DIFFERENCES IN ELASTASE HYDROLYSATES OF ELASTIN FROM LIGAMENTUM NUCHAE

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By means of molecular exclusion chromatography, a marked difference in the distribution of desmosine and isodesmosine in the products of proteolysis has been found between the partial enzymatic hydrolysates of elastin from the bovine nuchal ligament prepared by two different methods. In the preparation which was treated with hot NaOH the prevalling portion was localized in a macromolecular fraction corresponding to the void volume. An increased precipitation of this fraction with trichloracetic acid has been noticed. The autoclaved material forms substantial amounts of the coacervate during the digestion.

1. Introduction

Elastin requires the use of drastic procedures for solubilization and represents the material remaining after the application of procedures that extract other protein and nonprotein components of connective tissues. Therefore in the evaluation of the results obtained with different elastin preparations the manner of preparation should be considered.

The present paper shows how two elastin preparations differ in their resistance to elastase and in the composition of their hydrolysates.

2. Material and methods

Ligamentum nuchae of an adult cow was isolated, dried at 37° to constant weight, the hard mass was finely pulverized with the help of a file and delipidated with ether and acetone. The whole procedure was carried out with elastin of the same ligament.

Isolation and purification of elastin were performed by means of two methods:

a) According to Partridge et al. [1]. The bovine nuchal ligament was kept overnight in 0.9% NaCl at 37°, the salt solution was decanted, the ligament was washed twice with distilled water, 3 times autoclaved at 110-115° and dried.

b) According to McGavack and Kao [2]. The nuchal ligament was kept overnight in 0.1 N NaOH at 37°C, the supernatant was decanted, the ligament was boiled at 95°C in 0.1 N NaOH for 15 minutes 4 times, washed with water twice and dried.

Elastase (E.C. 3.4.4.7) was a commercial product (Koch-Light Laboratories Ltd., 50 mg of twice crystallized pancreatic elastase in 5 ml water suspension).

Enzymatic hydrolysis: 120 mg elastin were suspended in 4 ml 0.05 M NH_4HCO_3-buffer (pH 8.8) and 0.12 ml elastase suspension (= 1.2 mg elastase) were added. Digestion was carried out at 37°. The total period of digestion amounted to 22 hr. There was essentially no undissolved residue. Enzymatic hydrolysates were concentrated under reduced pressure at a temperature not exceeding 80° to volumes of 0.8 - 1.0 ml.

This volume was applied on Sephadex G-10 or G-100 columns. Ammonium bicarbonate buffer, 0.05 M, pH 8.8, was poured through the column and the sample was eluted with the same buffer at laboratory temperature.

The effluents were evaluated by means of
1) UV absorption measurements [3] at 274 nm (after 2 : 1 dilution with water)

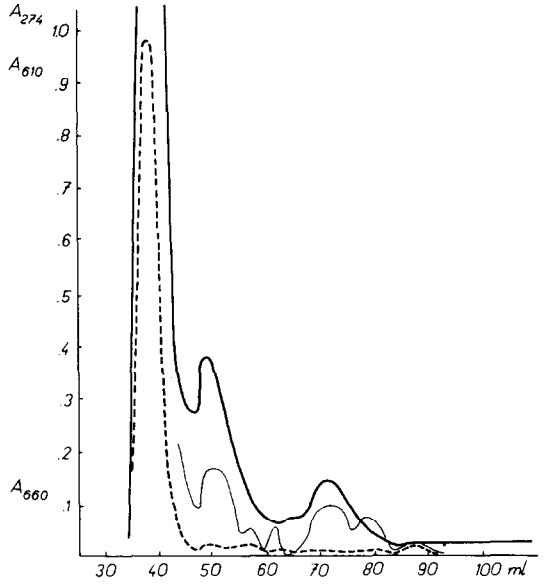

Fig.1. Analysis of the enzymatic hydrolysate of elastin prepared according to Partridge et al. on Sephadex G-10. Column size: 12 × 857 mm, V = 97.2 ml. Elution with 0.05 M ammonium bicarbonate buffer pH 8.8. Amount of elastin: 120 mg. The samples diluted 2 : 1 with water. Heavy full line: Absorbance at 274 nm, 1 cm cells. Thin full line: Folin's reaction (A_{660}, 1 cm cells); the samples with elution volumes < 44 ml are turbid. Dashed line: TCA-turbidity (A_{610}, 1 cm cells).

