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The determination of lysine and ornithine using acid ninhydrin

A recent note[1] described a rapid chromatographic method for the determination of lysine. *Inter alia* a linear relation was reported between optical density (O.D.) and lysine concentration in the range 0–3.5 μmoles per 6.4 ml aliquot. This note reports further work on the relationship between O.D. and lysine and ornithine concentration; and described chromatographic modifications which permit estimation of both ornithine and lysine.

Experimental

Standard curve reagents. The composition of buffers and acid ninhydrin was as described by BELL AND MASON[1]. Reagents other than BRIJ 35 were of AnalaR grade; ninhydrin was supplied by Koch-Light (Puriss grade, Cat. No. 4182h). Standard lysine (BDH Cat. No. 37129) and ornithine (Merck Cat. No. 6906) were prepared in pH 3.1 buffer. Lysine and ornithine solutions contained 0.25–2.5, and 0.1–0.5 μmoles of amino acid per 2 ml, respectively.

Standard curve procedure. Two ml aliquots of buffer and standard amino acid solutions were pipetted in triplicate into hard glass test tubes of three sizes; nominally 127 × 12.7 mm, 152 × 15.9 mm, and 152 × 25.4 mm. Four ml aliquots of acid ninhydrin were added and the solutions mixed; the tubes were then capped with aluminum foil and placed in vigorously boiling water for exactly 15 min. Developed solutions were cooled in running water for 15 min, and the optical densities read against distilled water in 1 cm cuvettes on a Unicam SP 600 spectrophotometer. Wavelengths used were 440 nm and 515 nm for lysine and ornithine respectively.

Chromatography. Resin, Biorad Aminex Q-50–particle size, 25–31 μ; working column height × diameter, 13.5 × 1.1 cm; working pressure, 0.7 kg/cm²; column temperature, 45°; elution rate, 50 ml per h; fraction volume, 2 ml. A chromatographic standard containing 0.4 μmoles proline, 0.4 μmoles ornithine, 8.0 μmoles histidine, and 4.0 μmoles lysine per ml was prepared in pH 2.0 buffer.

Results and discussion

Standard curves. The mean optical densities developed by buffer and standard amino acid solutions are presented in Table I.

Lysine. The data and accompanying Fig. 1 show a sigmoidal relationship between O.D. and lysine concentration in the range 0–2.5 μmoles per 6 ml; and illustrate that the precise shape of the curve is influenced by the diameter of the tube used for colour development.

Linear and quadratic regressions were generated from the data by the least squares method, and the optical densities predicted from these regressions compared with those obtained experimentally. A predicted O.D. was considered satisfactory if it lay within ±3% of the observed value. Statistically, quadratic regressions fitted the observed data better than did linear regressions for both the 127 × 12.7 mm and 152 × 15.9 mm tubes ($P < 0.05$: $F = 20.5, 8.7$ respectively on 1, 4 degrees of freedom); but when the ±3% limit was applied, none of the regressions satisfactorily predicted O.D. over all observed lysine concentrations.

TABLE I

STANDARD AMINO ACID CONCENTRATIONS, AND OPTICAL DENSITIES DEVELOPED IN TEST TUBES OF THREE SIZES

Amino acid (μmoles per 6 ml)	*Optical density*		
	127 × 12.7 mm	*152 × 15.9 mm*	*152 × 25.4 mm*
Lysine			
0	0.022	0.022	0.024
0.25	0.211	0.206	0.201
0.5	0.366	0.346	0.320
1.0	0.630	0.575	0.495
1.5	0.800	0.747	0.686
2.0	0.917	0.892	0.887
2.5	1.097	1.097	1.113
Ornithine			
0	0.004	0.006	0.005
0.1	0.247	0.247	0.251
0.2	0.470	0.485	0.496
0.3	0.731	0.738	0.752
0.4	0.920	0.985	1.007
0.5	1.171	1.233	1.260

Ornithine. In 152 × 15.9 mm and 152 × 25.4 mm tubes a linear relation was established between O.D. and ornithine concentration in the range 0–0.5 μmoles per 6 ml. In 127 × 12.7 mm tubes there was some departure from linearity at concentrations above 0.3 μmoles per 6 ml.

