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Amino Acid Composition of Elastoidin

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The name elastoidin was given to the fibrous protein of the ceratotrichia or fin-rays of the elasmobranch, *Mustelus laevis*, by Krukenberg (1885). He noted its similarity to collagen, but was of the opinion that it differed from the latter in essential respects, particularly in not yielding gelatin on boiling with water. Except for an elementary analysis made by Krukenberg, the only chemical study recorded on elastoidin is that by Engeland & Bastian (1938), who qualitatively identified glycine, alanine, serine and hydroxyproline in the acid hydrolysate. However, the material was made the subject of several physical studies (see Champetier & Fauré-Fremiet, 1937; Astbury & Lomax, 1935) and on the basis of its wide-angle X-ray diffraction it was classified as a collagen by Astbury (1938).

Ceratotrachia of selachians afford a convenient material for investigation of collagen because of the simplicity of their anatomical disposition in the fins and the ease with which they can be obtained in homogeneous form. When a shark fin sectioned at the base is split open longitudinally by pulling out the cutaneous sides from each other, the ceratotrichia are exposed as long translucent fibres embedded in the fleshy tissue. If the halves of the fins are soaked in water for some time, the fleshy material is sufficiently softened for the fibres to be pulled out intact. From large fins, fibres reaching 6–9 in. in length and 1–2 mm. in diameter are often obtained in quantity; they are of a translucent light-yellow colour, morphologically homogeneous and sufficiently rigid to be easily washed free from

any adhering tissue, the last traces of which can be completely removed by digestion with trypsin.

The amino acid composition of the elastoidin of the fin-rays of *Carcharinus melanopterus* is described here. The amino acid distribution follows the general pattern of typical collagens, but also shows certain significant differences which might explain the peculiarities in physical behaviour observed with elastoidin.

EXPERIMENTAL

Preparation of elastoidin. Elastoidin fibres were isolated from the dorsal fin of the shark *Carcharinus melanopterus*. The fin was obtained fresh within 12 hr. of the catch, split into two halves and soaked for 48 hr. in water under toluene, the water being periodically changed. The fin-rays were pulled out of the fleshy portion of the fin individually and washed free from adhering tissue.

The isolated fibres (12 g.) were immersed in 600 ml. of 2% NaHCO_3 solution containing 600 mg. of trypsin (British Drug Houses, Ltd., L.R.) protected by a toluene layer and left for 48 hr. at 37°. The enzyme solution was then poured off and the rays were washed repeatedly with distilled water. The fibres were preserved in water under toluene in the cold. For analysis the fibres were snipped and macerated in a Worthing Blendor with water at 0°, and the disintegrated material, consisting of fine fibres, was filtered off, washed with acetone and defatted by Soxhlet extraction with light petroleum (b.p. 40–60°).

Analysis

Moisture, ash, nitrogen and sulphur. Moisture was determined by drying to constant weight over P_2O_5 at 110° and 1 mm. pressure. The N content of the protein

(corrected for moisture 5.27%, ash 0.73%) was 17.52% (micro-Kjeldahl). S content determined according to the procedure described by Pirie (1932) was 0.51%. Ester-bound S was estimated as sulphate-S after hydrolysis with 5N-HCl for 20 hr. at 100° (Lugg, 1938*a*) and found to be 0.03%.

Hydrolysis. The disintegrated and defatted fibre (1 g.) was dissolved on the water bath in 200 ml. of 6N-HCl (redistilled twice in glass) and refluxed for 20 hr. in an oil bath maintained at 135–140°. The excess of HCl was removed by repeated concentration under reduced pressure and finally by leaving over KOH pellets in a vacuum desiccator. The residue was dissolved in 0.5N-HCl and made up to volume with water.

Chromatographic techniques. The amino acid separations were carried out by chromatography on Dowex-50 (200–400 mesh, 12% cross-linked) columns operated in the sodium form essentially as described by Moore & Stein (1951). A 1 cm. $\times$ 100 cm. column was used for quantitative determination of acidic and neutral amino acids and for a qualitative picture of the components in the basic amino acid range. The basic amino acids were estimated in separate runs on a 1 cm. $\times$ 15 cm. column. The rate of flow of solvent was maintained by applying a positive or negative pressure to the top of the separating funnel containing the buffer through an empty 10 l. aspirator bottle maintained at the proper pressure. Effluent fractions (1 g.) were collected on a Shandon automatic fraction collector. The effluent fractions were analysed by the photometric ninhydrin method of Moore & Stein (1948), hydroxyproline and proline being estimated at 440 m μ . in the Beckman Model DU quartz spectrophotometer and the other amino acids on a Klett-Summerson photoelectric colorimeter, with a 540 m μ . green filter. Identification of substances responsible for the various peaks was carried out in preliminary column runs by the use of paper chromatograms, after pooled samples of fractions comprising the peak points had been desalted in the way described by Stein (1953).