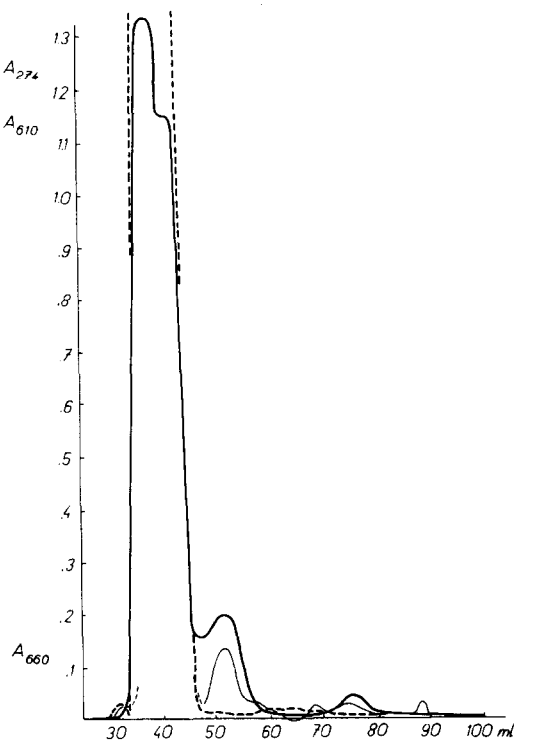

Fig. 2. Analysis of the enzymatic hydrolysate of elastin prepared according to McGavack and Kao on Sephadex G-10. Conditions as in fig. 1.

2) Folin's phenol reagent (after dilution with 20% soda) by measuring the absorption at 660 nm
3) measurement of turbidity with trichloracetic acid (TCA) at 610 nm (1.90 ml effluent was mixed with 0.95 ml water and 1.0 ml 10% TCA; turbidimetry was carried out in 1 cm cells for 30 minutes).

3. Results

Fig. 1 shows the resolution on Sephadex G-10 of the hydrolysate of elastin prepared by means of the Partridge method. The prevailing portion of the UV absorbing material is found to have the molecular weight above 600-700 (approximate exclusion limit 700). This also applies to fig. 2 with the distribution of the hydrolysate of elastin isolated by means of the McGavack method. In addition, there are peaks with the elution volume of about 50 and 75 ml which correspond to substances of low molecular weight. There is a marked difference in the content of TCA-precipitable material which is many times larger with McGavack's preparation.

The same hydrolysate underwent gel filtration on Sephadex G-100 as well (figs. 3 and 4). The difference between both preparations was still more pronounced here. The hydrolysate of elastin prepared by means of the Partridge method showed a marked macromolecular fraction (with a molecular weight above 100,000), the largest portion of the UV-positive material exhibiting, however, smaller molecular size corresponding to low peptides and UV-positive amino acids. The TCA-turbidity was distributed almost evenly within the whole range of molecular weights. By contrast, the hydrolysate of McGavack's material revealed the bulk of UV-absorbing substances with the elution characteristics of macromolecular substances and a lower degree of proteolysis with a com-

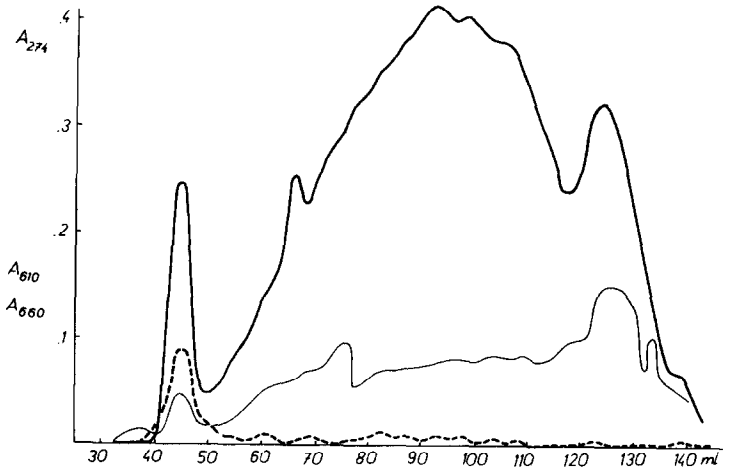

Fig.3. Separation of the enzymatic hydrolysate of elastin prepared by the method of Partridge et al. on Sephadex G-100. Column size: 13 × 777 mm, V = 131 ml. The samples diluted 2 : 1 with water. Other conditions as in figs. 1 and 2.

