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Stereoselectivity in Dipeptide Complexes of Cobalt(III)[1]

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Received January 26, 1973

The product 2:1 dipeptide–cobalt(III) complexes obtained by oxygenation of solutions containing Co(II) and dipeptides with at least one asymmetric center consist of a mixture of diastereomers. Proton magnetic resonance results indicate that the ratio of the pair of diastereomers obtained with L-alanylglycine varies from 2.1 to 0.7 as the pH is changed. Chemical shift differences are employed to identify the isomers and the visible circular dichroism associated with each isomer is estimated. Stereoselectivity is also observed in the formation of Co(III) complexes of glycyl-L-alanine, L-valylglycine, and L-phenylalanylglycine formed by the oxygenation route. It is suggested that stereoselectivity may originate in the formation of a binuclear peroxocobalt(III) intermediate. Glycyl-L-histidine appears to serve as a quadridentate ligand with Co(II) and oxygenation yields a binuclear peroxocobalt(III) complex that also possesses a hydroxo bridge. Mononuclear Co(III) product complexes that are formed slowly may not always reflect the composition of the predominant Co(II) or oxygenated species.

Research in several laboratories has shown that light pink or yellow Co(II) complexes may undergo reversible oxygenation to yield brown binuclear complexes, best described as peroxo complexes of Co(III), which decompose, usually irreversibly, to give red mononuclear complexes of Co(III). It is often convenient to refer to the brown intermediate complex as an oxygenated complex of Co(II). This description points up the rapid rearrangements possible due to easy access to the labile Co(II) species in the reversible first step. For those complexes that liberate protons in the oxygenation step, admission of nitrogen or other inert gas is insufficient to reclaim the initial Co(II) complex since the appropriate number of equivalents of acid must also be added.[2] Whether or not acid is liberated in the oxygenation step, acidification of a solution containing the brown, binuclear complex releases oxygen and regenerates Co(II) starting materials. Though only two nitrogen donors are sufficient for oxygenation in bis(salicylaldehyde imine) complexes of Co(II), three nitrogen donors seem necessary with amine and dipeptide ligands if significant amounts of oxygenated or product Co(III) complexes are to be formed in a reasonable length of time at room temperature.[2,3] Whether an appreciable concentration of the oxygenated complex builds up and how rapidly the product Co(III) complex is formed depend upon the ligands used and the pH at which the reactions are conducted. In the case of dipeptides appreciable buildup of intermediate occurs only at high pH.[2] Because of its transitory existence in dipeptide complexes, a red hydroperoxo-bridged dimer intermediate is not considered in this paper.[4]

With those hexacoordinate transition metal ions able to promote amide hydrogen ionization dipeptides yield 2:1 complexes with two pairs of amino and ionized amide nitrogen and carboxylate oxygen donor atoms. Planarity of the amide bond enforces a meridional structure for each set of three donor atoms from a terdentate dipeptide ligand. This structure has been found by X-ray diffraction analysis of crystals of the 2:1 glycylglycinate complexes of Ni(II)[5] and Co(III).[6] The same backbone structure has been inferred in solution by titration studies for a variety of dipeptide complexes of Ni(II)[7] and Co(II).[2,8] It is due to the Co(II)-promoted amide hydrogen ionization that at least three nitrogen atoms serve as donors making oxygenation possible.[2] Oxygenation of dipeptide complexes of Co(II) eventually yields mononuclear oxidized Co(III) complexes with the same backbone structure.[2,4,6,8] Though both Cu(II) and Pd(II) promote amide hydrogen ionization, tetragonality effects are so strong that axial positions are not occupied in peptide complexes of these metal ions.[9,10] Ni(II) also yields planar complexes with tri- (and higher) peptides.[7,11]

