Journal of Chromatography, 305 (1984) 461—464
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1919

Note

Separation of the cross-linking amino acids of elastin on thin-layer plates

STEPHEN KELLER*, ANJAN K. GHOSH, AJIT K. GHOSH, GERARD M. TURINO and INES MANDL

Columbia University College of Physicians and Surgeons, New York, NY 10032 (U.S.A.)

(First received June 6th, 1983; revised manuscript received July 29th, 1983)

There has long been a need for a simple and rapid method of separating the cross-linking amino acids of elastin. Such a procedure would greatly assist both structural and metabolic studies of this important protein, which is present in most types of connective tissue. Until recently the only methods available for separating the cross-linking amino acids in elastin hydrolyzates have been modified amino acid analyzer programs [1—3] and one-dimensional thin-layer chromatography (TLC) [4]. The original paper chromatographic procedure [5] as well as more recent electrophoresis methods [6, 7] do not separate desmosine from isodesmosine. Even though the analyzer procedures [1—3] separate the four cross-links, desmosine (DES), isodesmosine (IDE), merodesmosine (MD) and lysinonorleucine (LNL), from each other, these methods are complex and expensive. The previously reported TLC procedure [4] separates all four cross-links from one another but is not able to separate the cross-links from certain other amino acids, such as lysine, arginine and proline. To resolve this problem we have now devised a two-dimensional TLC procedure which completely separates the four cross-linking amino acids from each other and from all other amino acids present in elastin hydrolyzates.

EXPERIMENTAL

The *n*-propranol, *n*-butanol, concentrated ammonia (29%), glacial acetic acid and acetone were reagent grade obtained from Fisher Scientific (Springfield, NJ, U.S.A.). The E. Merck silica gel G powder and the 20 × 40 cm blank glass plates were obtained from Brinkmann Instruments (Westbury, NY, U.S.A.); the precoated 20 × 40 cm E. Merck silica gel G plates (250 μm layer) were obtained from Analtech (Newark, DE, U.S.A.). The desmosine and iso-

0378-4347/84/$03.00

desmosine standards were obtained from Elastin Products (Pacific, MO, U.S.A.); the merodesmosine and lysinonorleucine were kindly provided by Dr. Barry Starcher then at Washington University (St. Louis, MO, U.S.A.). The other amino acids used as standards were obtained from Nutritional Biochemicals (Plainview, NY, U.S.A.). The ninhydrin was obtained from Pierce (Rockford, IL, U.S.A.) and the glass atomizer for spraying the TLC plates as well as the 5-μl microcapillary pipettes were obtained from Fisher Scientific.

The cross-link standards were put in aqueous solutions at concentrations of 2 mg/ml and stored at −20°C. The remaining amino acid standards were made up to concentrations of 4 μM. Bovine ligamentum nuchae elastin (250 mg) was hydrolyzed in five sealed ampoules with a total volume of 15 ml of 6 M hydrochloric acid at 100°C for 18 h. After evaporation to dryness on a steam bath, the hydrolyzates were made up to a total volume of 5 ml with water and stored at −20°C. Thin-layer plates were prepared by suspending 30 g of silica gel G in 67 ml water and spreading a 250-μm layer onto 20 × 40 cm glass plates. The solvent for the first (20 cm) dimension was n-butanol—acetic acid—water (4:1:1). The second dimension solvent was n-propanol—water—ammonia (8:11:1). The amino acid standards, as well as the elastin hydrolyzate were all spotted on the silica gel plates in 3-μl aliquots, with a total of four applications per spot, using cool forced air drying between applications. Chromatography in the first dimension in the short direction of the plates was carried out in a polyethylene tank, 37 × 47 × 30 cm, height, width, depth. Solvent (100 ml) was placed into a parafilm-lined trough, and the glass plate was carefully inserted into it. Chromatography in the second dimension was carried out in the long direction, using a cylindrical glass jar 46 cm high, 25 cm diameter. When the run was completed, plates were dried at room temperature for 24 h, and then sprayed with a solution of 1 g of ninhydrin in 100 ml of acetone.

