CHROM. 4501

A rapid chromatographic method for the estimation of lysine

Lysine is frequently the most limiting essential amino acid in human and non-ruminant diets based on plant foods, though these foods are usually adequate in content of the remaining basic amino acids[1]. For this reason, plant breeders and animal nutritionists have shown considerable interest in this amino acid. Many of these scientists work in small laboratories without access to the more sophisticated equipment necessary for automated amino acid analyses, and in these situations there is a requirement for rapid methods employing simple apparatus.

Several methods for estimating lysine are presently in use. These are based on ion-exchange chromatography[2-9], gas–liquid chromatography[10], microbiological assay[11], enzymatic decarboxylation[12,13], paper chromatography[14], thin-layer chromatography[15], reaction with fluorodinitrobenzene[16,17] and reaction with picric acid[18]. Undoubtedly, the ion-exchange techniques, properly applied, are the most dependable and subject to least error, but with these methods the large number of ninhydrin-positive compounds normally present in hydrolysates of feeds or physiological solutions sets a lower limit to the time necessary for a clean separation of lysine from the remaining compounds on the column, particularly ornithine. Only when weak cation exchange resins have been employed has this latter separation been satisfactory in runs of less than 1 h duration[6], but these resins are not as dependable as strong cation-exchange resins for routine analysis[19].

This paper describes a simple manual chromatographic method which permits at least one lysine estimation per hour. By completely or partly automating the procedure this performance is improved. The method has been employed to estimate lysine in rumen liquor, silage liquor, deproteinized plasma, and hydrolysates of feed, faeces and bacteria.

Materials and methods

Column conditions. Resin type, Amberlite IR-120; resin particle diameter, 40 ± 7 μ; column resin height, 15 cm; column resin diameter, 0.6 cm; column temperature, 35°; rate of elution, up to 60 ml/h.

Buffer solutions. (i) pH 2.0 buffer (1.0 *N* sodium concn., 0.1 *M* citrate concn.). Per litre: sodium hydroxide (97%), 41 g; concentrated hydrochloric acid, 84 ml; citric acid, 21 g; phenol, 1 g. (ii) pH 3.1 buffer (1.0 *N* sodium concn., 0.1 *M* citrate concn.). Per litre: sodium hydroxide (97%), 41 g; concentrated hydrochloric acid, 53 ml; citric acid, 105 g; phenol, 1 g; BRIJ 35, 5 g. Adjust with conc. HCl as necessary.

Acid ninhydrin reagent. Dissolve 2.5 g ninhydrin (indantrione hydrate) in 160 ml of glacial acetic acid. Add 40 ml of 6 *M* phosphoric acid[20-23].

Procedure. Before use, the column is washed with 1 *N* NaOH and then equilibrated with pH 2.0 buffer. Of the sample 0.5–10.0 ml in pH 2.0 buffer are applied to the column, and 1-ml portions of pH 2.0 buffer are used to wash the walls of the column before pH 3.1 buffer is introduced and the elution commenced. The elution buffer is forced through the column at a rate of 40–60 ml/h, using nitrogen pressure or a simple pump. The eluate is collected in 2.4-ml fractions. To each fraction is added 4 ml of the acid ninhydrin reagent, and the mixture is heated in boiling water for

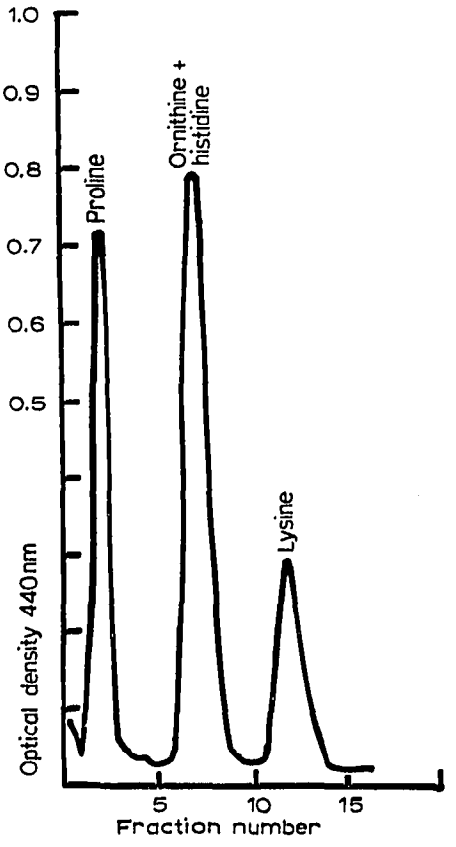

Fig. 1. Chromatogram of deproteinized calf plasma.

exactly 15 min. Each sample is then rapidly cooled to room temperature, and the optical density (O.D.) of the yellow lysine–ninhydrin complex is measured at 440 nm in 1-cm cuvettes on a Unicam SP 600 spectrophotometer.