With glycylglycine the 2:1 complex with hexacoordinate metal ions possesses only C_2 symmetry and equal amounts of two optical isomers occur. When a dipeptide is composed of one or two L-amino acid residues, its 2:1 complex gives rise to diastereoisomers which may not be produced in equal amounts. Previous work from this laboratory has indicated by proton magnetic resonance a nonexclusive preference for one diastereomer in solutions containing 2:1 complexes of Co(III) with both L-alanylglycine and L-phenylalanylglycine prepared by the oxygenation route.[8] One of the purposes of this paper is to utilize pmr to determine the isomeric ratios of a variety of dipeptide complexes of Co(III) and to report the circular dichroism of these solutions of known compositions. This study of 2:1 dipeptide complexes of Co(III) prepared by oxygenation of Co(II) complexes may be viewed as complementary to the work of Gillard and coworkers who have synthesized the same complexes from Co(III) starting materials and isolated the diastereomers.[12]

Due to the basic imidazole side chain glycyl-L-histidine offers modes of chelation in addition to those of other dipeptides that chelate as substituted glycylglycines. With Cu(II), Ni(II), and Pd(II) Gly-L-His chelates *via* amino and ionized amide nitrogen and carboxylate oxygen donor

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atoms.[10,13] At pH >9 a polymeric species, probably a tetramer, is formed.[14] Compared to the relatively strong interaction with the above three divalent transition metal ions, the reaction of Gly-L-His with Co(II) appears notably weaker. A 2:1 solution undergoes a shift of the absorption maximum to longer wavelengths at about pH 13 suggesting substitution by hydroxide.[15] However, the system does take up oxygen[2] and this paper reports properties of the Co(II), oxygenated, and product Co(III) complexes of Gly-L-His produced at room temperature. Results for the Co(III) complexes may be compared with those obtained at higher temperature.[16]

Results

Proton magnetic resonance spectra of solutions prepared by oxygenation and oxidation of solutions containing 2:1 molar ratios of L-alanylglycine and Co(II) show the presence of two isomers of Co^{III} (L-AlaGly)$_2^-$. The pmr spectra are exceptionally clean for this dipeptide, indicating the absence of other species. Two pairs of methyl doublets (J = 7.2 Hz) are separated by 0.025 ppm, two methine quartets by 0.19 ppm, and two glycyl methylene singlets by 0.06 ppm. From heights of the methyl peaks relative populations of each isomer may be estimated. As shown in Table I, the relative percentages of the two isomers vary softly with the pH that is maintained during the course of the reactions that form the Co(III) complex. Table I also lists the molar absorptivity of each solution where it absorbs maximally at 520 nm and reports the wavelengths and magnitudes of the CD extrema from 330 to 700 nm. Tight isosbestic points occur at 445 and 532 nm in the family of CD curves as the pH is varied.

Unfortunately the isomeric ratios of 2:1 product Co(III) complexes of other dipeptides exhibit insufficient pH dependence to permit an analysis similar to that made for L-AlaGly. Nevertheless information concerning stereoselectivity was obtained in a variety of 2:1 dipeptide complexes of Co(III) prepared by the oxygenation route. The final complex with Gly-L-Ala is formed slowly over a period of days. At pH 9.5 relative peak heights yield 58% for the isomer with the upfield methyl doublet (J = 7.1 Hz) and 42% for the isomer with the doublet 0.027 ppm downfield (J = 7.0 Hz). The absorption maximum at 520 nm gives ϵ 350 and CD extrema appear at 382 and 510 nm with $\Delta\epsilon = -0.3$ and -2.1, respectively. The Co(III) complexes of other glycylamino acid dipeptides form slowly by the oxygenation route at room temperature and do not yield clear-cut pmr spectra.

