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REACTIONS OF METAL CHELATES. II. HYDROLYTIC CLEAVAGE OF PEPTIDES BY BIS(SALICYLALDEHYDATO)-COPPER(II), -NICKEL(II) AND -COBALT(II)

A. NAKAHARA, K. HAMADA, Y. NAKAO AND T. HIGASHIYAMA

Institute of Chemistry, College of General Education, Osaka University, Toyonaka, Osaka (Japan)

A. INTRODUCTION

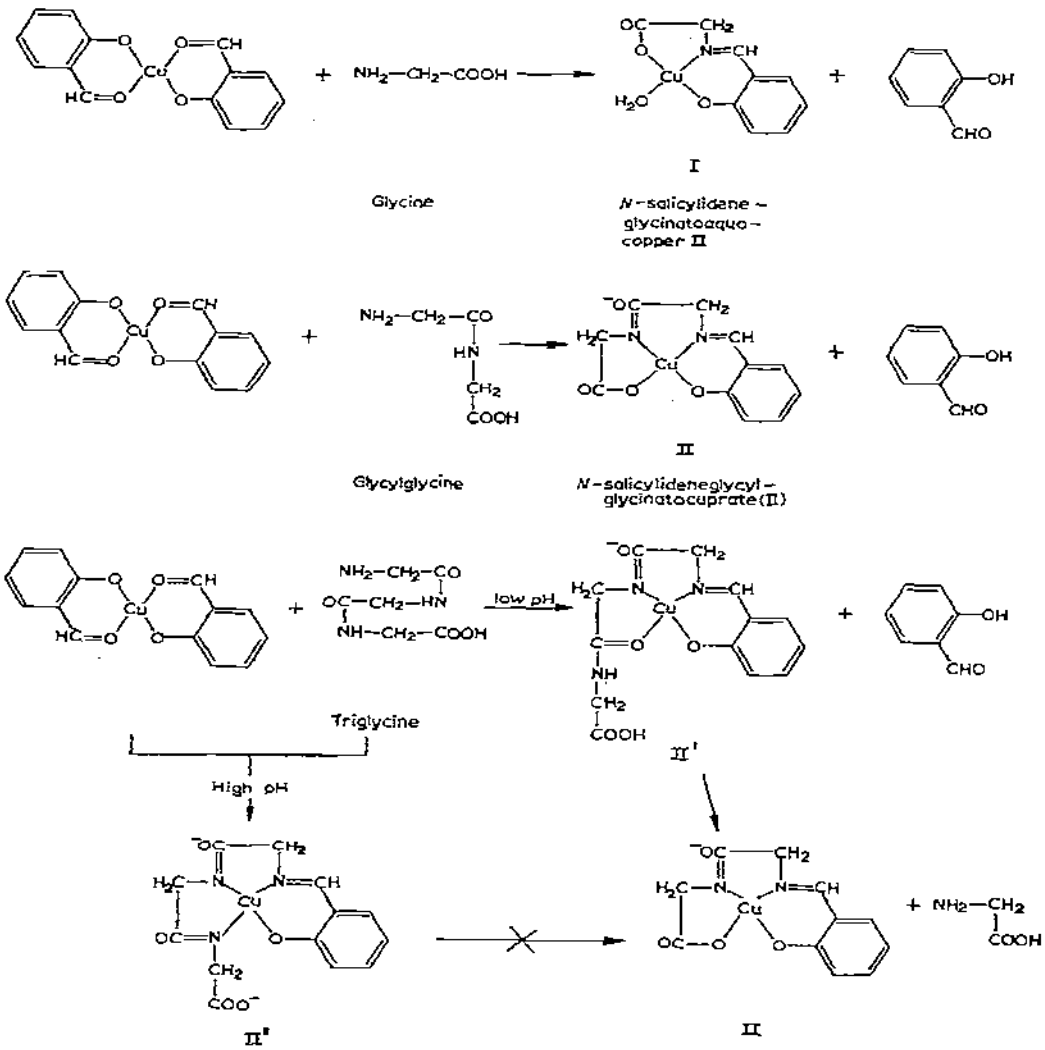
It has been demonstrated by Buckingham and Collman[2,3] that peptides are hydrolyzed in dilute aqueous solutions at pH values near 7 by stoichiometric reaction with the *cis*-β-hydroxyaquotriethylenetetraminecobalt(III) ion. They have reasoned that the hydrolytic reaction proceeds through a mechanism involving chelation of the terminal amino acid.