Estimation of cystine, methionine and tryptophan. Cystine was estimated polarographically by the procedure of Stern, Beach & Macy (1939) in samples hydrolysed with HCl-formic acid (Block & Bolling, 1951) under N₂. Methionine was determined by the volatile-iodide method of Baernstein (1936), in which the protein was hydrolysed and demethylated simultaneously by boiling under reflux with conc. HI under N₂. Tryptophan was estimated by the *p*-dimethylaminobenzaldehyde method of Horn & Jones (1945) in alkali hydrolysates of the protein (Lugg, 1938*b*).

Ammonia. The NH₃ content of elastoidin hydrolysates was determined by the microdiffusion method of Conway (1947).

RESULTS

Hydrothermal behaviour

The shrinkage temperature of the elastoidin fibre as measured in the Theis Shrinkage Tester was found to be 63–64° in water, the fibre contracting to about 30% of its original length. On cooling and applying longitudinal tension, the shrunk fibre became re-elongated to about 85% of its initial length. These properties are in accord with the results reported by Fauré-Fremiet (1937).

As observed by this author, prolonged treatment with steam is necessary to get elastoidin into solution. By repeated autoclaving with water at 120° (seven extractions for 4 hr. each) 98% of the material was obtained in solution. The solution on concentration and cooling set to a gel.

Optical properties

Native and re-elongated elastoidin fibres showed strong birefringence under the polarizing microscope, whereas the shrunk fibre had only a feeble birefringence.

Fig. 1. Electron micrograph of elastoidin fibres treated with trypsin and fixed in formalin. Chromium-shadowed.

For electron-microscopic examination the intact fibres were treated for 48 hr. with trypsin, fixed for 24 hr. in 10% formalin and dispersed in water in the Waring Blendor. Samples were shadowed with chromium at an angle of 20°. Band spacings (Fig. 1) varied between 750 and 880 Å in preparations examined by this method. A periodicity of 600 Å has been reported in dry elastoidin fibrils by Bear (1952) from low-angle X-ray studies.

Action of enzymes

The intact elastoidin fibre resisted the action of crude or crystalline trypsin (British Drug Houses Ltd., L.R. and Armour and Co., cryst.), was slightly acted on by papain (crude latex suspension) and was completely dissolved by crude or crystalline pepsin (British Drug Houses Ltd. and Armour and Co., cryst.). The shrunk fibre was completely digested by all three enzymes.

Fig. 2. Separation of amino acids from 5 mg. of elastoidin hydrolysate on a 1 cm. x 100 cm. column of Dowex-50.

Fig. 3

Fig. 3. Separation of hydroxyproline, aspartic acid, threonine and serine; 5 mg. of elastoidin hydrolysate on a 1 cm. x 100 cm. column of Dowex-50.

Fig. 4

Fig. 4. Separation of basic amino acids; 5 mg. of elastoidin hydrolysate on a 1 cm. x 15 cm. column of Dowex-50.

Amino acid analyses

Five different types of chromatograms were employed in the analysis of elastoidin hydrolysates. Threonine, serine, glutamic acid, proline, valine, isoleucine, leucine, tyrosine and phenylalanine were estimated on the 100 cm. column with a 5 mg. load and the usual buffer systems (Moore & Stein, 1951). A typical effluent curve is shown in Fig. 2. Hydroxyproline and aspartic acid, which were not well separated at pH 3.42, were resolved by the method of Moore & Stein (1951) by using a buffer of pH 3.20 (Fig. 3). Since the 5 mg. load was too high for satisfactory resolution of glycine and alanine, the two were separated with 1 mg. of hydrolysate. Lysine and arginine were determined on the 15 cm. column with a 5 mg. load (Fig. 4) according to the procedure of Moore & Stein (1951), and histidine and hydroxylysine were separated on the 15 cm. column with a 10 mg. load by the method of Piez (1954). A value of 0.87% on a nitrogen basis was found for hydroxylysine, compared with 0.73% reported by Ramachandran (1953). The ninhydrin-positive peaks, peak *A* (Fig. 2) and peak *B* (Fig. 4), which appear in addition to those expected at the positions of emergence of the common amino acids, could not be identified in paper chromatograms, as the amount of material was too low. The position of emergence in the two columns, however, indicates β -alanine (Moore & Stein, 1951).

The destruction of serine and threonine was estimated after refluxing known quantities with 6N-HCl for 20 hr. The losses were found to be 3.5% for serine and 1.0% for threonine. No corrections were applied for these losses in tabulating the analytical values. The stability of serine and threonine to a 22 hr. period of acid hydrolysis has also been reported by Dustin, Czajkowska, Moore & Bigwood (1953).

