ELASTOIDIN - A MIXTURE OF THREE PROTEINS

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Introduction. The properties of elastoidin, the insoluble fibrous protein from fins of sharks, are similar to those of collagen in many respects (Fauré - Fremiet, 1936). However the high tyrosine content, the presence of cystine and methionine, and a low hydroxyproline content serve to distinguish elastoidin from collagen (Damodaran, Sivaraman and Dhavalikar, 1956). The X-ray pattern of elastoidin resembles that of collagen in some ways, and yet differs in other respects (Burge, Cowan and McGavin, 1958). On autoclaving, the fibres yield a tyrosine-rich residue and a gelatin (Gross and Dumsha, 1958) which in amino acid composition (Gross and Piez, 1960) closely resembles collagen except in a higher content of tyrosine. We present here evidence for the presence of tryptophan, not recognized heretofore, in elastoidin and on the presence of as many as three protein species in the fibres.

Experimental. Elastoidin was a sample prepared according to Damodaran, et al (1956). Analyses for hydroxyproline were done by the method of Neuman and Logan (1950). Tyrosine and tryptophan were determined by a spectrophotometric method (Goodwin and Morton, 1946). Total carbohydrate, as glucose, was measured by the anthrone reaction (Morris, 1948). In the carbohydrate determination blank corrections were only

approximate, both due to color in the samples which darkened further with sulphuric acid and due to difficult solubility in the acid. This comment applies to elastoidin and Fraction A, and values for carbohydrate in these two materials are therefore only approximate.

Measurements of the UV-spectra of elastoidin and other fractions, in 0.1 N NaOH, were done with a Beckman DU spectrophotometer.

Total N-terminal groups were determined using 1-fluoro-2,4-dinitrobenzene.

Fractionation. Elastoidin (8.5 g. dry wt.) was suspended in 330 ml. of 90% formic acid. The fibres swelled rapidly, twisted and turned, and numerous smaller fibrils stripped themselves off the parent ones. This process continued for some time and a viscous fibrous mass resulted. Such "solutions" were stirred at room temperature (around 30°C) for about 18 days. To this was added 700 ml, or more, of ether with good stirring. The precipitated fibrous mass (very sticky and hygroscopic) was washed with ether and suspended in 500 ml. of water. Dilute NaOH was added to give a pH of 8.5. After 3 hr. dilute HCl was added to pH 3-4 and the insoluble fibrous mass was centrifuged down, washed with water and dried in air (Fraction A, 2.56 g. dry wt.). Soluble proteins left in the supernatant, after removal of fraction A, were fractionated as below. To the solution was added Na_2SO_4 to 0.2 saturation. The material that separated out was collected, dissolved in water at pH 6.5, and repricipitated with Na_2SO_4. The protein in solution at pH 6.5 was finally dialyzed against distilled water (Fraction B,

lg. dry wt.). The supernatant left, after the removal of Fraction B, was now saturated with Na_2SO_4. The viscous oily protein that separated was dissolved in water, reprecipitated with Na_2SO_4, and finally dissolved in water and dialyzed against distilled water (Fraction C, 2.7 g dry wt.). Total weight of the isolated fractions was 6.3 g. (74%).

Results and discussion. The data on the composition of elastoidin and of fractions A, B, and C are indicated below:

Constituent: g. per 100g.	Elastoidin*	Fractions A	B	C
Carbohydrate	0.41(not reported)	0.41	0.79	0.97
Tyrosine	6.62(7.15)	21.02	None	0.35
Tryptophan	1.62(not reported)	None	10.20	0.04
Hydroxyproline	8.45(8.76)	3.19	3.97	10.41

* Values in parentheses are from Damodaran, et al (1956)

The UV-spectra depicted in Fig. 1 are consistent with the contents of tyrosine and tryptophan in the four samples. The weight yield and the analytical data would indicate that Fraction A accounts for nearly 94% of the tyrosine in elastoidin. Similarly, fraction B would account for about 80% of the tryptophan in elastoidin. It is noteworthy that fractions A and B contain tyrosine and tryptophan, respectively, in amounts higher than is known to exist in other proteins. Fraction C which is almost devoid of tyrosine and tryptophan contains more hydroxyproline than elastoidin and thus resembles collagen in its composition.

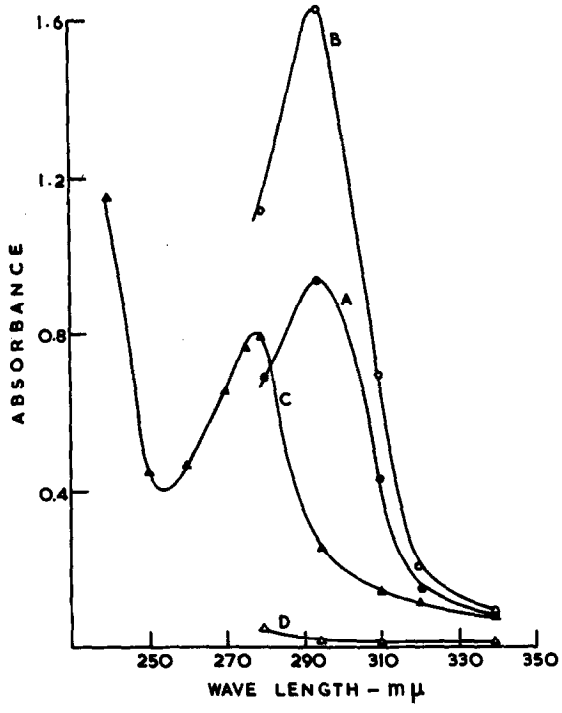

Fig. 1 - UV-spectra of proteins in 0.1 N NaOH
A- Elastoidin, 0.68 mg/ml. B-Fraction A, 0.495 mg/ml.
C- Fraction B, 0.283 mg/ml. D-Fraction C, 0.529 mg/ml.

Elastoidin suspended in 90% formic acid for 2 and 200 hrs. (resulting in solubilizations of the order of 13 and 55 per cent) showed as end groups only 0.4 and 0.44 moles, respectively, of amino acids per 10^5 g. protein. Cleavage of peptide bonds by the acid would, therefore, appear to be negligible. Significantly, fraction C, like the collagens had almost no end groups (0.05 moles per 10^5 g.). Total N-terminal groups detected in fractions A and B amounted to 0.82 and 0.61 moles, respectively, per 10^5 g. protein.

In the initial stages of contact of elastoidin fibres with formic acid only hydrogen bonds, perhaps, are broken causing

the smaller fibrils laid along the fibre axis to strip themselves off. Thus fibres dipped in the acid for only brief periods show the loss of the typical 2.9 A reflection found in wide angle X-ray photographs, but this characteristic is easily restored by promptly rinsing the fibres with water (Ramachandran, 1960). Only on prolonged contact the acid appears to leach out the soluble components. O-formylation does probably occur during prolonged contact of elastoidin with acid, but the three fractions isolated will contain few such groups because the isolation procedure involves treatment at pH 8.5 which is adequate for the hydrolytic removal of such ester formyl groups. It is conceivable, however, that the three components of elastoidin are held together by ester bonds, which the formic acid breaks. Anhydrous formic acid acting on proteins appears to promote only O-formylation and no peptide bond cleavage (Narita, 1959; Smillie and Neurath, 1959).

In X-ray and electron microscope studies of elastoidin (viz., Burge et al, 1958) it has been noted that there is a resemblance to the regular collagen pattern while there are also distinct differences. It is a matter only for speculation at present whether the elastoidin X-ray pattern is the result of superposition of the images from the three distinct protein species or arises from a unique interaction involving the three.

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