoulder

When the pH rises above 11, oxygen-free solutions containing cobalt(II) ion and excess ligand turn violet in the case of L-histidine and blue with glycylglycine or glycyl-L-alanine. Spectra of dipeptide chelates of

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Table III. Oxygenated Cobalt(II) Chelates (Circular Dichroism and Absorption Spectra) and Cobalt(III) Chelates (Circular Dichroism)

		Oxygenated cobalt(II)				Cobalt(III)	
		CD		Absorption		CD	
Ligand	pH	mμ	Δε	mμ	ε	mμ	Δε
L-Histidine	10.0	354	+8.4	325	3240	<350	+
		465	−3.0[a]	383	3000	508	−0.94
						583	+0.35
L-Histidinamide	10.0	355	+9.2	325	3280	<350	+
		452	−2.5	375	2900	477	−0.37
						544	+1.12
L-Histidylglycine	9.0			285 S[c]	4100	<350	+
				330 S	3500	436	−0.08
						530	+2.28
L-2,3-Diamino-propionic acid	8.3	350	−2.8	315	3290	350	+0.67
		507	+0.9[a]	383	2720	500	−0.83
						560	+1.30
Glycyl-L-alanine	12	354	+0.2	315[b]	S	387	−0.19
		535	−0.9[a]	355 S	~4000	505	−1.18
L-Alanylglycine	12	<400	−			596	+0.75
		496	−0.3			502	−2.26
		>600	+				

[a] Broad band. [b] Glycylglycine absorption spectrum. [c] S = shoulder.

cobalt(II) in the absence of oxygen at two pH values are shown in Figure 1 in the presence of excess ligand. The glycylglycine complex at pH 9.0 is a typical pink octahedral one and also exhibits an absorption maximum at 1100 mμ with ε 6. The blue solutions obtained at pH 11.5 exhibit two visible absorption peaks of low intensity, have an absorption maximum at 1000 mμ with ε 10, and are not similar to solutions containing the more intensely colored violet tetrahedral cobalt(II)–histidine complexes. Sarcosylglycine gives results similar to glycylglycine in Figure 1. The properties of these reversibly formed dipeptide complexes exhibit no dependence on total concentrations. The midpoint in growth of the peak at 605 mμ occurs at about pH 10.7. The circular dichroism of the glycyl-L-alanine complex is reported in Table II. The magnetic susceptibility of a solution containing 1 *M* glycylglycine and 0.05 *M* $Co(NO_3)_2$ at pH 12.5 and 25° is 4.1 BM.

All the results described above involved solutions scrupulously free of oxygen. When oxygen is bubbled through the pink solutions containing fully formed cobalt(II) chelates at about pH 9, a yellow color forms instantly, followed in most cases by intensification to brown within a few minutes. Absorption spectra of these differently colored complexes differ only in absorption intensity and not in other characteristics of the spectra, which exhibit one or two charge-transfer bands between 300 and 400 mμ with $\epsilon > 10^3$ and some tailing at longer wavelengths above 450 mμ. Absorption spectra and circular dichroism results for some of these oxygenated complexes are reported in Table III where weak, presumably d–d transitions in the visible region are evident from the CD results. Except for the last two entries where it is 20:1, the molar ratio of ligand to cobalt ion is 2:1.

Oxygenation of cobalt(II) chelates is rapid, and the rate-limiting step in the slower intensification of the brown color in our experiments is due to relatively slow passage of O_2 across the gas–solution interfacial boundary. On a Warburg apparatus standardized by the known result that one molecule of O_2 is taken up for every two cobalt(II) ions in a solution containing a 20:1 molar ratio of glycylglycine and cobalt(II) ions,[3] the cobalt(II) to O_2 ratio for production of oxygenated complexes was determined to be 2.0 ± 0.1 for all of the ligands listed in Table IV plus glycylhistidine. This result is in agreement with earlier work with histidine and histamine ligands.[2] Thus the oxygenation reaction also involves a dimerization of two cobalt(II) complexes to form a binuclear complex.

Bubbling of nitrogen gas through solutions containing oxygenated complexes results in a decrease in absorption in the 300–400-mμ region, and in the cases of histidine and dipeptides, complete reversibility of the oxygenation reaction is observed as indicated by production of light pink solutions identical with those of the starting cobalt(II) complexes. Addition of HCl to solutions containing oxygenated complexes until the pH is about 3 immediately yields pink solutions which revert to oxygenated complexes upon subsequent addition of base to pH ~12. This acid–base cycling is not always completely reversible; in the case of ethylenediamine some cobalt(III) complex is produced during each cycle.

