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Note

A simple and rapid chromatographic adsorption procedure for the isolation of arginine

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Isolation of arginine as its crystalline monopicrate, as its copper nitrate and as a diflavianate complex is already known[1]. These methods of isolation involve prolonged manipulations, procedural complications, uncommon and corrosive reagents, and manual labor too. Moreover, neither the yield nor the purity of the isolated product is encouraging, since the complex-forming agents used for isolation have the intrinsic property to cause indiscriminate precipitation of the complex(es) of other basic molecule(s), in addition to that of a desired one. Furthermore, traces of complex-forming agent, *viz.* flavianic acid, do not permit the use of isolated arginine in metabolic studies due to its adverse effects upon enzyme studies[2]. On this background, the present communication, describing a column chromatographic method utilizing thymol–amylose gel for preferential adsorption and quantitative elution of arginine, is of significance. It affords rapid isolation of pure arginine and is a reproducible alternative procedure to the ion-exchange chromatographic method which has stood the test of time. Finally, it has the positive advantage over all these methods that it neither requires costly chemicals not easily available nor needs sophisticated equipment not readily available in many laboratories.

EXPERIMENTAL

Preparation of hydrolysate

100 g of gelatin were dissolved and hydrolysed in 300 ml of 9.0 *N* sulfuric acid for 15 h. After a negative biuret test had indicated completion of hydrolysis, excess of sulfuric acid was removed as barium sulfate by adding a hot paste of barium hydroxide (prepared in distilled water) to the boiling hydrolysate. The bulky precipitate of barium sulfate was removed by centrifugation. The residue was then washed with boiling distilled water so as to remove any mechanically retained amino acids on it. The supernatant and washings were concentrated under vacuum and the resultant concentrate was adjusted to pH 4.6.

Preparation of thymol–amylose

Thymol–amylose was prepared by the procedure of Bourne *et al.*[3]. About 100 g defatted potato starch (BDH, Poole, Great Britain) suspended in 500 ml glass distilled water was slowly, yet continuously, added with continuous stirring, to 3 l

0.02 *M* boiling sodium chloride solution. After continuous stirring of the boiling solution for 30 min, the system was gradually cooled to 70° and then passed through a Sharples centrifuge at a rate of 3.5 l per h (25,000 rpm). The paste was allowed to come to room temperature when 8 g powdered thymol was added with stirring. This whole system was kept at room temperature for 72 h during which insoluble thymol–amylose complex settled slowly. The mother liquor, containing opalescent amylopectin–thymol complex, was decanted gradually. The thymol–amylose complex was washed three to four times with water saturated with thymol. The resulting precipitate at the end of the fourth wash was the adsorbent used in the present work.

Estimation of arginine contents

The arginine content of the concentrate was determined by the Sakaguchi reaction[4]. This reaction was employed at each further step to determine the progress in adsorption and elution as well as to estimate operational losses.

Batchwise procedure

Adsorption. The whole concentrate, adjusted to pH 4.6, was added to 50 ml semisolid mass of retrograded three-week-aged thymol–amylose kept near 10°. This was centrifuged after 24 h. There was no adsorption of arginine or any other amino acids on thymol–amylose cake as checked by the Sakaguchi and ninhydrin reactions[4,5], respectively. When the same procedure was repeated twice by keeping the supernatant with a fresh lot of thymol–amylose, almost all arginine and a very small amount of ninhydrin-positive material was found to be adsorbed.

Elution. The amylose cakes were collected by centrifugation and arginine was eluted by repeated extraction in 5.0 *N* ammonia. This was achieved by mechanical stirring of the system at 27° for 2 h followed by centrifugation. Thus, arginine adsorbed on thymol–amylose was quantitatively eluted along with a small amount of mechanically retained ninhydrin-positive material. The whole effluent (supernatant) was then concentrated under vacuum until dry and then extracted in a minimum amount of distilled water and adjusted to pH 4.6.

Column procedure

The whole concentrated supernatant, adjusted to pH 4.6, as above, was loaded onto a thymol–amylose column (90 cm × 2.5 cm I.D.) at 27° and percolated at the rate of 15 ml/h. The effluent was collected and the column was washed with at least three bed volumes of water (adjusted to pH 4.6) to remove any loosely retained amino acid entities. The adsorbed amino acids were then desorbed by eluting the column with 5.0 *N* ammonia. The fractions, each of 5.0 ml, were collected and assayed for arginine content.

The fractions containing Sakaguchi-positive material were concentrated under vacuum until dry and then extracted in 95% ethanol. The resulting white precipitate was allowed to settle and the supernatant was discarded. The residue was washed with methanol and dried in vacuum.

RESULTS

The Sakaguchi reaction indicated that arginine obtained by the batchwise procedure was more than 90% pure. It contained histidine as a major impurity as

judged by a diazotization reaction[6] and by R_F value determinations on Whatman No. 1 paper in *n*-butanol–acetic acid–water (4:1:5) and phenol–water (8:2) phases[7,8]. Further, it seemed to contain tryptophan as another impurity, as revealed from estimation of R_F values in the above-mentioned phases. 8.6 g of arginine was isolated as against the expected value of 9.2 g.

The Sakaguchi reaction showed that arginine was further purified by subjecting it to the column procedure after a batchwise operation and was about 98% pure. It appeared to be free from tryptophan and histidine impurities, as revealed by paper chromatographic homogeneity in both the phases. Internal standard run (*i.e.*, paper chromatographic analysis after spotting a mixture of isolated product plus authentic arginine sample) further confirmed the homogeneity of isolated arginine. During this purification step 8.2 g of arginine was isolated as against the starting 8.6 g of semipurified arginine preparation.

PRECAUTIONS

During the course of this investigation, the following precautions were found to be utmost necessary. Excess of Ba^{2+} ions in the solution is not desirable as it gets precipitated under the conditions of the Sakaguchi reaction, making centrifugation prior to colorimetric readings inevitable. This might reduce the efficiency and correctness in estimations due to losses in transfers. pH 4.6 was found to be most suitable for adsorption on thymol–amylose. Pyridine-like impurities in retrograded amylose were found to inhibit adsorption of arginine. Traces of amylose in the supernatant after elution cause precipitation, rendering crystallization of arginine difficult. Finally, arginine being highly soluble in water, final operations are to be carried out with comparatively smaller volumes to avoid losses during transfer.

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