THE PRESENCE OF COLLAGEN IN PROTEINPOLYSACCHARIDE FROM SHARK CARTILAGE

T. A. Mashburn, Jr. and P. Hoffman
Research Institute for Skeletomuscular Diseases
Hospital for Joint Diseases and Medical Center,
New York, N. Y. 10035

Received November 2, 1967

Proteinpolysaccharides isolated from acetone-dried bovine nasal septum cartilage by high speed homogenization in the cold (Malawista and Schubert, 1958) or by extraction with buffer at 37° (Hoffman, et al., 1967) have been reported to be chemically indistinguishable except for the slightly lower viscosity of the 37° extracted product (Hoffman, et al., 1967). Free-flow electrophoresis (Mashburn and Hoffman, 1966) of proteinpolysaccharide obtained by either method readily separates free or extraneous protein from proteinpolysaccharide, and provides a bellshaped distribution curve of proteinpolysaccharide which may be sectioned arbitrarily into a slowly migrating tail fraction (protein-rich, polysaccharide-poor), the major amount of the material in a mid fraction, and a rapidly migrating fraction (protein-poor, polysaccharide rich) (Hoffman, et al., 1967). In the present work, similar 37° extraction and purification of the proteinpolysaccharide of shark or stillborn human cartilage shows similar behavior with, however, some striking differences. Both these proteinpolysaccharides contain substantial amounts of bound collagen (as measured by hydroxyproline and glycine levels), which cannot be removed by free-flow electrophoresis or alcohol fractionation. The shark proteinpolysaccharide contains more collagen than the stillborn human, and occasionally the product obtained from bovine nasal septum contains small amounts of collagen

which, however, can represent a significant fraction of the protein.

METHODS

Proteinpolysaccharide was isolated from acetone-dried cartilage powder of the Blue shark (Prionace glauca - 26% of dry weight) and of stillborn human costal cartilage (15% of dry weight) by extraction with pH 5 acetate at 37° (Hoffman, et al., 1967) or by the high speed homogenization method (Malawista and Schubert, 1958). Preparative free-flow electrophoresis at pH 5 and analytical procedures were carried out as described previously (Hoffman, et al., 1967). Hydroxyproline was determined either by the method of Stegemann (1958) or by use of a Beckman amino acid analyzer. The collagenase digestions were performed by incubating, at 37° for 24 hrs, a 0.6 percent solution of the substrate in 0.05 M tris buffer, pH 7.5, containing 0.01 M calcium acetate with a 60:1 ratio of substrate to collagenase (Worthington).

RESULTS AND DISCUSSION

Proteinpolysaccharide extracted from shark cartilage at 37°, unlike that from bovine cartilage, contains considerable protein which may be readily removed either by alcohol fractionation or by free-flow electrophoresis. The rapidly migrating proteinpolysaccharide from electrophoresis of either crude or alcohol fractionated shark proteinpolysaccharide can be divided into fractions with essentially identical analyses. Table 1 (before) shows the analytical results of the major fraction from such an electrophoresis. For comparison the results of similarly treated bovine nasal septum and stillborn human costal cartilage proteinpolysaccharides are also shown.

It is immediately apparent that the shark and stillborn human proteinpolysaccharides contain substantial amounts of a collagen like protein as indicated by the presence of hydroxyproline and by the elevated glycine values.

TABLE 1

Action of Collagenase on Proteinpolysaccharide.
Major Fractions After Free-Flow Electrophoresis.

	Shark			Calf Nasal Septum		Stillborn Human	
	Before	After		Before	After	Before	After
		a	b				
Uronic acid, %	29	26	24	25	27	21	27
Glucosamine, μmole/g	61	42	21	37	34	37	15
Galactosamine, μmole/g	887	1141	1023	755	1051	888	1261
Amino Acids, %	7	2.5	3.4	9.3	6.8	11.6	5.0
Amino Acids							
Hypro	45	c	c	trace	c	45	c
Ser	69	109	118	111	137	81	133
Glu	115	167	152	139	150	118	135
Pro	131	153	147	115	107	127	103
Gly	266	133	150	157	128	248	154
Ala	74	40	60	74	71	90	68
Leu	45	40	56	70	72	61	89

a - major fraction from electrophoresis.
b - material from a after chromatography on a Bio-Gel P-100 column.
c - not measurable.

Although interactions between collagen and chondroitin sulfate are well known (DiSalvo, 1966), in all cases the collagen is the major component interacting with relatively small amounts of chondroitin sulfate (Lowther, 1963; Wood, 1964). In the present case, essentially homogeneous electrophoretic fractions having polysaccharide to collagen weight ratios of approximately ten, migrate similarly to proteinpolysaccharide containing no collagen-like material. Mathews (1965) studied collagen-chondroitin sulfate interactions in Tiselius free cell electrophoresis and demonstrated the appearance of a complex which migrated much more slowly than chondroitin sulfate and obviously consisted mainly of collagen. Complexes containing collagen associated with small amounts of proteinpolysaccharide would be readily separated from uncombined proteinpolysaccharide in our preparative electrophoretic system.