When the dark brown solutions containing oxygenated cobalt(II) complexes are permitted to stand, red or orange colors appear at rates characteristic of the ligand involved. Spectra of resulting octahedrally coordinated cobalt(III) chelates display one or two absorption maxima from 360 to 550 mμ and $\epsilon \sim 10^2$ and charge-transfer absorption at <330 mμ with $\epsilon > 10^3$. Visible absorption spectra and circular dichroism results for some cobalt(III) chelates obtained from oxygenated complexes are presented in Tables III and IV. Even though the dipeptide complexes of cobalt(III) chelates were prepared from solutions containing a 20:1 molar ratio of ligand to metal ion, most chelates are thought to contain two molecules of ligand for each cobalt ion.

In contrast to the rapid oxygenation reaction, dissociation of the oxygenated complexes to yield cobalt(III) complexes is slow, with rates dependent upon the ligand present and almost independent of pH from pH 9 to 12. The half-life for the dissociation reaction is noted after each ligand in the following list: glycylglycinamide, 3 min; histidylglycine, 16 min; diethylenetriamine, 18 min; glycylglycine, 40 min;[4] glycin-

Table IV. Results of Proton Balance Studies and Visible Spectra of Co(III) Products[a]

$$2Co^{II}L_2^{w} + O_2 \rightleftarrows [(Co^{III}L_2^{z})_2O_2^{2-}(OH^-)_b]^y + nH^+$$
$$+$$
$$2Co^{III}L_2^{z} + H_2O_2 \leftarrow mH^+$$

	w	n	(y)	(b)	x	m	$n-m$	z	N donors	mμ	ϵ
Glycyl (pH <9.5)	0	4	−4	0	−1	2	2	−1	4	520	350
(pH >11)	−2	0	−4	0	−1	2	−2	−1	4	386	122
Ethylenediamine	2	1	3	1	3	(−1)	(2)	1	4	515	100
										370	115
Histamine	2	1	3	1	3	(−1)	(2)	1	4	485	80
Histidinamide	2	2	2	0	2	0	(2)	1	5	483	130
Histidylglycine	0	2	−2	0	0	0	(2)	−1	5	493	220
Glycinamide	2	5	−1	1	1	(−1)	(6)	−1	4	500	115
										360	130
Glycylglycinamide	2						6	−1	6	478	360
										360	190
Diethylentriamine	2						−2	3	6	465	130
Diaminopropionic acid	0	0	0	0	1	2	(−2)	1	4	510	90
Histidine	0	0	0	0	1					493	120

[a] Parentheses indicate calculated value.

amide, 4 hr; histidinamide, 6 hr; glycylhistidine, 7 hr; histidine, 9 hr; 2,3-diaminopropionic acid, 24 hr; ethylenediamine, 8 days; histamine, 12 days. The slowest rates are found with those oxygenated complexes that possess the greatest effective positive charge on the metal ion, the smallest ligand field strength, and negligible steric effects that would otherwise favor decomposition of a binuclear complex. Final solutions containing cobalt(III) chelates are completely unaffected by bubbling of nitrogen gas.

Addition of HCl to pH 3 usually has no effect on the visible spectra of product cobalt(III) chelates, indicating that all six coordination positions are occupied by ligands other than hydroxide. With glycinamide and en ligands, however, addition of HCl shifts the absorption maxima 5–20 mμ to shorter wavelengths with some reduction in the molar extinction coefficient. We obtain for glycinamide ϵ_{495} 107 and ϵ_{350} 124, and for en ligand ϵ_{495} 80 and ϵ_{350} 74 after acidification. These changes are instantaneous and reversible on subsequent addition of NaOH. Irreversible, time-dependent spectral changes are observed upon addition of HCl to the cobalt(III) chelate of glycylglycinamide.

