The Irreversible Redox Rearrangement of Cobalt Oxygen Complexes of Dipeptides[1a]

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Abstract: The oxidative rearrangement of binuclear cobalt dioxygen complexes to mononuclear cobalt(III) chelates in aqueous solution at 25 °C and 0.10 M ionic strength has been investigated by spectroscopic, potentiometric, and polarographic techniques for the series of ligands: glycylglycine, glycyl-L-alanine, glycyl-L-serine, L-serylglycine, L-alanyl-L-alanine, L-alanylglycine, and glycyl-L-tyrosine. The reaction was found to proceed in two first-order steps. The first is the more rapid ($t_{1/2} \approx$ minutes) and probably involves the oxidation of one ligand molecule per two cobalt atoms and the conversion of the bridging dioxygen moiety to water. The second step is slower ($t_{1/2} \approx$ hours) and probably involves displacement of the oxidized ligand by the excess dipeptide which is present in solution. Rate constants are reported for both steps of these rearrangement reactions.

Despite the tremendous amount of attention which has been focused on the μ-peroxo bridged complexes of cobalt with a wide variety of ligands, the irreversible conversion of such complexes to cobalt(III) chelates remains a very poorly understood process. Since the rate of this side reaction is usually the limiting factor in determining the number of reversible oxygenation cycles through which a cobalt(II) complex can go, the study of this phenomenon is of primary importance. With dipeptide ligands this oxidation is frequently so rapid that one cannot study the oxygenation of these chelates without also dealing with the oxidative rearrangement reaction.

Early workers in the field of cobalt oxygen complex chemistry of dipeptides believed the final red cobalt(III) chelates to be the oxygenated species still containing the peroxo bridge.[2-6] This mistaken idea arose because of two rather unusual characteristics of these complexes. First, the reaction of these complexes to form unoxygenated cobalt(III) compounds is unusually rapid. In addition, the necessity of deprotonating the amide protons of the coordinating dipeptides as a condition of oxygenation prevents the formation of appreciable equilibrium amounts of the dioxygen complex below pH 9.[7,8] Tanford et al.[4] noted the formation of a "brown intermediate" at high pH during a study of the cobalt glycylglycine system. This brown complex has now been characterized for several systems by elemental analysis,[2,9,10] visible spectroscopy,[4,7,8,10,11] and proton stoichiometry studies[7,8] and shown to correspond to the general formula $(H_{-1}L)_2Co\text{-}O_2\text{-}Co(H_{-1}L)_2^{4-}$, where $H_{-1}L$ refers to an amide deprotonated dipeptide ligand. The brown color is due to a ligand to metal charge transfer band tailing out of the UV into the visible region of the spectrum and is a characteristic feature of cobalt dioxygen complexes.

Eventually Cagliotti et al.[9] proposed that the red compound isolated from oxygenated solutions of cobalt and glycylglycine was not the oxygenated species, but bis(glycylglycinato)hydroxocobalt(III). Their conclusion was based on the polarographic detection of H_2O_2 in the reaction mixture and the presence of an OH stretching band in the IR spectrum. Soon afterward this red compound was identified crystallographically by McKenzie[12] as $Co(H_{-1}L)_2$. The crystal structure shows both glycylglycine ligands to be terdentate, binding through the carboxylate oxygen, deprotonated amide nitrogen, and terminal amino group. No hydroxo group was coordinated, but McKenzie's crystals were isolated from strongly acidic solutions, so the presence of hydroxo complexes in solution at high pH's cannot be dismissed.

Thus the early work of Tanford et al.[4] on what they believed to be the formation of the μ-peroxo bridged cobalt glycylglycine complex must be reinterpreted as a study of the oxidative rearrangement of this complex to a red, monomeric, unoxygenated cobalt(III) chelate. Their study showed this reaction to be first order in oxygen complex concentration and strongly pH dependent from pH 9.5 to 12.

Michailidis and Martin[7] also published approximate half-lives for the oxidation of several cobalt dipeptide oxygen complexes based on the assumption of a single step oxidation process to yield cobalt(III) chelates and hydrogen peroxide. In a more recent paper Stadtherr et al.[13] proposed a revised scheme in which the oxidation of cobalt(II) to cobalt(III) does not necessarily go through the oxygen complex. In such systems the oxygenated species would be a relatively unreactive side product, with the main reaction pathway being direct oxidation of the cobalt(II) chelate. The relative importance of the two pathways is presumably indicated by the production of oxygen or peroxide. In systems where these compounds are detected during oxidation, the oxygen complex would be considered the reactive species. In those systems where neither oxygen nor peroxide is observed, the main pathway proposed is direct oxidation of the cobalt(II) chelate.

