EFFECT OF OTHER AMINO ACIDS ON RECOVERY OF TRYPTOPHAN FOLLOWING ACID HYDROLYSIS*

By L. C. GRUEN†

Abstract

The effect of the other commonly occurring amino acids, taken one at a time, on the recovery of tryptophan has been studied by following the conventional hydrochloric acid hydrolysis procedure. Hydrolysis in the presence of cystine results in substantial loss of tryptophan and some destruction of cystine. The only other amino acids which interfere with tryptophan during this procedure are serine, hydroxyproline, and tyrosine. These observations are discussed in terms of a feasible mechanism for tryptophan degradation in a protein.

Introduction

The most commonly adopted procedure (Spackman *et al.* 1958; Moore and Stein 1963) for amino acid analysis of a protein involves acid hydrolysis in the absence of oxygen, resolution of the amino acids by ion-exchange chromatography using appropriate buffers, and colorimetric estimation with ninhydrin. Although difficulties in the estimation of various amino acids, due mainly to either their lability toward acid hydrolysis or to difficulty in hydrolysing certain peptide bonds, have now been largely overcome, the situation with tryptophan remains unsatisfactory even with improved hydrolysis methods (Liu and Chang 1971).

In spite of considerable research seeking new or better techniques for the estimation of tryptophan, relatively little effort has been devoted to studies of the origin of tryptophan destruction arising from acid hydrolysis. Previous reports (Sahyun 1948; Spies and Chambers 1949; Monier and Jutisz 1950) have variously indicated that the presence of cystine, cysteine, threonine, or serine in the acidic hydrolysis mixture results in significant reduction in tryptophan recovery. It should be noted that in these studies tryptophan was assayed spectrophotometrically via a coloured reaction product. The specificity of such methods for tryptophan has recently been questioned (Gruen and Rivett 1971), since many other indoles and likely tryptophan oxidation products also give a positive colour reaction. Furthermore, little or no data were given concerning degradation of other amino acids apart from tryptophan. Recently Mondino and Bongiovanni (1970) observed that cystine, tryptophan, and to a lesser extent threonine and serine were degraded when a mixture of 18 amino acids was hydrolysed in 6N HCl by an open-flask technique and analysed on an amino acid analyser.

The present work was undertaken to clarify the effect of the other commonly occurring amino acids, taken one at a time, on the recovery of both tryptophan and the other amino acid following conventional acid hydrolysis procedures (Moore and Stein 1963).

* Manuscript received 1 August 1972.

† Division of Protein Chemistry, CSIRO, Parkville, Vic. 3052.

Materials and Methods

The amino acids used were supplied by Nutritional Biochemical Co. or Calbiochem and were chromatographically homogeneous. Stock solutions of each amino acid (*c.* 1 mg/ml) were prepared in doubly glass-distilled 6N HCl immediately before acid hydrolysis. In a typical experiment, approximately 1 mg of each of the appropriate amino acids in a total volume of 3 ml of 6N HCl was hydrolysed according to the recommended procedure (Moore and Stein 1963).

The hydrolysate was then lyophilized and stored at −10°C until just prior to analysis. Amino acid analyses were performed in the usual manner (Spackman *et al.* 1958) using a Beckman–Spinco 120B analyser.

Results and Discussion

Initially it was important to establish if tryptophan itself is stable under these conditions of acid hydrolysis. This proved to be so (see Table 1) and confirmed the work of others (Sahyun 1948; Monier and Jutisz 1950; Matsubara and Sasaki 1969; Mondino and Bongiovanni 1970) who have used a variety of conditions and acid strengths. The presence of leucine neither affected the yield of tryptophan nor was it

TABLE 1

RECOVERY OF AMINO ACIDS FOLLOWING ACID HYDROLYSIS IN 6N HYDROCHLORIC ACID

With the exception of the first two experiments, *c.* 1 mg of each of tryptophan, leucine, and the amino acid listed was hydrolysed in 3 ml of 6N HCl for 24 hr at 110°C in a sealed, evacuated pyrex tube

Other amino acid	Recovery (%)*		Other amino acid	Recovery (%)*	
	Other amino acid	Tryptophan		Other amino acid	Tryptophan
—	—	100	Aspartic acid	101	100
Leucine	100	100	Glutamic acid	101	101
Glycine	98	97	Lysine	100	97
Alanine	98	98	Arginine	102	101
Valine	99	97	Histidine	100	100
Isoleucine	101	98	Cystine†	87	59
Phenylalanine	100	99	Cysteine‡	32	99
Serine	94	92	Methionine	100	97
Threonine	100	98	Proline	99	98
Tyrosine	100	94	Hydroxyproline	94	94

* Amino acid recoveries were normalized to a leucine recovery of 100% (see text).
† 9% of the original cystine was recovered as cysteine.
‡ 43% of the original cysteine was recovered as ½cystine.

degraded (see Table 1). In several duplicate experiments the recovery of both of these amino acids was within 2% of the original amount used. Thus leucine was employed as an "internal standard" in all subsequent experiments, enabling corrections to be made for any physical losses occurring during the course of hydrolysis and analysis. This correction, where necessary, did not exceed 4%. For ease of comparison, all results listed in Table 1 have been normalized to a leucine recovery of 100%.