It can be shown that the collagen-like protein is not present as a reversibly bound protein by the following observations. Electrophoresis of a crude 37° extract demonstrates the presence of free, unbound protein which migrates either very slowly or not at all and is easily separable from the rapidly migrating proteinpolysaccharide. Alcohol fractionation of the proteinpolysaccharide gives the major fraction at 45% alcohol. Subsequent electrophoresis shows little or no slowly migrating protein and a proteinpolysaccharide peak which is analytically identical to that from the crude extract.

Soluble bovine achilles tendon (Worthington) or gelatin when mixed with proteinpolysaccharide do not result in the incorporation of hydroxyproline containing material in electrophoretic peak fractions. Such experiments would appear to preclude a reversible binding of collagen. Hence, it is very likely that the collagen-like residues are an integral part of the proteinpolysaccharide, even though nothing is known at present about the nature of the linkage.

To demonstrate that this hydroxyproline containing protein is collagen, three proteinpolysaccharides (Table 1) were treated with collagenase and fractionated by electrophoresis. Analytical values for the major fractions after electrophoresis are shown in Table 1. About 75% of the protein of shark proteinpolysaccharide, 50% of the stillborn human and 30% of the calf nasal septum proteinpolysaccharide was removed by collagenase digestion. The resulting products were found to be free of hydroxyproline. In each case, it can be seen that those amino acids such as gly, hypro, and ala, which predominate in collagen, were decreased in amounts compatible with the removal of collagen.

The shark proteinpolysaccharide after collagenase digestion contained only 2.5%-3.4% of amino acids; about the same amount as after extensive papain digestion of either shark or mammalian proteinpoly-

saccharide. However, unlike papain digested mammalian proteinpolysaccharide (Hoffman, et al., 1967), the shark proteinpolysaccharide even with this low level of amino acid is still a high molecular weight entity being eluted in the excluded volumn of a Bio-Gel P-100 column (Table 1, fraction B). Low molecular weight single chain polysaccharides, as demonstrated by their elution in the maximally retarded volume from a Bio-Gel P-60 or P-100 column, are obtained by either alkali treatment or papain digestion of shark or mammalian proteinpolysaccharides. Obviously, the protein remaining after collagenase digestion of shark proteinpolysaccharide still serves as a core to tie a number of single chain polysaccharides together into a macromolecule.

The changes shown in Table 1 are compatible with a loss of collagen. We have pointed out (Hoffman, Mashburn and Greenberg, submitted for publication) the notable similarities in the amino acid patterns of fractionated proteinpolysaccharides isolated from various mammalian cartilages. In no case has it been possible to state from which tissue or animal a proteinpolysaccharide was isolated based simply on amino acid analysis. The markedly higher proline and lower alanine and leucine values gives an indication that shark cartilage product is different. More work will have to be done to determine whether the differences are related to the differences in total amount of protein present or to fundamentally different protein cores. Whether the collagen is present as intact molecules, as α or β chains or as smaller subunits is not known.

The extraction of shark proteinpolysaccharide by the method of Malawista and Schubert (1958) leads to a product having a similar electrophoretic pattern to the 37° extracted material and containing comparable amounts of hydroxyproline. In contrast, the cold extraction of human stillborn costal cartilage (Rosenberg, et al., 1965) led to a product which had no detectable amount of hydroxyproline and we have confirmed this finding. The reason for this difference is not under-

stood. Proteinpolysaccharide from mature bovine nasal septa extracted at 37° contains much smaller amounts of hydroxyproline, being undetectable in some samples, and it would appear that the extraction of bound collagen with proteinpolysaccharide has a phylogenetic and chronological age dependency.

ACKNOWLEDGMENT

This work was supported by a grant from the NIH (AM 08482) and done during the tenure of an Advanced Research Fellowship (to TAM) of the American Heart Association. We thank Dr. Karl Meyer for a gift of stillborn human costal cartilage.

REFERENCES

DiSalvo, J., and Schubert, M., Biopolymers, 4, 247 (1966)
Hoffman, P., Mashburn, T. A. Jr., Meyer, K., and Anderson-Bray, B., J. Biol. Chem., 242, 3799 (1967)
Lowther, D. A., in Hall, D. A., International Review of Connective Tissue Research, Vol. 1, Academic Press, N. Y., p. 91, 1963
Malawista, I., and Schubert, M., J. Biol. Chem., 230, 535 (1958)
Mashburn, T. A. Jr., and Hoffman, P., Anal. Biochem., 16, 267 (1966)
Mathews, M. B., Biochem. J., 96, 710 (1965)
Rosenberg, L., Johnson, B., and Schubert, M., J. Clin. Invest., 44, 1647 (1965)
Stegemann, H., Hoppe-Seyler's Z. Physiol. Chem., 311, 41 (1958)
Wood, G. A., in Hall, D. A., International Review of Connective Tissue Research, Vol. 2, Academic Press, N. Y., p. 21, 1964