A FIBROUS PROTEIN FROM THE SLIME OF THE HAGFISH

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The hagfishes are well known for the copious amounts of slime, or "mucus," which they secrete when disturbed or annoyed (1). In sea water, a hagfish can produce in a few seconds several times its own volume of slime. The slime is colorless and transparent, and, although extremely dilute, has a tough, coherent, stringy consistency. It is highly viscous, and also possesses rigidity, exhibiting "elastic recoil." This material, obtained from the Pacific hagfish, *Polistotrema stouti*, has now been examined in connection with a general study of mechanical properties of protein, mucoprotein, and polysaccharide systems. It has been found to be heterogeneous in composition, containing insoluble protein fibers which are largely responsible for its peculiar consistency. The present paper describes the microscopic structure of the slime and the composition of the fiber protein.

Materials—Specimens of *Polistotrema stouti* were obtained from Monterey Bay. The animals survived for several weeks without food in tanks of running sea water, and could be stimulated repeatedly for the production of slime. I am greatly indebted to Dr. Berry Campbell for advice and aid in the handling of hagfish and in devising suitable methods of stimulation.

Behavior of Slime Secreted in Sea Water—The animals were stimulated in sea water by agitation or local pressure. The voluminous transparent slime thus produced contained a loosely tangled mass of fibers, each about 1.3 μ in diameter and several cm. long. This mass later contracted spontaneously and ir-

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reversibly, expelling water, to about a fiftieth of its original volume. From the resulting compact mat of fibers, strands could be drawn several feet in length and variable in thickness (0.05 to 0.5 mm.). The strands were soft and elastic when wet, and strong and flexible when dry. The yield of fibers was about 0.25 gm. from a liter of slime. The sea water expelled from the original slime contained about 0.01 per cent of a dissolved protein which could not be precipitated by ammonium sulfate or trichloroacetic acid, but could be recovered in a denatured form by dialysis, electrodialysis, and evaporation to dryness.

FIG. 1. Secretion from the slime gland, diluted somewhat with water, showing fiber coils (about 0.11 mm. in length).

Behavior of Slime Secreted in Absence of Water; Fiber Coils—In order to avoid the presence of a large excess of sea salt, as well as occasional particles of foreign matter present in sea water, the secretion was also obtained undiluted by stimulating the animals out of water. The hagfish was anesthetized with ether, suspended vertically, and wiped clean. Upon local electrical stimulation, the slime glands discharged white drops of secretion. The latter contained no extended fibers, but fibers in tightly rolled coils of uniform elliptical shape about 0.11 mm. in length and 0.06 mm. in width (Fig. 1). Several dozen of these coils were discharged simultaneously from a single duct, as could be observed with the microscope. When the secretion was diluted with either sea water or distilled water, and slightly agitated, the coils unrolled

to produce extended fibers (Figs. 2 and 3) and the suspension acquired the characteristic appearance and consistency of the slime as ordinarily secreted in water.

Most of the coils unrolled immediately, but some remained coiled and could not be unrolled even by vigorous agitation. When secretion took place directly in water, on the other hand, the unrolling was almost complete, although microscopic examination of the slime revealed an occasional intact coil.[1]

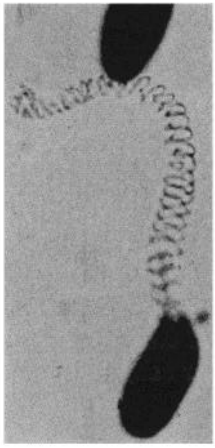

FIG. 2

FIG. 3

FIG. 2. Fiber coil unrolling upon agitation.

FIG. 3. Fibers (about 1 μ in diameter) almost completely unrolled, forming a concentrated slime.

Isolation of Soluble Protein from Slime Secretion—Several hagfish were stimulated electrically and the drops of secretion washed down with distilled water, producing a concentrated slime, which was filtered on a Buchner funnel. The filtrate, acidified with acetic acid, formed a thixotropic gel. Upon dilution with several volumes of alcohol, the gel shrank; it was then repeatedly washed with alcohol and finally dried. The yield was very small, amounting to about 5 per cent of the accompanying fiber protein. The

[1] The mechanism of this curious process of secretion of fibers in preformed coils is being investigated by Professor W. W. Newby of the University of Utah, who is studying the histology of the slime glands.

nitrogen content of this soluble material (by digestion and nesslerization) was 12.7 per cent and the carbohydrate content[2] (as glucose, by the method of Sørensen and Haugaard (2)) was 2.9 per cent. The dried protein swelled enormously in water, and was dissolved by treatment with ascorbic acid-hydrogen peroxide, suggesting that its rigidity might be associated with a phosphoric acid component.[3]

Purification of Fiber Protein—The mat of fibers collected from the filtration above was resuspended in distilled water, stirred thoroughly, and then run through rubber rollers and squeezed dry. The resulting films were cut in small pieces and washed twice by soaking an hour in distilled water and squeezing with a stirring rod. They were finally washed with alcohol and ether and dried, yielding a fluffy mass of pure white fibers. The ash content of this material, estimated by igniting with sulfuric acid at 500°, was 1.1 per cent. A portion was later suspended in phosphate buffer (ionic strength 0.2) at pH 7.7 to dissolve possible traces of remaining soluble protein, washed phosphate-free, electrodialyzed, and redried with alcohol and ether. The fiber protein thus purified had an ash content of 0.9 per cent, and contained 17.5 per cent nitrogen (corrected for ash) and 1.4 per cent carbohydrate.