At about the same time that Michailidis and Martin[7] reported their kinetic data, Gillard and Spencer[10] proposed a multistep oxidation mechanism, based on eletronic spectra, manometric data, and CD-ORD spectra. The first two steps in their reaction scheme are the protonation and rearrangement of the peroxo bridge, and the release of peroxide to form monomeric cobalt(III) chelates. At this stage of the reaction, they propose that the peroxide may either disproportionate or oxidize the excess ligand which is usually present in studies of dipeptide complexes. In order to explain the lack of stereoselectivity in the formation of the oxygen complex, Gillard and Spencer[10] also proposed that the dipeptide ligands in the brown complex are bound in a bidentate fashion through the deprotonated amide nitrogen and the terminal amino groups. They reported the isolation of the brown oxygen complex, a red protonated intermediate, and the red bis(dipeptide)cobalt(III) chelate from reaction mixtures at various stages in the oxidation process of the glycyl-tyrosine complex. They also observed that in some systems there was remittal of oxygen during the latter stages of the oxidation, while with other ligands there was either no change in the pressure of oxygen or a slow continuous uptake of oxygen in excess of the amount required for complete oxygenation. It was proposed that the remittal of oxygen was the result of the disproportionation of hydrogen peroxide released during the oxidation of the oxygen complex, and that the slow uptake of oxygen was indicative of oxidation of the ligand.

We now report extensive spectroscopic, polarographic, and potentiometric data on the rates of oxidative rearrangement

of the oxygen complexes of the cobalt(II)-dipeptide complexes of glycylglycine, glycyl-L-alanine, glycyl-L-serine, glycyl-L-tyrosine, L-alanyl-L-alanine, L-alanylglycine, and L-seryl-glycine. The data show that the oxidation occurs in at least two steps. The first step is the faster and involves the uptake of hydrogen ion from solution. The second step is slower and is apparently independent of pH. The polarographic data also show that neither oxygen nor hydrogen peroxide are released in significant amounts during the oxidation process.

Experimental Section

Spectral Studies. Electronic spectra were recorded on a Cary 14 spectrophotometer. Solutions were maintained at 25.0 °C by using a jacketed cell holder connected to a thermostated water bath. The ionic strength of each solution was adjusted to 0.10 M by the addition of KNO_3. In order to minimize precipitation of the metal, a tenfold excess of ligand was present in all solutions. Kinetic runs were initiated by the addition of cobalt to a high-pH, oxygen-saturated solution of the ligand. Spectral measurements were begun within 1 min of mixing. Except with glycyltyrosine, the formation of the oxygen complex was treated as an instantaneous process, so that the time required for the formation was not considered in the treatment of the data. The pH values of these kinetic solutions were measured 10–15 min after mixing using a Corning Model 12 Research pH meter equipped with a combination electrode and standardized with dilute HCl to read $-\log [H^+]$ directly.

pH Studies. In order to accurately determine the changes in pH that occur during the oxidation process, reactions were run in a sealed, water-jacketed titration cell, and the hydrogen ion concentration was measured using a Beckman Research Model pH meter equipped with glass and calomel electrodes and standardized as described above. Solutions were maintained at 25.0 °C and 0.10 M (KNO_3) and were protected by an atmosphere of CO_2-free nitrogen.

Polarographic Studies. Differential pulse and sampled dc polarographic measurements were made using a Princeton Applied Research Model 174A polarographic analyzer. All measurements were made against a saturated calomel reference electrode. Solutions contained 0.10 M KNO_3 and a 30- to 40-fold excess of ligand and were protected from the atmosphere by a layer of oxygen-free nitrogen flowing across the surface of the solution.

Results

The absorbance spectrum as a function of time on an oxygenated 10:1 Co:glycylglycine solution is shown in Figure 1. The strong absorbance below 400 nm is due to a charge transfer band characteristic of the oxygenated species and shows a continuous decrease with time. The asorbance at 520 nm, however, initially decreases and then increases to its maximum value at infinite time. The presence of a minimum in the plot of the absorbance at 520 nm vs. time requires the existence of at least two, and possibly more, separate steps in the overall oxidation process, with an intermediate compound having a molar absorptivity at 520 nm which is less than that of either the oxygen complex or the final product. The rate of the first step, which involves the disappearance of the brown oxygen complex, can be conveniently followed by measuring the absorbance at 350 nm. It is obvious from a comparison of the initial and final spectra (Figure 1) that the final cobalt(III) chelate makes a very minor contribution to the absorbance at this wavelength. Although a complete spectrum of the intermediate species was not obtained, the fairly minor changes in the 350-nm region of the spectrum that result from the conversion of the intermediate to the final product as compared to the changes in the 530-nm region suggest that the intermediate in the reaction is also an unoxygenated cobalt(III) chelate, and thus would be expected to have a relatively small extinction coefficient at 350 nm. Thus the change in the absorbance spectrum at 350 nm provides an accurate measure of the rate of the initial step in the oxidation process, regardless of the relative rates of subsequent reactions. It was found that the spectral changes can be described by a first-order rate

