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Short Communications

Separation on a Preparative Scale of Amino Acids from a Protein Hydrolysate

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In the course of a feeding experiment with cows, the ^{15}N content of amino acids of milk proteins had to be estimated [1]. Since the heavy isotope was expected to occur in low concentrations, it was intended to perform all measurements on relatively large amounts of nitrogen (approx. 2 mg). This quantity allows some 5 or 6 measurements to be made per sample with a high accuracy. For this purpose a separation of the amino acids was attempted on a preparative scale.

The milk protein precipitated with 15 % TCA was dialyzed, freeze-dried, extracted with ether in order to remove fatty material, and hydrolyzed with 6 N hydrochloric acid at 105°C for 24 h. Since the "humine" retained part of the amino acids, it was extracted twice with boiling water. The mixture was freed from HCl by adsorption on IR-120 and elution with piperidine [2].

The hydrolyzate from 7 g of protein, dissolved in 50 ml of 0.5 N acetic acid was poured onto a column of Dowex 1-X8 [3] with a cross-sectional area of 12.5 cm², and a height of 30 cm. The flow rate was 40 ml/h and fractions of a volume of 15 ml were collected (Fig. 1).

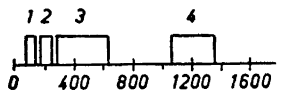

Fig. 1. Elution of the Dowex 1 column. (1) Neutral and basic amino acids; (2) Tyr; (3) Glu; (4) Asp.

The neutral and basic amino acids from the Dowex 1 column were rechromatographed on a Dowex 50-X4 column [3] with a cross-sectional area of 50 cm² and a height of 80 cm. First, the column was eluted with 4 800 ml of 1 N hydrochloric acid. Then gradient elution was applied, the gradient being of the shape [4]

$$C = C_2 - (C_2 - C_1)\,(1 - v)^{A_2/A_1}$$

where $A_1 = A_2$; A_1 and C_1 correspond to the cross-sectional area and the concentration of the mixing chamber, A_2 and C_2, respectively, to the vessel containing the hydrochloric acid of the higher concentration. The total volume of the system was 11 litres; C_1 = 1 N HCl, C_2 = 4 N HCl. In order to obtain an approximately constant gradient, A_2 and C_2 were changed to respectively $A_1/5.7$ and 5 N HCl when v/V had reached the value 0.8, v standing for the volume delivered, and V for the total volume of the system.

The column was closed with a rubber stopper through which a glass tube, adjustable in height, reached down to about 1 cm above the level of the resin. Since it was considered advisable to avoid another mixing of eluents of different strengths in the

Table 1. Yield of amino acids from 7 g of protein (in mg).

Glu	513	Val	163	His	98
Asp	346	Pro	243	Phe	91
Tyr	97	Lys	226	Arg	66
Ala	142	Cys	17		
Ser + Thr	374	Met + Ileu + Leu	492		
Gly	65				
			In total 2 933 mg		

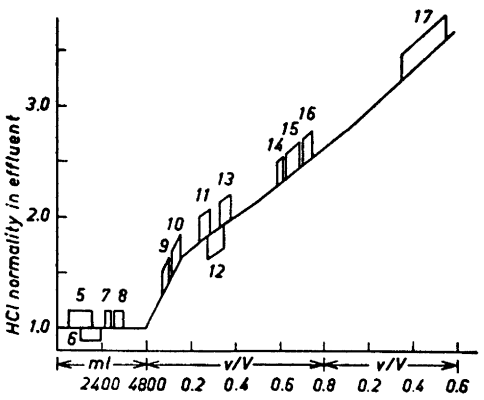

Fig. 2. Elution of the Dowex 50 column. (5) Ser; (6) Thr; (7) Gly; (8) Ala; (9) Val; (10) Pro; (11) Ileu; (12) Met; (13) Leu; (14) Cys; (15) Lys; (16) His; (17) Arg + Phe.

volume above the resin, this volume was kept at a minimum. This was achieved by application of a pressure equivalent to the difference in level between the mixing chamber and the top of the resin, *via* a side tube to the air space above the resin before the eluent was allowed to flow into the column. The flow rate was continuously kept at 108 ml/h by means of a capillary. The results are plotted in Fig. 2. Phenylalanine and arginine were separated on a cellulose column.

Purity of compounds rather than a high yield was the main aim in the separation. From Table 1 the total yield from 7 g of protein is seen to be about 40 %.

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Crystalline Leghemoglobin

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The red pigment from the soya bean root nodules was first demonstrated by Kubo [1] to have a hemoglobin nature. Ellfolk and Virtanen [2] showed that this pigment was not electrophoretically homogeneous after repeated ammonium sulphate fractionations, but contained two hemin components which seemed to occur in approximately equal concentrations. This was recently confirmed by Thorogood [3] who also found a third component in addition to the above two components and possibly a fourth.

Studying different techniques the present author has found that a cellulose ion exchange column was the most convenient one to separate these different hemin components. Diethylaminoethylcellulose according to Peterson and Sober [4] was used as adsorbent. The preparation of the individual components in this way was as follows:

After crushing the nodules, leghemoglobin was extracted twice into distilled water and fractionated once with ammonium sulphate between 55–80 % saturation (23°C). After dialysis against phosphate buffer (pH 8) and then against distilled water the protein solution was put on a cellulose ion exchange (DEAE-SF) column buffered to pH 5.2 with an acetate buffer of ionic strength 0.01 μ. All the hemin proteins remain on the top of the column as a sharp band. By elution with an acetate buffer (pH 5.2, μ = 0.01) one component moved down the column. This component was found to be the electrophoretically slowest one. Using buffer of this ionic strength the other components hardly moved at all and therefore, in order to increase the elution speed, the ionic strength of the acetate buffer was increased to 0.02 μ or 0.03 μ by addition of sodium chloride.

The second component was found to be the electrophoretically faster component. Between these two bands moved a faint, broad band which electrophoresis showed to be a mixture of several proteins with no

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