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# Structure of Elastin

## 2. Yellow Coloration

AIMO KÄRKELÄ and EINO KULONEN

*Department of Medical Chemistry, University of Turku, Finland*

For the investigation of its yellow pigment, elastin was partially hydrolyzed with acids, bases and enzymes under varying conditions and the hydrolyzates submitted to several fractionations. The best result was obtained by hydrolysis in 2 N sulfuric acid for 96 h at +95°C with subsequent adsorption on aluminium oxide and elution with ammonia.

Data on the infra-red absorption spectra of elastin and an enriched yellow preparation are presented. This preparation could not be degraded further without loss of the colour. The yellow pigment is tightly bound to the peptide chain and could not be identified as a bile pigment or by ultraviolet absorption spectrum.

The yellow colour was intensified in an unexplained manner by dialysis or treatment with tungstate.

The yellow pigment of elastin has recently attracted considerable interest [1-3] and several classes of compounds have been suggested as possible chromophores.

The first aim was to isolate the yellow component, but only enriched preparations could be obtained. The elastin was broken down with several enzymes, acids and bases. All methods of hydrolysis were handicapped by the formation of humins. In this respect barium hydroxide and papain were the most favourable agents. A complete hydrolysis of elastin was not achieved without concurrent destruction of the pigment.

The partial hydrolysates were fractionated by dialysis, by ion-exchange chromatography (Dowex-50) using different buffer systems, by preparative silica gel electrophoresis, by adsorption chromatography and by peptide-precipitating agents.

The best yellow fractions were obtained by adsorption on aluminium oxide and elution with ammonia. The yellow color seems to be tightly bound to the peptide chain (*cf.* Partridge and Davis [4]) and it could not be separated from the amino acids. Under certain conditions the colour is intensified, for example during dialysis or after addition of tungstates. This

effect is quite unexplained. An unsaturated bond suggested by Labella [2] is possible.

The main results are the preparation of the enriched fraction, the infra-red data of elastin and analysis of the peptide to which the yellow component is attached. It seems that there exists a yellow "core" which is gradually broken down to smaller non-coloured fragments, of which some amino acids were identified.

The attempts to identify the yellow component as a bile pigment or porphyrin derivative were not successful.

## Material and preparation

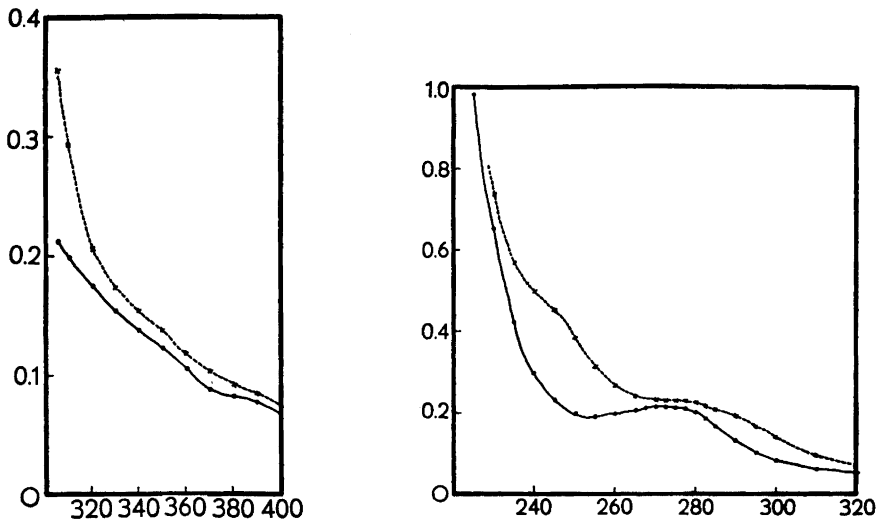
The preparation of elastin has been described in our earlier paper on the structure of elastin [5].

The *best enriched yellow preparation* was designated as R-5/$Al_2O_3$ and obtained as follows: Fifty g of elastin was suspended in 1 000 ml of 2 N sulfuric acid and kept at +95°C for 48 h with occasional shaking. Already after the first hours the fluid acquired a reddish-brown colour and at the end of the heating the fluid was rather dark. After filtration the fluid was neutralized with solid barium hydroxide. The precipitate adsorbed a part of the dark-brown colour but the filtrate was still rather brown, like concentrated urine.

