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TRANSITION METAL COMPLEXES OF SMALL PEPTIDES

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Work on the complexes of biologically significant ligands with transition metal ions has been primarily concerned with biological effects. Although the metabolic activity of a number of metal peptide complexes has been studied, there is little chemical or structural information available, other than that reported by Freeman from crystallographic studies of copper(II) peptide systems[1]. In parallel with a chemical and X-ray structural investigation[2] of the non-haem iron-containing protein, ferredoxin, we are investigating the metal complexes of small sulphur-containing peptides, with emphasis on their role as model systems. Because of the scarcity of structural data on complexes of amino-acids and peptides, we have also studied the simple dipeptides as ligands, and report here some of our results.

Complexes of simple dipeptides with cobalt have been known for some time. Following some early work by Smith[3] and others, Gilbert, Otey and Price[4] studied the oxidation of cobaltous glycylglycine complexes by air in aqueous solution, and showed that a red complex is formed, where [Co]:[GG] = 1:2, with absorption maxima at 520 and 398 mμ. They further isolated a solid complex with the same absorption spectrum, to which the formula $[(GG)_2CoO_2Co(GG)_2]$ was assigned. (Throughout this paper, unsubstituted glycylglycine is abbreviated GGH_2, and its mono- and di-anions as GGH^-, and GG^{2-}, respectively). Later, kinetic studies[5] showed that an intermediate brown complex was formed at high pH; this was converted to the red complex by lowering the pH. A recent polarographic study[6] found no evidence for the presence of a peroxo group in the red compound; it was therefore suggested[6] that it was a hydroxy compound, either $[Co(GGH)_2(OH)_2]^-$ or $[Co(GGH)_2(OH)]$.

While attempting to prepare the red "irreversible oxygen-carrying" complex of Gilbert, Otey and Price[4] for crystallographic purposes, we isolated a large number of red complexes of cobalt(III). There are various possibilities for the mode of attachment of the glycylglycine ligand (GGH_2, GGH^-, or GG^{2-}; bi- or terdentate; and, according to which of the four possible donor atoms is used, with

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five or six-membered chelate rings). We have therefore studied these complexes of glycylglycine with cobalt in detail.

THE BIS(GLYCYLGLYCINATO)COBALTATE(III) ANION, $[Co(GG)_2]^-$

When an aqueous solution (pH 6.5 to 9.0) containing cobalt(II) ions and glycylglycine is treated with oxygen, the major product (which we call α) is a purple-red compound, which, as shown later, contains the complex anion $[Co(GG)_2]^-$. This compound has the absorption spectrum reported by Gilbert, Otey and Price[4] for the alleged "irreversible oxygen-carrier." Its reversible reaction with acid is discussed later. The ammonium salt of the same anion was made by the reaction of glycylglycine with the hexamminecobalt(III) ion.

A three dimensional X-ray structural analysis of this ammonium salt (β form) $(NH_4)\,[Co(GG)_2]2H_2O$, has now been completed. The crystals are orthorhombic prisms, with $a = 8.54$, $b = 10.89$, and $c = 15.16$ Å. The observed density is 1.75 g/cm^3, giving a calculated molecular weight (4 molecules per unit cell) of 371. The theoretical value for $(NH_4)\,[Co(GG)_2].\,2H_2O$ is 373. The space group, *Iba*2, requires that the cobalt atoms and the nitrogen atoms of the ammonium ions each lie on a two-fold crystallographic axis. The complex anions contain two planar terdentate glycylglycinate ligands, as shown in Fig. 1. The R factor is at present 10%.

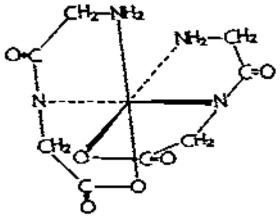

Fig. 1. The stereochemistry of the complex anion $[Co(GG)_2]^-$ in the salt $(NH_4)\,[Co(GG)_2]2H_2O$.

When glycylglycine reacts with the hexamminecobalt(III) cation, the complex anion is obtained as its ammonium salt (β-form). When this salt is treated with sodium hydroxide, ammonia is lost, and the sodium salt may be crystallized from the resulting solution. The ammonium salt can also be converted to the α-form, and both α- and β-salts, treated with tetraethylammonium hydroxide, give the same tetraethylammonium his(glycylglycinato)cobaltate(III) hydrate (δ-form). The sodium salt of the same anion may also be obtained by the reaction of glycylglycine with sodium triscarbonatocobaltate(III).