Chemical shift nonequivalence is displayed in pmr spectra by the glycyl methylene hydrogens of the 2:1 cobalt(III) dipeptide complexes of Gly-L-Ala and GlyGly. In all cases $J \simeq 16.4$ Hz. The chemical shift difference is 0.26 ppm for the major isomer and 0.15 ppm for the minor isomer of the Gly-L-Ala complex. The latter value is identical with that observed for the amino terminal glycyl hydrogens of the 2:1 GlyGly complex. Therefore, for the Gly-L-Ala complexes, we assign the minor isomer as the diastereomer with the methyl side chains near the carboxylate group of the other dipeptide ligand and the major isomer to the structure with the methyl side chains near the amino nitrogens. A corollary to this assignment, useful later, is that the methyl resonances of the major isomer appear at higher field due to their shielding by the nearby carboxylate groups.

Table I. Properties of Solutions Containing Co^{III}(L-AlaGly)$_2^-$

pH	% isomers I	% isomers II	Absorption ϵ at 520 nm	Circular dichroism $\Delta\epsilon$	Circular dichroism λ, nm	Circular dichroism $\Delta\epsilon$	Circular dichroism λ, nm	Circular dichroism $\Delta\epsilon$	Circular dichroism λ, nm
7.5	68	32	423	+0.91	575	−3.48	501	+1.31	401
9.5	59	41	406	+0.57	579	−2.97	502	+1.08	400
11.5	51	49	423	+0.31	583	−2.73	503	+0.93	399
13	41	59	333			−1.82	505	+0.55	396

Three diastereomers are expected for the bis(sarcosylglycine)cobalt(III) complex and three methyl peaks separated by 0.09 and 0.06 ppm are found in the pmr spectrum. However the relative peak heights of 1.1:1.4:1.0 do not correspond well to the 1:2:1 ratios expected for a *RR,RS* = *SR,SS* system.

Two isomers, the major one twice as common as the minor isomer, are also indicated by pmr of the 2:1 L-valylglycine complex of Co(III). Each isomer exhibits two pairs of methyl doublets with J = 7.1 ± 0.1 Hz. With reference to downfield from external TMS at 100 MHz, centers of the methyl doublets for the major isomer occur at 142.4 and 128.6 Hz and for the minor isomer at 139.5 and 118.0 Hz. Centers of the α-methine doublets occur at 361.3 Hz (J = 3.6 Hz) for the major isomer, much upfield from those for the minor isomer at 393.5 Hz (J = 2.4 Hz). The two α,β hydrogen coupling constants quoted are notably less than the value J = 6.1 Hz found in uncomplexed anionic ligand.[17] Singlet glycyl methylene peaks occur at 478.9 and 485.7 Hz for the major and minor isomer, respectively. By an argument similar to that employed above for the L-AlaGly complex, the marked separation of 0.32 ppm for the α-methine doublets suggests that the major isomer is the one with the L-valyl side chains nearer to each other and to the amino nitrogens rather than the carboxylate oxygens. At pH 8 the absorption maximum at 525 nm yields ϵ 455 and CD extrema appear at 407, 509, and 591 nm with the $\Delta\epsilon = +1.5, -5.4$, and +0.8, respectively.

The major isomer constitutes about 70% of the 2:1 L-phenylalanylglycine complex of Co(III). The complex pmr spectrum of the methylene-methine portion of the phenylalanyl residue was successfully interpreted in terms of two overlapping ABC systems. Chemical shifts of the C, A, and B protons occur at 386.9, 344.7, and 334.9 Hz and at 409.4, 362.9, and 323.7 Hz for the major and minor isomers, respectively. The chemical shift difference between the geminal A and B protons is 0.10 ppm in the major isomer and a much greater 0.39 ppm in the minor isomer. For the geminal coupling constant, $J_{AB} = -14.5$ Hz throughout. For the vicinal coupling constants, J_{AX} and J_{BX} are 3.8 and 7.2 Hz for the major and 4.0 and 9.3 Hz for the minor isomer. These vicinal coupling constants are comparable to those found in unbound anionic dipeptide.[17] At pH 9.3 the absorption maximum occurs at 526 nm with ϵ 435 and CD extrema appear at 407, 506, and 580 nm with $\Delta\epsilon = +1.1, -3.8$, and +1.1, respectively. Other aminoacylglycyl-dipeptide complexes of Co(III) form more slowly at room temperature by the oxygenation route and do not yield clear-cut pmr spectra.

