GENERAL CHARACTERISTICS OF IMMUNOMODULATOR BIOGLYCANS FROM INVERTEBRATES OF THE SEA OF JAPAN

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Bioglycans have been isolated from 23 species of marine invertebrates from the Sea of Japan and a comparative study of them has been made. It has been shown that these bioglycans possess immunomodulating activity and consist of complexes of polysaccharides with a protein component.

We have shown previously that marine invertebrates possess the capacity for producing bioglycans (polysaccharides and glycoconjugates), which are stimulators of the immune system of man and the higher animals.

In the present paper we give the results of the screening of 23 species of marine invertebrates from the Sea of Japan as sources of immunomodulator bioglycans. A comparative study has been made of the fractions containing bioglycans. Their isolation was achieved by a standard procedure which we had developed previously [2]. The treatment of all the invertebrates was carried out monotypically under standard conditions. In all cases the total bioglycan fractions were purified with the aid of dialysis and were subjected to lyophilization. The total fractions obtained were studied in the comparative aspect. Their yields, carbohydrate, protein, and nucleic acid contents, other analytical characteristics, and their monosaccharide compositions were determined. In the gel filtration of the total fractions on Sephadex G-75 it was found that they all, as a rule, were separated into two components: high-molecular-mass, issuing with the free volume, and low-molecular-mass. Both components contained polysaccharide and protein constituents and they did not differ in monosaccharide composition. Figure 1 shows for the case an extract from Urechis unicinctus, a typical elution curve in the gel filtration of the extracts obtained on Sephadex G-75. And only in the case of preparations from the coelomic fluid of U. unicinctus, octopus stomach, and the viscera of the sea cucumber and the ascidian was the high-molecular-mass component observed

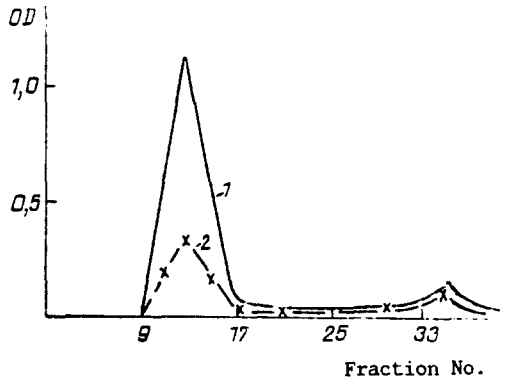

Fig. 1. Gel filtration of an extract from Urechis unicinctus (coat): Column of Sephadex G-75, elution with water, rate of elution 10 ml/h; 1) OD_{490}; 2) OD_{750}.

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TABLE 1. General Characteristics of Bioglycans from Invertebrates of the Sea of Japan