Results and discussion

Fig. 1 shows a chromatogram of deproteinized calf plasma. Of the more commonly occurring acid ninhydrin positive amino acids, most difficulty was experienced in separating lysine from both ornithine and histidine. At citrate concentrations less than 0.5 *M*, lowering the pH improved the ornithine–lysine separation, but a less satisfactory separation of lysine and histidine was achieved. Using the procedure described above, proline, diaminopimelic acid and hydroxylysine were eluted before histidine and ornithine which eluted together, cleanly separated from lysine. Under these conditions, the "pH 5.5 ninhydrin" reagent of MOORE AND STEIN[24] was found to be unsatisfactory, probably due to the strong buffering capacity of the elution buffer.

As shown in Fig. 2, several amino acids formed a coloured complex when reacted with acid ninhydrin under the conditions described earlier. The E^{M}_{440} for arginine was found to be about 1.2 moles^{-1} cm^{-1}, but as suggested by CHINARD[20], this colour was probably due to contaminants. During the first 15 min of heating, the yield of the lysine–ninhydrin complex increased rapidly, but only a slow increase in colour yield was obtained thereafter. Accordingly, a 15-min heating period was adopted as standard procedure. The coloured complex thus formed was stable at room temper-

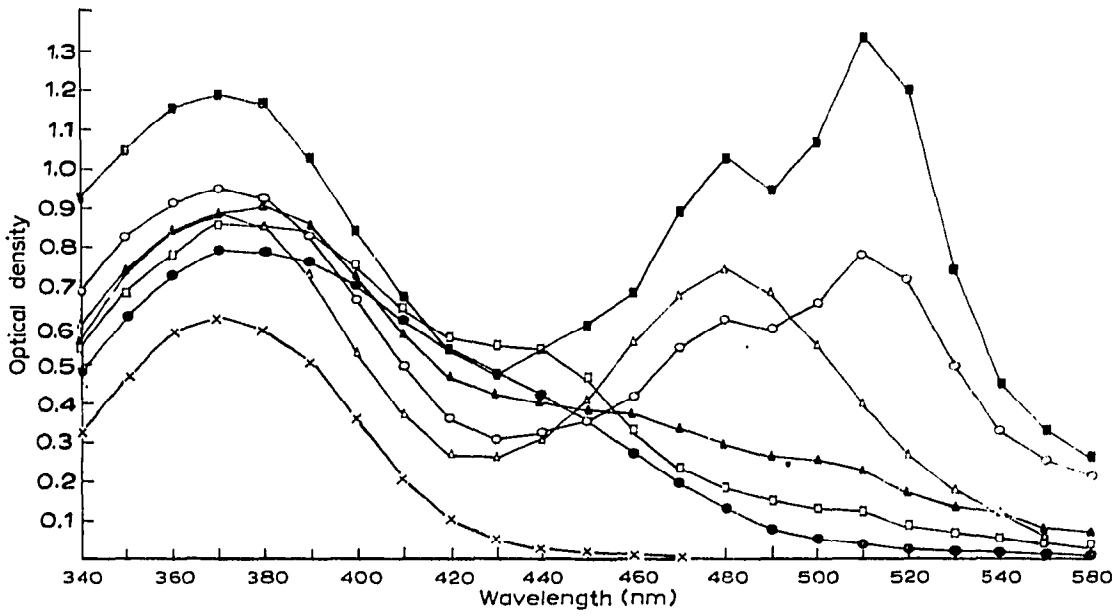

Fig. 2. Absorption spectra of products obtained by heating several amino acids with acid-ninhydrin reagent for 15 min at 100°. Symbols (in brackets μmoles/6.4 ml): ○—○, proline (0.5); ●—●, diaminopimelic acid (DAPA) (1.0); □—□, hydroxylysine (1.0); ■—■, ornithine (0.5); △—△, histidine (20.0); ▲—▲, lysine (1.0); × — ×, pH 3.10 buffer (blank) (—).

ature for at least 60 min. Under these conditions a straight-line relationship was established between O.D. and lysine concentration, in the range 0–3.5 μmoles lysine/6.4 ml, the molar extinction coefficient, E^{M}_{440}, for the lysine–ninhydrin complex being 2.64×10^3 moles^{-1} cm^{-1}. The recovery of added standard lysine from the column was 100%.

