クジラ鼻軟骨proteinpolysaccharide成分としてシアル酸の存在

誌名	日本水産學會誌
ISSN	00215392
巻/号	373
掲載ページ	p. 192-196
発行年月	1971年3月

The Occurrence of Sialic Acid as a Component of Whale Cartilage Proteinpolysaccharide

Hiroki NAKAGAWA*

(Received September 5, 1970)

It was undertaken to ascertain whether sialic acid found in cartilage was a component of proteinpolysaccharide (PP) or of cartilage residue which mainly consisted of collagen.

The remains of cartilage left after the extraction of PP were fractionated into three fractions. These and PP were analyzed for their sugar components and collagen. The sialic acid content was high with increase in the uronic acid content, while low with increase in the collagen content. Since the uronic acid and collagen were indices of PP and cartilage residue respectively, it was concluded that the sialic acid was a component of PP.

Proteinpolysaccharides(PP) are the protein complexes of acid mucopolysaccharides, in which the mucopolysaccharides are covalently bound to the protein[1,2]. They are widely distributed in animal connective tissues and seem to play important roles in the tissues[3]. It has been found that the acid mucopolysaccharides closely relate to many diseases[4,5,6], such as Hurler's syndrome, skin diseases, etc. PP isolated from bovine or human cartilage have often been investigated. Nasal septa of whales are also good materials for PP, since a large amount of pure cartilage is easily obtained.

The ground substance of cartilage consists of PP and collagen. The former is the complex of chondroitin sulfate, keratosulfate, and non-collagenous protein[1,2]. The structure of PP has remained unsettled, though the chondroitin sulfate-protein linkage region was recently elucidated[7,8,9,10].

The author[11] pointed out that dried nasal cartilage of whales contained about 1.3% sialic acid which was identified as N-acetylneuraminic acid. SENO *et al.*[12] isolated keratosulfates containing 0.2–5.3% sialic acid from several cartilage specimens. The sialic acid content of whale cartilage, however, was too high to assume that all the sialic acid occurred as a component of the known keratosulfate. Moreover, sialic acid naturally occurs as a component of hexosamine-containing oligo- or polysaccharides which contain neither uronic acid nor sulfated sugars[13,14]. It seems, therefore, that either keratosulfate containing a large amount of sialic acid, or unknown oligo- or polysaccharide exists in cartilage. In order to contribute to the elucidation of the structure and function of connective tissues, the auther intended to isolate sialic acid-containing oligo- or polysaccharide from whale cartilage, and to investigate its structure.

It was ascertained in the present paper that the sialic acid found in cartilage occurred

* Lab. of Fish. Tech., Fac. of Agr., Kyushu Univ., Fukuoka, Japan. (中川浩毅: 九州大学農学部水産製造学教室)

in PP, but not in cartilage residue which mainly consisted of collagen.

Experimental

Material. Nasal cartilage of a sei whale, *Balaenoptera boraelis*, which had been stored at −25°C was freed from adhering connective tissue, finely chopped, and washed with water.

Extraction of PP. The extraction procedure was based on the method described by MALAWISTA and SCHUBERT[15]. Five grams of the chopped cartilage was vigorously homogenized in a homogenizer (Nihon Seiki Seisakusho Ltd.) with 250 ml of cold water at top speed for a total of 80 min. During homogenization, the temperature of the mixture was maintained at 5–15°C. To the homogenate was added 500 ml of cold ethanol, and after standing for 3 hr at 5°C, the mixture was centrifuged at 8000 rpm (10000×g) for 30 min. During centrifugation, the temperature was maintained at 5°C. The opalescent supernatant was carefully decanted, and the residue was washed with 250 ml of 70% (v/v) ethanol in the centrifuge tubes. The washings were added to the supernatant, and to the combined solution was added 10 g of anhydrous sodium acetate. The precipitated PP was collected by centrifugation, washed three times with 80% ethanol to remove sodium acetate, then with absolute ethanol and ether, and dried *in vacuo* over phosphorus pentoxide at 60°; yield 320 mg. This perfectly white product was designated PP I.