The tyrosine content was estimated by the method of Lugg (3). Duplicate samples were hydrolyzed in 3.3 N sodium hydroxide. Values of 5.3 and 5.6 per cent tyrosine were obtained. The tryptophane content, according to Lugg's method, was less than 0.2 per cent. The cystine content was estimated by the method of Folin and Marenzi (4). Duplicate samples were hydrolyzed in 6 N sulfuric acid; the humin formation was so slight that no decolorizing was necessary. Values of 0.38 and 0.46 per cent cystine were obtained. No great accuracy is claimed for these analytical figures, in the absence of parallel analyses upon proteins of known composition by the same procedures, especially in hydrolysis. However, they serve to characterize the composition of the fibers, and to differentiate them clearly from other fibrous proteins.

The fibers were insoluble in dilute acid, alkali, and salt solu-

[2] I am much indebted to Miss Sue Y. Green for carbohydrate analyses.

[3] Robertson, W. van B., personal communication.

tions, and in 6 M urea. In any of these solutions they softened and formed a swollen mat, from which strands could be drawn; but the high dilution and characteristic properties of the original slime were not regained. Upon being autoclaved with water at 120° for 4 hours, the substance was still undissolved, but its tensile strength was much impaired.

DISCUSSION

The composition of the fibrous protein from hagfish slime may be compared with that of collagen, myosin, fibroin, and the keratins (5). The absence of tryptophane, within the limits of the analysis, and the low cystine content distinguish the protein from myosin and the keratins. Its moderately high tyrosine content differentiates it from collagen, assuming the composition of the latter to be similar to that of its derivative gelatin, which is tyrosine-free. On the other hand, it contains much less tyrosine than does silk fibroin. Thus it appears to be quite unlike any of these fibrous proteins. The name *mitin* (μίτος, thread) is suggested for the fiber protein from *Polistotrema* and for similar substances which may subsequently be obtained from related animals. It will be of interest to compare the configuration of mitin as studied by x-ray diffraction[4] with similar results for natural and derived protein fibers (6).

The heterogeneity of the original slime and its irreversible contraction render it unsuitable for study of mechanical properties in relation to its composition and structure. However, it might serve as a large scale model to illustrate structures which have been proposed for certain gels with no optically visible structure. It consists of a tangle of threads, whose ratio of length to diameter is of the order of 10^5, and whose surfaces are lyophilic in character, suspended in a medium of relatively low viscosity. Shear is accompanied by straightening and aligning the tangled threads to some extent, while recovery from shear ("elastic recoil") involves resumption of the random entanglement. This is not unlike the supposed molecular structure of a gel of rubber, or better, polyisobutylene or polystyrene (in which there are no cross links joining

[4] Dr. R. B. Corey of the California Institute of Technology is investigating the x-ray diffraction of mitin fibers, and has observed patterns which differ from those of other fibrous proteins.

the molecular chains), swollen in a non-polar solvent (7). On the other hand, the unrolling of coils of mitin, with the formation of an elastic slime, might be a model for the denaturation of a globular protein (6), which under certain conditions transforms a solution of low viscosity into a rigid gel.

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SUMMARY

The slime of the hagfish contains a fibrous protein secreted in the form of coils which unroll to form extended fibers. The composition and properties of this protein, which differ widely from those of other fibrous proteins, are described.

BIBLIOGRAPHY

1. Parker, T. J., and Haswell, W. A., Text-book of zoology, London, **2**, 138 (1910).
2. Sørensen, M., and Haugaard, G., *Compt.-rend. trav. Lab. Carlsberg*, **19**, No. 12 (1933).
3. Lugg, J. W. H., *Biochem. J.*, **31**, 1422 (1937); **32**, 775 (1938).
4. Folin, O., and Marenzi, A. D., *J. Biol. Chem.*, **83**, 103 (1929).
5. Cohn, E. J., *Chem. Rev.*, **24**, 203 (1939).
6. Astbury, W. T., *Ann. Rep. Progress Chem.*, **35**, 198 (1938); in Luck, J. M., Annual review of biochemistry, Stanford University, **8**, 113 (1939).
7. Sparks, W. J., Lightbown, I. E., Turner, L. B., Frohlich, P. K., and Klebsattel, C. A., *Ind. and Eng. Chem.*, **32**, 731 (1940). Whitby, G. S., *Tr. Inst. Rubber Ind.*, **6**, 55 (1930); *J. Physic. Chem.*, **36**, 198 (1932).