Steric requirements for production of oxygenated complexes were investigated spectrophotometrically for a series of glycyl dipeptide complexes in a 2:1 molar ratio near pH 9. Because of little build-up of oxygenated complexes, production of cobalt(III) complexes was measured, the over-all rate constant for which is a product of an equilibrium constant for dimerization to yield an oxygenated complex and a rate constant for decomposition of the latter. The approximate half-life for the over-all reaction is noted after each glycyl dipeptide in the following list: Gly-Gly, <1 hr; Gly-Ala, ~10 hr; Gly-Leu, ~5 days; Gly-Val, ~3 months; Val-Gly, <1 hr. Oxygenated complexes become more stable near pH 12, where it is necessary to employ higher ligand to metal ratios to prevent precipitation. An outstanding exception is glycylsarcosine as a ligand, which never yields oxygenated cobalt(II) or cobalt(III) complexes under any conditions of this study even after standing more than 1 month.

A main effort of this research was to determine the number of equivalents of hydrogen ion liberated or consumed during oxygenation and oxidation of cobalt(II) complexes. A summary of the results with the chemical equations involved is presented in Table IV. Positive values of n indicate oxygenated chelates that are not completely reversible to N_2 without addition of acid.

Determination of the evenness or oddness of equivalents of acid liberated in the oxygenation reaction indicates whether a negatively charged bridging group as well as an oxygen bridge exists in the binuclear oxygenated complex. Unit positive values of b shown in Table IV suggest hydroxo bridges in the oxygenated complexes of ethylenediamine, histamine, and glycinamide. The binuclear complex is written as a peroxo complex of cobalt(III) rather than as an oxygenated complex of cobalt(II); employment of the latter description would make all values of x in Table IV one unit less positive. Throughout the conditions of $8 < pH < 11$, not all complexes listed in Table IV will contain two ligands per cobalt(II) ion, but this number of ligands determines the stoichiometry because the binuclear oxygenated complexes cannot contain more than two chelating ligands per cobalt ion. If the oxygenated complex is not built up during the reaction, only the total number of protons liberated in the over-all oxidation reaction, $n - m$, may be determined.

For the reactions studied and summarized in Table IV, numbers without parentheses indicate experimentally determined quantities. The numbers surrounded by parentheses may be calculated from demands of conservation of mass and electroneutrality in the reactions at the top of Table IV. The equations so derived are as follows: $y = 2w - n$, $b = 2(x - 1) - y$, $y + m = 2z$, and $2w = 2z + n - m$.

Glycylglycine, the first ligand shown in Table IV, presents the most complex case. The rate of the second, decomposition step is known to be first order in binuclear oxygenated complex and pH dependent from pH 9 to 11.[4] Some interference in the two steps occurs at the lower end of the pH range, and rate data were used to correct for the occurrence of both reactions. Since the ligand to cobalt(II) ion ratio is 20:1, it was also necessary to correct for free ligand hydrolysis at high pH. From the results of measurements in the pH-stat at 13 pH values between pH 9.5 and 11.3, the value of n varies from nearly zero at pH 11.3 to a value ap-

proaching 4 at pH 9.5. The value of $m = 2.0 \pm 0.1$ throughout this pH range and the independently measured $n - m$ varies from $+2$ to -2 as the pH increases. Above pH 11.3 ionization of product H_2O_2 results in an observed decrease in the value of m.

Titration of cobalt(II) and glycylglycine in oxygen-free solutions described above gives evidence for two additional ionizations ascribed to amide hydrogens, but the 20:1 ligand to metal ion ratio rendered difficult calculation of pK_a values for the ionizations. With the assumption that only cobalt(II)–glycylglycine complexes with two ionized amide hydrogens pick up oxygen, apparent pK_a values for amide hydrogen ionization may be calculated from the variation of n with pH with the aid of a pM *vs.* $\bar{h}$ plot[16] for two ionizable groups. From the plot we obtain $pK_1 = 9.85$ and $pK_2 = 10.15$. For two equivalent and independently ionizing groups pK_2 should be 0.6 log unit greater than pK_1 barring any electrostatic consideration which should provide a further separation. Thus the ionizations from two glycylglycine molecules about a cobalt(II) ion occur in a cooperative manner: ionization of the first amide hydrogen promotes ionization of the second. Cooperative amide hydrogen ionizations also appear in nickel complexes of peptides where a change from high-spin octahedral to low-spin square-planar complexes accompanies the ionizations.[15]

Decomposition of oxygenated complexes with ethylenediamine or histamine ligands is so slow that m was not determined experimentally. The values of n listed in Table IV were calculated from pH 9 to 10 for both ligands after corrections were made for incomplete formation of cobalt(II) complexes from known formation constants.[17]

Proton balance studies were performed with histidinamide as ligand from pH 7.7 to 9.5. Slow decomposition of the oxygenated complex permitted a correction by absorption measurements for its incomplete formation at the lower end of the pH range. From five determinations $n = 2.00 \pm 0.02$. From four measurements for histidylglycine from pH 9.4 to 10.2, $n = 2.0 \pm 0.1$. More rapid decomposition of the oxygenated complex precluded the above correction at pH <9.4 for incomplete complex formation. For both ligands no change in pH occurs in the second decomposition reaction.

