Journal of Chromatography, 166 (1978) 333–334
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 11,373

Note

Analysis of "labile" arginine

LEWIS B. JAMES

Department of Biochemistry, John Curtis School of Medical Research, Australian National University, P.O. Box 334, Canberra City (Australia)

(Received July 21st, 1978)

The action of alkali on arginine, giving rise to ornithine, citrulline and ammonia, has been reported in the literature[1]. However, there is still in common practice a sample purification procedure that recommends the use of ammonia for the elution of amino acids from ion-exchange resin, before analysis by automated equipment. For example, during preparation of plant extracts the material is applied to Dowex 50(H^+) resin, and after washing the column with water, the amino acids are eluted with ammonium hydroxide.

The present communication is concerned with showing the effect of alkali on arginine in the presence and in the absence of Dowex 50 resin.

Three experiments were carried out. These were:

(1) A stock solution or arginine (1 μmole/ml) in pH 2.2 sodium citrate buffer was made up and a 200-nmole aliquot was subjected to analysis. Further aliquots of this solution were analysed after being allowed to stand for 3 h in 1 *M* and 5 *M* ammonia (30 ml), respectively. The ammonia was removed by rotary evaporation. Two further aliquots of the stock solution were applied to 3 cm columns of Dowex 50-X8 (H^+) 50–100 mesh resin and after washing the columns with water the samples were eluted with 30 ml of 1 *M* and 5 *M* ammonium hydroxide, respectively.

(2) The latter part of the first experiment was repeated using Beckman amino acid analyzer calibration mixture containing 1000 nmoles per ml of amino acid, in place of the arginine solution.

(3) The Dowex 50-X8 resin was replaced with Dowex 50-X2 (H^+) 100–200 mesh. Aliquots of calibration mixture were analysed after elution from the Dowex 50-X2 resin with 1 *M* or 5 *M* ammonium hydroxide, respectively.

The analyses were carried out with a modified Beckman 120B analyzer using titanous chloride-reduced ninhydrin and 9.9 mm pathlength cuvettes in the colorimeter[2]. The instrument calibration constant for each amino acid was established before commencement of the work. The constants obtained were: arginine 92, lysine 98, histidine 95 and ornithine 97. Beckman M81 spherical resin (7.5-cm bed) was used for the determinations. Elution of the amino acids from the column was achieved with pH 5.28 sodium citrate buffer. It has been established previously that lysine and ornithine were not resolved when these conditions of analyses were adopted. Table I shows the results obtained in this investigation. In the first experiment 200 nmoles of arginine had been applied to the analyzer column and using the constant, mentioned above, a recovery of 199 nmoles was obtained for the control. A slight loss

TABLE I

RECOVERY OF ARGININE

Expt. 1: data of recovery of a standard arginine solution; Expt. 2: Data of recovery of arginine from an analyser calibration mixture (Dowex 50-X8 50–100 mesh); Expt. 3: Data of recovery of arginine from an analyser calibration mixture (Dowex 50-X2 100–200 mesh).

Treatment of Arg	*Recovery (nmoles)*		
	Expt. 1	*Expt. 2*	*Expt. 3*
Control	199	153	153
Dowex 50, 5 *M* ammonia elution	114	78	86
Dowex 50, 1 *M* ammonia elution	99	76	25
Stored in 1 *M* ammonia	188	—	—
Stored in 5 *M* ammonia	183	—	—

was experienced with samples that had been standing in 1 *M* and 5 *M* ammonia for 3 h. From the samples that had been passed through Dowex resin, 50% of the arginine had been lost in the case where 1 *M* ammonia had been used for elution of arginine, and 43% of arginine had been lost during elution with 5 *M* ammonia. A peak (corresponding to 12 nmoles of amino acid) in the lysine location on the chromatograms was also evident.

In the second experiment, stock amino acid calibration mixture (1000 nmoles/ml) had been passed through Dowex resin. For the analytical determination an aliquot containing 150 nmoles of arginine was used because of the presence of lysine in the mixture; a loading of 200 nmoles would place the tip of the lysine peak off the most accurate region of the recorder chart scale, thereby making any addition to the lysine peak, from arginine degradation more difficult to detect.

The analytical determination of the calibration mixture showed that a loss of approximately 49% had occurred for arginine. The recovery for histidine, as was to be expected was 149 nmoles, but for lysine there was an apparent increase in the original loading of 150 nmoles to 173 nmoles, presumably due to the presence of ornithine in the analysis mixture.

In the third experiment the difference from the previous experiments is solely one of resin cross linkage and mesh size. With this finer mesh Dowex resin, the passage of the sample solution through the column was much slower.

In the analytical determination the recovery of arginine, for the amino acid mixture that had been eluted from the Dowex resin with 1 *M* ammonia, was calculated to be 16% of the control. For the mixture that had been eluted with 5 *M* ammonia, arginine recovery was 57% of the control. In the sample that provided a 16% recovery of arginine, the lysine peak showed a larger increase (to 183 nmoles) than had been previously obtained, and when an aliquot of this sample was analysed using Beckman PA-35 resin (14 cm resin bed) with a programmed 30°–52° temperature and pH 4.26–5.28 buffer change, the presence of ornithine was detected. As expected the amount of ornithine detected was not equivalent to the loss of arginine experienced.

REFERENCES

1 J. P. Greenstein and M. Winitz, *Chemistry of the Amino Acids*, Vol. 2, Wiley, New York, 1961, p. 1612.
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