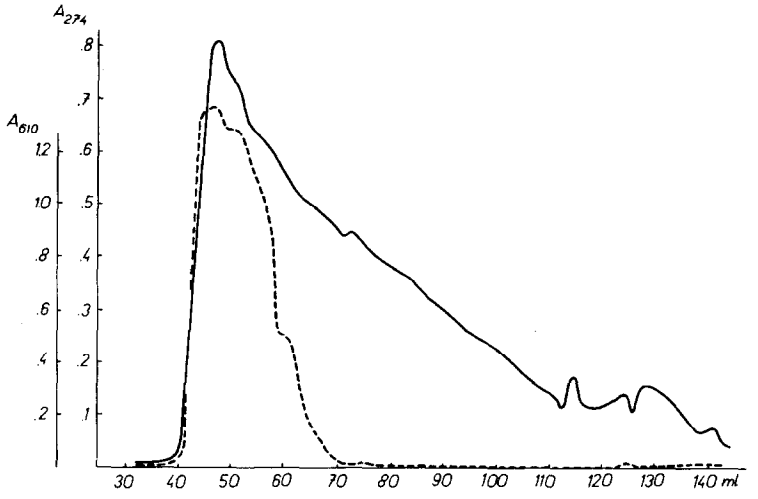

Fig.4. Separation of the enzymatic hydrolysate of elastin prepared by the method of McGavack and Kao on Sephadex G-100. Conditions as in fig. 3. Note change in scale of the abscissa!

paratively small content of low peptides. High turbidity with TCA is in agreement with these findings. The curve with Folin's phenol reagent, indicating the amount of tyrosine residues, shows a similarity to the UV-absorption curve (fig. 3).

Differences between both preparations are still more pronounced with regard to the development of turbidity during elastase digestion which is far more rapid and stronger with the Partridge preparation (fig. 5). The authors [1,4] call this turbidity formation coacervation. The coacervation is a function of pH, temperature, ionic strength and protein concentration.

4. Discussion

According to the opinion of Thomas and Partridge [5,6] the cross-links of elastin are formed mainly by desmosine and isodesmosine. One should realize that UV-absorption at 274 nm is caused mainly by these two amino acids (52% of the total absorption), and

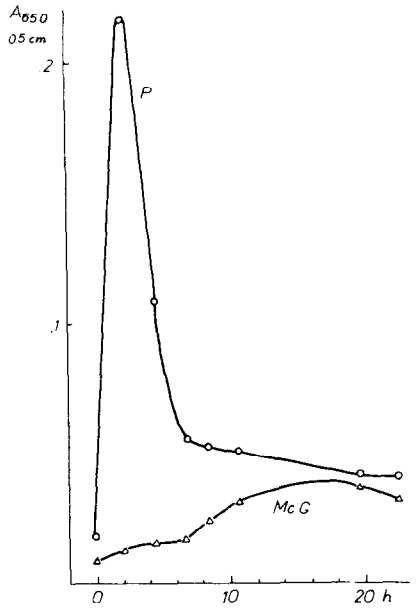

Fig. 5. Turbidity of elastins prepared by the methods of Partridge et al. (P) and McGavack et al. (McG) during the enzymatic elastolysis. 30 mg elastin suspended in 2 ml 0.05 M NH_4HCO_3-buffer (pH 8.8) and 0.6 mg crystalline elastase added. Digestion at 37°. Before turbidimetry shaken and 8 min free sedimented.

only in smaller part by the content of tyrosine or further amino acids and any other substances of the hydrolysate. From the given figures it is possible to infer where the cross-links of elastin remain in the course of enzymatic proteolysis. After complete elastolysis with elastase, most of the cross-links in the Partridge preparation (with mere autoclaving of the ligament) pass into poly- and oligopeptides. In the McGavack preparation, after an action of hot NaOH, the cross-links remain in large fragments, i.e. in proteins and polypeptides.

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