For glycinamide formation of the oxygenated complex from pH 9.8 to 11.1 gives $n = 5.0 \pm 0.2$ from seven determinations with a 53-fold excess of ligand over cobalt(II) ion. Since dissociation of the binuclear oxygenated complex with glycylglycinamide occurs rapidly, only the total number of protons liberated in the over-all reaction was measured. Values of $n - m$ obtained are 5.7, 5.5, and 4.9 at pH 9.02, 9.30, and 9.78, respectively.

L-Histidine and L-2,3-diaminopropionic acid, two ligands of identical charge type, give similar cobalt(II) complexes, both of which undergo oxygenation without a change in pH of the solution to give stable binuclear oxygenated complexes. For diaminopropionic acid as a ligand, determinations at pH 8.3 and 8.8 gave $m = 2$. For histidine the apparent m varies from zero at pH 9.4 through -1 near pH 10.5 to -1.7 at pH 11.2 as protons are liberated to the solution at high pH. Addition of acid to this last high pH solution at completion of the oxidation gave an equivalence point after the addition of 1 equiv per cobalt(III) ion with a midpoint near pH 10.5. Since the dissociation reaction with histidine is slow and is accompanied by additional oxygen uptake,[2,18] the second equation atop Table IV is not applicable and the reaction is not considered further.

Solutions containing 2 moles of diethylenetriamine (dien) per one of cobalt(II) ion consume hydrogen ion in the over-all oxidation reaction to yield $n - m = -2.0 \pm 0.1$ from pH 10.0 to 11.1. Due to the relatively rapid rate of decomposition of oxygenated complex no separation into individual contributions was attempted. The spectrum of the oxygenated complex gave a maximum at 298 mμ with ε 2840 while that observed in a solution containing a 3:2 dien:Co ratio at pH 10 gave a maximum at 305 mμ with ε 2220. Only in the 3:2 case was an initial drop in pH observed on formation of oxygenated complex. The corresponding cobalt(III) complexes give maxima at 465 mμ with ε 130 in the 2:1 ratio and at 470 mμ with ε 87 in the 3:2 case. Only in the last instance does a shoulder also appear at 550 mμ with ε 46.

Discussion

The larger formation constant for L-histidine over L-histidinamide may be ascribed to the greater basicity of histidine and to the greater chelating ability of a carboxylate group over an amide carbonyl oxygen. No evidence of cobalt(II) ion promoted amide hydrogen ionization appears below pH 10 for either histidinamide or histidylglycine so that chelation through the amide nitrogen does not occur. L-Histidine also exhibits a larger formation constant than L-2,3-diaminopropionic acid, despite the greater basicity of the latter. This inversion is due to the relatively high metal ion binding capability of an imidazole group[19] and provides an additional argument for its involvement in histidine chelation in solution.

Despite a blue color and a magnetic susceptibility in the appropriate range, the cobalt(II) complexes of dipeptides like glycylglycine at high pH are not tetrahedral because of the low molar extinction coefficients shown in Figure 1. Small amounts of cobalt(III) complexes are also inconsistent with the spectra. At pH <9, before amide hydrogen ionization has occurred, chelation in the high-spin octahedral complex occurs only at the amino nitrogen and the amide carbonyl oxygen. Upon amide hydrogen ionization chelation takes place at the amino nitrogen, amide nitrogen, and carboxylate group of dipeptides so that at pH >11.5 the absorption maximum of the high-spin octahedral cobalt(II) complex for the transition $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ in O_h has shifted from 497 mμ at low pH to 480 mμ at high pH as shown in Figure 1. Concurrently the absorption maximum for the $^4T_{1g} \rightarrow {}^4T_{2g}$ transition undergoes a shift from 1100 to 1000 mμ. Thus the deprotonated amide nitrogen exhibits a greater ligand field strength than the amide oxygen. Analysis[20] yields for the low

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pH solution a ligand field parameter $Dq = 1025\ cm^{-1}$ and a Racah parameter $B = 815\ cm^{-1}$, while for the high pH solution $Dq = 1125\ cm^{-1}$ and $B = 805\ cm^{-1}$.