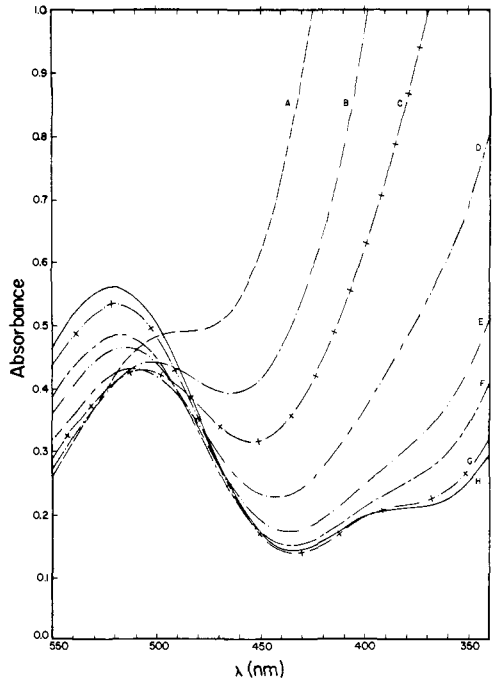

Figure 1. Absorbance spectrum of the bisglycylglycine–cobalt–dioxygen complex as a function of time: A, 8.3 min; B, 17.8 min; C, 27.7 min; D, 46.2 min; E, 77.7 min; F, 107.7 min; G, 188.7 min; H, 276.7 min; [Co] = 1.50×10^{-3}; [GG] = 1.50×10^{-2}; pH 10.50; μ = 0.10 M (KNO_3); t = 25 °C.

equation, which after integration is written as eq 1:

$$k_1 t = -\ln (A_t - A_i) + \ln (A_0) \quad (1)$$

where A_0, A_t, and A_i are absorbances at 350 nm at the time of mixing, any intermediate time t, and infinite time, respectively. Values of k_1 were determined by plotting $\ln (A_t - A_i)$ vs. t. Both the linearity of these plots and the invariance of k_1 to changes in the initial concentration of the oxygen complex support the proposed order of the reaction. Both the rate and pH dependence of the initial step vary considerably with changes in the ligand. The variation of k_1 as a function of pH is shown in Figure 2, and sample values are given in Table I.

The spectra of glycyltyrosine-cobalt-dioxygen systems are anomalous in two respects. The absorbance at 350 nm does not reach a maximum until about 10 min after the addition of cobalt to alkaline, oxygen-saturated solutions of the ligand. The value of k_1 reported for this ligand represents the eventual rate of decrease of the 350-nm absorbance without any consideration of data obtained prior to the absorbance maximum. It was also found that after standing for 2–3 weeks, the glycyltyrosine reaction mixtures begin to show an increase in the residual absorbance at 350 nm. The value of k_2 reported below for this system was determined only from data collected prior to the beginning of this increase.

It was found that the second step in the oxidation process is appreciably slower than the first, so that for at least one half-life of the initial brown to red conversion, one is dealing with essentially only the first step in the oxidative rearrangement. By using the rate constant of the initial reaction and the molar absorptivities of the initial brown dioxygen complex and of the final red complex at 520 nm, determined by the extrapolation of the absorbance at 520 nm to zero and infinite times, the molar absorptivity of the intermediate complex B was calculated by the use of eq 2.

$$\epsilon_B = 2\left[\frac{\text{Abs} - \epsilon_A A_0 e^{-k_1 t}}{T_M(1 - e^{-k_1 t})}\right] - \epsilon_C \quad (2)$$

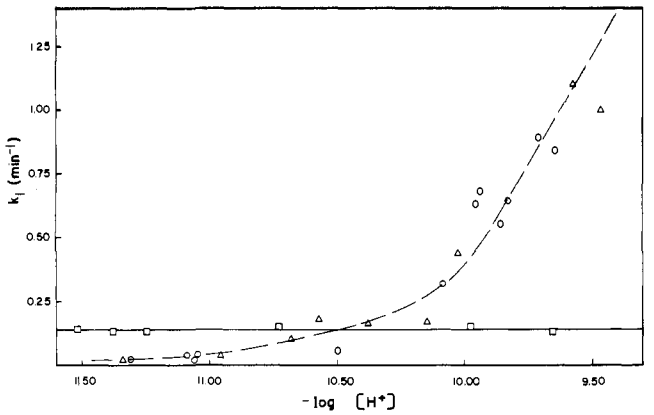

Figure 2. Variation in calculated values of k_1 as a function of pH for the cobalt dioxygen systems with the ligands glycylglycine (○) alanylglycine (△), and alanylalanine (□): μ = 0.10 M (KNO_3); t = 25 °C.

Table I. Rate Constants for the Initial Step in the Oxidative Rearrangement of Cobalt–Bis(dipeptide)–Dioxygen Complexes[a,b]

−Log [H$^+$]	$10^2 k_1$ (s^{-1})	−Log [H$^+$]	$10^2 k_1$ (s^{-1})	−Log [H$^+$]	$10^2 k_1$ (s^{-1})
Gly-gly		Gly-L-ala		L-Ala-gly	
9.65	1.4	9.68	0.15	9.47	1.7
9.71	1.5	9.96	0.15	9.58	1.8
9.83	1.1	10.00	0.097	10.03	0.73
9.96	1.1	10.25	0.20	10.15	0.28
10.09	0.53	10.26	0.20	10.38	0.27
10.50	0.092	10.60	0.18	10.68	0.17
11.09	0.063	10.86	0.15	10.96	0.062
11.31	0.043	11.76	0.12	11.34	0.033
Gly-L-ser		L-Ser-gly		L-Ala-L-ala	
9.87	0.11	8.82	0.088	9.66	0.22
9.92	0.080	9.76	0.097	9.98	0.25
10.28	0.11	10.00	0.087	10.73	0.25
10.30	0.11	10.37	0.057	11.25	0.22
10.63	0.10	10.79	0.030	11.38	0.22
11.70	0.088	11.05	0.022	11.52	0.23
		Gly-L-tyr			
		9.25	0.043		
		9.97	0.043		
		9.98	0.032		
		10.25	0.028		
		10.54	0.030		
		10.55	0.018		

