The Effect of Complex Charge on the Racemisation of L-alanine in Co(III) Complexes*

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Abstract

The effect of charge on the racemisation of L-alanine in Co(III) complexes has shown that racemisation rates are directly proportional to the charge on the complex from a −2 through zero to a +2 charge. Although Co(III) complexation increases the rates of racemisation of amino acids, complexation of dipeptides to Co(III) reduces the rates of racemisation. The larger the positive charge on the complex, however, the faster is the rate of racemisation.

Introduction

The increased chemical reactivity [1] which occurs at the α-carbon of the ligand in the metal amino acid complexes has been recognized, for sometime and studied very recently in base-catalyzed Knoevenagel-type condensations [2], isotope exchange [3] and racemisation [4] reactions. This enhanced reactivity in these complexes is thought to be due to the metal ligand bonding [5] and the net charge on the complex [6].

Recently, we reported that of all the metal ions studied, Co(III) showed the greatest enhancement on the rate of racemisation of L-alanine [7]. Earlier reports, on base catalyzed aldol-type condensations [6, 8] and deuterium isotope exchange [9] on the Co(III) glycinato complexes, indicate that the reactivity increased with increasing positive charge on the complex. Based purely on qualitative results the order of reactivity has been reported to be $[Co(en)_2(gly)]^{+2} > [Co(gly)_3] \gg [Co(ox)_2(gly)]^{-2}$. Norman and Phipps [10] obtained quantitative data on the deuterium isotope exchange for three Co(III) glycinato complexes and noted a similar trend between charge and reactivity. There is, however, no quantitative or even qualitative report on the effect of the complex on the racemisation of complexed amino acids. Therefore, we have directed our studies to investigating the effect of overall charge on the complex and the nature of the metal ion on the racemisation of L-alanine in Co(III) and Cu(II) complexes. We have employed a simple, fast and quantitatively accurate gc method, developed in our laboratory [11] for determining the racemisation rate in these complexes.

Experimental

The syntheses and purification of the complexes were carried out according to previously reported methods [12]. All the complexes gave satisfactory analytical and spectral data.

Sample Preparation and Racemisation

In each case 25 ml of a 0.03 *M* solution of the complex was prepared and the pH was adjusted to a desired value by the addition of an appropriate volume of sodium hydroxide (0.1 *M*) or hydrochloric acid (0.1 *M*) solutions. One ml aliquots of the solution containing the complex was sealed in pyrex glass tubes and racemised in a constant temperature oil bath for the required time intervals. The tubes were cooled, opened and samples were decomposed.

Decomposition and Derivatisation to N–Trifluoroacetyl O–2-Propyl Esters

The metal ions [Co(III) and Cu(II)] were freed from their complexes by the addition of a few drops of 0.1 *M* sodium sulphide in acidic or basic medium depending on the nature of the complex. Hydrolysis to the free amino acids was affected in case of Co(III) dipeptide complexes by heating the samples for 20 h with 6 *M* hydrochloric acid at 110 °C. The water from the sample tubes was evaporated under an air stream at 80 °C. The traces of moisture were removed by azeotropic distillation with dichloromethane followed by vacuum dessication. To each dried amino acid residue was added 1 ml of 4 *N* 2-propanol/HCl. The tubes were resealed and heated in an oil bath for 2 h at 110 °C. The excess 2-propanol was removed from the tubes, as outlined above, by a stream of air at 80 °C. Derivatisation was completed by the

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TABLE I. Reaction Rate Constants for the Racemisation[a] of L-alanine in Co(III) Complexes.

Complex Entry	Complex Structure	Charge on the complex	10^7 k, s^{-1}
1.	$K_2[Co(ox)_2(L\text{-}ala)]$[b]	−2	0.27
2.	$K[Co(ox)(L\text{-}ala)_2]$[c]	−1	3.08
3.	$[Co(L\text{-}ala)_3]$[d]	0	36.0
4.	$[Co(en)(L\text{-}ala)_2]ClO_4$[e]	+1	87.5
5.	$[Co(tren)(L\text{-}ala)](ClO_4)_2$[f]	+2	138.0
6.	$[Co(NH_3)_5(L\text{-}ala)](BF_4)_3$	+3	[g]

[a]Racemisation conditions; pH, 9.5 and temperature, 75 °C. [b]Optical rotation of this complex M_{589} = +400. [c]*trans* (N)-isomer. [d]Δ isomer. [e]*trans*(O) isomer. [f]*trans* isomer. [g]An accurate rate constant was not obtained due to decomposition.

addition of 0.5 ml of 30% trifluoroacetic anhydride in dichloromethane. After standing at room temperature for 2 h, the excess reagent was removed by evaporation. The derivatized amino acid residue was taken up in dichloromethane (0.5 ml) and transferred to small vials for g.c. analysis.