The cooperative manner in which the amide hydrogen ionizations occur from the two glygly ligands in each complex suggests a profound change about the metal ion in agreement with appearance of some low-spin octahedral cobalt(II) complex. The longer wavelength position of the absorption maximum at 608 mμ in Figure 1 is consistent with its assignment to the transition $^2E_g \rightarrow {}^2T_{1g}$, $^2T_{2g}$ in O_h in a low-spin octahedral complex. The transitions in both the high-spin and low-spin forms are magnetic dipole allowed in agreement with $\Delta\epsilon/\epsilon$ values near 0.01 for the glycyl-L-alanine complex in Table II. Magnetic susceptibilities for octahedral cobalt(II) chelates range from 4.8 to 5.2 (max) BM in high-spin to 1.8–1.9 BM in low-spin complexes. The observed value of 4.1 BM for the $Co^{II}(glygly)_2^{2-}$ chelate then implies about 70% high-spin and 30% low-spin forms. This conclusion is consistent with the relatively low extinction coefficient for the 480-mμ absorption in the dipeptide chelates. Thus both the intermediate value of magnetic susceptibility and the presence of two transitions in the visible region shown in Figure 1, for bisdipeptide chelates of cobalt(II) ion observed at high pH after cooperative amide hydrogen ionization has occurred, are compatible with an equilibrium between high and low spin states[21] with a single tridentate conformation for the pair of ligands arranged in near D_{2d} symmetry (actually C_2).

A minimum of three nitrogen donors per cobalt ion appears necessary to form an oxygenated complex as suggested earlier.[22] All the cobalt(III) complexes listed in Table IV possess at least four nitrogen donors, and except for the two cases with six N donors these complexes probably reflect the mode of binding in the oxygenated complexes. When glycine is the ligand, little build-up of an oxygenated complex is observed, though oxidation does occur. At pH ~8.5, glycinamide is similar to glycine, as chelation occurs through N and O donors in each ligand. At higher pH, ~11, oxygenation becomes complete for glycinamide only, where amide hydrogen ionization leads to two N donors for each glycinamide molecule. With the exceptions of glycylsarcosine and histidylglycine, which binds like histidine, all dipeptide ligands yield greater build-up of oxygenated complexes at high pH. Amide hydrogen ionization yields one more N donor atom per ligand molecule, resulting in stabilization of the oxygenated complex. We conclude that amide hydrogen ionization must occur in simple dipeptide complexes for the formation of oxygenated complexes. Because the amide nitrogen cannot become a donor atom in glycylsarcosine, no oxygenated or cobalt(III) complexes are produced with this ligand. Evidently, oxygenation must be coupled with at least three nitrogen donors in order to yield the diamagnetic binuclear oxygenated complexes. If the oxygenated complexes cannot be formed, oxidation of cobalt(II) to cobalt(III) complexes by molecular oxygen cannot occur.

Binuclear oxygenated complexes resulting from the combination of 1 mole of oxygen with 2 moles of cobalt(II) complex might be described as an oxygenated complex of cobalt(II) or a peroxo complex of cobalt(III) ions. Because of the diamagnetic character of the binuclear complexes and an O–O distance comparable to that in peroxides, the peroxocobalt(III) description is often assumed. The well-known inertness of cobalt(III) complexes makes the latter description seem inconsistent with immediate regeneration of pink cobalt(II) complexes on addition of acid to solutions of oxygenated complexes and upon bubbling nitrogen through solutions containing oxygenated complexes of histidine and dipeptides. Insofar as it is useful to distinguish between the two descriptions of oxygenated complexes, the facile kinetic effects may be incorporated into the cobalt(III) presentation if the reversibility of the first equation atop Table IV is kept in mind. In this convenient oxidation–reduction reaction, an inert cobalt(III) complex becomes a labile cobalt(II) complex *via* a pathway not available to most cobalt(III) complexes. Though the ligand field transitions of the oxygenated complexes are obscured by charge-transfer bands, the magnitudes and wavelengths of circular dichroism peaks observed near 500 mμ are consistent with the cobalt(III) complex description.

