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Technical Note

Spectrophotometric Determination of Tyrosine in Proteins

ABSTRACT

A new spectrophotometric method for the determination of tyrosine in proteins has been developed. Tyrosine forms a red coloured coupled product with 4-aminophenazone-sodium metaperiodate reagent in an ammoniacal solution which absorbs at 470 nm. Beer's law is obeyed in the range 1·5–15 μg/ml. The method is simple, sensitive, reproducible and accurate within ± 1·5 % and applicable to the assay of tyrosine in protein hydrolysates.

INTRODUCTION

Quantitative separation and determination of tyrosine, 2-(*p*-hydroxyphenyl)-1-aminopropionic acid in proteins or biological fluids are very important, because normal growth is impossible in diets deficient in tyrosine (Hassan, 1975). Spectrophotometric methods reported earlier for its determination include chromogenic reagents such as diazotized sulfanilic acid (Hanke *et al.*, 1922), Folin's phenol reagent (Folin & Denis, 1912), Millon's reagent (Brand & Kassel, 1939), α-nitroso-β-naphthol (Ottaway, 1958), nitration reaction followed by alkali treatment (Hassan, 1975) and ceric ammonium nitrate (Chrastil, 1975). Many of these methods are found to be tedious and/or time-consuming (Folin & Denis, 1912; Hanke *et al.*, 1922; Brand & Kassel, 1939; Chrastil, 1975).

The purple-red coloured dye formed on the condensation of 4-aminophenazone with phenols in the presence of potassium hexacyano ferrate (III) under alkali conditions has been reported by Emerson (1943).

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Later, Vinod *et al.*, (1974) studied the reaction of *p*-cresol with 4-aminophenazone. Based on these observations, a new method has been described for the spectrophotometric determination of tyrosine in proteins using 4-aminophenazone and sodium metaperiodate in ammoniacal solution.

MATERIALS AND METHOD

Spectral and absorbance measurements were made with a Systronics model 105 (MKI) spectrophotometer.

All the solutions were prepared in distilled water using AR grade chemicals. Aqueous solutions of 4-aminophenazone (0·5% in 3:1 methanol:water), ammonium hydroxide (3M), sodium metaperiodate (0·1M) and tyrosine (50 μg/ml) were prepared.

Preparation of standard curve

An aliquot of tyrosine (0·3–3 ml) was taken into a 10-ml volumetric flask and made up to 7·9 ml with distilled water, then ammonium hydroxide (0·1 ml), sodium metaperiodate (0·5 ml) and 4-aminophenazone (1·5 ml) were added successively and mixed. The absorbance was measured at 470 nm against a corresponding reagent blank during the stability period (5–40 min). The amount of tyrosine was deduced from the standard curve.

Method for protein hydrolysates

The protein (0·2 g) was hydrolysed by the procedure of Udenfriend & Cooper (1952) for 24 h in 20 ml of 20% (m/m) HCl in a sealed Pyrex-glass tube immersed in a boiling-water bath. The acid was removed by evaporation under vacuum at room temperature over phosphorus pentoxide and sodium hydroxide. After appropriate dilution ($\sim$50 μg of tyrosine per millilitre), the above procedure was carried out for the estimation of tyrosine.

RESULTS AND DISCUSSION

Experiments on the variation of ammonia strength showed that maximum absorbance was obtained when 0·1 ml of 3M ammonium

hydroxide was taken. The other oxidizing agents, such as potassium persulphate, potassium hexacyano ferrate (III) and potassium iodate were tried in place of IO_4^- and were found to be inferior. The best order of addition of reactants for obtaining maximum colour was found to be that given in the procedure described above.

Beer's law was valid over a concentration range of 1·5–15 μg/ml of tyrosine. The molar absorptivity and Sandell's sensitivity were found to be $6{\cdot}88 \times 10^3$ litre mol^{-1} cm^{-1} and 0·026 μg cm^{-2}, respectively. Precision and accuracy were found by analysis of eight separate samples containing known amounts of tyrosine. The per cent relative standard deviation and per cent standard error were found to be 1·56 and 1·2, respectively. Comparison of values in the recovery experiments of tyrosine from various protein hydrolysates with that of the reported method (Ottaway, 1958) revealed good recovery and accuracy (Table 1).

More than 25-fold excess quantities of cysteine, cystine, methionine, aspartic acid, glutamic acid, asparagine, glutamine, serine, threonine, glycine, valine, alanine, leucine and isoleucine, a 20-fold excess of arginine, histidine, lysine, proline and hydroxy proline and a 16-fold excess of phenylalanine and tryptophan did not interfere with the determination.

The advantage of the method is the stability of the coloured product and the sensitivity, which is more than that of many of the reported methods. None of the other amino acids interfere with the determination of tyrosine. Hence, it can be used in the determination of tyrosine directly in protein hydrolysates without separating other amino acids.

TABLE 1
Estimation of Tyrosine in Protein Hydrolysates

Protein	*Tyrosine content (mg/100 mg)*		*Recovery experiments*	
	Proposed method	*Reported method*	*Amount added to protein (mg)*	*Per cent recovery*
β-lactoglobulin	3·03	3·12	2	96·8
Bovine serum albumin	3·90	4·0	3	96·4
Egg albumin	2·78	2·85	3	95·7
Vitellin	2·81	2·9	2	95·1
Lysozyme	2·73	2·81	2	95·3
Conalbumin	3·75	3·82	2	97·0

REFERENCES

Brand, E. & Kassel, B. (1939). The photometric determination of tryptophan, tyrosine, diiodotyrosine and thyroxine. *J. Biol. Chem.*, **131**, 489–502.

Chrastil, J. (1975). Colorimetric estimation of phenols and tyrosine. *Anal. Chem.*, **47**, 2293–96.

Emerson, E. (1943). The condensation of aminoantipyrine. II. A new colour test for phenolic compounds. *J. Org. Chem.*, **8**, 417–28.

Folin, G. & Denis, W. (1912). *J. Biol. Chem.*, **12**, 245–51.

Hanke, M. T. & Koessler, K. K. (1922). *J. Biol. Chem.*, **50**, 235–8.

Hassan, S. S. M. (1975). New spectrophotometric methods for simultaneous determination of tryptophan and tyrosine. *Anal. Chem.*, **47**, 1429–32.

Ottaway, J. H. (1958). Colorimetric method for the estimation of tyrosine. *Biochem. J.*, **68**, 239–44.

Udenfriend, S. & Cooper, J. R. (1952). The chemical estimation of tyrosine and tyramine. *J. Biol. Chem.*, **196**, 227–33.

Vinod, D., Stathers, J. B. & Warnhoft, E. W. (1974). On the reaction of *p*-cresol with 4-aminophenazone. *Can. J. Chem.*, **52**, 2932–5.

C. S. P. Sastry, P. Satyanarayana & M. K. Tummuru
Food and Drugs Laboratory,
School of Chemistry,
Andhra University,
Waltair 530 003, India

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