Recently, we have also found that bis(salicylaldehydato)-copper(II) severs the *N*-terminal dipeptides from some oligopeptides. Though the mechanism of the present reaction may not differ essentially from that of the reaction presented by Buckingham and Collman, it is of special interest to note that the hydrolytic reaction displayed by the present chelate is specific for the *N*-terminal dipeptide. In this paper we will describe the hydrolytic cleavage reactions of some oligopeptides under the influence of salicylaldehydato-copper(II), -nickel(II) and -cobalt(II).

B. THE REACTIONS OF BIS(SALICYLALDEHYDATO)COPPER(II) WITH GLYCINE, GLYCYLGLYCINE OR TRIGLYCINE

As was reported earlier[1], bis(salicylaldehydato)copper(II) reacts readily with glycine and glycylglycine in aqueous media to give the *N*-salicylideneglycinatoaquocopper(II), *I*,[4,5] and *N*-salicylideneglycylglycinatocuprate(II) ion, *II*[5], respectively. On the other hand, the reaction of bis(salicylaldehydato)copper(II) with triglycine in acidic media results in the hydrolysis of one of the two peptide linkages, producing the chelate *II* and free glycine.

The effective degradation of triglycine into glycylglycine and glycine is quite sensitive to pH; the optimum pH for the hydrolytic reaction has been found to be near 4.5, on the basis of a series of experiments (Fig. 1). For example, almost no hydrolysis has been observed for the reaction at pH 8.5. This may be because of formation



of a different intermediate as illustrated in II''. There are some reasons supporting the explanation that a different chelate is produced at a different pH. Pyridine-2-carboxamide can, for example, be coordinated around the metal as a bidentate ligand in two ways: at low pH by pyridine nitrogen and carboxamide oxygen, and at high pH by the pyridine nitrogen and the amide nitrogen[6–8]. The structure of the 3NlO-type chelation as shown in II'' is supposed to be less favourably affected by the hydrolytic attack of water molecule or hydroxyl ion, since the peptide linkage is involved in the metal-chelate ring. These are the reasons why the structure of the intermediate chelate must he II', as far as the successful hydrolytic reactions are concerned.

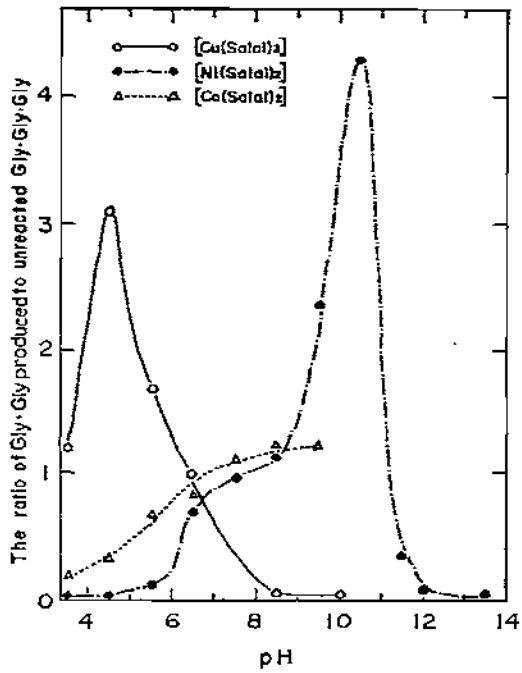


Fig. 1. The pH-dependence of the hydrolytic reaction of tripeptides by $[Cu(Salal)_2]$, $[Ni(Salal)_2]$ and $[Co(Salal)_2]$.

Analyses of the amino acids and small peptides, which were indispensable for estimating the catalytic effect of the chelate, were successfully achieved by the use of an amino acid analyzer. The sample solutions for analysis were prepared by precipitating copper(II) as copper(II) sulfide from the reaction products which had, for example, been treated at pH 4.5 and 70 °C for three hours. A typical example of the analysis of a reaction product is shown in Fig. 2.