The first three rows of Table II summarize results of titrations of glycyl-L-histidine hydrochloride in the presence

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Table II. Equivalents of Base to End Point in Titrations of Glycyl-L-histidine Hydrochloride (GH_3^+), Diaminoethane Dihydrochloride (enH_2^{2+}), and Co(II)

GH_3^+: Co(II): enH_2^{2+}	Equiv	pH	GH_3^+: Co(II): enH_2^{2+}	Equiv	pH
1:1:0	3	8.9	1:1:1	5	10.0
2:1:0	5	9.5	1:1:2	6	9.5
3:1:0	7	9.5	2:1:1	7	10.0

of Co(II). Under nitrogen equimolar mixtures titrate 3 equiv of base by pH 8.9 and admission of oxygen results in the uptake of 0.5 more equiv to maintain the same pH. In 2:1 and 3:1 mixtures 2 and 4 additional equiv are required to yield end points at a higher pH implying that the second and third ligands are only weakly bound if at all to Co(II). In all three cases 0.5 equiv of base is required to maintain the pH upon admission of oxygen. Nearly identical absorption and CD spectra appear for all three ligand to Co(II) ratios at the end points for both the Co(II) complexes on the one hand and their oxygenated complexes on the other. The Co(II) complexes exhibit an absorption maximum at about 502 nm with ϵ 35 and their oxygenated complexes display a shoulder at about 355 nm where ϵ is ≃2400. In contrast the absorption spectra of the product Co(III) complexes that are formed slowly over a period of days at room temperature absorb at shorter wavelengths in the 2:1 and 3:1 mixtures than in the 1:1. The first two solutions are orange and absorb maximally at about 482 nm with ϵ 220 while the last solution is red and displays a maximum at 508 nm with ϵ 170. The CD of the 1:1 Co(III) complex exhibits a positive extremum at the same wavelength as the absorption maximum.

Titrations with Gly-L-His·HCl and Co(II) were also performed with added diaminoethane dihydrochloride (en·2HCl). Table II shows that addition of 1 mol of en·2HCl results in the titration of 2 more equiv of base by pH 10 in both 2:1 and 1:1 mixtures of Gly-L-His·HCl and Co(II). Addition of 2 mol of en·2HCl to a 1:1 mixture titrates 6 equiv of base by pH 9.5. In all three cases just described the titrations were performed under nitrogen. When the titrations were performed throughout under oxygen the same number of equivalents was required in all three cases as with nitrogen, but the end points were achieved at about 0.5 unit lower pH.

Slow addition of standard base to solutions containing 1:1 or 2:1 molar ratios of glycylglycine and Co(II) through which oxygen gas is bubbled yield end points near pH 9 after the addition of 2.5 and 3.0 equiv of base per mole of metal ion, respectively. At higher pH values a fleeting yellow color may be observed but in both cases the solutions at the end points exhibit an absorption maximum at 520 nm with twice the molar absorptivity in the 2:1 solutions.

Several titrations under oxygen were performed with 1:1:1 molar ratios of GlyGly, Co(II), and a second ligand. End points are achieved near pH 9.7 after addition of 4 equiv of base with diaminoethane dihydrochloride, picolinic acid hydrochloride, or dipicolinic acid as the second ligand. Faint brown or yellow colors appeared briefly as intermediates. In all three cases red solutions indicate that the final products are mononuclear Co(III) complexes. The requirement of 4 equiv of base indicates amide hydrogen ionization and demonstrates again the adequacy, for the last two ligands, of three nitrogen donors for oxygenation and oxidation. The mononuclear Co(III) product obtained with dipicolinate absorbed at 520 nm with about half the intensity of 2:1 GlyGly complex, suggesting that disproportionation may have occurred.