The red ornithine complex was found to be unstable under room conditions. The mean O.D. of triplicate solutions containing 0.5 μmoles per 6 ml fell by an estimated 2.5% in the first hour after removal from boiling water. In each of the following two hours the mean O.D. fell by a further 9%.

Chromatography. A standard chromatogram showing the resolution of 0.4 μmoles of proline, 0.4 μmoles of ornithine, 8.0 μmoles histidine, and 4.0 μmoles of lysine is presented in Fig. 2. These results differed from those presented by BELL AND

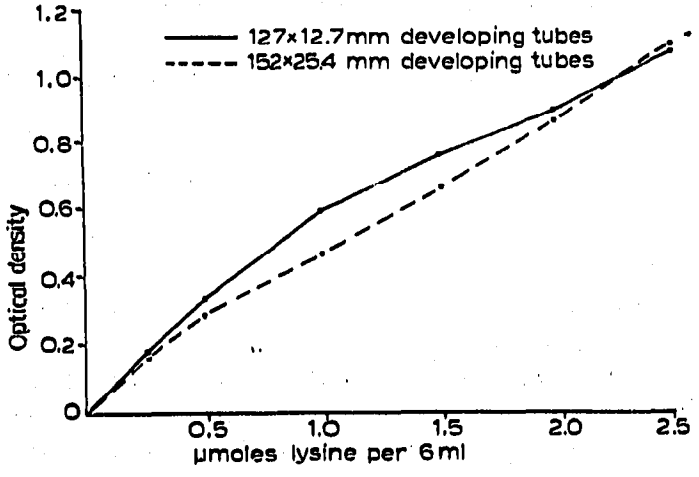

Fig. 1. Standard lysine curves (blank corrected).

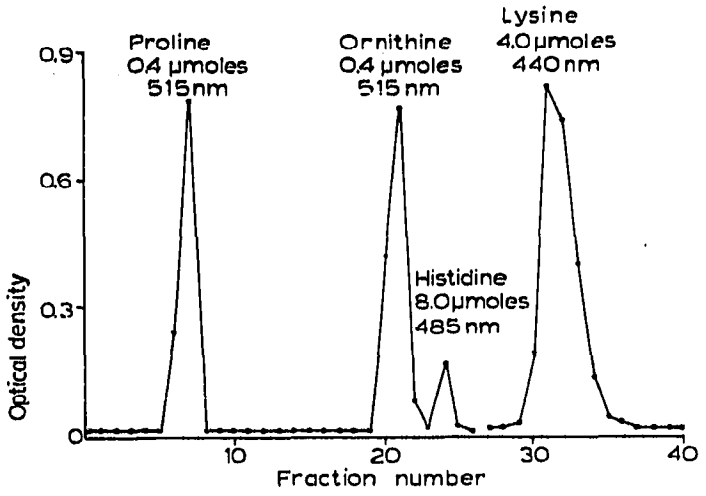

Fig. 2. Standard amino acid chromatogram.

MASON[1] in the separation of ornithine from histidine, and in the volume of buffer required to elute the amino acids.

The separation of ornithine from histidine provides the basis for the use of this technique to determine ornithine and lysine. There are two potential advantages of acid ninhydrin over the conventional acetate buffered ninhydrin[2]. Ornithine develops up to five or six times as much colour with acid ninhydrin as an equimolar lysine solution, whereas the colour yields with acetate buffered ninhydrin are similar[2]. Because ornithine concentrations in natural materials are normally considerably lower than those of lysine, acid ninhydrin is more likely to give similar colour yields for both amino acids from a single chromatogram. In addition, a number of compounds which produce intense colour by reaction with acetate buffered ninhydrin give little or no colour with acid ninhydrin[3,4]. Colorimetric interference is therefore less likely in samples containing "non-protein" amino acids.

Using Amberlite IR-120 resin, BELL AND MASON[1] eluted the lysine peak of a chromatogram with approximately 30 ml of buffer. This allowed at least one lysine determination per hour. Under the conditions of the present work, lysine peaks were eluted with approximately 60 ml of buffer. Even with two columns and staggered loading, one operator could fully process only three chromatograms per day.

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