DISCUSSION

The results of the amino acid analyses of elastoidin are summarized in Table 1. The composition found exhibits the main features considered characteristic of collagens (Kendrew, 1954). Thus there is a high content of glycine, amounting to about 32%, and of hydroxyproline and proline, together amounting to about 17% of the total residues. Residues of non-polar amino acids total 64%. The hydroxy amino acids hydroxylysine, threonine and serine are present in about the proportions usually found in mammalian collagens (Bowes & Kenten, 1948; Neuman, 1949); the hydroxyproline content is lower, but the quantity is comparable to that found in fish collagen (Neuman & Logan, 1950; Gustavson, 1955). These chemical characteristics explain the

molecular arrangement which elastoidin and collagens have in common, as shown by their similar wide-angle X-ray patterns.

A striking deviation from collagen composition is the high tyrosine content of 7.15% by weight, compared with the usual value of about 1% for collagens. Significance is also to be attached to the presence of 0.35% of cystine which is not present in cattle-hide collagen, and according to most analyses is present only in insignificant amounts in other collagens. These two differences in chemical composition should be relevant to the properties that distinguish elastoidin from collagens in general.

Table 1. *Amino acid composition of elastoidin*

	g./100 g. of protein	N as % of total N	Residues/ 10 ⁵ g. of protein
Alanine	11.40	10.23	128.0
Glycine	25.37	27.04	338.0
Valine	2.72	1.86	23.2
Leucine	2.62	1.59	20.0
Isoleucine	2.69	1.64	20.5
Proline	13.27	9.22	115.3
Hydroxyproline	8.76	5.35	66.8
Phenylalanine	2.10	1.02	12.7
Tyrosine	7.15	3.16	39.5
Tryptophan	0.00	0.00	0.0
Serine	3.31	2.52	31.5
Threonine	2.42	1.62	20.3
Cystine	0.35	0.23	1.5
Methionine	1.78	0.96	12.0
Arginine	8.61	15.81	49.5
Histidine	1.73	2.68	11.1
Lysine	3.74	4.09	25.6
Hydroxylysine	0.88	0.87	5.4
Aspartic acid	6.40	3.85	48.1
Glutamic acid	11.01	5.98	74.9
Amide N	—	3.40	(42.5)
Total	116.3	103.1	1045.3

Lloyd (1941), in comparing the swelling behaviour of collagen, elastoidin and ovokeratin, was of the opinion that with increase in sulphur content these substances showed increasing resemblance to keratin, probably because of disulphide bridges between polypeptide chains. The similarity to keratin in chemical composition, shown by the presence of tyrosine and cystine, may also afford an explanation for the observed hydrothermal properties of elastoidin. Gustavson (1955) advanced the hypothesis that the hydrothermal properties of the collagens depend on the hydrogen-bond linkage of the OH group of the hydroxyproline to the keto imide group of the peptide chain. This was based on the finding that skin collagens of cod fish and pike, which have a shrinkage temperature of 40° and 55° respectively, compared with 65° for bovine collagen, have also lower contents of hydroxyproline (5.8 and 7.9% respectively) than bovine collagen (12.7%). This does not apply to

elastoidin, which has a high shrinkage temperature of about 64°, but a low hydroxyproline content similar to that of fish collagen. The reversibility of thermal shrinkage shown by elastoidin in its natural state also indicates the possibility of the presence in elastoidin of cross-linkages that do not occur in other collagens, since these manifest reversible shrinkage only after treatment with formalin (Gerngross & Katz, 1926). The cross-linking may also afford an explanation for the slowness with which elastoidin is converted into gelatin even under drastic treatment with steam under pressure.

SUMMARY

1. The amino acid composition of elastoidin from the ceratotrichia of *Carcharinus melanopterus* has been determined.

2. The amino acid distribution follows the general pattern characteristic of collagens, and justifies the inclusion of elastoidin in the collagen group of proteins made on the basis of physical studies.

3. Elastoidin differs from other collagens, being rich in tyrosine and containing a significant amount of cystine. These differences may explain the peculiar hydrothermal behaviour of elastoidin which distinguishes it from other collagens.

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Comparison of the Metabolism of Uniformly ¹⁴C-labelled L-Phenylalanine, L-Tyrosine and L-Tryptophan in the Rat

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In the adult animal whose metabolism is in the steady state the amount of ingested tryptophan incorporated into body protein should be balanced by liberation of an equivalent amount of endogenous tryptophan by protein breakdown. The observed degradation products of tryptophan should therefore account for an amount of tryptophan equal to that ingested. In fact, as is shown in the subsequent discussion, this is very far from being the case. Our knowledge of the metabolism of

tryptophan differs from that of the metabolism of phenylalanine and tyrosine in a most important respect. Phenylalanine is metabolized via tyrosine to the metabolically highly reactive acetoacetic and fumaric acids, so that metabolism of large amounts of phenylalanine and tyrosine can be readily accounted for. But there is no pathway at present known for conversion of the aromatic ring of tryptophan into metabolically reactive aliphatic compounds.