Discussion

The diastereomers of 2:1 complexes of terdentate dipeptides containing L-amino acid residues and coordinated meridionally about a hexacoordinate metal ion have markedly different structures. The L-amino acid side chains are directed toward the amino nitrogen of the other chelated dipeptide in diastereomer A and toward the carboxylate oxygens in isomer B. Consequently, the methine hydrogens are nearer to carboxylate oxygens in isomer A than in isomer B. If the carboxylate oxygen results in greater shielding of the methine hydrogens in isomer A these hydrogens should undergo an upfield shift.

The marked upfield position of the methine hydrogen in the major isomers of three dipeptide complexes of Co(III) provides a basis for assigning structures of the diastereomers. For the 2:1 dipeptide complexes of Co(III), the methine hydrogen resonance occurs at 0.19–0.32 ppm higher field in isomer I, the major one at low pH, for L-AlaGly and for the major isomers of L-ValGly and L-PheGly. Thus the major isomer observed in solution is identified with the structural diastereomer A until a definitive X-ray structure analysis of a diastereomer is performed.

If the foregoing structural assignment is correct, in the major isomer the amino acid side chains are directed toward the amino nitrogens (isomer A) rather than the carboxylate oxygens (isomer B). Removal of one of these donor oxygens is required for formation of a binuclear peroxo complex. Side chains in diastereomer B limit access of oxygen to the Co(II). Similarly attack by oxygen is restricted in the 2:1 glycylamino acid dipeptide complexes of Co(II) because removal of the carboxylate oxygen by rotation is impeded by the side chain. These results suggest that formation of oxygenated complexes is stereoselective. The same steric factors that inhibit formation of some oxygenated complexes also favor break-up of those binuclear complexes to mononuclear Co(III) products. Thus the stereoselective preference observed in a more rapidly formed product Co(III) complex probably reflects equilibrium and kinetic features of the oxygenated complex rather than the thermodynamically favored distribution of isomers of the Co(III) product. Additional considerations favoring this conclusion have been presented.[8] In those cases where long times are required for dissociation of binuclear to mononuclear Co(III) complexes stereoselective preferences observed in the latter complexes may more nearly reflect the equilibrium distribution of diastereomers in the products.

It is possible to combine the relative populations of the two diastereomers of the 2:1 L-AlaGly complex of Co(III) determined from pmr with the CD results in Table I to obtain an estimate of the CD due to each pure isomer I and II. All the CD results were scaled to ϵ 415 at 520 nm so that the complex formed over more than 1 month at pH 13 is comparable to those formed in 1–2 days at pH 7.5–11.5. Similar results are obtained if the results at pH 13 are not included in the analysis. Solution of simultaneous equations of the form

$$\Delta\epsilon_{obsd} = \Delta\epsilon_{I}(I) + \Delta\epsilon_{II}(II)$$

yields for $\Delta\epsilon_I$ and $\Delta\epsilon_{II}$ respectively +2.1 and −0.3 at 400 nm, −4.8 and −0.5 at 500 nm, and +2.0 and −1.5 at 580 nm. Thus isomer II with the methyl groups further apart yields the weaker CD that is also negative throughout the visible region. The CD of isomers I and II calculated from the results on solutions that had undergone oxygenation of Co(II) and reported in Table I are in good agreement with values

obtained for nearly pure isomers prepared directly from Co(III) starting materials.[12]

The CD of isomers I and II might be considered as the sum of a vicinal contribution due to the methyl side chains of the two ligands and a configurational contribution due to the disposition of the two ligands about Co(III). By solving simultaneously at the three wavelengths the two equations

$$\Delta\epsilon_I = 2\Delta\epsilon_v \pm \Delta\epsilon_c$$

$$\Delta\epsilon_{II} = 2\Delta\epsilon_v \mp \Delta\epsilon_c$$

we obtain at 400, 500, and 580 nm respectively, for the vicinal contribution, $\Delta\epsilon_v = +0.45, -1.3$, and $+0.1$ and, for the configuration contribution, $\Delta\epsilon_c = \pm 1.2, \mp 2.1$, and ± 1.75. The values for the vicinal contribution may be compared with the CD of Co(III) complexes containing only one L-AlaGly as a ligand and with three NH_3 or one dien at the other positions.[18] Though the shift to shorter wavelengths in the last pair of complexes appears to yield a different sign pattern, a net negative CD of comparable magnitude is obtained for the two complexes as well as for the calculated vicinal contribution.