Examination of the results reveals that most of the amino acids do not affect the tryptophan recovery, within the generally accepted experimental error of 3%. Of the hydroxyamino acids, only threonine has no observable effect. Tyrosine has a small influence and the presence of serine or hydroxyproline leads to decreased yields of both tryptophan and of these amino acids. This result with serine is not surprising in view of its known deamination in acid to produce pyruvic acid (Abderhalden and Broich 1933; Damodaran and Ramachandran 1941). Experiments in this laboratory have verified the strong affinity of α-keto acids (e.g. glyoxylic acid, pyruvic acid) for tryptophan (Olcott and Fraenkel-Conrat 1947). Finally, dealing with the sulphur-containing amino acids, tryptophan survives acid hydrolysis in the presence of methionine or cysteine. Although most of the lost cysteine is recovered as cystine, a large excess of cystine relative to cysteine does not occur in the latter hydrolysis. However, when tryptophan is hydrolysed with cystine there is substantial loss of tryptophan and some destruction of cystine, with nearly all of the lost cystine being recovered as cysteine. In this hydrolysis there is, of course, a vast excess of cystine present relative to cysteine. Thus there seems little doubt that a major factor in the loss of tryptophan accompanying acid hydrolysis of a protein is degradative oxidation by cystine. Recent studies have, in fact, demonstrated enhancement of the tryptophan recovery from a protein when the acid hydrolysis is performed under reducing conditions (Matsubara and Sasaki 1969; Gruen and Nicholls 1972) or in the presence of an antioxidant (Dreze 1960; Oelshlegel *et al.* 1970; Liu and Chang 1971; Hugli and Moore 1972).

In summary, although the claim (Mondino and Bongiovanni 1970) that tryptophan can be recovered quantitatively from hydrochloric acid hydrolysates of proteins containing no cystine appears to be over-optimistic, the effect of cystine is certainly much greater than that displayed by the other interfering amino acids, viz. serine, hydroxyproline, and tyrosine. Even with the adoption of a better acid hydrolysis medium (Liu and Chang 1971), difficulties in tryptophan determination result from the presence of aldehydes, α-keto acids (as derived, for example, from serine), or from the well-known influence of carbohydrate (Lugg 1938). In the presence of these substances it is unlikely that tryptophan can be quantitatively estimated by an acid hydrolysis procedure and as yet there appears to be no satisfactory alternative to alkaline hydrolysis.

Acknowledgments

The author thanks Dr. C. M. Roxburgh and Messrs. A. S. Inglis and P. W. Nicholls for their assistance and for the amino acid analyses.

References

ABDERHALDEN, E., and BROICH, F. (1933).—*Biochem. Z.* **262**, 321.
DAMODARAN, M., and RAMACHANDRAN, B. V. (1941).—*Biochem. J.* **35**, 122.
DREZE, A. (1960).—*Bull. Soc. Chim. biol.* **42**, 407.
GRUEN, L. C., and NICHOLLS, P. W. (1972).—*Analyt. Biochem.* **47**, 348.
GRUEN, L. C., and RIVETT, D. E. (1971).—*Analyt. Biochem.* **44**, 519.
HUGLI, T. E., and MOORE, S. (1972).—*J. biol. Chem.* **247**, 2828.
LIU, T-Y., and CHANG, Y. H. (1971).—*J. biol. Chem.* **246**, 2842.

LUGG, J. W. H. (1938).—*Biochem. J.* **32**, 775.
MATSUBARA, H., and SASAKI, R. M. (1969).—*Biochem. biophys. Res. Commun.* **35**, 175.
MONDINO, A., and BONGIOVANNI, G. (1970).—*J. Chromat.* **52**, 405.
MONIER, R., and JUTISZ, M. (1950).—*Bull. Soc. Chim. biol.* **32**, 228.
MOORE, S., and STEIN, W. H. (1963).—*Meth. Enzym.* **6**, 819.
OELSHLEGEL, F. J., SCHROEDER, J. R., and STAHMANN, M. A. (1970).—*Analyt. Biochem.* **34**, 331.
OLCOTT, H. S., and FRAENKEL-CONRAT, H. (1947).—*J. biol. Chem.* **171**, 583.
SAHYUN, M. (1948).—*Stanford med. Bull.* **6**, 303.
SPACKMAN, D. H., STEIN, W. H., and MOORE, S. (1958).—*Analyt. Chem.* **30**, 1190.
SPIES, J. R., and CHAMBERS, D. C. (1949).—*Analyt. Chem.* **21**, 1249.