HYDROLYSIS OF PHENYLTHIOHYDANTOINS OF AMINO ACIDS

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The Edman (1950) proposal for sequential determination of the amino acids in a peptide chain has been applied in a direct or subtractive manner. In the direct manner, the nature of the N-terminal amino acid is determined by identification of the liberated phenylthiohydantoins of the N-terminal amino acids by paper or column chromatography (Sjöquist, 1955, 1960; Edman and Sjöquist, 1956). In the subtractive procedure, the N-terminal amino acid is identified by the difference in amino acid composition of the peptide before and after application of the degradation reaction (Fox, et al., 1951; Hirs, et al., 1960). In a modified direct procedure, the liberated phenylthiohydantoin is subjected to various hydrolytic treatments to release the constituent amino acid which is identified by paper chromatography (Edman, 1950; Ingram, V. M., 1953; Levy, 1954). The present report explores the use of the amino acid analyzer to identify and quantitate the amino acids released upon hydrolysis of 3-phenyl-2-thiohydantoins of various amino acids. The thiohydantoins have been subjected to several types of hydrolytic treatment in an attempt to determine optimum conditions for cleavage to the constituent amino acids. The hydrolytic conditions included an alkaline hydrolysis similar to that recently proposed by Stark and Smyth (1963) for the hydrolysis of the hydantoins of amino acids.

EXPERIMENTAL

The 3-phenyl-2-thiohydantoins of the amino acids were prepared in this laboratory and characterized by melting point, elementary analyses and ultraviolet absorption spectra. Amino acid analyses were determined by the method of Moore, et al. (1958), and Spackman, et al. (1958), using a Beckman/Spinco Amino Acid Analyzer.

Solutions of the thiohydantoins were prepared to contain 1 to 1.5 μmoles of compound per ml. A 1-ml aliquot of each solution was introduced into a hydrolysis tube (micro-Carius), and the solvent was removed under a stream of nitrogen to yield the solid phenylthiohydantoin which was subjected to acid or basic hydrolysis. The solutions prepared were: (1) three solutions in ethyl acetate, each containing the phenylthiohydantoins of aspartic acid, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine; (2) two solutions (in acetone containing 1% of water), each containing the phenylthiohydantoins of arginine hydrochloride, histidine hydrochloride, and N$^{\varepsilon}$-phenylthiocarbamyl-lysine; (3) two solutions (in ethyl acetate with 5% acetone) of each of the single phenylthiohydantoins of serine and threonine.

Hydrolyses were performed by adding either 3 ml of 0.1 N NaOH or 2 ml of 6 N HCl (metal-free) to each tube containing the solid phenylthiohydantoin. The contents were frozen and the tubes were evacuated on an oil pump and sealed. The tubes were heated in an oil bath at 120 or 150° for 24 or 48 hours. The hydrolysates (acidified with 6 N HCl in the case of the alkaline hydrolysis) were concentrated to dryness on a rotary evaporator. The evaporation was repeated twice after the addition of water. The solid residue (except for precipitated silica) was dissolved in 5 ml of the pH 2.2 citrate buffer (Moore and Stein, 1954), the mixture was centrifuged (when necessary to remove suspended silica), and the supernatants were stored at -10° until subjected to amino acid analyses.

The results shown in Table I are average values obtained from the two or three completely separate determinations. Values agreed within $\pm$ 3% of the mean.

TABLE I

HYDROLYSIS OF PHENYLTHIOHYDANTOINS

Phenylthiohydantoin hydrolyzed	Per cent of phenylthiohydantoin recovered as amino acid			
	0.1N NaOH, 120° 12 hours	6N HCl, 120° 24 hours	6N HCl, 150° 24 hours	6N HCl, 150° 48 hours
Lysine	72			
Histidine	70			
Arginine as Ornithine	< 2 53			
Aspartic acid	89	48	88	80
Threonine as Glycine	< 1 67		< 1	
Serine	< 1		< 1	
Glutamic acid	66	39	81	72
Proline	96	64	88	80
Glycine	96	70	104	99
Alanine	86	40	78	66
Valine	98	12	88	83
Methionine	84	29	67	52
Isoleucine as Alloisoleucine	43 58	4 6	39 51	37 44
Leucine	71	22	59	50
Tyrosine	97	16	76	66
Phenylalanine	95	16	86	78

RESULTS

The recovery of amino acids upon hydrolysis of their phenylthiohydantoins is shown in Table I. In general, the phenylthiohydantoins were only partially hydrolyzed in 6 N HCl at 120° for 24 hours--conditions which give complete hydrolysis of most proteins. Upon increasing the temperature to 150°, the yields of amino acids from the hydrolysis in 6 N HCl were much improved and, in some cases, approached 90% of the theoretical value. Extending the reaction time to 48 hours at 150° resulted in lower values than those obtained in 24 hours. Both serine and threonine were completely destroyed in the acid hydrolysis. With the exception of the phenylthiohydantoin of glutamic acid, all of the other phenylthiohydantoins studied gave a larger yield of the constituent amino acids under the alkaline hydrolytic conditions (0.1 N NaOH for 24 hours at 120°) than under any of the acidic conditions. Again serine was completely destroyed, but threonine was largely recovered as glycine (67%). Arginine was recovered in 53% yield as ornithine. In both the acidic and basic hydrolysis, isoleucine was recovered in part as alloisoleucine. The total of isoleucine plus alloisoleucine approached the theoretical amount in the basic hydrolysis. In general, our results are consistent with the earlier studies (Edman, 1950; Ingram, 1953; Levy, 1954) which used paper chromatographic techniques to detect and quantitate the amino acid recoveries, and are in close agreement with the results of Stark and Smyth (1963) on the hydrolysis of the hydantoins of amino acids. With the exceptions noted above, the recoveries of the constituent amino acids in the alkaline hydrolysis of the 3-phenyl-2-thiohydantoins of the amino acids were sufficiently high that the values could be used without correction in the determination of an N-terminal amino acid.

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