ACID HYDROLYSIS OF PHENYLTHIOHYDANTOINS OF AMINO ACIDS

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Abstract

The phenylthiohydantoins (PTHs) derived from amino acids were hydrolysed in boiling hydriodic acid for 24 hr. Good yields of free amino acids were obtained for all PTH derivatives except methionine. In contrast to hydrolysis with hydrochloric acid, hydrolysis with hydriodic acid converts PTH-threonine, PTH-serine, and PTH-tryptophan respectively to α-amino-n-butyric acid, alanine, and a mixture (approx. 2 : 1) of glycine and alanine. This procedure provides a useful adjunct to thin-layer chromatography and ultraviolet spectroscopy for quantitative identification of the PTH derivative.

I. Introduction

In 1950 Edman described a method for degradation of peptides with phenyl isothiocyanate. Acid cleavage of the peptide bond of the *N*-terminal amino acid resulted in the formation of a 3-phenyl-2-thiohydantoin (PTH) via an unstable 2-anilino-5-thiazolinone (Edman 1956). With the advent of the protein sequenator (Edman and Begg 1967) the problem of rapid identification of the amino acid cleaved from the peptide chain has increased in significance because the sequenator separates an amino acid (as a 2-anilino-5-thiazolinone) every 90 min. One approach to this problem (Edman and Begg 1967; Edman 1970) is to convert the thiazolinone to the PTH derivative, identify it by thin-layer chromatography, and quantitate the yield from the ultraviolet spectrum. This is undoubtedly the method of choice for single residues because of the great advantage of the thin-layer method with respect to rapidity and multiple processing of samples. If more than one residue is present, or some ambiguity remains, quantitation and confirmation can be obtained from gas chromatography (Pisano and Bronzert 1969) or amino acid analysis after hydrolysis. Van Orden and Carpenter (1964) and Africa and Carpenter (1966) established conditions for hydrolysis of the PTH derivatives of amino acids to the free amino acids by acid and alkali respectively. This is one method of identifying the *N*-terminal amino acid of peptides and proteins after Edman (1950) degradation. As a sequel to our earlier work (Inglis, Nicholls, and Roxburgh 1971*a*, 1971*b*) we investigated the possibility of using hydriodic acid instead of hydrochloric acid for the hydrolysis.

An alternative approach for protein sequencing has been made by Smithies, Gibson, and Fanning (personal communication), and it also incorporates hydriodic acid hydrolysis. They propose the use of two hydrolytic procedures—one acid, the

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other alkaline—on the thiazolinone for an unequivocal and quantitative determination of the *N*-terminal amino acid. This procedure has the advantage that it eliminates the manipulations involved in converting the 2-anilino-5-thiazolinone to the PTH derivative. On the other hand it requires two samples and neglects the information that can be derived from the non-destructive spectral and thin-layer analyses of the more stable PTH derivative. Besides the hydrolytic results for PTH derivatives of amino acids in hydriodic acid, results for hydrobromic acid hydrolysis, and hydriodic acid hydrolysis of thiazolinones are also discussed in this paper.

II. Experimental

All procedures and reagents were the same as described previously (Inglis, Nicholls and Roxburgh 1971*a*).

The PTH amino acids were as supplied in the kit by Pierce Chemical Co. and were not purified further.

III. Results and Discussion

The rapid hydrolysis of peptide bonds with hydriodic acid (Inglis, Nicholls, and Roxburgh 1971*a*) is not paralleled for the hydrolysis of all PTH derivatives of amino acids. In general we found that it was better to hydrolyse for 24 hr even though some PTH derivatives gave excellent yields after quite short hydrolysis times (e.g. PTH-glycine gave 100% recovery of glycine after 4 hr hydrolysis). Reasonable yields (49%) of lysine were obtained after 4 hr refluxing of the PTH of ϵ-phenylthiocarbamyllysine with constant-boiling hydriodic acid. Attempts to increase these yields for short reaction times by raising the hydrolysis temperature were unsuccessful and led to lower yields of lysine with a concomitant increase in ammonia.

When the PTH derivatives were refluxed in hydriodic acid for 24 hr, the yields of the free amino acids were usually good and showed some improvement over the hydrolysis in 6N HCl in a sealed tube at 150°C (Van Orden and Carpenter 1964). The results for hydriodic acid hydrolyses are listed in Table 1 alongside the acid (Van Orden and Carpenter 1964) and alkaline (Africa and Carpenter 1966) hydrolysis results previously published.

Results for some PTH amino acids were also higher for hydriodic acid hydrolysis than for alkaline hydrolysis. Two of these in particular are important because they are for serine and threonine, amino acids which are generally obtained in low yield after the degradation procedure. Serine is not recovered at all after either HCl or NaOH hydrolysis while threonine is recovered as glycine in 67% yield after alkaline hydrolysis. Furthermore, the conditions for alkaline hydrolysis are the more demanding; much lower results than those shown in Table 1 are obtained if oxygen is not rigorously excluded. In hydriodic acid PTH-threonine is converted in high yield to α-amino-n-butyric acid and so results in an unambiguous identification on the amino acid analyser. As with serine in proteins, PTH-serine is largely converted to alanine. This, of course, could lead to an ambiguity in identification. Alanine is also formed on hydrolysis of the PTH of *S*-carboxymethylcysteine. However, neither PTH-serine nor PTH-*S*-carboxymethylcysteine would be confused with PTH-alanine after thin-layer chromatography using system H of Edman (1970) since the R_F values are widely different. In addition after a degradation PTH-serine is

characterized both by additional spots on the chromatogram and by the ultraviolet spectrum which shows some absorption at 320 nm. The amino acid analysis would therefore confirm the amount of these PTH amino acids present unless, of course, they appear at the same step in the degradation.