Fractionation of residue. The cartilage residue left after the extraction of PP was homogenized with 250 ml of cold water as described above, and centrifuged at 15000 rpm (25000×g) for 30 min. The precipitated residue was washed with ethanol and ether, and dried as above; yield 330 mg. This product was designated R. To the supernant was added 500 ml of cold ethanol, and the mixture was centrifuged at 8000 rpm (10000×g) for 30 min. The precipitate was washed with ethanol and ether, and dried; yield 110 mg. This was designated SC. To the ethanolic supernatant was added 7.5 g of sodium acetate. The resulting precipitate was collected by centrifugation, washed, and dried in the same way as PP I; yield 70 mg. This was designated PP II. Fig. 1 shows the schematic procedure for the extraction of PP and the fractionation of residue.

Analytical methods. Sialic acid was determined by the thiobarbituric acid method of AMINOFF[16] after hydrolysis in 0.1 N H_2SO_4 for 1 hr at 80°C. N-acetylneuraminic acid isolated from whale cartilage[11] was used as a standard. Hexosamine was determined by the method of SVENNERHOLM[17] after purification of hydrolyzate with a Dowex 50 column.[18] Samples were hydrolyzed with 4 N HCl for 12 hr in a sealed tube at 100°C. Uronic acid was determined by the carbazole method of BITTER and MUIR[19]. Hexose was determined according to the anthrone method[20] by using galactose as a standard.

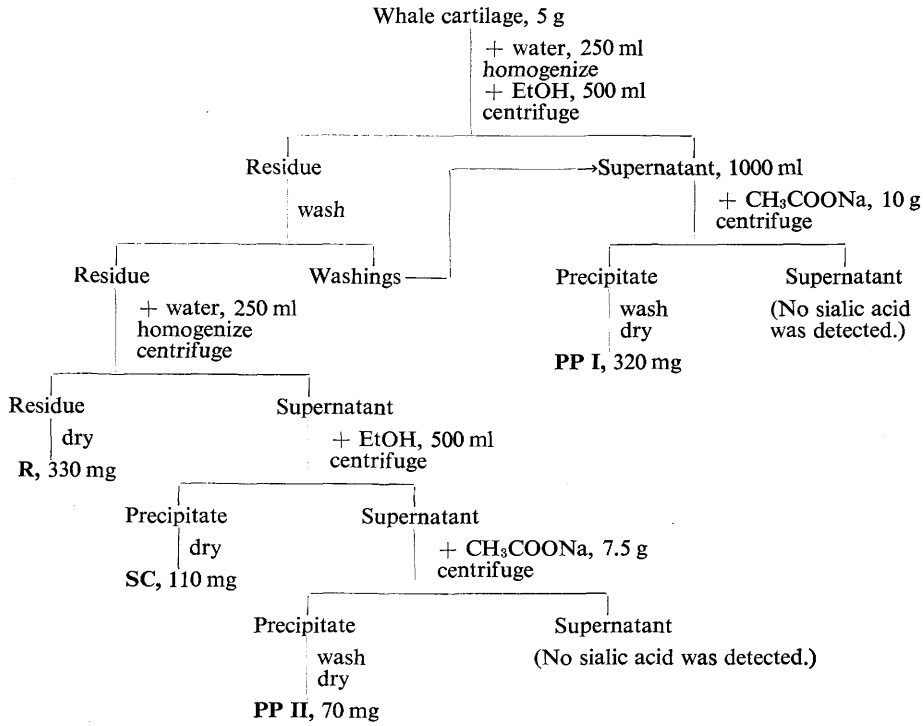

Fig. 1. Schematic procedure for extraction of proteinpolysaccharide and fractionation of residue.

Collagen was estimated by multiplying the content of hydroxyproline by 7.7, assuming that collagen contains 13% hydroxyproline. Hydroxyproline was determined by the method of BERGMAN and LOXLEY[21] after hydrolysis in 6 N HCl for 20 hr at 100°C.

Results and Discussion

Analytical data of PP I, PP II, R, and SC fractions are shown in Table 1. PP I was a PP fraction. PP II was a fraction mainly consisting of PP which was less readily water-extractable. These fractions contained a considerable amount of collagen, most of which could be removed by treatment with a CM-cellulose column[22]. R was the water-insoluble

Table 1. Analyses of fractions

Fraction	Sialic acid	Uronic acid	Hexosamine	Hexose	Collagen	Uronic acid / Sialic acid
PP I	2.9%	15.1%	17.6%	7.2%	7.6%	5.2
PP II	2.4	11.5	13.5	6.8	17.7	4.8
R	0.7	3.6	5.7	4.6	38.0	5.1
SC	0.3	1.4	2.2	1.1	69.0	4.7
Cartilage	1.3	6.6	9.3	5.7	33.3	5.1

residue fraction left after the extraction of PP, and its main component was collagen. SC was the soluble collagen fraction.