The hydrolysis was continued at +95°C for an additional 48 h in 2 N sulfuric acid and more dark-brown colour was formed. The precipitation with barium hydroxide was repeated and the supernatant centrifuged clear. For removal of the dark colour additional barium sulfate was suspended and after 2 ½ h agitation the supernatant was collected by centrifugation. It resembled normal urine in colour. At a lower temperature (+37°C)

*Table 1.* The intensity of the yellow colour in some elastin preparations.

| Method of preparation | Nitrogen mg/ml in solutions with $E^{1\,cm}_{330} = 1$ |
|---|---|
| Oxalic acid-soluble elastin | 0.82 |
| Hydrolyzate with $Ba(OH)_2$ | 2.36 |
| fraction obtained with Dowex-50, pH 3.42 | 0.57 |
| Hydrolyzate (partial) with 4 N HCl, | |
| fraction obtained with Dowex-50, pH 3.42 | 0.42 |
| » » » » pH 6.7 | 0.23 |
| Hydrolyzate (partial) with 4 N $H_2SO_4$ | 0.85 |
| outside the bag after dialysis | 0.52 |
| R-5 | 1.60 |
| R-5/$Al_2O_3$ (adsorbed on $Al_2O_3$ and eluted with $NH_3$) | 0.12 |
| Hydrolyzate with papain | 1.05 |
| supernatant after precipitation with 10 % trichloroacetic acid | 0.81 |
| supernatant after precipitation with tungstic acid (final conc. 1.67 % sodium tungstate in 0.11 N $H_2SO_4$, supernatant neutralized to pH 5; the colour not quite elastin-like) | 0.14 |
| Humic acid (Light & Co., Ltd., in 0.1 N HCl) | 0.093 |




*Figs. 1—2.* The absorption spectra of elastin in 0.1 N hydrochloric acid (solid) and in 0.1 N sodium hydroxide (dotted).

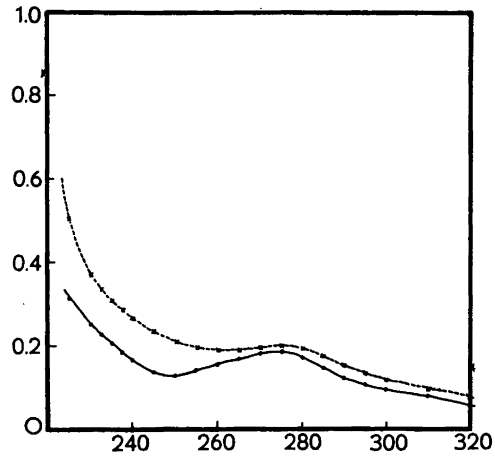



*Fig. 3.* The ultraviolet absorption spectrum of the preparation R-5/$Al_2O_3$ in 0.1 N hydrochloric acid (solid) and in 0.1 N sodium hydroxide (dotted).

less brown and red substances were formed, but hydrolysis lasted much longer and was more incomplete.

The hydrolysate was allowed to drain through a 2 cm layer of aluminium oxide (Merck, "nach Brockmann"). The yellow colour was retained in the surface of the aluminium oxide, which was washed with water until the washings were ninhydrin-negative. Only very little of the yellow colour was removed. The bulk of the yellow substance was eluted with 1 M ammonia, which was removed by evaporation in vacuum. The residue was brownish-yellow and the yield was 0.7 g.

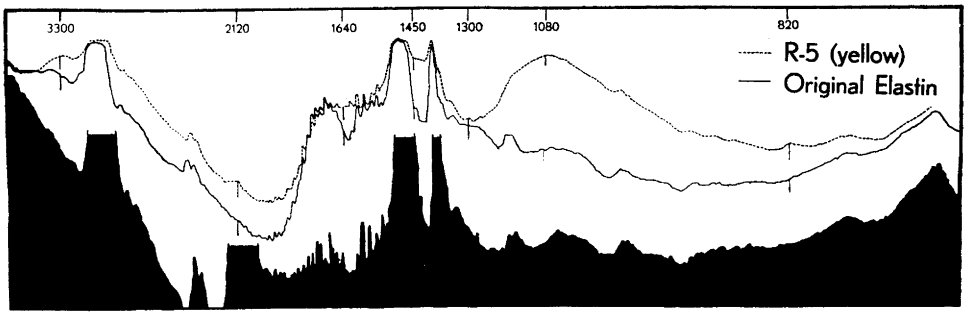



*Fig. 4.* The infra-red absorption spectra of oxalic acid-soluble elastin and of the enriched preparation R-5/$Al_2O_3$. (Perkin-Elmer single-beam instrument, sodium chloride cell of 0.05 mm, concn. 10 mg in 0.05 ml liquid paraffin, the borderline of the black area indicates the spectrum of the paraffine.) The differences in the spectra are pointed out with their wave numbers ($cm^{-1}$).

