The Kinetics of Base Hydrolysis of the Peptide Bond in the Glycylglycine Methyl Ester Complex of β-(Triethylenetetramine)cobalt(III)

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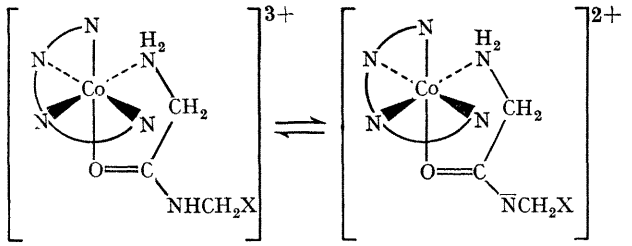
Summary Base hydrolysis of the peptide bond in the glycylglycine complex of β-(triethylenetetramine)cobalt(III) occurs about $6{\cdot}5 \times 10^4$ times as fast as in glycylglycine.

BUCKINGHAM *et al.*[1] have described the use of the β-hydroxoaquotriethylenetetraminecobalt(III) ion as a reagent for the specific cleavage of *N*-terminal peptide bonds. Collman and Kimura[2] have also shown that peptide bond formation can occur if glycine methyl ester is treated with *cis*-α- or *cis*-β-[Co trien Cl_2]Cl in the presence of a weakly co-ordinating amine such as diethylamine. The product of this reaction which can be readily isolated as the orange perchlorate β-[Co trien GlyglyOMe] $(ClO_4)_3,H_2O$ provides a suitable substrate for kinetic studies of the peptide bond cleavage reaction. (An intermediate of this type has been suggested by Buckingham *et al.*[1] to account for the peptide bond cleavage.)

The crystal structure of the complex β-[Co trien GlyglyOEt]$(ClO_4)_3,H_2O$ has recently been determined[3] (I; X = CO_2Et). The peptide ester is co-ordinated through the terminal amino-group and the carbonyl oxygen of the peptide bond, as suggested by Collman and Kimura.

Kinetic studies of the base hydrolysis were carried out using a pH-stat in the pH range 10—11 and at $I = 0{\cdot}1$ M and 25°. One mole of base was consumed for hydrolysis of one mole of (I; X = CO_2Me). At constant pH the reaction is pseudo-first order in the complex. The plots of log $(V_\infty - V_t)$ against time exhibited generally good linearity, although in some runs a little initial curvature was observed possibly due to a trace contaminant or conformer. Paper chromatography (phenol–water) of the reaction products obtained

after base hydrolysis confirmed the presence of glycine. The visible spectrum of the complex ion produced on hydrolysis was consistent with the expected *cis*-β_2-[Co trien gly]$^{2+}$ ion (II), λ_{max} 480 nm (ϵ 128) and λ_{max} 348 nm (ϵ 136).†



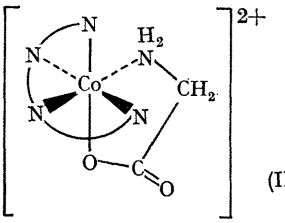
(I)"CoHA^{3+}" $+H^+$ "CoA.$^{2+}$"

Since the ester function in the complex ion (I) is also expected to undergo hydrolysis in the presence of base (k_{OH} for glycine methyl ester = 77 M^{-1} min.$^{-1}$ at 25° and I = 0·1 M, $t_{\frac{1}{2}}$ = 6·9 min. at pH 11),[5] experiments were carried out to determine the rate of methanol release. Samples of the complex (I; X = CO_2Me) were added to a borax buffer (0·05 M, pH 9·18) at 25° and the release of methanol followed by g.l.c. The half-life for ester hydrolysis under these conditions was not more than 2—3 min. This result is consistent with the pH-stat observations that consumption of a single mole of base per mole of complex occurred in the pH range 10—11. Presumably, the ester function was completely hydrolysed during the period required to reach the reaction pH of 10—11. This is not unexpected, since base hydrolysis of an ester ligand in the co-ordination sphere of an ion carrying a tripositive charge would be expected to occur much more rapidly than in the uncomplexed ligand.

The pH-stat kinetics obviously refer to the hydrolysis of (I; X = CO_2^-). Potentiometric titration indicates that the pK_a for the ionisation of the peptide hydrogen of (I; X = CO_2^-) is 9·41 [practical ionisation constant at I = 0·1 M (KCl) and 25°].‡ Thus, in the pH range studied both the protonated (CoHA^{2+}) and unprotonated (CoA$^+$) species occur, so that at constant pH,

$$k_{obs}([CoHA^{2+}] + [CoA^+]) = k_1[CoHA^{2+}][OH^-] + k_2[CoA^+] \times [OH^-]$$

A typical set of k_{obs} values is shown in the Table, (at pH 11·10 the half-life of the reaction is 5·3 min.). The trend in the values of $k_{obs}/[OH^-]$ is understandable on the basis of the suggested kinetic scheme. Values of k_1 and k_2 were evaluated from plots of $k_{obs}/[OH^-]$ against α, where α is the degree of ionisation to CoA$^+$. A reasonable fit to the data is given by k_1 = 162 M^{-1}min.$^{-1}$ and k_2 = 86M^{-1}min.$^{-1}$ at 25° and I = 0·1M. The constants are somewhat



(II)

approximate due to the limited percentage of CoHA^{2+} present in the pH range used. For the base hydrolysis of glycylglycine k_{OH} is *ca.* 2·4 × 10^{-3}M^{-1}min.$^{-1}$ at 26° (determined in 2M-sodium hydroxide).[6] Thus the peptide bond cleaves *ca.* 6·5 × 10^4 times faster in CoHA^{2+} than in the free dipeptide.

Recent studies[7] of carboxypeptidase A (*M* 34,300), which contains one zinc atom per molecule, have indicated that the metal ion is located at the active site. Molecular models (prepared from the *X*-ray data), of the enzyme–substrate complex with *N*-glycyl-L-tyrosine indicate that the carbonyl group of the peptide bond can co-ordinate with the zinc ion. The present system, though stoicheiometric rather than catalytic, provides an excellent model for a metallo-enzyme of this type.

TABLE

pH	$10^2 k_{obs}$ (min.$^{-1}$)	$t_{\frac{1}{2}}$(min.)	$k_{obs}/[OH^-]$ (M^{-1}min.$^{-1}$)
10·10	1·61	43·0	98·00
10·20	2·02	34·8	97·34
10·40	3·06	22·7	93·10
10·60	4·86	14·3	93·48
10·80	7·22	9·6	87·61
11·10	13·02	5·3	79·15

Rate constants determined at 25° and I = 0·1 M (KCl). Values of [OH$^-$] calculated from the pH using a value of 0·772 for the activity coefficient and pK_ω = 13·9965 at 25°.

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† The spectrum reported by Marzilli and Buckingham (ref. 4) for *cis*-β_2 [Co trien gly]$^{2+}$ is in fact that of *cis*-β_1[Co trien gly]$^{2+}$ and *vice versa*. We are grateful to Dr. Buckingham for confirming this point.

‡ The equilibrium may be represented, CoHA^{3+} ⇌ CoA^{2+} + H$^+$. The practical ionisation constant is defined $K_a = [CoA^{2+}]\{H^+\}/[CoHA^{3+}]$, where braces are used to represent activities.

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