Stepwise Degradation of Peptides

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In the stepwise degradation of peptides according to Ottesen's modification of Edman's method (Edman [1], Fraenkel-Conrat [2], Ottesen and Wollenberger [3], Christensen [4], the phenylthiocarbamido derivative formed in the reaction of a peptide with phenylisothiocyanate, is brought to react in aqueous solution to form thiohydantoin and a peptide with one amino acid less than the original peptide. In spite of the mild conditions maintained during the ring closure, 0.1 *N* HCl, 65° C during 2—24 hours, undesired side reactions sometimes occur *e.g.* hydrolysis of peptide bonds along the peptide chain. We have therefore considered it of interest to investigate the possibility of finding milder conditions for the ring closure. Among the preliminary results of these investigations we may mention that certain thiohydantoins form readily at 60—70° C in 0.05 *M* aqueous citrate buffer at pH 4.5—5 and may be extracted with an organic solvent. In order to reduce the possibility of a back-reaction between thiohydantoin and peptide and of a decomposition of the former, ring closure was effected at the given conditions but with simultaneous extraction with the organic solvent in a modified Steudel-Kutcher apparatus.

For lower peptides the following procedure has proven satisfactory. One ml of a 0.02 *M* solution of the peptides is reacted with phenylisothiocyanate at pH 8 according to Ottesen and Wollenberger. After removal of dioxane and excess of reagent by extraction with cyclohexane, 1 ml citrat buffer of the desired pH is added and continuous extraction with benzene at 70° is performed. After 10—24 hours extraction practically all thiohydantoin is found in the benzene. The quantity obtained is determined by U.V. absorption measurements after evaporation of the benzene and dissolution in ether. Since the uncharged phenylisothiocarbamido derivatives are slightly soluble in benzene pH should not be too low. These compounds have a maximum of absorption at $\lambda = 245$ mμ where the hydantoins have a minimum. A comparison of the extinction at the maxi-

Table 1.

Peptide	Time of extraction 70° (hours)	pH	Yield * %
Alanylglycine	5	5	98 alanine
Glycylaspargine	24	4.4	83 glycine, 80 aspargine **
Glycylglycine	18	5	94 glycine
Alanylglycylglycine	18, 17, 20	4.5	98, ala, 92, gly_1, 93 gly_2

* All hydantoins isolated were hydrolyzed in barium hydroxide and the amino acids involved investigated by filter paper chromatography. No other spots than the expected ones appeared.

** Determined by the ninhydrin method in the aqueous solution after extraction of the glycine hydantoin.

mum and minimum, 268 mμ and 245 mμ respectively may therefore give information about the purity of the extracted material. Above pH 4.3 only hydantoins are found in the benzene. Isopropylether (proposed by A. L. Levy) is probably a better solvent for thiohydantoins but it is not as selective as benzene and extracts appreciable amounts of the phenylisothiocarbamido derivatives of shorter peptides.

The role of the citrate buffer is as yet unclear, but definitely less satisfactory results were obtained in extraction experiments where no buffer was used and pH was continuously adjusted by addition of acid. Oxalate buffer may replace citrate but its buffer capacity is inferior to that of citrate in the important range pH 4—5.

The presence of buffer will cause the salt concentration of the reaction medium to rise from step to step in the degradation of peptides and will make the evaluation of the titration curves[3] difficult. Hence the yield in the reaction of phenylisothiocyanate can only be judged from the yield of thiohydantoin.

The table shows some results with simple peptides.

As regards the application of this method to proteins we may just point out that the phenylisothiocarbamido derivatives of these substances are insoluble in organic solvents and that lower pH-values and other extraction media (*e.g.* isopropylether) may be adopted. The stability of the protein chain naturally sets the ultimate limit for such modifications.

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