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Spectrophotometric Determination of Arginine in Proteins

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ABSTRACT

A simple, sensitive and rapid spectrophotometric method for the direct determination of arginine in protein hydrolysates is described. It is based on the formation of stable yellow coloured species (λ_{max}, 440 nm) on the addition of thymol-sodium hypobromite reagent under alkaline conditions. Beer's law is obeyed in the range 1–13 μg/ml with a percentage recovery range from 98·5 to 100. The different variables which may interfere in the reaction have been studied and the most suitable conditions for quantitative application have been determined.

INTRODUCTION

Arginine (monosubstituted guanidine derivative) is a vital constituent of proteins and is indispensable in human nutrition to establish and maintain a positive nitrogen balance. Sakaguchi (1925) developed a method using α-naphthol-sodium hypohalite as chromogenic reagent for the determination of arginine. Subsequently several modifications have been made using different chromogenic agents: sodium hypohalite-7-chloro oxine (Janus, 1956); 8-hydroxy quinoline (Ceriotti & Spandrio, 1957); diacetyl monoxine (Rendi, 1957); diacetyl-1-naphthol (Goldschmidt *et al.*, 1971); 2-methyl-1-naphthol (Casadebains *et al.*, 1979); and 5-chloro-7-iodo 8-hydroxy quinoline (Khramov *et al.*, 1980).

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Most of these methods suffer from such disadvantages as blank colour, instability of coloured species and tediousness. The scope of Sakaguchi's reaction has been reinvestigated. A new method, overcoming the above disadvantages, based on the same principle using thymol (2-propyl 5-methyl phenol)-sodium hypobromite as a chromogenic agent is reported here.

MATERIALS AND METHODS

The absorbance measurements were made on a Systronics model 105 (MK1) spectrophotometer. All the chemicals used were of CP or AR grade.

Aqueous solutions of thymol (0·02 % in 1N alkali), sodium hypobromite (2 %: 0·68 ml of bromine in 100 ml of 5 % sodium hydroxide) and arginine (0·1 %) were prepared. A sample solution containing 25 μg arginine/ml was prepared by proper dilution of stock solution.

Preparation of standard curve

Into a 5 ml standard volumetric flask an aliquot of arginine solution (0·2–2·6 ml), thymol (2 ml) and sodium hypobromite (0·1 ml) were added successively, and mixed and diluted to the mark with distilled water. The absorbance was measured at 440 nm against a corresponding reagent blank during the stability period (1 min–1 h). The amount of arginine was deduced from a standard calibration curve.

Estimation of arginine in protein hydrolysates

A known weight (100 mg) of the protein was hydrolysed in 6N HCl (2 ml) in a sealed tube for 8 h at 110 °C. After hydrolysis the product was neutralised with sodium hydroxide and diluted to 100 ml with distilled water. After appropriate dilution (25 μg arginine/ml) the above procedure was adopted for the estimation of arginine. Recovery experiments were also performed under these conditions. Pure arginine was added to the proteins before hydrolysis and arginine determination was done as before. With protein hydrolysates 0·2 ml of sodium hypobromite was used instead of 0·1 ml. The results given in Table 1 are an average of five determinations and are in good agreement with the reported methods (Lewis *et al.*, 1950; Hanumantha Rao & Subrahmaniam, 1976).

TABLE 1
Estimation of Arginine in Protein Hydrolysates (100 mg)

Protein	*Arginine content (found) (mg)*		*Recovery experiments*	
	Proposed method	*Reported method*	*Amount added to protein (mg)*	*Percentage recovery*
Bovine serum albumin	5·58	5·7	3	98·5
Egg albumin	5·79	5·9	3	98·7
β-Lactoglobulin	2·8	2·88	2	99·1
Vitellin	7·9	8·0	4	99·2
Ovomucoid	3·63	3·7	2	98·9
Arachin	8·26	8·39	4	99·0

RESULTS AND DISCUSSION

Experiments on the variation of alkali strength showed that maximum absorbance was obtained when thymol was dissolved in 0·75–1·25N sodium hydroxide. The order of addition of reactants had a marked influence on the development of colour (both maximum intensity and stability). Yellow colour was developed only when the order of addition of reactants was arginine–thymol and sodium hypobromite. The absorbance readings remained constant in the temperature range 10–50 °C.

Beer's law is valid over the concentration range 1–13 μg arginine/ml. Molar absorptivity and Sandell's sensitivity are $7{\cdot}01 \times 10^3$ litres/mol/cm and 0·025 μg/cm^2, respectively. The slope and intercept obtained by using linear least squares treatment of the results of the system involving arginine are $9{\cdot}3 \times 10^{-3}$ and $2{\cdot}62 \times 10^{-3}$, respectively, with a correlation coefficient equal to 0·996.

Precision and accuracy were found by analysis of eight separate samples containing known amounts of arginine. The percentage relative standard deviation and percentage error were found to be 1·05 and 0·867, respectively.

In order to assess the possible analytical applications of arginine, the effects of the 20 most important amino acids which often accompany arginine in protein hydrolysates were studied. The following amounts of other amino acids did not interfere with the determination of arginine

(40 μg): glycine, alanine, phenylalanine, valine, leucine and isoleucine up to 0·8 mg; proline, hydroxyproline, cysteine, cystine and methionine up to 1·3 mg; glutamic acid, glutamine, aspartic acid and asparagine up to 1 mg; serine, threonine, lysine and tyrosine up to 0·7 mg; histidine up to 0·5 mg; tryptophan up to 0·15 mg. Sucrose, fructose and phosphate did not interfere up to 1 mg. However, the other guanidines did interfere.

The reaction product of arginine with hypobromite develops a yellow colour with thymol, analogous to that of Sakaguchi's reaction. The advantage of the method lies in the stability of the coloured product and the sensitivity which is more than that of many of the reported methods. In the present method the colour develops immediately and the influence of temperature is limited. Furthermore, the method does not involve the elaborate clean-up procedures mentioned in the other methods. Hence the method can be used in the determination of arginine in bulk samples and protein hydrolysates with reasonable precision and accuracy.

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