The minimum of three nitrogen donors per cobalt(II) ion required to form diamagnetic binuclear oxygenated complexes appears incompatible with their description as peroxocobalt(III) complexes when even oxygen donor atoms are sufficient to form low-spin cobalt(III) complexes. This inconsistency may be resolved by postulating a low-spin oxygenated cobalt(II) complex as an intermediate en route to the diamagnetic binuclear peroxocobalt(III) complex.

Two possible pathways exist for oxidation of a high-spin cobalt(II) complex ($t_2^5e^2$) to a low-spin cobalt(III) complex (t_2^6). In one case, loss of an electron might occur to give an unstable cobalt(III) complex with two unpaired spins (t_2^5e) which later undergoes spin pairing. In the second pathway spin pairing occurs first to yield a low-spin cobalt(II) complex (t_2^6e) followed by loss of the e level electron. Oxygenation along with at least three nitrogen donors provides a mechansim for some stabilization of the low-spin cobalt(II) complex in the latter pathway. When an oxygenated complex does not form as with glycylsarcosine, no cobalt(III) complex appears even after long standing indicating that the former pathway is a much higher energy one. For practical purposes then a low-spin oxygenated cobalt(II) intermediate is obligatory for oxidation of cobalt(II) complexes by molecular oxygen.

The rates of formation of cobalt(III) complexes of dipeptides exhibit pronounced steric inhibition in the C-terminal residue, as indicated by the 3-month half-life for glycylvaline, while the <1-hr half-life for valylglycine demonstrates little steric inhibition associated with the N-terminal position. The reported rates are products of the equilibrium constants for oxygenated complex formation and rate constants for their decomposition to cobalt(III) complexes. Bulky substituents should only promote decomposition to monomers; hence steric inhibition should originate in the rate step to form binuclear oxygenated complex. Examination of molecular models shows that if a carboxylate group is detached in formation of oxygenated complex, substitution on the C-terminal residue sterically impedes development of an oxygen bridge and dimer formation.

(21) R. C. Stoufer, D. W. Smith, E. A. Clevenger, and T. E. Norris, *Inorg. Chem.*, 5, 1167 (1966).
(22) S. Fallab, *Angew. Chem. Intern. Ed. Engl.*, 6, 496 (1967).

Three ligands, en, histamine, and glycinamide, yield $b = 1$ in Table IV, indicating a dibridged oxygenated species with a hydroxo in addition to an oxygen bridge. Since a second bridge should serve to stabilize the oxygenated complex, and because the hydroxo bridge occurs only with bidentate chelates, the absence of hydroxo bridges with the other ligands is evidently due to steric effects. This conclusion is supported by examination of molecular models which indicate that in these last cases dibridged species could form only with difficulty or not at all.

Values listed in Table IV for z, the charge on the product Co(III) complexes, are consistent with the results of the proton balance studies and the visible absorption spectra as indicated by the number of nitrogen donor atoms. In one case, spectra of the Co(III) products may be identified with complexes made by other routes. The Co(III) complex spectrum for ethylenediamine recorded in Table IV corresponds to that of *cis*-dihydroxobis(ethylenediamine)cobalt(III). Upon acidification the spectrum obtained is consistent with that assigned to the *cis*-diaquo complex.[23] The ready reversibility of these acid–base effects on the spectrum indicates that Co(III)–ligand bonds are not made or broken in the transformations. Similar rapid reversibility was also observed on acidification of the dihydroxobisglycinamide complex to give the *cis*-diaquo complex. Both cobalt(III) complexes of glycinamide possess two ionized amide hydrogens.

Examination of the product cobalt(III) chelates listed in Table IV suggests that they are derived from the binuclear oxygenated complexes by filling the coordination position vacated by the departing peroxo group with an additional chelating group, if one is available, otherwise by a hydroxo group. Hydroxo groups are evident from a rapid, reversible shift of the visible spectrum to shorter wavelengths on mild acidification to yield coordinated water molecules.

According to the above view, the cobalt(III) complex composed of two glycylglycine molecules, the visible spectrum of which is unaffected by addition of HCl to pH 3, should contain two tridentate ligands chelated through amino and ionized amide nitrogens and the carboxylate group, $Co(glygly^{2-})_2^-$, to yield a complex with C_2 symmetry. This formulation is consistent with a -1 over-all charge indicated by an ion-exchange study[8] and the high $\Delta\epsilon$ value for glycyl-L-alanine reported for the cobalt(III) complex in Table III suggestive of chelation of the carboxyl terminal residue. In addition, such a structure has been found in an X-ray diffraction study though the reported absorption[24] is less than half that recorded in Table IV. The amide nitrogens bound in the kinetically inert cobalt(III) complex are less basic than the amide oxygens, and any rapid protonation taking place on addition of excess acid occurs at the unbound amide oxygens. Thus the red complex isolated[3,10] on addition of excess H_2SO_4 should be reformulated as $[Co(glygly^-)_2^+]_2\,SO_4^{2-}\cdot 2H_2O$. Hydrogens on the protonated amide oxygens are quite acidic, and in neutral solutions the species with a -1 over-all charge would be obtained.[24a]

