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Note

Contribution to clean-up procedures for serum amino acids

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Most studies dealing with gas chromatographic determinations of amino acids in biological samples involve proteinaceous starting material. Physiological fluids requiring analysis for free amino acid are usually pretreated with a denaturating agent such as picric acid[1,2], sulfosalicyclic acid[3–6], ethanol[7,8] or chloroform[9]. Following removal of the precipitated protein by centrifugation the amino acids are isolated by cation-exchange clean-up. However, variable recoveries of the free plasma amino acids, caused mainly by co-precipitation of the basic amino acids ornithine, lysine and arginine, found by other workers[10–12], led to a search for an alternative procedure.

A simple handy technique, involving dilution of the sample with acetic acid and, thus, elimination of the protein precipitation, was suggested by Pellizzari *et al.*[12], and in combination with cation-exchange clean-up it was used for determination of free amino acids in physiological fluids[12–14]. Even when the results obtained with the acetic acid method proved to be more consistent with those obtained by the amino acid analyzer technique than those obtained by the picrate denaturation[12], the yields of some plasma amino acids were higher with the picrate method, making the acetic acid procedure less attractive[12].

This fact led us to investigate the acetic acid procedure more comprehensively, *i.e.* with respect to the ion-exchange material and the inherent isolation process. It was found that the rate of cross-linking of the ion-exchange polystyrene matrix with the divinylbenzene (DVB) influenced yields of some amino acids markedly, and that by reversing the mechanism of uptake toward the elution a further improvement occurred. Moreover, pieces of polyethylene tubing were found to be a convenient substitute for the ordinary glass columns.

METHOD

Thin-wall polyethylene tubing (3–3.2 mm I.D.), commonly available, was cut into pieces *ca.* 25 mm long, and a plug of glass wool (3–4 mm) was placed in one end. A slurry of 100–200 mesh Dowex 50W-X2 (H^+) p.a. resin (Serva, Heidelberg, G.F.R.) in water was sucked in the tubing with help of a syringe to form a 15-mm high column of the wet resin. A peristaltic pump SJ-1211H (Atto, Tokyo, Japan), with a variable flow-rate of 7–700 ml/h, was used to wash the resin bed with 1 ml of 1 *N* hydrochloric acid and 2 ml of distilled water and for the subsequent uptake of the amino acids by suction, as follows.

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A 50–100-μl volume of serum (or urine) was placed in a 1.5 ml Silli-Vial (Pierce Eurochemie, Rotterdam, The Netherlands) and mixed with ten times the volume of 25% (v/v) aqueous acetic acid, containing internal standards (25 nmol/ml)[15]. The mixed solution was sucked slowly (*ca.* 0.5 ml/min) through the activated resin in the plastic column, which was immersed in the liquid by the open end and connected by a piece of glass stem (3.2 mm O.D. and 2 mm I.D.) to the silicone tubing of the peristaltic pump. After the fluid had been sucked off completely, 250–500 μl of water were added and sucked in the resin at a flow-rate increased by two- to three-fold. This step was repeated. For elution of the amino acids it was necessary to place in the lower "suck-in" end of the column a piece of polypropylene tip (a conventional conical tip, used for the push-button pipettes, was cut on both sides to *ca.* 3.2 and 2 mm O.D., respectively) filled with glass wool. The "suck-off" end of the column was then set on a PTFE luer (8 mm × 3.2 mm O.D.) of a 1 ml syringe (Pressure-Lok®, Pierce Eurochemie No. 19352), which was just filled with 0.7 ml of 2 *N* aqueous ammonia, and the amino acids were eluted by pushing the syringe plunger down for 15–20 sec. The process is illustrated in Fig. 1.

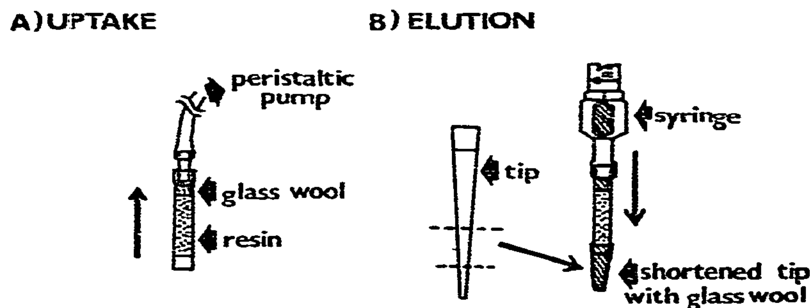

Fig. 1. Schematic illustration of the uptake and elution processer.

RESULTS AND DISCUSSION

Regardless to the kind of sample pretreatment, *i.e.* whether the proteins were precipitated or not, in all the previous procedures[1,2,6–9,12–14] the strongly acidic cation-exchangers of Dowex 50W or Amberlite CG or IR type with higher percentage of DVB-cross-linking (8 or 12%) were used. The higher cross-linkage of the resin provides a doubled resin capacity as compared with the lower one, and this is probably the reason for persistent use of the X8 type resin.

We have tested the Dowex 50W p.a. resin (Serva) with 2–12% of cross-linkage, together with the acetic acid procedure, and found a continuous decrease in uptake of some amino acids as the percentage of cross-linkage increased. When the X8 resin was used instead of the X2 resin, the recoveries for the aromatic (phenylalanine, tyrosine) and long-chain aliphatic (α-aminocaproic acid, being the internal standard in our case) amino acids were lowered to 70–80% and those for arginine and tryptophan to 40–50% only. The yields were even smaller when the X12 resin was employed.

In contrast, the X2 resin improved recoveries for arginine and tryptophan to 70–80% and recoveries of the other protein amino acids were close to 100%. In all these studies the usual procedure was employed, *i.e.* uptake and elution proceeded in

the same direction by forcing the substances through the resin bed under a slightly increased pressure. Under such conditions 0.8–1.0 ml of aqueous ammonia was required to elute amino acids from the resin completely.

The use of reversed flow for the uptake brought a further improvement in the recovery for arginine (more than 90%) whereas that for tryptophan remained unaltered (*ca.* 70%). Recoveries of all protein amino acids proved to be more reproducible under these conditions, and the total amount of the eluant could be reduced to two-thirds of the volume required for the former technique. This method of sample clean-up was used routinely for screening of serum amino acids during thyroid disorders[16]. The amino acids were estimated by means of gas chromatography after their conversion into cyclic derivatives at room temperature[15]. The elaborated technique based on the flow/counter-flow principle might also be useful for other kinds of application.

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