Selective Cleavage of Peptides by Alkali in the Presence of Transition-metal Ions

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DURING attempts to prepare a Cu^{II}-hexapeptide complex for an *X*-ray structure analysis, violet–pink crystals separated from an alkaline solution containing Cu^{II} and Gly-Gly-Gly-Gly-Gly-Gly. Identity of *X*-ray diffraction patterns proved that the compound was the Cu^{II} complex of the pentapeptide Gly-Gly-Gly-Gly-Gly (which had recently been characterised[1]). Apparently one residue of the hexapeptide had been selectively cleaved.

As a consequence of this chance observation, rate studies of the alkaline hydrolysis of pentaglycylglycine in the presence of a variety of metals have been made. Conditions of temperature, concentration, and pH were chosen so that the reactions could be followed easily during a 2 hr. period. In

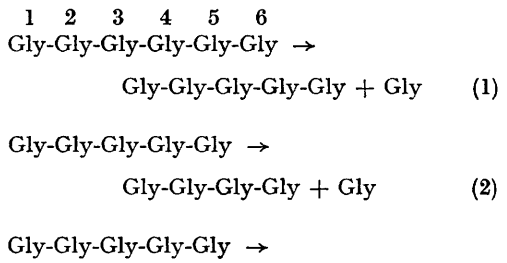
each experiment, metal chloride solution (0·02 ml., 1·4 M) was added to the peptide (*ca.* 8 mg.). The volume was made up to 1 ml. with 1M-NaOH and the reaction mixture was heated on a steam bath. Aliquots of 5 μl. were withdrawn at intervals and applied to Whatman No. 1 paper. After being dried, the spots were acidified with a microdrop of 5M-HCl to decompose any metal complexes. Descending chromatography for 2 days (after equilibration with solvent vapour) using t-butyl alcohol : formic acid : water (70 : 15 : 15) gave excellent separations of all possible components. These were detected and qualitatively estimated by the usual ninhydrin procedure.

Under the above conditions and in the absence of metal ions, complete hydrolysis rapidly occurred. No hexa-, penta-, or tetra-peptide was detectable after 15 min. Of 18 metal ions examined, only Cu^{II} and Ni^{II} gave clear-cut evidence that they inhibited hydrolysis in a selective way. In their presence, the reaction mixture contained mainly tetraglycylglycine and glycine after 15 min. Glycylglycine became detectable only after 30 min. Even after 120 min. the pentapeptide was still present although the major components were then triglycylglycine and glycine. Diglycylglycine was never present in detectable amounts. These observations, and parallel experiments with tetraglycylglycine, indicate that the initial steps in the hydrolysis of pentaglycylglycine are described by equations (1) and (2), in which the role of the metal ion is neglected for the moment. Equation (3) can occur only to a small extent, if at all. The stabilities of the Cu^{II}– and Ni^{II}–tetrapeptide complexes in alkali are well known,[2,3] and the tetrapeptide is ultimately hydrolysed only because some of the free ligand must be in equilibrium with the complex in solution. In the case of the glycylglycine–Cu^{II} system, Jones *et al.*[4] have shown by a detailed kinetic study that only the free peptide in equilibrium with the Cu^{II} complex undergoes hydrolysis.

In order to define whether the initial step (1) occurs at the NH_2- or CO_2H-terminal residue of the hexapeptide, pentaglycylglycine was synthesised with a ^{14}C label in the terminal carboxyl group and subjected to the same procedure. The only radioactive spots on the chromatograms were those of starting material and glycine. Activity was confined to the latter component by the time the former had disappeared. Selective cleavage therefore takes place specifically at the bond between residues 5 and 6, and equation (3) is not detectable within the limits of our analytical technique.

Pentaglycyl-DL-alanine, m.p. 262—264°, was synthesised and selective fission of its CO_2H-terminal residue was shown to occur in the presence of Cu^{II} and Ni^{II}. After 15 min., when most of the hexapeptide had disappeared, no glycine was detected in the hydrolysate and the major components were alanine and tetraglycylglycine. In contrast with the previous case, some of the CO_2H-terminal dipeptide was also released in the initial stages, Gly-Ala being observed in small quantities even before glycine became detectable.

The known crystal structures of the Cu^{II}–triglycylglycine[5] and Cu^{II}–tetraglycylglycine[1] complexes isolated at high pH leave little doubt that the complex species in solution have Cu^{II} atoms coordinated by four nitrogen donors. Our findings demonstrate that chelation involving deprotonated peptide nitrogen atoms specifically protects sequences of four to five residues of a peptide against alkaline hydrolysis. Despite the well known disadvantages of decomposition and racemisation attending alkaline hydrolysis of proteins,[6,7] this effect warrants further exploration as a potential aid in sequence determination.



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[4] M. M. Jones, T. J. Cook, and S. Brammer, *J. Inorg. Nuclear Chem.*, 1966, **28**, 1265.
[5] H. C. Freeman and M. R. Taylor, *Acta Cryst.*, 1965, **18**, 939.
[6] R. L. Hill, *Adv. Protein Chem.*, 1965, **20**, 37.
[7] Copper complexes have been used to prevent racemisation in the alkaline hydrolysis of peptides (V. Bruckner, K. Kovács, J. Kovács, and A. Kótai, *Acta Chim. Acad. Sci. Hung.* 1955, **5**, 267; J. Nyilasi and Z. Kovats, *ibid.*, 1952, **2**, 451; 1953, **3**, 273) and to prevent transpeptidation in the alkaline hydrolysis of γ-esters of glutamic acid peptides (V. Bruckner, A. Kótai, and K. Kovács, *ibid.*, 1959, **21**, 427).