Protonated forms

When a mineral acid is added to aqueous solutions of the purple red complexes (λ 520, 398 mμ), there is a marked colour change to a paler, brick-red (λ 500, 395 mμ). Protonation becomes detectable at pH *ca* 2.8, and is not complete till quite high acid concentrations are reached (~ 6 *M* acid). The process occurs extremely rapidly, and, at a constant concentration of cobalt(III), isosbestic points are observed, as shown in Fig. 2.

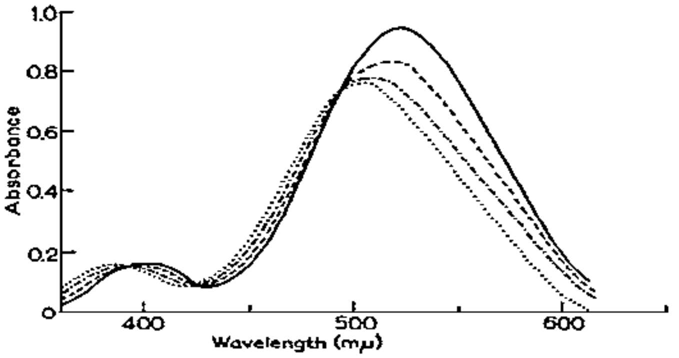

Fig. 2. Spectral changes during the conversion of $[Co(GG)_2]^-$ to $[Co(GGH)_2]^+$; ——— pH 9; - - - - - - pH 2; -.-.-.-.-.-. pH 0.5; .... In 6*N* H_2SO_4.

Crystalline complexes may be obtained from acid solutions of any of the purple–red salts (α-, β-, or δ-forms) by adding a large amount of acetone (an adduct with sulphuric acid was already known[5]). The resulting brick-red complexes are most likely cationic, since the anion of the relevant mineral acid is always present. With sulphuric acid, both α- and β-forms give the same adduct. However, the products obtained by treating the α- and β-forms with hydrochloric acid have differing X-ray powder patterns; this may be due to the presence of co-crystallized ammonium chloride in the adduct from the β-form (ammonium salt). Protonated products from the δ-form (tetraethylammonium salt) are usually deliquescent, probably through a co-precipitation of the tetraethylammonium salts of the mineral acid.

The reversible protonation may be represented by:

$$[Co(NH_2CH_2CO \cdot N \cdot CH_2COO)_2]^- + 2H^+ \rightleftharpoons [Co(NH_2CH_2CONHCH_2COO)_2]^+$$

The deprotonated amide nitrogen has a position in the spectrochemical series lower than that of protonated amide, since the band positions are 520 mμ for the alkali complexes, and 500 mμ for the acid complex.

Stereoselectivity

The analogous purple-red complexes (λ 520 mμ) of the optically active dipeptides L-alanylglycine, glycyl-L-leucine, and L-alanyl-L-alanine have been made by the reaction of the peptide with sodium triscarbonatocobaltate(III) in the presence of active charcoal. The electronic spectra of all these complexes are similar to the spectrum of the glycylglycine complex, so that structural similarity is also likely. This is also demonstrated by studies of the rotational strengths of the *d–d* transitions. All three complexes of these asymmetric peptides show a high ratio of rotational strength to optical density. This ratio is about the same for the complexes of glycyl-L-leucine and L-alanylglycine, while the complex of L-alanyl-L-alanine shows a ratio of rotational strength to optical density about twice as large.

Until pure isomers (*i.e.* $\mathrm{D_L}$ and $\mathrm{L_L}$; $\mathrm{D_{LL}}$ and $\mathrm{L_{LL}}$*) have been characterized the extent of stereoselectivity in these reactions is not certain; however, from the large Cotton effects shown by the reaction mixtures, stereoselectivity is marked. The asymmetric centre of L-alanylglycine is in the N–N′ chelate ring, whereas that of glycyl-L-leucine is in the N–O chelate ring. Similar degrees of stereoselectivity are found for both complexes, so that it seems likely that both amino-acid residues, whether forming N–N′ or N–O chelate rings, are involved in chelation, *i.e.* that these optically active dipeptides also act as terdentate ligands.

