Tetrahedron Letters No. 18, pp. 1501-1504, 1970. Pergamon Press. Printed in Great Britain.

A SIMPLE AND NOVEL PEPTIDE BOND FORMATION BY COPPER(II) COMPLEX

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(Received in Japan 24 February 1970; received in UK for publication 13 March 1970)

Acceleration of hydrolysis rates of amino acid esters,[1] amides[2] and peptides[3] has been frequently observed in the presence of Cu(II) ion and cobalt(III) complex ion. Furthermore, peptide bond formation by cobalt(III) complexes and amino acid esters were independently found by Buckingham[4] and Collman.[5] A different type of peptides formation was recognized by Nakahara et al[6] using a Cu(II) complex of the Schiff base derived from salicylaldehyde and glycinamide. However, no isolation of the peptide formed from these metal complexes has been reported in any of the papers cited above,[4,5,6] and it was described[5] that racemization of asymmetric centers in the peptides formed would easily occur on the metal because of labilization of the α-hydrogen in the metal complex.

We wish to report simple and novel peptide bond formation in non-aqueous solutions at room temperature using Cu(II) ion and the successful isolation of peptides from the metal complex without racemization. Treatment of the alcoholic solution of amino acid esters with the Cu(II) ion has resulted in the formation of dipeptides, and in case of glycine esters tri- and tetrapeptides were isolated in a single step reaction besides dipeptide.

$$\text{H-Gly-OEt} \xrightarrow[\text{2) Ph}\cdot\text{CH}_2\cdot\text{OCO}\cdot\text{Cl, Et}_3\text{N in CHCl}_3]{\text{1) Cu}^{++}\text{ in EtOH}} \text{Z-Gly}_2\text{-OEt} + \text{Z-Gly}_3\text{-OEt} + \text{Z-Gly}_4\text{-OEt}^{7)}$$

An ethanolic solution (60 ml) containing H-Gly-OEt·HCl (60 mM) and Et_3N (60 mM) was added to an ethanolic solution (10 ml) of anhyd. $CuCl_2$ (5 mM).

The blue solution which was obtained, was stirred at room temperature for 3 hr. The Cu(II) complex was decomposed by adding ethanol saturated with hydrogen chloride. After Cu(II) ion was removed as CuS by treatment with H_2S, a mixture of peptide ester hydrochlorides obtained by the evaporation of the ethanolic solution was treated with carbobenzyloxy chloride-Et_3N in $CHCl_3$ in order to characterize the peptides which formed. From the reaction mixture, Z-Gly_4-OEt[7] precipitated in 2.7% yield based on H-Gly-OEt (8% yield based on $CuCl_2$ used[8]). The structure of this compound was confirmed from its melting point (mp 209°),[9] IR spectrum and elemental analysis. After evaporation of the $CHCl_3$ solution, an oily substance which formed was purified by column chromatography on silica gel ($CHCl_3$: EtOH = 97 : 3), giving Z-Gly_2-OEt[7] (mp 77-79°) and Z-Gly_3-OEt[7] (mp 162-164°) in 28.1% and 22.7% yields based on H-Gly-OEt (169% and 91% yields based on $CuCl_2$[8]). These peptides were identified with authentic samples.

Examination of this novel reaction using other Cu(II) salts have disclosed that $CuSO_4$, $Cu(OAc)_2$ and CuO are also effective for this peptide formation. It should be noted that no appreciable amount of diketopiperazine is observed in any of these reactions.

Next, this peptide formation reaction was examined using several optically active amino acid esters. As shown in the Table, various kinds of dipeptides with the same amino acid sequences were obtained without racemization. Unlike the H-Gly-OEt reaction, formations of tri- and tetrapeptides were not observed in these cases. This is probably due to the steric effect of the side chain of amino acid esters. It is also characteristic that only α-esters show peptide formation in case of H-L-Asp(OEt)-OEt and H-L-Glu(OCH_3)-OCH_3.