RESULTS AND DISCUSSION

When a one-dimensional separation of standard amino acids in the first system, n-butanol—acetic acid—water, is carried out the elastin cross-links all stay at, or near the sites of application, while all other amino acids placed on the plate migrate much farther. It is likely that the strong binding to the stationary phase is a result of the higher charge density of the cross-links which along with their relatively high pK values renders them extremely hydrophilic. To enable these substances to migrate and separate in the second dimension, a high pH provided by ammonia was applied. The replacement of n-butanol by n-propanol which is miscible with water in all proportions allowed the formulation of a solvent system containing more water than is usual for amino acid separation. In this system the cross-links moved away from the origin [4]. After one-dimensional separation of the amino acids in the n-propanol—water—ammonia system, along the long axis of the plates, the four cross-links were seen to have very different R_F values. However, in the one-dimensional system lysine has the same R_F value as LNL, proline has an R_F value similar to IDE and arginine has an R_F value similar to that of MD. The two-dimensional separation of a standard amino acid mixture containing the four cross-links, along with lysine (LYS), arginine (ARG), proline (PRO), glycine, alanine,

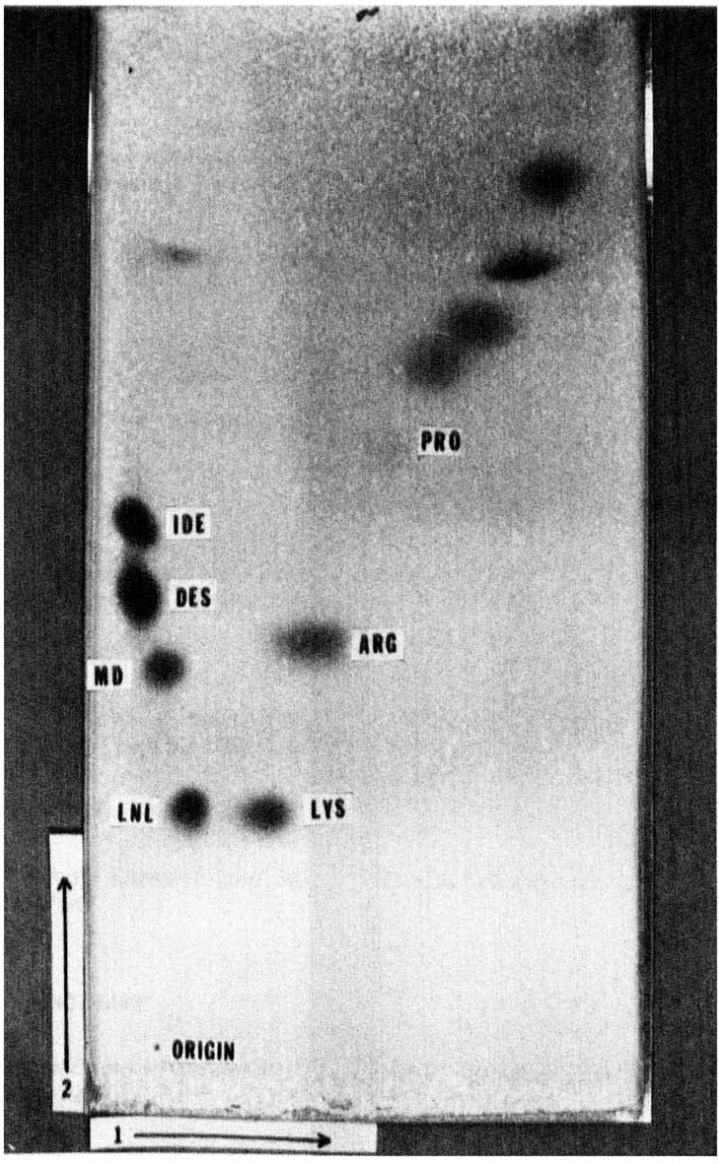

Fig. 1. Separation of standard amino acids on a silica gel plate by the two-dimensional method using *n*-butanol—acetic acid—water (4:1:1) in the first dimension and *n*-propanol—ammonia—water (8:1:11) in the second.

valine and leucine by a combination of the two systems is shown in Fig. 1. The four cross-links are seen to be completely separated along the left border of the plate, and well removed from all the other amino acids contained in the mixture. Gly, Ala, Val and Leu are the four spots located above and to the right of Pro. The same separation procedure was used to resolve the components of a hydrochloric acid hydrolyzate of bovine ligamentum nuchae elastin. Fig. 2 shows that the cross-links are again situated on the left border of the plate, completely separated from each other and from all other amino acids in the hydrolyzate. The amount of merodesmosine in elastin is much lower than that of the other three cross-linking amino acids which accounts for the fact that it is not visible on the chromatogram.