Invertebrate	Yield* of bioglycan, %	Composition of the bioglycan, %+					Monosaccharide composition‡						
		carbohydrates	protein	nucleic acids	amino sugars	ash	Glc	Gal	Man	Rha	Fuc	Xyl	Ara
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Bread crust-sponge** Halichondria panicea	0,6	12,6	18,6	4,7	3,2	9.0	2+	2+	±	—	±	—	—
2. Metridium starcheskii**** Metridium senile	1,6	36,1	54,3	6,1	11 8	4 7	3+	±	±	—	±	—	—
3. Serpula cherveobraznaya Serpula vermicularis	2,3	12,1	57,0	6,0	2,7	3,6	3+	±	±	—	±	—	—
4. Urekhis odnopoyaskovyi Urechis unicinctus (viscera)	1,2	63,6	44,0	12,7	5,5	6,1	4+	—	—	±	±	—	±
5. Faskolozoma yaponskaya Sipunculidae	2,0	10,8	64,7	3,8	2,3	6,3	4+	—	—	—	+	—	±
6. Khetopter raznonogii Chaetopetrus variopedatus	0,7	30,1	51,9	5,7	6,1	8,5	3+	±	±	—	±	±	±
7. Krab pribrezhnyi Hemigrapsus sanguineus	3,25	21.4	42,5	3,0	4,8	4,7	-	+	+	—	—	+	±
8. Lepidozona Al'brekhta Khiton Lepidozona albrechti Chiton	2.8	18,7	64,8	2,5	3,6	6,1	5+	—	—	—	—	—	—
9. Umbonium rebristyi Umbonium costatum	0.7	10,6	53 7	6,1	5,2	7,0	2+	2+	±	—	±	—	—
10. Grebeshok primorskii (a scallop) Patinopecten yessoensis (mantle)	1,0	28,0	52,0	6,4	4,2	4,5	5+	—	—	—	—	—	—
11. Maktra sakhalinskaya Spisula sachalinensis (mantle)	0,7	28,5	54,2	6,8	3,4								
12. Japanese buttersweet shell Glycymeris yessoensis	1,7	26,7	32.4	2,7	3,9	3,0	4+	—	—	—	+	—	—
13. Os'minog peschanyi Octopus conispadiceus	n.d	14,0	64,4	4,5	4,7	6,8	5+	—	—	—	—	—	—
14. Kal'mar tikhookeanskii (a squid) Todarodes pacificus (body)	1,8	4,0	52,7	6 4	1,0	6,9	5+	—	—	—	—	—	—
15. Koptotiris Greya Coptothyris grayi transversa	0,5	24,8	44,4	3,0	5,0	6,0	4+	—	—	—	+	—	±
16. Kukumariya yaponskaya (a sea cucumber) Cucumaria japonica	1,2	26,9	51,3	9,5	4,9	5,7	3+	±	±	±	2+	±	±
coat viscera	1,8	28,6	52,5	10,0	5.7	1,2	3+	—	+	—	+	—	—
17. Trepang dal'nevostochnyi Stichopus japonicus	0,3	26,5	65,4	5,8	4,7	6,9	+	—	±	—	3+	—	±
18. The sea urchin Skafekhipus seryi Scaphechinus griseus	n.d	7,9	37,3	3,3	2,0	14,6	+	—	+	—	±	—	+
19. Globular sea urchin Stronglyocentrouts nudus	n.d	11,7	71.2	5.4	3,8	4,8	+	+	+	—	+	+	+
20. Heart sea urchin Echinocardium cordatum	n.d	12,9	54,6	6,6	6.5	5,2	2-	—	—	—	+3		—
21. Grebeshkovaya patiriya Patiria pectinifera	3,6	11,7	58,1	2 5	2,1	8,5	2+	+	+	—	—	—	+

alone on the elution curves. The analytical results for the total extracts obtained are given in Table 1. As can be seen from this table, the amount of the protein component was greater than that of the polysaccharide fraction. In all cases traces of nucleic acids were detected, their amount varying over wide limits according to the species of animal used as the source of bioglycan. It was impossible to find any regular feature whatever in the ratio of the carbohydrate and protein components of the bioglycan according to the species of invertebrate.

Analysis of the monosaccharide compositions of the extracts with the aid of paper and gas-liquid chromatographies of the hydrolysates (Table 1) showed that the carbohydrate component of the bioglycans isolated form mollusks was a D-glucan: D-glucose was the only monosaccharide identified in hydrolysates of these bioglycans. It was the common monosaccharide for all the biolgycans with the single exception of the bioglycan from the crab. The

TABLE 1 (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14
22. Khalotsintiya purpurnaya Halocynthia aurantium													
coat	0,01	7,9	24,4	—	—	17,3	2+	2+	—	—	—	—	—
viscera	2,2	89,5	15,5	2,8	2,3	6,6	5+	—	—	—	—	—	—
23. Stiela purpurnaya Styela clava	H. o.	82,1	34,9	2,1	8,7	7,7	4+	—	—	—	+	—	—

*On the dry weight.
†A total of less than 100% is probably due to the difficulty of hydrolyzing the glycosaminoglycans; a figure above 100% shows the presence of colored impurities of undetermined nature.
‡Symbols: −) absent; +) present in trace amounts; 1+ − 5+) increasing amounts up to 100% (5+).
**Where no English name has been found a transliteration of the Russian name is given in parentheses [Translator].