[a] Values obtained at μ = 0.10 M (KNO_3) and 25 °C. [b] Values listed are from single determinations.

in which Abs represents the total absorbance at 520 nm, ϵ_A, ϵ_B, and ϵ_c are the molar absorptivities of the oxygen complex A, the cobalt(III) intermediate B, and the bis(dipeptide)-hydroxocobalt(III) chelate C, respectively, T_M represents the total analytical concentration of cobalt, and A_0 is the initial concentration of oxygen complex. By working at high pH, the complete formation of the oxygen complex is assured and A_0 is equal to $\frac{1}{2}T_M$. Equation 2 is based on the reaction mechanism outlined below, in which the initial step results in the formation of equal amounts of the intermediate B and the final product C. The molar absorptivities determined by this method agree with all previously reported values and are listed in Table II.

After a set of molar absorptivities and k_1 had been calculated, the concentrations of the three proposed species in solution were determined as a function of time from the absorbance at 520 nm and the following set of equations.

Table II. Molar Absorptivities at 520 nm of Cobalt Dioxygen Complexes and Oxidative Rearrangement Products[a]

Ligand	ϵ_A	ϵ_B	ϵ_C	ϵ_C (lit.)	Ref
Gly-gly	710	80	400	384	4
Gly-ala	360	19	300	310	11
Gly-ser	478	63	347		
Gly-tyr	450	32	390		
Ala-gly	615	123	405	392	11
Ser-gly	530	45	363		
Ala-ala	435	15	326	326	11

[a] Values determined at μ = 0.10 M (KNO_3) and 25 °C.

Table III. Average Values of k_2, Rate Constant for Conversion of Intermediate to Final Product[a]

Ligand	$10^4 k_2$ (s^{-1})	Ligand	$10^4 k_2$(s^{-1})
Gly-gly	1.6 ± 0.5	Gly-tyr	0.035 ± 0.017
Ala-gly	1.1 ± 0.2	Gly-ser	1.0 ± 0.3
Ser-gly	0.14 ± 0.03	Ala-ala	2.3 ± 1

[a] Values determined at μ = 0.10 M (KNO_3) and 25 °C.

$$\text{Abs} = \epsilon_A[A]l + \epsilon_B[B]l + \epsilon_C[C]l \quad (3)$$

$$T_M = 2[A] + [B] + [C] \quad (4)$$

$$[A] = \tfrac{1}{2}T_M e^{(-k_1 t)} \quad (5)$$

In the systems

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

where the rate of A → B is large compared to that of B → C the concentration of A quickly becomes negligible, so that the data from later times may be treated as a simple conversion of B to C. It was found that these data fit a first-order plot of ln (B) vs. t. The rate of the second step appears to be independent of pH, and the average values obtained for k_2 are listed in Table III.

The oxidation process was also studied polarographically. The differential pulse polarogram of a bis(glycylglycinato)-cobalt(II) solution at pH greater than 11 shows an oxidation peak at −0.53 and a reduction peak at −1.46 V vs. SCE. Oxidation and reduction peaks are distinguished by running sampled dc polarographs where they exhibit negative and positive current, respectively. As oxygen is bubbled through this solution, the peak at −0.53 V decreases. As oxygenation reaches completion, a reduction peak for free oxygen appears at −0.15 V, along with a broad peak centered around −0.95 V which is due to the reduction of hydrogen peroxide formed in the electrochemical reduction of oxygen.

As the brown oxygen complex begins to rearrange, a new reduction peak develops at −0.73 V. However, in the presence of free oxygen, this new peak is partially obscured by the peroxide peak. In order to follow the development of this peak without the peroxide interference, nitrogen was bubbled through freshly prepared solutions of the oxygen complex at pH 11–11.4. At such high pH's the formation of the oxygen complex is sufficiently irreversible to degassing with nitrogen to allow essentially complete removal of free oxygen from solution. Polarograms obtained by this method are shown in Figure 3. The peak at −0.73 V increases initially, but then decreases as the reduction peak at −0.53 V continues to develope. The rate of decrease of the −0.73-V peak can be described by a first-order rate expression, with a calculated rate constant of 0.54×10^{-4} s^{-1}, which is roughly equivalent to the

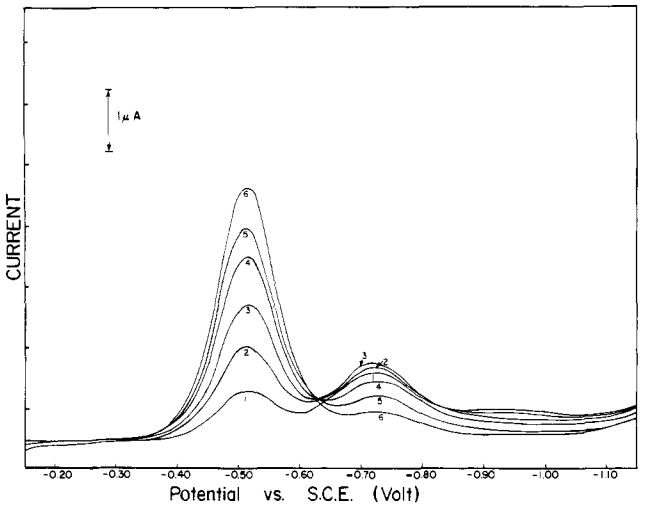

Figure 3. Differential pulse polarograms of a degassed solution of the glycylglycine dioxygen complex as a function of time at pH 11.3: 1, 18 min; 2, 35 min; 3, 53 min; 4, 83 min; 5, 121 min; 6, 188 min; $[Co]_t = 1.50 \times 10^{-3}$; $[GG] = 4.00 \times 10^{-2}$; $[KNO_3] = 0.100$; $t = 25$ °C.