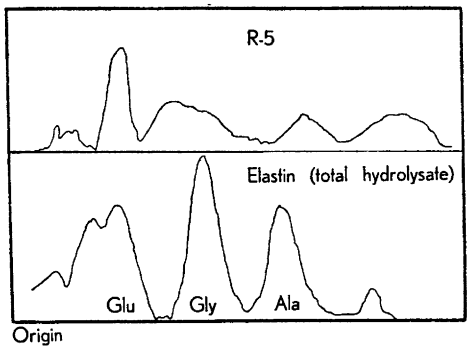



*Fig. 5.* Comparison of "total hydrolyzates" of elastin and of R-5/$Al_2O_3$ (densitograms from one-dimensional paper chromatograms run with phenol/$NH_3$). Note the absence of glycine in R-5.

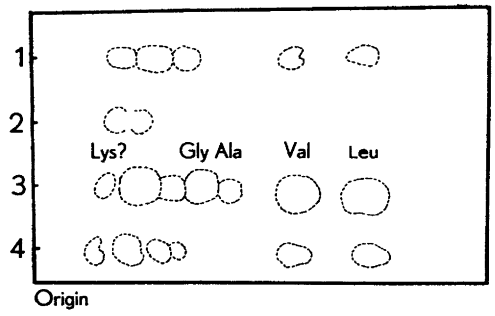



*Fig. 6.* Comparison of the butanol/acetic acid chromatograms of R-5 and original elastin. 1 = R-5/$Al_2O_3$, "total hydrolyzate"; 2 = Asp + Glu; 3 = elastin, "total hydrolyzate"; 4 = yellow "start" spot of No. 1 (the hydrolyzed R-5), rehydrolyzed.

*Hydrolysis with papain* was carried out at +37—38° C for 24 h. The digest contained 40 ml of 2.5 % elastin solution, 5 ml of 0.25 M citrate buffer (pH 5) and 5 ml of $H_2S$-activated papain (0.626 mg N/ml). The liberated amino nitrogen amounted to 18 % of the total.

*Hydrolysis with saturated Ba (OH )$_2$* was performed in a solution containing 5 % elastin which was kept for 3—4 days at +37°C. The fluid remained "purely" yellow.

The *R-4-hydrolysate* was made at +37°C in 4 N sulfuric acid for 14 days and finally the mixture was boiled under a reflux condenser for 24 h.

The intensity of the yellow colour was expressed as the concentration of nitrogen per ml solution having the extinction coefficient $E^{1\,cm}_{330} = 1$.

Some data are presented in the Table 1. Specimens of the absorption spectra are given in Figs. 1—3. There was no evidence to support the hypothesis of bile pigment origin (attempts were made with chloroform extraction and oxidation with hydrogen peroxide.) The comparison with a "humic acid" sample remained inconclusive.

In the many fractionation experiments with Dowex-50 it was observed that after the change of any buffer a yellow fraction appeared. Two sources of error were recognized: (1) some yellow colour can be eluted from the resin itself and (2) other proteins, for example gelatin, form during the hydrolysis yellow artefacts which appear similarly. The significance of these yellow fractions remains unknown.

## Analysis of the preparation R-5/$Al_2O_3$

*Infra-red absorption* (Fig. 4). Absorptions at 3 300 $cm^{-1}$ and 1 640 $cm^{-1}$ are due to amide groups, at 2 120 $cm^{-1}$ to amino acids and at 1 450 $cm^{-1}$ perhaps to carboxyl groups. Peaks at 1 300 $cm^{-1}$ and 820 $cm^{-1}$ are so small that no conclusions, for example concerning double bonds, seem warranted. The broad peak at 1 080 $cm^{-1}$ may be interpreted in several different ways (C—O of ethers, alcohols, or anhydrides, molecular size).

*Amino-acid composition.* Although the yellow substance was of such lowmolecular weight that it was dialysable, it did not move in the chromatograms as a compact spot but the colour faded in these manipulations as also in column chromatography and in preparative electrophoresis. The comparison of the amino acids of R-5/$Al_2O_3$ and elastin is presented in Fig. 5.

It was found that on the origin of the chromatographed R-5-hydrolyzates there always remained a yellow ninhydrin-positive spot. When this "start" spot was rehydrolyzed, a pattern resembling original R-5 hydrolysate was obtained (Fig. 6). From this it is concluded that the yellow component is during acid hydrolysis gradually broken to non-coloured products and that the R-5/$Al_2O_3$ represents the smallest yellow unit for the present.

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## REFERENCES

1. With, T. *Acta Med. Scand.* **128** (1947) 25.
2. Labella, F. S. *Nature* **180** (1957) 1360.
3. Loomeijer, F. J. *Nature* **182** (1958) 182.
4. Partridge, S. M. and Davis, A. F. *Biochem. J.* **61** (1955) 11.
5. Kärkelä, A. and Kulonen, E. *Acta Chem. Scand.* **11** (1957) 1434.

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