Since only the imidazolium ($pK_a = 6.8$) and ammonium ($pK_a = 8.2$) protons are titrated in unbound Gly-L-His·HCl, titration of a third equivalent by pH 9 in a 1:1 mixture with Co(II) (Table II) indicates ionization of either a water molecule in the coordination sphere or the peptide hydrogen. The low pH by which the third ionization is complete and the fact that additional ligand titrates as if unbound suggest that peptide ionization is occurring. Gly-L-His contains all the features of GlyGly where amide hydrogen ionization does not occur with Co(II) until near pH 10[2,8] and hence must be bound more strongly. We suggest that GlyHis binds to Co(II) as a quadridentate ligand. Consistent with this view, addition of 1 or 2 mol of en·2HCl to 1:1 or 2:1 mixtures of GlyHis·HCl simply results in titration of 2 additional equiv (Table II) and binding of en to Co(II). When 2 mol of en·2HCl is added to a 1:1 mixture, only 6 equiv is titrated, indicating that both en molecules are bound to each Co(II) and that no amide hydrogen ionization has occurred from GlyHis so that it is at most a bidentate ligand through only one nitrogen donor atom.

The only permissible quadridentate ligand structure for a GlyHis and Co(II) complex contains the amino and ionized amide nitrogens and a carboxylate oxygen donor atom in a plane with the N-1 imidazole nitrogen bound in an axial position. This structure accounts for the difficulty in binding a second bulky GlyHis ligand. The proposed structure without all three nitrogen donors in one plane is consistent with the avoidance by Co(II) of all strong donor atoms in the coordination plane. For example, in contrast to the 1:1 tetragonal diamagnetic complexes formed by tripeptides with Ni(II),[7,11] tripeptides form with Co(II) 2:1 hexacoordinate complexes[19] with a structure similar to the 2:1 dipeptide complexes of Ni(II) and Co(II), all of which have not undergone tetragonal distortion. The quadridentate formulation of the GlyHis complex of Co(II) also accounts for the results obtained on forming the oxygenated complex.

Admission of oxygen to 1:1, 2:1, or 3:1 mixtures of GlyHis·HCl and Co(II) requires 0.5 equiv of base to reestablish the end point pH. This half-integral number of equivalents is indicative of formation of a hydroxo bridge in addition to the peroxo bridge in a binuclear complex of Co(III).[2,20] The requirement of 0.5 equiv for all three molar ratios supports formulation of the Co(II) complex under all conditions as effectively 1:1 with quadridentate GlyHis. Upon oxygenation the cis fifth and sixth positions participate in peroxo and hydroxide bridges forming a dibridged binuclear complex. Half-integral numbers of equivalents do not appear when en·2HCl is added because this bidentate ligand prevents formation of the hydroxo bridge in these modestly basic solutions.