value for k_2 determined spectroscopically. Therefore, the reduction peak at −0.73 V is assigned to the intermediate complex **B**, and the peak which develops at −0.53 V is assigned to the final cobalt(III) chelate. The region of the polarogram from −1.2 to −1.5 V is more difficult to evaluate because the peaks strongly overlap one another.

In following the complete oxidative rearrangement of the glycylglycine and alanylglycine dioxygen complexes, no appreciable amount of hydrogen peroxide was detected polarographically. When peroxide was added to the reaction mixture after the oxidation reaction had reached completion, a large peak appeared at about −1.0 V. From the slow decrease in this peak, it appears that hydrogen peroxide has a half-life of at least 20 h under the conditions at which the oxidation was studied. With such a long residence time in solution, any peroxide released during the reaction should be detected, unless it is rapidly consumed in some subsequent reaction in the oxidation process.

The polarograms of the final reaction mixtures formed from the dioxygen complexes also lack indication of the development of a free oxygen reduction peak. The detection of oxygen is complicated by the necessity of blowing nitrogen across the surface of the solutions to prevent diffusion of atmospheric oxygen into solution. It was found that the nitrogen blanket results in the slow loss of oxygen from air saturated KNO_3 solutions. This diffusion of oxygen out of solution is described by a first-order rate equation $-d[O_2]/dt = k_0[O_2]$. A plot of the natural log of the oxygen peak height vs. time gave a rate constant of 0.0051 min^{-1} corresponding to a half-life of oxygen in solution of about 2 h. Since the first step in the oxidation has a rate appreciably faster than this rate of diffusion, one would expect to see a definite oxygen peak if free oxygen were being released in the first step. One cannot, however, conclusively rule out the slow release of oxygen in the second step of the oxidation, since the rate of this step is comparable to the observed rate of oxygen diffusion.

The absence of either oxygen or peroxide in solution indicates that the bridging peroxide moiety is either released in solution as water, or is very rapidly reduced to water in solution. Since it is obvious that one cannot form the oxygen complex from cobalt(III) chelates and water, the step in the oxidation process in which the bridging peroxide is lost must be effectively irreversible. As discussed earlier, it is believed that the peroxide is lost in the first step of the oxidation, since the electronic spectrum of the intermediate complex appears to be a typical cobalt(III) spectrum. Therefore, the first step in the oxidative rearrangement must be essentially irreversible.

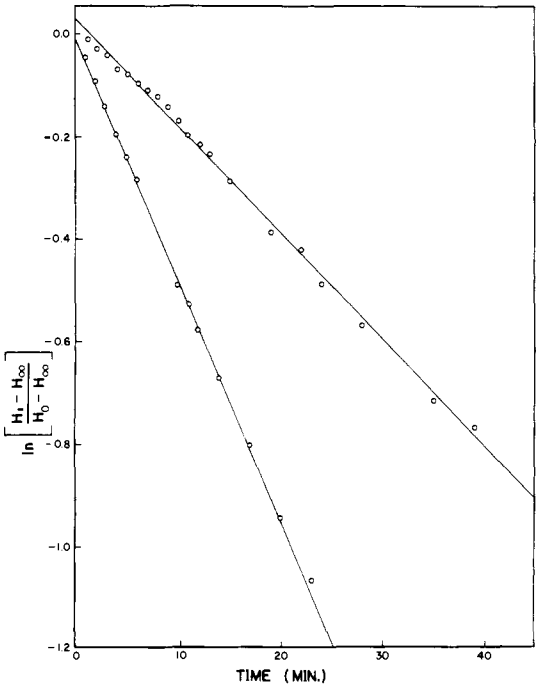

Figure 4. Normalized first-order plot of $\ln [(H_t - H_\infty)/(H_0 - H_\infty)]$ vs. time, where H_0, H_∞, and H_t represent hydrogen ion concentration at zero time, infinite time, and any intermediate time t, respectively, showing the rate of change in the hydrogen ion concentration during the oxidative rearrangement of the glycylglycine-cobalt-dioxygen complex when the initial pH's are 10.25 (lower line) and 10.60 (upper line): $\mu = 10$ M (KNO_3); $t = 25$ °C.