The analytical data showed that the sialic acid content was high with increase in the carbohydrate contents, while low with increase in the collagen content. This indicated that sialic acid did not relate to cartilage residue mainly consisting of collagen, but related to PP.

Uronic acid has not been detected so far in purified collagen, though hexoses are covalently attached to collagen molecule[23,24,25]. Therefore, it can be considered that almost all the uronic acid in cartilage is a component of chondroitin sulfate which is the main component of PP. As shown in Table 1, the content ratio of uronic acid to sialic acid in each fraction was approximately 5; this suggests that these carbohydrates were bound to the same molecule, i.e. PP. Thus, the sialic acid found in R and SC fractions was likely to be a component of the PP which existed in these fractions. Judging from the analytical values of PP I, the uronic acid content of pure PP, which does not contain collagen, was estimated to be about 16%. Since R and SC fractions contained 3.6% and 1.4% uronic acid, it was calculated that these fractions contained about 20% and 8% PP, respectively.

From the facts described above, it may be concluded that the sialic acid found in cartilage is a component of PP, and its existence in PP suggests that sialic acid-containing oligo- or polysaccharide occurs as a component of PP.

Acknowledgment

The author wishes to thank Prof. M. TOYOMIZU for many helpful discussions and suggestions during this work. Thanks are also due to the Fukuoka branch of Kyokuyo Co., Ltd. for supplying fresh nasal cartilage of whales.

References

1) M. SCHUBERT: in "Structure and Function of Connective and Skeletal Tissue", 124–131, Butterworth & Co., Ltd., London (1965).
2) B. M. SCHEINTHAL and M. SCHUBERT: *J. Biol. Chem.*, **238**, 1935–1940 (1963).
3) S. SUZUKI: in "Structure and Functions of Mucopolysaccharides" (in Japanese), 107–124, Nankodo Co., Ltd., Tokyo (1968).
4) K. TSURUMI and A. KIMURA: *Metabolism and Disease* (in Japanese), **6**, 91–98 (1969).
5) K. ASO: *ibid.*, **6**, 99–104 (1969).
6) Z. YOSIZAWA and T. OZEKI: *ibid.*, **6**, 105–111 (1969).
7) L. RODEN and R. SMITH: *J. Biol. Chem.*, **241**, 5949–5954 (1966).
8) L. RODEN and G. ARMAND: *ibid.*, **241**, 65–70 (1966).
9) B. ANDERSON, P. HOFFMAN, and K. MEYER: *ibid.*, **240**, 156–167 (1965).
10) L. RODEN: in "Structure and Function of Connective and Skeletal Tissue", 141–145, Butterworth & Co., Ltd., London (1965).
11) H. NAKAGAWA, N. ENOMOTO, and Y. TOMIYASU: This Bull., **30**, 580–584 (1964).

12) N. SENO, K. MEYER, B. ANDERSON, and P. HOFFMAN: *J. Biol. Chem.*, **240**, 1005–1010 (1965).
13) A. GOTTSCHALK: in "Glycoproteins", 20–28, Elsevier Publishing Co., Amsterdam (1966).
14) R. W. JEANLOZ: Advances in Enzymology, **25**, 442–456 (1963).
15) I. MALAWISTA and SCHUBERT: *J. Biol. Chem.*, **230**, 535–544 (1958).
16) D. AMINOFF: *Biochem. J.*, **81**, 384–392 (1961).
17) L. SVENNERHOLM: *Acta Soc. Med. Upsaliensis*, **61**, 287–306 (1956).
18) N. F. BOAS: *J. Biol. Chem.*, **204**, 553–563 (1953).
19) T. BITTER and H. M. MUIR: *Anal. Biochem.*, **4**, 330–334 (1962).
20) E. W. YEMM and A. J. WILLIS: *Biochem. J.*, **57**, 508–514 (1954).
21) I. BERGMAN and R. LOXLEY: *Anal. Chem.*, **35**, 1961–1965 (1963).
22) H. NAKAGAWA: This Bull., **37**, 197–202 (1971).
23) W. T. BUTLER and L. W. CUNNINGHAM: *J. Biol. Chem.*, **241**, 3882–3888 (1966).
24) R. J. SPIRO: *ibid.*, **242**, 4813–4823 (1967).
25) H. B. BOSMANN and E. H. EYLAR: *Biochem. Biophys. Res. Commun.*, **30**, 89–94 (1968).