Detailed studies on the reaction mechanism are under way in our laboratory, however, this new peptide bond formation is considered to be a reaction by Cu(II)-amino acid ester complex from the following reasons: 1) A formation of H-Gly_2-OEt and diketopiperazine was not observed when an ethanolic solution of H-Gly-OEt was stirred at room temperature for 3 hr. without $CuCl_2$. 2) The reaction of Cu(II) complex of H-Gly-OEt, $CuCl_2$(H-Gly-OEt)$_2$,[10] in which two amino groups were coordinated to the central Cu(II) ion, with H-Gly-OEt in an ethanolic solution gave the similar results as those described above. 3) In

Table Peptide Formation with Various Amino Acid Esters Using $CuCl_2$[a]

No	Amino Acid Esters	Solv.	Z-Peptides Isolated[b] Yields(%) Based on Amino Acid Esters[c]	M.p. °C	$[\alpha]_D$(Temp. Solv.)	Optical Rotation of Pure Sample[d] $[\alpha]_D$(Temp. Solv.)
1	H-L-Phe-OEt	EtOH	Z-L-Phe-L-Phe-OEt 18.2 (109)	130 -134	-16.6° (28, EtOH)	-17.6° (24.6, EtOH)
2	H-L-Ala-OMe	MeOH	Z-L-Ala-L-Ala-OMe 34.5 (207)	98 -101	-51.5° (16, MeOH)	-53.5° (16, MeOH)
3	H-L-Leu-OMe	MeOH	Z-L-Leu-L-Leu-OMe 27.1 (163)	94 -96	-34.2° (25, EtOH)	-35.5° (24, EtOH)
4	H-L-Ser-OMe	MeOH	Z-L-Ser-L-Ser-OMe 21.5 (129)	138 -141	-3.0° (22, MeOH)	-3.5° (22, MeOH)
5	H-L-Met-OMe	MeOH	Z-L-Met-L-Met-OMe 21.7 (130)	98 -100	-25.6° (19, MeOH)	-28.0° [e] (19, MeOH)
6	H-L-Asp(OEt)-OEt	EtOH	Z-L-Asp(OEt)-L-Asp(OEt)-OEt 10.8 (64.8)	81 -85	-12.2° (24, EtOH)	-12.3° (18, EtOH)
7	H-L-Glu(OMe)-OMe	MeOH	Z-L-Glu(OMe)-L-Glu(OMe)-OMe 7.4 (44.4)	105 -107	-20.7° (13, MeOH)	-21.0° (13, MeOH)

a) An equimolar mixture of amino acid ester hydrochloride and triethylamine in alcohol was stirred at room temperature for 3 hr. Molar ratio of $CuCl_2$ to amino acid ester is 1 : 12 in all cases. b) Measurements of melting point and optical rotation were performed using a sample isolated by column chromatography on silica gel. c) Figures in parentheses are yields of dipeptides calculated on $CuCl_2$ used.[e] d) Prepared independently using the DCCD method from Z-amino acid and amino acid ester. e) M. Brenner, R.W. Pfister, Helv. Chim. Acta, 34, 2085 (1951).

case of H-Gly-OEt, total yields of peptides (di-, tri- and tetrapeptides) are about 268% based on $CuCl_2$ used.[e] This implies that at least three peptide chains formed from one Cu(II) ion.

Investigation as to whether or not transition metal ions other than Cu(II) are effective for this peptide formation are also under progress.

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7) Z-Gly_2-OEt = Z-Gly-Gly-OEt, Z-Gly_3-OEt = Z-Gly-Gly-Gly-OEt
Z-Gly_4-OEt = Z-Gly-Gly-Gly-Gly-OEt (Z : $C_6H_5 \cdot CH_2 \cdot OCO$-)

8) The calculation is based on the assumption that one mole of peptides should be produced from one mole of $CuCl_2$.

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