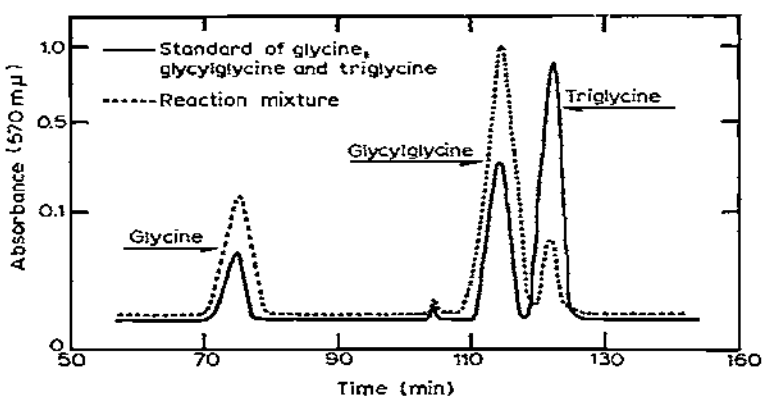


Fig. 2. Typical result of amino acid analysis.

C. THE REACTIONS BETWEEN BIS(SALICYLALDEHYDATO)COPPER(II) AND ALANYLGLYCYLGLYCINE OR GLYCYLGLYCYLALANINE

The hydrolytic reactions of alanylglycylglycine and glycylglycylalanine have also been investigated in order to confirm the validity of the proposed mechanism. If the mechanism presented above is valid, only alanylglycine and free glycine must be produced as the result of the reaction between bis(salicylaldehydato)-copper(II) and alanylglycylglycine. On the other hand, free alanine and glycyl-glycine must be obtained out of the reaction between the same chelate and glycyl-glycylalanine. The results of experiments have shown that the above expectations are mostly true.

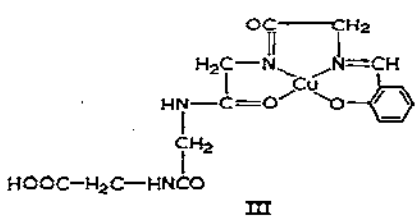
$[Cu(Salal)_2]+H\cdot Ala\cdot Gly\cdot Gly\cdot OH \rightarrow [Cu(Salal = Ala\cdot Gly)]+Gly+Salal$

$[Cu(Salal)_2]+H\cdot Gly\cdot Gly\cdot Ala\cdot OH \rightarrow [Cu(Salal = Gly\cdot Gly)]+Ala+Salal$

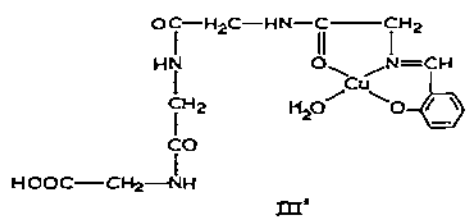
However, the main reaction as described here is always, more or less, accompanied by some unfavorable side reactions, which we will discuss in the next section. From the reaction system containing alanylglycylglycine and the copper(II) chelate, for example, a very small but still appreciable amount of alanine is detected together with large quantities of alanylglycine and glycine. Nevertheless it may be concluded that the tripeptides are hydrolyzed under the influence of the bis-(salicylaldehydato)copper(II), and that the reactions are nearly specific for the *N*-terminal dipeptide.

D. THE REACTION OF BIS(SALICYLALDEHYDATO)COPPER(II) WITH TETRAGLYCINE

The reaction of the same chelate with tetraglycine has also been investigated. The hydrolysis of tetraglycine also occurs efficiently like that of triglycine, but the reaction is less uniform than in the case of triglycine. In other words, the reaction product contains not only glycylglycine but also glycine and triglycine, although the yield of glycylglycine is much greater than the others. This kind of unfavorable hydrolytic reaction may be explained by the coexistence of a different complex species as illustrated in III', as well as the formation of the typical species, III. As for the structure of the intermediate III', the first peptide linkage from the *N*-terminal dipeptide may be much easier hydrolyzed than the other peptide linkages, since the linkage concerned is located close to the metal-chelate ring. We have



III



III'

succeeded in isolating a chelate of type III' in the case of glycylglycine. It has been confirmed that a chelate of type III' is readily converted to a chelate of type III (in the case of glycylglycine it corresponds to the chelate II) by adding a small amount of base. Moreover, it has also been confirmed that heating an aqueous solution containing a chelate of type III' results in the hydrolytic cleavage of glycylglycine at the expected linkage.

