TRITIATED LYSINE-VASOPRESSIN: METHOD OF PURIFICATION

J. VANĚČKOVÁ and T. BARTH

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 6

Received August 11th, 1972

The conditions of electrophoretic separation of tritiated lysine–vasopressin from the mixture of biologically inactive degradation products of the hormone were determined. The method is simple, not time-consuming and, permits pure $[H]^3$-lysine–vasopressin to be prepared in high yields.

The aqueous solutions of the neurohypophyseal hormone, lysine-vasopressin are very unstable, especially at higher concentrations. Disulfide interchange and deamidation take place and the arising products show little biological activity, if any at all. The problem of the purity of the preparation is especially important with the radioactively labeled preparations of lysine-vasopressin, predominantly as regards the determination of the specific radioactivity of the preparation. The solutions of tritiated or in another manner labeled hormone are contaminated – in addition to the products of the above described reactions – also by certain intermediary products of the synthetic procedure and alternatively also by side products of non-specific labeling.

In this paper we report on our experience with a technically simple and little time-consuming method of separation of biologically inactive components, resulting from the above described reactions, from biologically active lysine-vasopressin tritium-labelled at position 2 of the tyrosine residue of the peptide chain.

EXPERIMENTAL

Material. Tritiated lysine–vasopressin was prepared and purified by the method of Carlsson and coworkers[1–3]. We are indebted for a sample of the product to Dr V. Pliška, Eidgen. Techn. Hochschule, Zürich. The biological activity was determined by the pressor test on despinalized rat according to Sawyer[4]. The pressor activity of the preparation was 8·8 pressor unit per ml of sample, the specific labelling 5·2 μC per pressor unit. Unlabelled lysine vasopressin, a synthetic product, was kindly provided by Dr I. Krejčí, Research Institute for Pharmacy and Biochemistry. Its pressor activity varied between 100 and 150 pressor units per ml. A synthetic preparation of lysine-vasopressin (a solution containing $5 \cdot 10^{-3}$ mg/ml), prepared and provided by Dr E. Kasafírek, Research Institute of Pharmacy and Biochemistry, was employed as a standard.

Determination of pressor activity. The changes in blood pressure were measured by Stadham transducer and recorded by a linear compensating recorder EZ-2. The curves were compared with those obtained with the lysine-vasopressin standard (285 pressor units per mg of lysine vasopressin) by the four-point test.

Radioactivity measurement. To the sample measured (usually 100 μl), 5–10 ml of the scintillation solution of Bray[5] was added. The scintillation was measured in Packard-Tri-Carb-Scintillation Spectrometer 3 375. The effectiveness of the individual measurements was 20–25%. The radioactivity distribution on bands cut from paper electropherograms was determined in Model FH 452 apparatus, a product of Frieseke und Hoepfner, G. m. b. H. Erlangen-Bruck, equipped with Model FH 49 integrator of the same make.

Paper electrophoresis was carried out according to Durrum[6], in 1M-CH_3COOH at a potential gradient of 23 V/cm, 2·5 h on Whatman No 3 paper.

Gel filtration. Unpurified, radioactive lysine–vasopressin (5 ml of solution in 0·5M ammonium acetate) was chromatographed on a column (98 . 1·5 cm) of Biogel P-2 (Bio-Rad Laboratory). The column was eluted with water at a rate of 1 ml/min and fractions were collected at 5 min intervals.

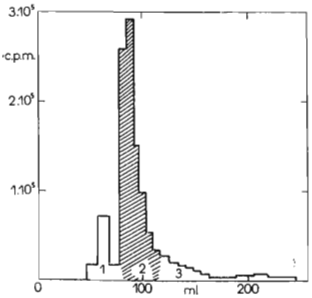

FIG. 1

Chromatography of Stock Sample of [H^3]-Lysine-Vasopressin on Column of Biogel P 2

The hatched area symbolizes the distribution of biological activity. See Experimental for details.

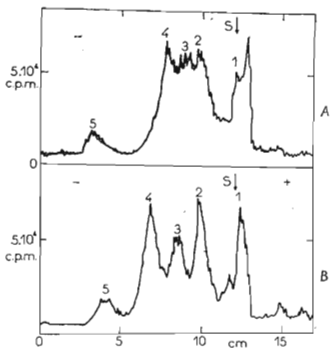

FIG. 2

Electrophoresis of [H^3]-Lysine-Vasopressin on Paper

An electrophoretic separation of 50 μl of concentrated fraction from the Biogel P-2 column on Whatman No 3 paper. The conditions are given under Experimental. *B* same as *A*, unlabelled lysine-vasopressin (50 μl, 5 pressor units) added to the radioactive sample.

RESULTS AND DISCUSSION

Gel filtration on the column of Biogel P-2 enabled us to separate from the sample of lysine-vasopressin the accompanying salts (in this case ammonium acetate used for elution of the CM-Sephadex column, ref.[3]) and the dimer of the hormone, a typical product of disulfide interchange occurring in aqueous solutions of lysine-vasopressin (Fig. 1). The fractions containing the biologically active product (hatched area of the diagram) were pooled and their volume reduced to 4–5 ml by freeze-drying. The dry product was submitted to paper electrophoresis under conditions given in the experimental part. The individual peptide components were resolved after 2·5 h of electrophoresis. The separation of the radioactive components was semi-quantitatively evaluated (Fig. 2*A*), the individual components were then eluted by water (or by 0·001M-HCl), and their biological activity was determined. The activity was located in the fastest component (designated No 5). The remaining fractions showing negligible biological activity and also lower electrophoretic mobilities (presence of free carboxyls) are designated No 1–4. The addition of unlabeled lysine-vasopressin as carrier to the mixture before the electrophoretic separation, results in an improved resolution of the individual components (Fig. 2*B*).

The reproducibility and yields of this method were examined with unlabeled lysine-vasopressin. Electrophoresis of 100–200 pressor units (approximately 0·5-1mg of the product) and elution of the electropherogram by 0·05M-CH_3COOH gave a yield of 76–82%. Labelled lysine-vasopressin can be still isolated under these conditions from a mixture in which its distribution (by weight) is several per cent. Our sample, which had been stored for several months at 2°C, contained only 8–10% of the original radioactive lysine-vasopressin.

We are indebted to Dr I. Krejčí and Dr E. Kasafirek, Research Institute for Pharmacy and Biochemistry, for kindly supplying us with the synthetic preparation of lysine-vasopressin, to Mrs H. Kovářová and Mr J. Hanzlik for skilled and expert technical assistance.

REFERENCES

1. Carlsson L., Sjöholm. J.: Acta Chem. Scand. *20*, 259 (1966).
2. Sjöholm I., Carlsson L.: J. Labell. Compounds *3*, 1 (1967).
3. Carlsson L., Pliška V., Thorn N. A.: Experientia *25*, 749 (1969).
4. Sawyer W. H. in the book: *The Pituitary Gland* (G. W. Harris, B. T. Donovan, Eds), Vol. 3, p. 288. Butterworths, London 1966.
5. Bray G. A.: Anal. Biochem. *1*, 279 (1960).
6. Durrum E. L.: J. Am. Chem. Soc. *72*, 2943 (1950).

Translated by V. Kostka.