The spectrum of the cobalt(III) complex of glycylglycinamide indicates six nitrogen donors corresponding to four ionized amide hydrogens. The pH dependence of $n - m$ reported in the Results section indicates that amide hydrogen ionization is promoted by cobalt(II) ion only above pH 9 and is only 28% complete at pH 9.8. Therefore, amide hydrogen ionizations are promoted by cobalt(II) ion most easily in glycylglycine followed by glycylglycinamide and do not occur at all below pH 11 in glycinamide.

No amide hydrogen ionizations have occurred below pH 10 in the cobalt(II) complexes of either histidinamide or histidylglycine. The results quoted in Table IV for these parallel cases show one amide hydrogen ionization per two ligand molecules in half of each binuclear complex formed upon oxygenation. Thus each cobalt ion is surrounded by two imidazole, two amino, and one amido nitrogen donors and one oxygen in the oxygenated complex. Interpretation of the results for the dissociation reaction may be in error if additional oxygen is being taken up, as in the case of histidine.

Different oxygenated complexes appear to form with dien depending upon whether the ligand to cobalt ion ratio is 2:1 or 3:2. The proton balance and spectrum results presented in Table IV are consistent with six nitrogen donors in the product cobalt(III) complex obtained in solutions with a 2:1 ratio. Presumably each cobalt ion in the intermediate oxygenated complex contains one oxygen molecule, five nitrogen donors, and n and $b = 0$. These results contrast with a report of no oxygenated complex formation in solutions containing a 2:1 ratio, while the report is in agreement with our observation of complex formation in solutions with a 3:2 ligand to metal ion ratio.[22] The spectrum of the cobalt(III) complexes obtained under the latter conditions indicates a mixture of approximately equimolar amounts of the 2:1 complex described above and a 1:1 complex with three nitrogen donors. The drop in pH observed upon oxygenated complex formation only in the solutions with a 3:2 ratio suggests that under these conditions one of the oxygenated complexes contains a hydroxide group.

For the cobalt(III) chelates the $A_{1g} \rightarrow T_{1g}$ transition (in O_h) at >450 mμ frequently yields oppositely signed CD peaks as the T_{1g} state is split in fields of lower symmetry. The $A_{1g} \rightarrow T_{2g}$ transition at 350–400 mμ is often obscured by charge transfer absorption at shorter wavelengths. Comparison of the CD magnitudes of the cobalt(III) chelates in Table III with those recorded for amino acid complexes[25] indicates that configurational as well as vicinal effects contribute to the magnitude.

(23) J. Bjerrum and S. E. Rasmussen, *Acta Chem. Scand.*, **6**, 1265 (1952).

(24) R. D. Gillard, E. D. McKenzie, and E. B. Robertson, *Nature*, **209**, 1347 (1966); R. D. Gillard, E. D. McKenzie, R. Mason, and G. B. Robertson, *Coord. Chem. Rev.*, **1**, 263 (1966); R. D. Gillard, P. M. Harrison, and E. D. McKenzie, *J. Chem. Soc., A*, 618 (1967).

(24a) NOTE ADDED IN PROOF. These conclusions are supported by recent X-ray crystal structure analysis of the complex $[Co(glygly^-)_2^+]ClO_4$, formed rapidly upon addition of perchloric acid to a solution containing $Co(glygly^{2-})_2^-$. The analysis indicates that the amide nitrogen atoms remain trigonal and coordinated upon protonation of the uncoordinated peptide oxygen atoms (M. T. Barnet and H. C. Freeman, personal communication).

(25) C. T. Liu and B. E. Douglas, *Inorg. Chem.*, **3**, 1356 (1964); T. Yasui, J. Hidaka, and Y. Shimura, *Bull. Chem. Soc. Japan*, **39**, 2417 (1966); R. G. Denning and T. S. Piper, *Inorg. Chem.*, **5**, 1056 (1966).