The Chemical Cleavage of the Tryptophyl Bond

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The chemical cleavage of a specified peptide bond in the polypeptide chain has been used for the sequential analysis of proteins as well as the enzymatic hydrolysis.[1] The oxidative cleavage of the tryptophyl peptide bond has been studied by using *N*-bromosuccinimide.[2]

In order to establish a different procedure which can be strictly responsible for the selective cleavage of the tryptophyl bond, the authors previously proposed the scheme shown in Fig. 1.[3] Another approach, utilizing the γ-carbonyl function in compound (II) for the present purpose, has also been presented by Previero *et al.*[4]

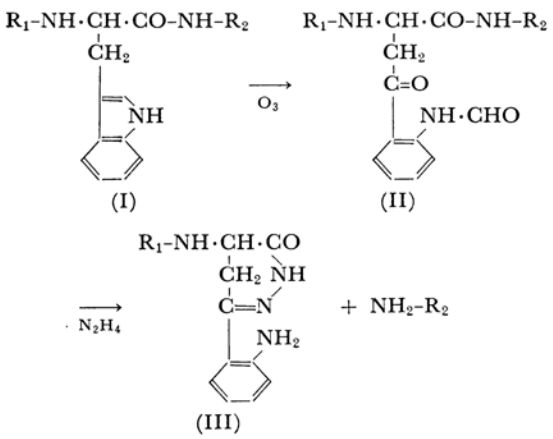

Fig. 1. The chemical cleavage of the tryptophyl bond.

The tryptophyl peptide (I) can be converted into the N'-formyl kynurenyl peptide (II) by ozone oxidation.[5] The cyclisation step leading to the specific cleavage from the II to the III was carried out by a modified method, using the hydrazine previously reported.[3]

A typical procedure for the chemical cleavage of the tryptophyl bond is as follows: the tryptophyl peptide (5 μmol) was dissolved in 99% formic acid (1 m*l*) containing resorcinol (1.1 mg). Ozone (1—2%) generated from oxygen was bubbled into the formic acid solution for half an hour at room temperature. A pipetted aliquot (0.1 m*l*, 0.5 μmol) was evaporated to dryness *in vacuo* at room temperature. The residue was dissolved in a 0.2 M hydrazine-acetate buffer (pH 3.6, 0.05 m*l*), and the aqueous solution was heated at 100°C for two hours. The amino acid released was assayed by paper chromatography and by a subsequent ninhydrin reaction.[6] The findings on the chemical cleavage of the synthetic tryptophyl peptide derivatives are summarized in Table 1.

TABLE 1. YIELD OF THE SPECIFIC CLEAVAGE AT THE TRYPTOPHYL BOND

Peptide derivative	Released amino acid by	
	The specific cleavage, %	The non-specific hydrolysis, %
Z[a]·Try·Gly·OH	35 (52)[b]	7
Z·Try·Ala·OH	57 (80)	5
Z·Try·Leu·OH	68 (95)	0
Z·Try·Phe·OH	49 (69)	0
Z·Try·Asp·OH	52 (53)	13
Z·Ala·Try·Leu·OH	54	—
H·His·Phe·Arg·Try·Gly·OH[c]	45	6
H·His·Phe·Lys·Try·Gly·OH[c]	43	7
H·His·Phe·Orn·Try·Gly·OH[c]	49	12

a) Z: Carbobenzoxy.
b) The yield shown in parenthesis was based on the kynurenine formed, which was determined by the ninhydrin reaction after acid hydrolysis of the oxidized peptide.
c) Supplied by Dr. H. Yajima of Kyoto University, Kyoto.

The formation of the pyridazone derivative (III) was confirmed by both spectroscopic and chromatographic comparisons with the authentic sample. The chemical cleavage of tyrocidin B[7] by the present procedure gave a new amino group, which was characterized as 2, 4-dinitrophenyl phenylalanine. The application of this method to the non-enzymatic cleavage of proteins is now in progress.

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5) B. Witkop, *Ann.*, **556**, 103 (1944); A. Previero, E. Scoffone, C. A. Benassi and P. Pajetta, *Gazz. chim. Ital.*, **93**, 849 (1963); A. Previero and E. Bordignon, *ibid.*, **94**, 630 (1964).
6) R. C. Canfield and C. B. Anfinsen, "The Proteins," Vol. 1, ed. by H. Neurath, Academic Press, New York (1963), p. 318.
7) Supplied by Dr. L. C. Craig of the Rockefeller Institute, New York.