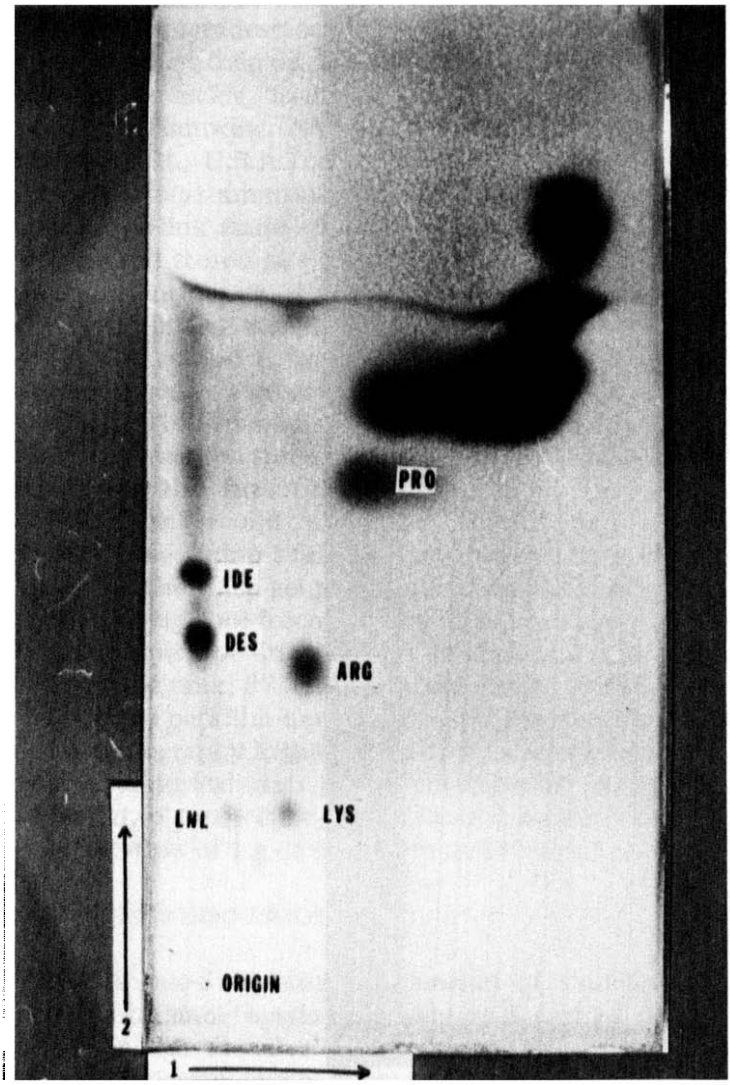

Fig. 2. Separation of the amino acids in an acid hydrolyzate of elastin by the two-dimensional method used in Fig. 1.

ACKNOWLEDGEMENT

This work was supported in part by USPHS Program Project Grant HL15832.

REFERENCES

1 M. Ledvina and F. Bartoš, J. Chromatogr., 31 (1967) 56.
2 B.C. Starcher, J. Chromatogr., 38 (1968) 293.
3 K.W. Corbin, Anal. Biochem., 32 (1969) 118.
4 S. Keller, G.M. Turino and I. Mandl, Conn. Tiss. Res., 8 (1981) 251.
5 S.M. Partridge, D.F. Elsden and J. Thomas, Nature, 197 (1973) 1297.
6 E. Moczar, B. Robert and L. Robert, Anal. Biochem., 45 (1972) 422.
7 R.A. Green, J A. Foster and L.B. Sandberg, Anal. Biochem., 52 (1973) 538.