NONENZYMATIC CLEAVAGE OF TRYPTOPHYL PEPTIDE BONDS IN PEPTIDES AND PROTEINS

I. Cleavage of C-tryptophyl peptide bonds in model peptides through their conversion into N'-formyl-kynurenine derivatives.

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Recently a number of nonenzymatic methods for preferential and selective cleavage of peptide bonds were reported and reviewed by Witkop (1961). Generally these methods involve a labilizing influence of suitable side chains on the adjacent peptide bond. Such conditions are sometimes the result of a specific chemical modification (Patchornik and Sokolowsky, 1964). In connection with the specific tryptophan ⟶ N'-formylkynurenine conversion in peptides and proteins (Previero and Bordignon, 1964), we investigated the labilizing influence of neighbouring γ-keto group on the hydrolysis of kynurenine peptides.

Materials and Methods

N-acylated tryptophyl peptides.

N-carbobenzyloxy-DL-tryptophylglycine ethyl ester, N-carbobenzyloxy-L-tryptophyl-L-leucine methyl ester, N-carbobenzyloxyglycyl-DL-tryptophan methyl ester and N-acetyl-DL-tryptophylglycine ethyl ester were synthetized from corresponding N-acylated amino acids and amino acid esters by the carbodiimide method. The acylated peptides with free carboxyl groups were obtained by saponification of corresponding ester derivatives.

Table I

Yields of amino acids liberated by preferential cleavage of tryptophyl peptides after their conversion into N'-formylkynurenine derivatives

Compounds	Time of hydrol. (min)	Extent of hydrolysis (%)					Cleavage product
		pH 8.3	pH 9	pH 9.4	pH 9.8	pH 10.4	
N-carbobenzyloxy-DL-tryptophylglycine	60	15.4	17	21.3	26.7	31.8	glycine
	240	30.8	42.5	46.7	53.0	59.2	
N-carbobenzyloxy-L-trytophyl-L-leucine	60	9.5	13.2	15.4	20.0	25.8	leucine
	240	21.0	30.0	33.4	40.0	48.8	
acetyl-DL-tryptophyl-glycine	60	13.2	15.5	21.0	24.2	30.0	glycine
	240	30.0	38.6	44.0	49.3	54.2	
N-carbobenzyloxyglycyl-DL-tryptophan							no detectable ninhydrin positive substances
N-carbobenzyloxy-DL-tryptophan	240	no estimable hydrolysis					
acetyl-DL-tryptophan							

N'-formylkynurenine derivatives.

5 ml of formic acid (98-100%) containing a tryptophyl peptide (10^{-4} mole) and 10 mg resorcinol were treated with a slow stream of ozone at 8-10°. The ozonizer was identical to the model described by Willard and Merrit (1942). At suitable intervals, 0.5 ml samples were withdrawn from the reaction mixture, transferred into 3 ml ethanol and the increase of optical density at 315 mμ was measured with a Beckman DU spectrophotometer. The ozone oxidation was stopped when the maximum increase in optical density was reached (Previero and Bordignon, 1964).

After evaporation of the formic acid, the residue was taken to dryness in vacuum over KOH and finally dissolved in aqueous water solutions (Table I).

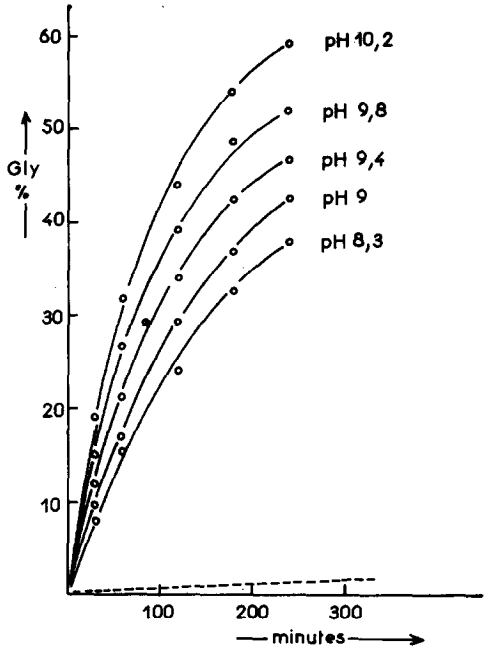

Figure 1

The liberation of glycine (by partial hydrolysis; different pH; 100°) from N-carbobenzyloxy-DL-tryptophylglycine before (----) and after (——) its conversion into N'-formylkynurenine derivative.

Hydrolytic cleavage.

N'-formylkynurenine derivatives (10^{-5} moles) were heated at 100° in 1 ml of 0.5 M $NaHCO_3$ and other buffer solutions ($NaHCO_3$-Na_2CO_3) of comparable ionic strenght at pH values listed in Table I. Aliquots of 0.1 ml were withdrawn at suitable intervals (see Table I) and tested with the ninhydrin reagent of Moore and Stein (1954) in order to determine the extent of the cleavage.

By paper chromatography of the reaction mixtures it was established that the only ninhydrin positive substance obtained from peptides was the liberated C-terminal amino acid.

When the tryptophyl peptides were submitted to the same hydrolytic procedures without previous oxidation, no hydrolysis occured.

Results

The data reported in Table I and plotted in Fig.1 indicate that the specific chemical conversion of tryptophan residues into N'-formylkynurenine residues increases the rate of hydrolysis of the adjacent C-peptide bonds in basic media. Preliminary experiments have shown that a similar increase occurs in acidic media but the use of too extensive degradation conditions does not allow useful applications.

It is reasonable to suppose an intramolecular catalysis involving the neighbouring γ-keto group of the N'-formylkynurenine residue or its addition product with a nucleophyle hydroxyl ion (Bender and Silve, 1962).

The yield (60-70%) of the tryptophan $\xrightarrow{O_3}$ N'-formylkynurenine conversion in proteins (Previero and Bordignon, 1964) and the conditions employed for the preferential hydrolytic cleavage of N'-formylkynurenine derivatives suggest fruitful applications in protein chemistry.

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