Despite the identity of the Co(II) and also the oxygenated (binuclear peroxo) complexes in 1:1, 2:1, and 3:1 mixtures of GlyHis and Co(II), the mononuclear oxidized Co(III) complex formed in the first case contains fewer nitrogen donors than in the last two cases. The Co(III) complexes form only slowly over a period of several days or more rapidly at high temperatures;[16] in either case opportunities for rearrangements exist. The 1:1 mixture yields a mononuclear Co(III) product complex with an absorption maximum at 508 nm consistent with the three nitrogen donors and the quadridentate ligand structure described above. The 1:1 Co(III) complex exhibits an absorption maximum and a CD sign pattern similar to those of the second violet complex of Gillard and Spencer.[16]

Introduction of 2 mol of GlyHis eventually yields a product Co(III) complex with an absorption maximum near 482 nm consistent with five or six nitrogen donors. The CD patterns of the mononuclear Co(III) products change over a period of days and do not correspond well to that of any of the isomers isolated by Gillard and Spencer.[16] Our absorption maximum at 482 nm is at shorter wavelength than any they reported suggesting that standing at room temperature may give different complexes than heating for a short time.

Regardless of the precise structures of the Co(III) complexes, absorption spectra indicate that the oxidized complex formed from 1:1 molar ratios of glycyl-L-histidine and Co(II) with oxygen is different from those formed at 2:1 or greater ratios. On the other hand, similarities in the absorption and CD spectra of the Co(II) and binuclear oxygenated complexes suggest that both kinds of complexes possess similar structures at 1:1 and higher ratios. Thus Co(III) complexes formed slowly or at high temperatures need not reflect structures of either the predominant Co(II) or oxygenated species.

It has been claimed that titration of a solution containing a 1:1 ratio of GlyGly and Co(II) in the presence of oxygen yields a binuclear peroxo complex with only one dipeptide ligand per metal ion and hence only two nitrogen donors are required for formation of the oxygenated species.[21] It is also claimed that only one amide hydrogen per metal ion has undergone ionization in the oxygenation reaction in a solution containing a 2:1 ratio of GlyGly to Co(II).[21] These claims are not substantiated by the results presented. Long times were noted for equilibria to be obtained during the course of titrations and no spectral evidence is offered to support the claim that a binuclear peroxo complex is the product under the titration conditions.

As described in the Results section of this paper, significant amounts of binuclear peroxo complexes do not appear in titrations of GlyGly and Co(II) in the presence of oxygen. Products of the titrations are red mononuclear Co(III) com-

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plexes. The overall titration equation for 2:1 solutions is

$$2Co^{II}(H_2L_2)^{2+} + {}^1/_2O_2 + 6OH^- \rightarrow 2Co^{III}L_2^- + 7H_2O$$

where L represents GlyGly with an ionized amide nitrogen coordinated to metal ion. The product Co(III) complexes for both 2:1 and 1:1 solutions exhibit an absorption maximum at 520 nm, identical with earlier results with excess ligand and consistent with four nitrogen donors.[2,8] The nearly half absorption magnitude for the 1:1 solution suggests disproportionation to yield the same Co(III) dipeptide complex as in the 2:1 solution. The disproportionation observed in the 1:1 solutions confirms earlier suggestions that at least three nitrogen donors are required for oxygenation and oxidation of Co(II) complexes in the presence of amine and peptide ligands.[2,3] Because ionization of amide hydrogens in a 2:1 dipeptide-cobalt(II) complex has been shown to be cooperative in the presence of oxygen,[2,8] both amide hydrogens undergo deprotonation in formation of binuclear peroxo and final red Co(III) complexes.

Experimental Section

Commercial dipeptides were weighed out as required and the purity was checked by titration on a Radiometer combination titrimeter-titrigraph. Analytical reagent grade $Co(NO_3)_2 \cdot 6H_2O$ and $Co(CH_3COO)_2 \cdot 4H_2O$ were employed. Titrations under nitrogen were performed as described previously.[8] CD spectra were recorded on a Durrum-Jasco 5 instrument. All molar absorptivities and differential molar absorptivities between left and right circularly polarized light are based upon the molar concentration of cobalt ion. Pmr spectra were recorded on a Varian HA-100 spectrometer and are reported in hertz downfield from external TMS. ABC pmr spectra were analyzed by the program LAOCOON II.[22] All pH values recorded are meter readings uncorrected for the presence of any D_2O. Two to one molar ratios of dipeptide and Co(II) (at 0.1 or 0.2 *M*) were used for the pmr experiments. Usually the D_2O solution for the pmr experiments was diluted with H_2O to 0.02 or 0.04 *M* cobalt for CD and absorption spectra. For the Gly-L-His studies cobalt concentrations of 2.5–10 m*M* were employed for each ratio of ligand to metal ion. All experiments were performed throughout at room temperature, near 25°.