In the glycyltyrosine system detectable amounts of peroxide and dioxygen are observed polarographically during the oxidation process. However, polarographic investigation of this system is hampered by the fact that passing nitrogen through the solution tends to dissociate the oxygen complex. Thus it is not possible to study the oxidation in the absence of atmospheric oxygen, and any conclusion that oxygen and peroxide are being released by the oxidation reaction would have to be based on the rate of change of the height of the peaks assigned to these compounds. The presence of atmospheric oxygen and the overlap of the oxygen and peroxide peaks with cobalt related signals would make conclusions based on these data somewhat speculative. However, it was observed that H_2O_2 added to glycyltyrosine-cobalt-dioxygen solutions had a residence half-life of only about 1 h compared to the 20-h half-life in the glycylglycine-cobalt-dioxygen systems. Thus there must be some interaction between peroxide and either free glycyltyrosine or the glycyltyrosine chelate.

The changes in pH of solutions of the glycylglycine-cobalt-dioxygen complexes were measured throughout the course of the oxidation process. It was found that at initial pH's below 10.7, about 0.5 mol of hydrogen ion was taken up per mole of cobalt in solution, and that the rate of change of hydrogen ion concentration corresponds to the rate of the first step in the oxidation process as measured spectrophotometrically. Plots of $-\log [H^+]$ vs. time at different initial pH values are shown in Figure 4. The values of k_1 obtained by this method are 7.7×10^{-4} s^{-1} at pH 10.4 and 3.4×10^{-4} s^{-1} at pH 10.65, compared to spectrophotometric values of approximately 1×10^{-3} and 7×10^{-4} s^{-1}. When the oxidation was carried out at pH 11.2 or higher, the pH remained essentially constant throughout the oxidation process. This result is due either to the formation of a different product (e.g., a hydroxocobalt(III) complex) not requiring hydrogen ion, or simply to the increased self-buffering of the solution. At pH 11.3, the calculated

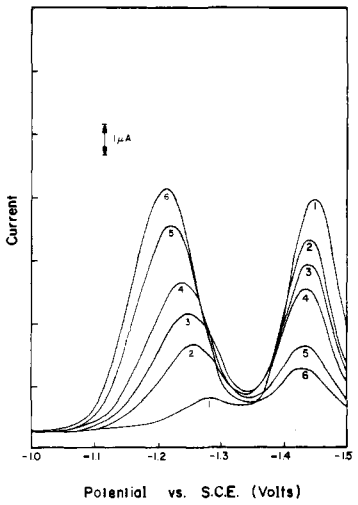

Figure 5. Differential pulse polarograms of a bis(glycylglycinato)cobalt(III) solution at the following pH values: 1, 11.24; 2, 11.13; 3, 11.06; 4, 10.97; 5, 10.73; 6, 10.13; $[Co]_t = 9.93 \times 10^{-4}$; $[GG] = 3.97 \times 10^{-2}$; $[KNO_3] = 0.100$; $t = 25$ °C.

change in pH is 0.06 pH units, which is well within the normal detection limits of the pH meter. The linearity of the plots in Figure 4 and the attainment of a constant pH after the first step in the oxidation has reached completion indicate that the second step does not involve the release or uptake of hydrogen ions.

The final product of the oxidative rearrangement of the glycylglycine dioxygen complex has been identified crystallographically as the bis(glycylglycinato)cobalt(III) chelate,[12] in which both amide groups are deprotonated except in strongly acid (ca. 1 M) solution. In acidic or slightly basic solutions, the product observed in this study has an NMR and visible spectrum and a polarogram, which agree with those previously reported for the glycylglycinecobalt(III) complex.[2,5,11,12] However, the oxidation kinetics are run at higher pH values than those employed in the previous studies, and there is strong polarographic evidence that hydrolysis of the complex occurs in the pH range 10–12. Figure 5 shows the polarogram of a solution of the cobalt(III) glycylglycine complex produced by the high pH oxidative rearrangement of the dioxygen complex and the changes that result from the addition of hydrochloric acid. The reduction peak at −1.46 V decreases with the addition of acid, accompanied by simultaneous development of a new peak at −1.22 V. This addition of acid also results in an anodic shift to −0.51 V and a slight decrease in the −0.53-V peak. The two potentials observed at lower pH agree with the values reported by Tang and Li.[5] This shift is completely reversible upon addition of strong base. Since there are no dissociable ligand protons, any reversible deprotonation is almost certainly indicative of a chelate hydrolysis reaction. Calculations based on the increase in the −1.22-V peak and the decrease in the −1.46-V peak both give a value of the log of the chelate hydrolysis constant of -10.8 ± 0.2. The agreement of the two calculations lends support to the proposal that the peaks represent two species linked by some proton dependent equilibrium. Thus it appears that the product of the high pH oxidative rearrangement is actually a mixture of normal and hydroxo complexes of the cobalt(III) dipeptide chelate.

Discussion

From an inspection of Figure 2, it is obvious that reaction rates of the systems investigated display wide range dependence on the concentration of hydrogen ion. In the only previous study of this specific subject, Tanford et al.[4] observed the same general pH dependence in the oxidation of the glycylglycine oxygen complex, although they obtained values of k_1 slightly lower than those reported here. A portion of this discrepancy is the result of plotting $\ln (A_t - A_i)$ in this work rather than $\ln (A_t)$. This was done to take into account the fact that the absorbance at 350 nm does not go to zero at the end of the first step in the oxidation, but rather falls to some nonzero minimum.