Gas Chromatography

GC analyses were performed on a HP 5830 A gas chromatograph under appropriate isothermal conditions using a stainless steel capillary column (150 ft × 0.02 in.), coated with a 1:1 mixture of N-docosanoyl-L-val-t-butyl amide and N-octadecanoyl-L-val-L-val-cyclohexyl ester [11]. In all cases baseline separation of D- and L-enantiomeric alanine was obtained. Reversible first order rate constants for the racemisation reaction were determined by plotting $0.5 \ln[(1 + D/L)/(1 - D/L)]$ *vs.* time, where D- and L- are the concentrations of D- and L-alanine, respectively.

Results and Discussion

The rate constants for the racemisation of L-alanine in Co(III) complexes with varying net complex charge are shown in Table I. The reaction rate constants increased >500 times as the net charge on the complex increased from −2 to +2. L-Alanine in $[Co(L\text{-}ala)_3]$ (complex 3) racemised approximately 12 times faster than in $K[(Co(OX)(L\text{-}ala_2)]$ (complex 2) and 135 times faster than in $K_2[Co(OX)_2(L\text{-}ala)]$ (complex 1). The relative racemisation rate of L-ala in these complexes was:

$$[Co(tren)(L\text{-}ala)]^{+2} > [Co(en)(L\text{-}ala)_2]^{+} >$$

$$[Co(L\text{-}ala)_3]^{0} > [Co(ox)(L\text{-}ala)_2]^{-} \gg$$

$$[Co(ox)_2(L\text{-}ala)]^{-2}.$$

These results clearly point out a substantial effect of complex charge on the relative reactivity at the α-carbon in the L-ala ligand and are in good agreement with the previous observations made by Dabrowiak and Cooke [6] and Norman and Phipps [10] in the aldol-type condensation and isotope exchange reactions of Co(III) glycinato complexes, respectively. In an effort to study the effect of +3 charge on the racemisation of L-ala, $[Co(NH_3)_5(L\text{-}ala)](BF_4)_3$ was racemised under similar conditions. This complex exhibited no appreciable racemisation. Also, it was noted that it partially decomposes at longer reaction times, as evidenced by the formation of a precipitate in the reaction tubes. Retardation of racemisation observed in this complex appears to be due to the fact that L-ala acts as a monodentate, where the amino nitrogen is not complexed to the metal [13]. Consequently, the electron withdrawing power [5] due to Co(III) is absent or less significant in the complex, which in turn may retard racemisation. This supports the assumption that the activation of the coordinated amino acids in the metal complexes results from the complexation of both the $-NH_2$ and $-COO^-$ groups [1].

We have extended these studies to Co(III) dipeptidato complexes. The rate constants for the racemisa-

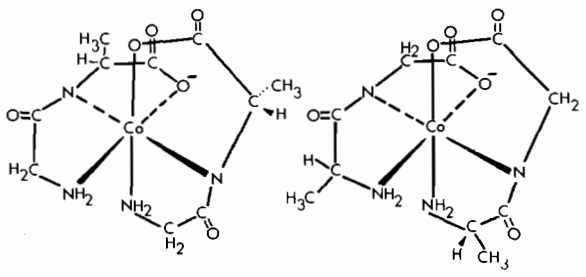
TABLE II. Reaction Rate Constants for the Racemisation[a] of L-Alanine in Co(III) Dipeptide Complexes.