Efforts were made to account quantitatively for dipeptides as Co(III) complexes by utilizing $Co(CH_3COO)_2 \cdot 4H_2O$ as the Co(II) starting material with twice as much dipeptide. Results of the comparisons of areas under the methyl peaks of dipeptides with that due to a known amount of acetate are not completely satisfactory but do appear to indicate some loss of dipeptide. For instance, in the first three solutions of Table I only 75–90% of the alanyl methyl groups are accounted for as Co(III) products yet no other peaks appear in the well-defined pmr spectra. Lower percentages were obtained by this method for Gly-L-Ala. Lower percentages are accompanied by a reduced molar absorptivity at 520 nm. In comparing CD results of dipeptide Co(III) complexes, the magnitudes should probably be scaled to the same ϵ value at 520 nm.

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Mono- and Dibridged Peroxo Complexes of Cobalt(III)[1]

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Received January 26, 1973

The binuclear peroxocobalt(III) complex formed by oxygenation of solutions containing 2 mol of L-2,3-diaminopropionate/mol of Co(II) becomes dibridged at pH >9 with formation of a μ-hydroxo bridge. The hydroxo bridge forms most rapidly at high pH. The suggestion that from 310 to 450 nm two maxima appear in monobridged peroxocobalt(III) complexes and only one maximum in dibridged complexes is supported. Similar circular dichroism patterns of peroxo bis complexes fall into oppositely signed sets with L-2,3-diaminopropionate and L-2,4-diaminobutyrate in one set and L-histidine in the other. In contrast the bis mononuclear Co(III) complexes of the three ligand systems exhibit similar patterns and identical signs. Conversion of the binuclear peroxo to mononuclear Co(III) complexes is facilitated by addition of H_2O_2 and, in separate experiments, unaffected by addition of the enzyme catalase. From CD and kinetic results, it is suggested that binuclear peroxo complexes may not be intermediates on the main pathway from Co(II) to mononuclear Co(III) complexes but only relatively unreactive complexes formed in a side reaction.

Introduction

When formation of binuclear peroxo complexes of Co(III) from oxygen and Co(II) complexes results in release of an odd number of equivalents of acid for each 2 mol of metal ion, it was suggested that a μ-hydroxo bridge in addition to the peroxo bridge is formed yielding a dibridged binuclear complex.[2] In the group of ligands first investigated an odd number of acid equivalents was observed and a dibridged binuclear peroxo complex suggested for the bis complexes of diaminoethane, histamine, and glycinamide. A hydroxo bridge was also implied for the 1:1 complex with dien. The absence of μ-hydroxo bridges with the other ligands investigated was ascribed to steric hindrance, on the assumption that μ-hydroxo bridges would form unless inhibited by steric effects or lack of suitable coordination sites about the metal ions.[2] Although no evidence was obtained for a hydroxo bridge in the binuclear bis(histidine)complex formed at pH 10, features of the uv spectra of the binuclear complex formed in 1 *N* base[3] suggested to us the presence of a hydroxo bridge in the more basic solutions.[4] At about the same time, a hydroxo bridge was suggested on the basis of titration evidence for the binuclear triethylenetetramine (trien) complex.[5] With four coordination positions about each metal ion occupied by amino nitrogen donors and the fifth position involved in a peroxo bridge, a μ-hydroxo group bridging the sixth positions of each Co(III) ion is in keeping with the above idea. The hydroxo bridge also aids interpretation of kinetics of the overall oxygenation reaction for the

(1) This research was supported by a grant from the National Science Foundation.

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