The nonintegral dependence on $[H^+]$ observed in most systems suggests that the initial step in the internal redox rearrangement can occur by more than one pathway. There are several alternatives available, including direct protonation of the peroxide bridge, dissociation of one of the cobalt chelates followed by rapid hydrolysis, or direct S_N2 attack by hydroxide ion on one of the cobalt centers. It is unlikely that any one reaction pathway can account for all of the observed results. One obvious pattern in the values of k_1 is that glycine C-terminal dipeptides exhibit a much more pronounced dependence on hydrogen ion concentration than do the remaining ligands. The presence of glycine in the C-terminal position apparently does not, however, produce a uniform effect on the magnitude of k_1, since both glycylglycine and alanylglycine react much faster than serylglycine. Alternatively, an alanine in the C-terminal position results in a comparatively high value of k_1, but no specific pH dependence.

It has been assumed throughout this study that the primary rearrangement reaction begins with the μ-peroxo-bridged oxygen complex. On the basis of our results, it does not appear that the proposal by Martin et al.,[13] in which the principle reaction pathway is direct oxidation of the cobalt(II) chelate with the oxygen complex present as a relatively unreactive side product, is valid for these systems. If this were the correct mechanism, one would expect the rate of oxidation to increase as the concentration of unoxygenated cobalt(II) chelate increases. Thus there should be an increase in the rate of oxidation with decreasing pH and some correlation between this rate and the equilibrium constant for oxygenation. This type of pH dependence is observed in only half the systems studied, and in some cases the dependence on hydrogen ion is in the opposite direction, with the rate of oxidation increasing with increasing pH. In addition, the value of k_1 does not appear to be related to the magnitude of the reported oxygenation constants for chelates of these ligands.[8]

There are several indications that the initial step in the oxidative rearrangement may involve oxidation of one of the ligand molecules. The strongest evidence for this is the absence of either peroxide or oxygen in the reaction mixtures. Since it is a certainty that the dioxygen moiety is lost from the cobalt chelate, the only remaining form in which it could enter solution is as water. The most reasonable source of the two additional electrons required to reduce the dioxygen bridge to water is the ligand.

Margerum has already demonstrated that Cu(III) and Ni(III) complexes of polypeptide ligands in aqueous solution decompose in the presence of oxygen via oxidation of the ligand.[14-16] Product analysis in the specific case of the Ni-tetraglycine system[14] shows that oxidation at each amide nitrogen-α carbon bond, which after hydrolysis yields the corresponding amide, accounts for 75% of the reaction product. Carbon dioxide, ammonia, and glyoxylic acid were also produced in significant quantities. An analogous scheme for the cobalt-dipeptide-dioxygen system is outlined below:

$$\underset{\text{A}}{Co_2(H_{-1}GG)_4O_2^{4-}} + H^+ \xrightarrow{k_1} \underset{\text{B}}{Co(H_{-1}GG)(GG_{ox})OH_2^-} + \underset{\text{C}}{Co(H_{-1}GG)_2OH^{2-}} \quad (6)$$

$$Co(H_{-1}GG)(GG_{ox})OH_2^- \overset{\text{fast}}{\rightleftharpoons} Co(H_{-1}GG)(GG_{ox})OH^{2-} + H^+ \quad (7)$$

$$Co(H_{-1}GG)(GG_{ox})OH^{2-} + GG^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Co(H_{-1}GG)_2OH^{2-} + GG_{ox} \quad (8)$$

where GG^-, $H_{-1}GG^{2-}$, and GG_{ox} represent normal, amide deprotonated, and oxidized forms of glycylglycine. As mentioned earlier, reaction 6 shows a nonintegral dependence on $[H^+]$, and the pH dependence varies widely with changes in the ligand. Thus it appears that the reaction A → B + C actually proceeds through several pathways which yield the same final products but differ in the pH dependence of the rate limiting step. In this case k_1 would be the sum of several microconstants, each multiplied by the appropriate power of the hydrogen ion concentration.

The proposal that both B and C are formed in reaction 6 is suggested by the fact that organic compounds usually undergo two electron oxidations or reductions. Since the reduction of peroxide to water requires the two electron oxidation of only one ligand molecule, it would be impossible to form two identical cobalt(III) complexes. The polarograms shown in Figure 3 provide evidence supporting this proposal. When the peak at −0.73 V, which corresponds to the intermediate B, reaches its maximum height, the bis(glycylglycinato)hydroxocobalt(III) peak is already at approximately half its maximum height, indicating the simultaneous formation of equimolar amounts of B and C in the first step of the oxidative rearrangement (eq 6). These polarograms then clearly show the interconversion of B to C, which has been identified both by this polarographic data and McKenzie's crystallographic study[12] as the bis(glycylglycinato)cobalt(III) chelate.

One possible mechanism involves monoprotonation of the peroxo bridge, facilitating heteronuclear O–O bond fission, as follows:

$$(H_{-1}GG)_2Co{-}O{-}O{-}Co(H_{-1}GG)_2 \xrightarrow{H^+} (H_{-1}GG)(GG_{ox})Co(OH_2)^- + (H_{-1}GG)_2CoOH^{2-} \quad (9)$$

Thus the results are compatible with an ionic O–O bond rupture and a concerted oxidation of one of the molecules of the coordinated ligand.