Complex Structure	Charge of the Complex	10^7 k, s^{-1}
$K[Co(L\text{-}ala\text{–}gly)_2]$[b]	−1	8.4[c]
$[Co(NH_3)_3(L\text{-}ala\text{–}gly)]ClO_4$	+1	22.8
$K[Co(gly\text{–}L\text{-}ala)_2]$[b]	−1	4.0
$[Co(NH_3)_3(gly\text{–}L\text{-}ala)]ClO_4$	+1	12.6[c]

[a]Racemisation conditions: pH = 8.6 and Temperature 119.2 °C. [b]Mixture of R and S isomers. [c]The reaction rate constants (k 10^7, s^{-1}) for racemisation of free dipeptides at pH 8.5 and 119.9 °C; L-ala–gly: 52.6; gly–L-ala: 71.4.

tion of L-ala in these complexes are presented in Table II. All of the complexes of dipeptides racemised slower than the corresponding free dipeptides [14]. We now realize that free dipeptides racemise faster due to intramolecular effects [15]. These effects are significant in uncomplexed dipeptides. As a result they racemise faster than the positively charged dipeptide complexes or the amino acid complexes of Ni, Cu or Pd. Intramolecular effects are not possible in the dipeptide metal complexes, but the charge effects appear to affect the racemization rates in these complexes.

As expected, L-ala in the dipeptide complexes with a +1 net charge racemised three times faster than it did in the complexes with a −1 net charge. These results clearly point out the effect of complex charge on the rate of racemisation in these complexes. L-Alanine is known to racemise faster at the carboxyl terminal position than the amino terminal position in free dipeptides [14]. A similar trend was noted by Gillard and Phipps [16] in their study on the deuterium exchange reaction in [Co(dipeptide)$_2$] and [Co(NH$_3$)$_3$(dipeptide)]$^+$. On the contrary, from Table II it can be seen that the complexed L-ala at $-NH_2$ terminal position racemised approximately two times faster (*e.g.* [Co(L-ala–gly)$_2$]$^-$ > [Co(gly–L-ala]$^-$) than it did at the –COOH terminal position. These interesting results may be explained by considering the fact that the racemisation site is adjacent to two negative charges (the deprotonated amido nitrogen and deprotonated carboxyl group), as in [Co(gly–L-ala)$_2$]$^-$ A and [Co(NH$_3$)$_3$(gly–L-ala)]$^+$ complexes, which would appreciably prevent the formation of the carbanion [7]. Only one negative charge is near the chiral carbon in the complexes [Co(L-ala–gly)$_2$]$^-$ B and [Co(NH$_3$)$_3$(L-ala–gly)] leading to a relatively less reactive site toward racemisation.



A B

The pronounced effect of complex charge was also observed in Cu(II) L-alanine complexes shown in Table III, from which it is evident that the amino acid in the complex [Cu(phen)(L-ala)]$^+$, with a net positive charge, has racemized about 6 times faster than the complex without charge. The racemisation of L-ala in the presence of Cu(II) ions is known to proceed through a less direct mechanism involving

TABLE III. Reaction Rate Constants for the Racemisation[a] of L-alanine in Cu(II) Complexes.

Complex Structure	Charge on the Complex	10^7 k, s^{-1}
[Cu(L-ala)$_2$][b]	0	6.6
[Cu(phen)(L-ala)] SO$_4$½·2H$_2$O	+	41.9

[a]Racemisation conditions: pH = 9.0; Temperature = 109 °C. [b]*cis* and *trans* isomers.

oxidation. Gillard and co-workers attributed this to the formation of pyruvate in the reaction mixture [17]. It has also been supported by Gillard and O'Brien [18] in their study on pyruvate mediated reactions of metal complexes L-ala. We have observed this to be true but only at longer reaction times and higher pH conditions.

The proposed mechanism for the racemisation process in the metal complexed amino acids involves the initial abstraction of the α-hydrogen by a base resulting in the formation of a carbanion [19]. If this is true, a formal positive charge on the complex would favour the racemisation reaction to proceed. In fact, L-alanine has been shown to racemise faster with increasing positive charge on the complex (Tables I–III). Similarly, the complexes with net zero charge exhibited enhanced reactivity toward racemisation when compared with the corresponding complexes with increasing negative charge. It is clear at this stage that the high formal overall positive charge on the complex results in enhanced electron withdrawing capabilities, which inturn greatly increases the acidity of α-carbon, thus favouring the reactions involving the carbanion mechanisms. Present study provides additional support to the mechanism of racemisation involving a carbanion intermediate in metal amino acid and dipeptide complexes. Further experiments to study these effects with other metals and ligands are underway.

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