Hydriodic acid hydrolysis also provides an unambiguous and quantitative determination of PTH-tryptophan. It yields relatively large amounts of glycine (43%) and alanine (26%). This fission between the α–β and β–γ carbon atoms of tryptophan is characteristic only of the PTH derivative and there was no evidence for it during hydrolysis of tryptophan alone or in peptide combination in a protein. Glycine and alanine, and trace amounts of glycine were also obtained as minor products of hydrolysis of PTH-tyrosine and PTH-phenylalanine respectively.

TABLE 1

HYDROLYSIS OF PHENYLTHIOHYDANTOIN (PTH) DERIVATIVES WITH 5N HI

X = α-amino-n-butyric acid; Y = homocysteine thiolactone; Z = allo-isoleucine; n.d., not determined

PTH	Amino Acid Recovered (%)			PTH	Amino Acid Recovered (%)		
	HI*	HCl†	NaOH‡		HI*	HCl†	NaOH‡
Trp	43	n.d.	83	Gly	98	104	77
	(as Gly)			Ala	85	78	83
	26			Val	66	88	102
	(as Ala)			Met	12	67	102
Lys	78	n.d.	81		(as Y)		
His	46	n.d.	100	Ile	31	39	41
Arg	70	n.d.	45		42	51	53
			(as Orn)		(as Z)	(as Z)	(as Z)
Asp	78	88	99	Leu	88	59	88
Thr	78	< 1	64	Tyr	81	76	96
	(as X)	(as Gly)	(as Gly)	Phe	76	86	103
Ser	97	< 1	< 1	CMCys	92	n.d.	n.d.
	(as Ala)				(as Ala)		
Glu	87	81	66	Met. oxide	66	n.d.	n.d.
Gln	52	n.d.	59	Asn	89	n.d.	88
	98				105		
	(as NH_3)				(as NH_3)		
Pro	87	88	91				

* 5N HI, 140°C, 24 hr, sealed tube.

† 6N HCl, 150°C, 24 hr, sealed tube (Van Orden and Carpenter 1964).

‡ 0·1N NaOH, 120°C, 12 hr, oxygen-free (Africa and Carpenter 1966).

Hydrolysis of PTH-histidine gave a low yield of histidine (46%) and ammonia (16%). Hydrolysis of PTH-methionine gave unacceptably low yields of homocysteine thiolactone (12%) and ammonia (17%). Slightly improved recoveries were obtained for hydrolysis times of 4 hr and 1 hr which yielded 24 and 16% respectively of the thiolactone. The preliminary chromatography using system D (Edman 1970) should clearly establish the identity of this amino acid derivative. Alternatively, it would be possible to oxidize PTH-methionine to the sulphone prior to hydrolysis in hydriodic

acid because this derivative affords a good yield (66%) of methionine sulphone on hydrolysis. PTH-glutamine yielded less glutamic acid than did PTH-glutamic acid but quantitative yields of ammonia were obtained. This is another amino acid derivative which gives higher recoveries of the amino acid after shorter hydrolysis times. Throughout this work there was no strong evidence to support either the use of colourless instead of coloured hydriodic acid or hydrolysis under vacuum instead of reflux, although a colourless hydriodic acid reagent in an evacuated sealed tube was used for the greater portion of the work.

Hydriodic acid acts similarly on the 2-anilino-5-thiazolinone of the amino acid (Smithies, Gibson, and Fanning, personal communication). We have not investigated the thiazolinone hydrolysis extensively. This is a less convenient task than analysing the purified PTH derivatives. Thiazolinone derivatives admixed with other amino acids were obtained by a manual Edman degradation on a number of dipeptides. Hydrolysis of the mixtures in hydriodic acid generally revealed either equivalent or lower yields of the amino acids from the thiazolinones than expected from the hydrolysis of the PTH derivatives and it should be stressed that, for derivatives of serine, threonine, and tryptophan which are likely to be obtained in low yield by Edman degradation, the preliminary quantitative (ultraviolet spectra) and qualitative (thin-layer chromatography) evidence can be invaluable. However, these hydrolysis results should not be taken as a general rule for all thiazolinones of amino acids. Most of the 2-phenyl-5-thiazolinone derivatives are hydrolysed readily to the free amino acid by hydrochloric acid (110°C, 22 hr) but not by hydriodic acid (Inglis and Maclaren, unpublished data).

Some PTH amino acids were also hydrolysed in hydrobromic acid (constant-boiling reagent). Yields of the free amino acids were generally lower than for hydriodic acid hydrolysis and there was considerable deamination of the derivatives. PTH-serine, for example, yielded 3% glycine, 9% alanine, and 58% ammonia; PTH-threonine gave 7% glycine and 8% α-amino-n-butyric acid, and PTH-arginine yielded 16% ornithine and approximately 200% ammonia.

Hydriodic acid therefore seems preferable to either hydrochloric acid or hydrobromic acid for specifically cleaving the thiohydantoin ring to produce the free amino acid in high yield.

IV. References

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