The exact nature of GG_{ox} is still uncertain. The two most probable oxidation routes involve the formation of an imine. The first route, involving oxidation at the amide nitrogen, would give $H_2NCH_2CON{=}CHCOO^-$ as GG_{ox}. The second, involving oxidation at the terminal amino group, would yield $HN{=}CHCONHCH_2COO^-$. Although the first alternative appears to be more compatible with Margerum's results,[14] there is nothing in the data presented here favoring one mechanism over the other. Margerum's results also indicate decarboxylation as a possible mode of oxidation, but previous tests by Gillard and Spencer[10] for the presence of carbon dioxide as a product of the oxidative rearrangement of the glycylglycine–cobalt–dioxygen complex were negative, indicating that decarboxylation is not a viable alternative.

If the oxidation of the dipeptide ligand is occurring at the amide nitrogen, it is also necessary to include hydrolysis of GG_{ox} to glycinamide and glyoxalic acid in the first step (eq 6) in order to explain the observed pH dependence of reaction 8. The coordination of the GG^- releases the amide proton into solution. However, release of $H_2NCH_2CON{=}CHCOO^-$ would not result in any balancing protonation reaction. Glycinamide, on the other hand, would be coordinated via a deprotonated amide nitrogen, so that release of this ligand into solution would result in no net change in the pH. Similarly, the release of coordinated $HN{=}CHCON^-CH_2COO^-$ would result in the necessary uptake of one proton to balance the deprotonation of the incoming GG^-.

There is no obvious explanation for the anomolous changes in the absorption spectrum of the glycyltyrosine complex. It has been proposed by Gillard and Spencer[10] that the slow development in the absorption at 350 nm is the result of protonation of the peroxide bridge subsequent to the oxygenation reaction. The formation of this protonated binuclear intermediate is then presumably followed by the release of peroxide into solution. As mentioned above, the reversibility of the glycyltyrosine system to degassing with nitrogen does not permit a reliable polarographic investigation to determine whether or not peroxide is released in the oxidative rearrangement of the cobalt glycyltyrosine dioxygen complex. However, even though this mechanism *may* be valid for the glycyltyrosine system, the definite lack of any oxygen or peroxide peaks in the polarograms of glycylglycine and alanylglycine complexes indicates that it is not valid as a general mechanism for the redox rearrangement of cobalt dipeptide dioxygen complexes. The previous report by Cagliotti et al.[9] of the detection of hydrogen peroxide in a glycylglycine reaction mixture is highly questionable, since the polarograms were run on air-saturated solutions, which always show a reduction wave for hydrogen peroxide resulting from the reduction of molecular oxygen.

Reaction 8 in the proposed mechanism must in principle be reversible. The fact that it was possible to treat it as an irreversible process is probably due to two factors. First, glycylglycine should be a considerably more strongly coordinating ligand than GG_{ox}, since the latter has two fewer electrons and thus would be expected to be a weaker base, so that $k_2 \gg k_{-2}$. Second, there is always at least a tenfold excess of glycylglycine in solution which pushes the equilibrium even farther to the right.

It is recognized that the data available at present do not constitute conclusive proof of the proposed mechanism. It is still necessary to determine which mode of oxidation is involved and if the oxidized ligand hydrolyzes during the course of the oxidative rearrangement. Further work on this subject is currently in progress.

References and Notes

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(2) (a) E. L. Smith, *J. Biol. Chem.*, **173**, 571 (1948); (b) J. B. Gilbert, N. C. Otey, and V. E. Price, *ibid.*, **190**, 377 (1951).
(3) G. W. Miller, B. T. Gillis and N. C. Li, *J. Biol. Chem.*, **325**, 2840 (1960).
(4) C. Tanford, D. C. Kirk, Jr., and M. K. Chantooni, Jr., *J. Am. Chem. Soc.*, **76**, 5325 (1954).
(5) P. Tang and N. C. Li, *J. Am. Chem. Soc.*, **86**, 1293 (1964).
(6) G. W. Miller and N. C. Li, *Trans. Faraday Soc.*, **57**, 2041 (1961).
(7) M. S. Michailidis and R. B. Martin, *J. Am. Chem. Soc.*, **91**, 4683 (1969).
(8) W. Harris, G. McLendon, and A. E. Martell, *J. Am. Chem. Soc.*, in press.
(9) V. Cagliotti, P. Silvestroni, and C. Furlani, *J. Inorg. Nucl. Chem.*, **13**, 95 (1960).
(10) R. D. Gillard and A. Spencer, *J. Chem. Soc. A*, 2718 (1969).
(11) P. J. Morris and R. B. Martin, *Inorg. Chem.*, **10**, 964 (1971).
(12) E. D. McKenzie, *J. Chem. Soc. A*, 1655 (1969).
(13) L. G. Stadtherr, R. Prados, and R. B. Martin, *Inorg. Chem.*, **12**, 1814 (1973).
(14) E. B. Paniago, D. C. Weatherburn, and D. W. Margerum, *Chem. Commun.*, 1427 (1971).
(15) D. W. Margerum, K. L. Chellappa, F. P. Bossa, and G. L. Burce, *J. Am. Chem. Soc.*, **97**, 6894 (1975).
(16) F. P. Bossa and D. W. Margerum, *J. Am. Chem. Soc.*, **98**, 4003 (1976).