When the lysine contents of various acid hydrolysates and deproteinized physiological solutions were estimated by both the present method and the standard automated Technicon amino acid analyser, no significant difference was found between the values obtained.

One of the main advantages of this method is that although it was developed as a manual technique employing very simple and general laboratory equipment, it is faster than most of the more sophisticated automated procedures and no less accurate. Indeed, by increasing the number of columns to three and using a staggered loading procedure it is possible to perform one analysis every 30 min. Furthermore, the fact that ammonia and many other "pH 5.5 ninhydrin" positive compounds give no coloured complex under these low pH conditions means that the method is less prone to interference from unidentified compounds in complex media.

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1 B. O. EGGUM, *Dansk Pelsdyravl*, 31 (1968) 18.
2 D. H. SPACKMAN, W. H. STEIN AND S. MOORE, *Anal. Chem.*, 30 (1958) 1190.
3 D. R. KOMINZ, *J. Chromatog.*, 9 (1962) 253.

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4 A. WYNSCH, *J. Chromatog.*, 30 (1967) 225.
5 A. ROBSON, M. J. WILLIAMS AND J. M. WOODHOUSE, *J. Chromatog.*, 31 (1967) 284.
6 R. A. WALL, *J. Chromatog.*, 37 (1968) 549.
7 B. C. STARCHER, *J. Chromatog.*, 38 (1968) 293.
8 G. ERTINGSHAUSEN, H. J. ADLER, AND A. S. REICHLER, *J. Chromatog.*, 42 (1969) 355.
9 A. D. HUGHES, *J. Agr. Sci.*, 72 (1969) 459.
10 D. ROACH AND C. W. GEHRKE, *J. Chromatog.*, 43 (1969) 303.
11 B. S. MILLER, J. Y. SEIFFE, J. A. SHELLENBURGER AND G. D. MILLER, *Cereal Chem.*, 27 (1950) 96.
12 E. F. GALE, in D. GLICK (Editor), *Methods of Biochemical Analysis*, Vol. 4, Interscience, New York, 1954, pp. 285–306.
13 Anon., *Lysine*, Technicon Autoanalyzer Methodology, Bulletin L-1 (1959), Technicon Comp., Ardsley, New York.
14 Y. POMERANZ AND B. S. MILLER, *J. Assoc. Offic. Agr. Chemists*, 46 (1963) 399.
15 J. G. HEATHCOTE AND C. HAWORTH, *J. Chromatog.*, 43 (1969) 84.
16 A. S. M. SELIM, *J. Agr. Food Chem.*, 13 (1965) 435.
17 N. A. MATHESON AND M. SHELTAWY, *Biochem. J.*, 98 (1966) 297.
18 A. S. M. SELIM AND N. H. MESSIHA, *Analyst*, 91 (1965) 261.
19 C. W. H. HIRS, S. MOORE AND W. H. STEIN, *J. Biol. Chem.*, 200 (1953) 493.
20 F. P. CHINARD, *J. Biol. Chem.*, 199 (1952) 91.
21 E. WORK, *Biochem. J.*, 67 (1957) 416.
22 K. EL-SHAZLY AND R. E. HUNGATE, *Appl. Microbiol.*, 14 (1966) 27.
23 V. C. MASON, *J. Agr. Sci.*, 73 (1969) 99.
24 S. MOORE AND W. H. STEIN, *J. Biol. Chem.*, 211 (1954) 907.

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Behaviour of histones in exclusion chromatography and gel electrophoresis in relation to their molecular weights

The system of gel exclusion chromatography introduced by PORATH AND FLODIN[1] with dextran gels and later extended to polyacrylamide gels has been used with success in separating fractions of the histones[2–8]. As far as the authors are aware, however, none of these investigations was carried out to establish the molecular weights in the manner described by WHITAKER[9] and ANDREWS[10] for many well-characterised proteins. This study was therefore undertaken because knowledge of this parameter is essential information for understanding the structure of deoxyribonucleohistone.

Experimental

As standards, the following proteins were used (with their molecular weights given in parentheses): bovine serum albumin (67 000 and containing a little dimer); hen ovalbumin (45 000); bovine chymotrypsinogen (25 670) and trypsin (24 300); horse heart myoglobin (17 800); egg lysozyme (14 400); bovine pancreatic ribonuclease (13 700); horse heart cytochrome *c* (12 450); bovine insulin (5900) and salmine (about 4000). The following histone fractions were prepared from calf thymus (for nomenclatures see ref. 11): F1, (I); F2B, (IIb2); F2A2, (IIb1); F3, (III); and