The rotational strengths also suggest that the protonated forms contain terdentate ligands. It has previously been pointed out[7] that the size of Cotton effects in chelated compounds is a function of the number of chelate rings present. The rotational strengths of the longest wavelength absorption band in the protonated and deprotonated complexes are of the same order. No major rearrangement of the ligands occurs on protonation.

Other compounds

The X-ray data on salts of the major product of reactions of glycylglycine with cobalt-containing species are collected in Table I. The cation present in the α-form has not yet been identified; a hydrated hydroxonium ion may well be present, although the crystals can be obtained from almost neutral aqueous solution.

A large number of minor products has been identified; the available data are in Table II, which also describes the various protonated forms. Dr. Freeman (in Sydney) and Dr. van der Helm (in the United States) have kindly given us details of the unit cells of complexes containing cobalt and glycylglycine which they are studying crystallographically. The great complexity of this apparently simple system is apparent from the fact that neither of their specimens have been obtained here!

By analogy with other reversibly oxygenated complexes of cobalt, we suggest that the oxygen-carrying complex in the cobalt–glycylglycine system is the brown intermediate recognized[5] by Tanford. We are currently attempting to synthesize this compound, in order to study its reactions and structure.

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* The optical configuration of the whole complex is given as D or L, while those of the asymmetric carbon atoms in the ligands are given as subscript D or L.

TABLE I

PURPLE–RED BISGLYCYLGLYCINATOCOBALTATE(III) SALTS

Salt	*Morphology*	*Unit cell*	*Space group*
α-form*	Six-sided or diamond shaped plates; heavily twinned	Monoclinic $a = 13.0$, $b = 23.0$, $c = 6.0$; $\beta = 117°36'$. $Z = 4$	$P2_1/c$
β-form (NH_4^+ salt)	Long prisms with six-sided cross section	Orthorhombic $a = 8.5$, $b = 10.9$, $c = 15.2$, $Z = 4$	*Iba* 2
δ-form (NEt_4^+ salt)	Flat needles	Monoclinic $a = 10.5$, $b = 12.15$, $c = 20.0$; $\beta = 92°12'$. $Z = 4$	$P2_1/c$
Na^+ salt*	[a]	—	—
λ-form	Long prisms-six-sided cross section	Monoclinic $a = 18.3$, $b = 12.2$, $c = 15.0$; $\beta = 123°20'$. $Z = 8$	$C2/c$ or Cc
μ-form	Needles[b]	Probably monoclinic with $b \sim 11$ Å	—

[a] No single crystal information is yet available on this salt, which has been characterized through its X-ray powder pattern only.

[b] These crystals changed in appearance during some weeks; the data available were obtained on aged samples.

* The α-form has been shown to be the sodium salt; the latter ions apparently originating from the alumina used for "purification".

TABLE II

DATA ON MINOR PRODUCTS IN THE COBALT-GLYCYLGLYCINE SYSTEM

γ-form	From a reaction mixture following ref. 4. Probably a protonated form, as the sulphate. Characterized by its X-ray powder pattern.
χ-form	Brick-red rectangular prisms, from a reaction mixture following ref. 4. Possibly same as *o*-form. Orthorhombic: $a = 13.8$, $b = 18.0$; $c = 10.8$. Space group *Pbca*. Density = 1.79 g/cm^3, whence mol.wt. ($Z = 8$) = 362.
ε-form	Brick-red needles; the protonated product of the α-form with HCl. Characterized by X-ray powder pattern.
η-form	Orange to brick-red long prisms; a minor product of the reaction of $[Co(NH_3)_6]^{3+}$ with GG. Monoclinic; $a = 11.7$, $b = 19.5$, $c = 9.8$; $\beta = 99°50'$. Space group $P2_1/c$. Mol.wt ($Z = 4$) = 530. Calc. for $[Co(GG)(GGH)(NH_3)_2]9H_2O = 516$.
θ and ι-forms	These have been observed as contaminants of the α-form in some powder photographs.
ω-form	Prismatic needles; the sulphuric acid adduct obtained from α- or β-form. Characterized by the X-ray powder pattern.

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