E. THE REACTIONS OF BIS(SALICYLALDEHYDATO)-NICKEL(II) AND -COBALT(II) WITH SOME PEPTIDES

The above described reactivity of bis(salicylaldehydato)copper(II) in hydrolyzing peptides has been compared with that of the corresponding nickel(II) and cobalt(II) chelates. In the case of nickel(II) and cobalt(II) chelates, the reactivity is less sensitive to pH than in the case of copper(II) chelate, as is clear from Fig. 1. The optimum pH for the hydrolytic reaction also differs from that for the copper-(II) chelate, and is found to be near 10. The results of reactions at pH values near 10 between bis(salicylaldehydato)-nickel(II) or -cobalt(II) and tripeptides are not very different from the best results of the copper(II) chelate. However, reactions between these nickel(II) and cobalt(II) chelates with tetraglycine are considerably inferior than those of the copper(II) chelate. In other words, the reaction is more difficult to occur and less uniform. Details of the experimental results are summarised in Table 1.

TABLE 1

COMPARISON OF THE REACTIVITIES OF BIS(SALICYLALDEHYDATO)-COPPER(II),-NICKEL(II) AND -COBALT(II) IN THE HYDROLYTIC REACTION OF TETRAGLYCINE

chelate	*pH*	*unreacted*	*hydrolyzed product*		
		4G	*3G*	*2G*	*G*
[Cu(Salal)$_2$]	4.5	1	0.75	2.64	1.07[a]
[Ni(Salal)$_2$]	8.5	1	0.03	1.09	0.02
[Co(Salal)$_2$]	8.5	1	0.19	0.66	0.10

[a] The values indicate the ratio of each species after the reaction at 70 °C for three hours.

Though the experimental data we now have are not sufficient to establish a practical method for the hydrolysis of peptides, further pursuit of this kind of work might be helpful in understanding the mechanism of reactions of various peptidases in biological systems.

F. EXPERIMENTAL

Hydrolytic Reactions of Peptides with Metal Chelates: one-to-one, two-to-one and five-to-one molar mixtures of metal chelates and peptides were dissolved in aqueous ethanol (50% by volume mixture) or aqueous dimethylformamide (50% by volume mixture), and were adjusted to the respective pH by dilute solutions of hydrochloric acid and sodium hydroxide. The mixtures were stirred and heated at about 70 °C for three hours. After they had been cooled at room temperature, metals were precipitated as sulfides and filtered. The filtrates were diluted to suitable concentrations for analysis.

. Analyses of amino acids and small peptides: A Yanagimoto LC-5 Amino Acid Analyzer was used to estimate the hydrolytic reactions. A typical analysis is shown in Fig. 2. All peaks for glycine, alanine, glycylglycine, triglycine and tetraglycine were separately obtained according to the standard operating procedure. Under the same conditions, the quantitative analyses of alanylglycine and glycylalanine were not attained because of their close retention time with those of unreacted alanylglycylglycine and glycylglycylalanine, respectively. For that reason, the hydrolytic reactions of the peptides concerned were estimated only on the basis of the analytical data of glycine and alanine, respectively.

ACKNOWLEDGEMENT

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REFERENCES

1 The first paper of this series of work will appear in *Bull. Chem. Soc. Japan*, 40 (1967) 2826.
2 J. P. COLLMAN AND D. A. BUCKINGHAM, *J. Am. Chem. Soc.*, 85 (1963) 3039.
3 D. A. BUCKINGHAM, J. P. COLLMAN, D. A. R. HAPPER AND L. G. MARZILLI, *ibid.*, 89 (1967) 1082.
4 G. L. EICHHORN AND N. D. MARCHAND, *ibid.*, 78 (1956) 2688.
5 A. NAKAHARA, *Bull, Chem. Soc. Japan*, 32 (1959) 1195.
6 K. YAMASAKI AND M. SEKIZAKI, *ibid.*, 38 (1965) 2206.
7 A. MASUKO, T. NOMURA AND Y. SAITO, *ibid.*, 40 (1967) 511.
8 Y. NAWATA, H. IWASAKI AND Y. SAITO, *ibid.*, 40 (1967) 515.