TABLE 2. Fractionation of Total Preparations of the Bioglycans from DEAE Cellulose

Fraction	Composition, %		Monosaccharide compositions of the fractions
	carbohydrates	protein	
A 1	26,2	59,4	Fuc : Ara : Xyl : Man+Gal : Glc (5 : 1 : 1 : 17 : 11)
A 2	26,2	32,0	Glc
A 3	25,6	48,0	Fuc : Ara : Xyl : Man+Gal : Glc (5 : 1 : 2 : 8 : 5)
A 4	83,6	12,6	Xyl : Man : Glc (1 : 2 : 24)
B 1	78,8	21,9	Glc
B 2	30,1	43,6	Glc
B 3	19,1	58,2	Glc

*Bioglycans: A) from the squid; B) from the scallop.

polysaccharide moieties of the bioglycans of other invertebrates, including crabs, consist of heteropolysaccharides incorporating the usual monosaccharides that are widely distributed in nature. In all cases, the analysis showed the presence in the total hydrolysates of amino sugars in amounts varying between 5 and 12%, but most probably these were impurities of the accompanying glycosaminoglycans which, after gel filtration on Sephadex, sepaated from the main bioglycans. Finally, in all the total extracts we observed the presence of an appreciable amount of ash impurities (up 15%) separation from which required special purification. But even in the purified fractions inorganic impurities were present, which may show a substantial role of individual cations and anions in the architectonics of the higher structures of the bioglycans.

In this connection, we made an attempt to purify the total preparations and to fractionate them with the aid of ion-exchange chromatography on DEAE-cellulose. We used the total bioglycans from squid viscera and from scallop coat. The analytical results for the fractions obtained are given in Table 2. It is not difficult to see that fractionation took place in both cases; the fractions obtained differed with respect to their quantitative contents of the carbohydrate and protein components. In the case of the squid, it was possible to separate the glucan from the heteropolysaccharides and to separate the latter into individual fractions.

Unfortunately, we were unable to find any characteristic law whatever in the manifestation of the immunomodulating activities of the bioglycans as functions of the species of invertebrate and its position in the evolutionary system. All the bioglycans studied intensified the phagocytosis of the invertebrates and increased their survival rate in experimental infections.

Thus, marine invertebrates possess the capacity for producing immunomodulator bioglycans which consist of complexes of polysaccharides with a protein component. It is not excluded that in the formation of the complexes an essential role is played by inorganic cations and anions.

EXPERIMENTAL

Materials and Methods. For paper chromatography we used Filtrak FN 3, 12, and 15 and the solvent n-butanol–pyridine–water (6:4:3). Monosaccharides were detected with aniline phthalate or a solution of silver nitrate. GLC was conducted on a Pye-Unicam 104 chromatograph (with a flame ionization detector) with a glass column (0.4 × 150 cm) containing 3% of QF-1 on Gas-Chrom Q (100-120 mesh). Analysis was conducted within a program from 175 to 225°C (5°C/min).

To determine the monosaccharide composition, 5-10 mg of a total preparation in 1 ml of 2 N H_2SO_4 was heated in a sealed tube at 100°C for 5-6 h, and the hydrolysate was neutralized with $BaCO_3$, deionized with cation-exchange resin KU-2 (H^+ form), and investigated by PC. For analysis by the GLC method, the sugars were converted into polyol acetates [3]. The amounts of carbohydrates were determined by Smith's method [4], the amounts of protein by Lowry's method [5], and the amounts of nucleic acids as described by Spirin [6].

Isolation of the Bioglycans. The marine invertebrates wer collected in the summer in the sublittoral of the Dea of Japan. The bioglycans were isolation by salt extraction, for which 500 g of freshly collected animals (or individual organs) was homogeneized and extracted with 2.5 liters of extractant. The subsequent working up was carried out as described in [2].

Gel Filtration of the Bioglycans. Solutions of 10 mg of a bioglycan were deposited on a column (1.5 × 60 cm) containing Sephadex G-75, and elution was carried out with water at the rate of 10 ml/h. Fractions with a volume of 2.5 ml were collected, and aliquots of each fraction were analyzed for the presence of carbohydrates and proteins.

Fractionation of the Bioglycans on DEAE-Cellulose. Solutions of 200 mg of a bioglycan in 10 ml of 0.02 M acetate buffer, pH 4.5, were deposited on a column (3 × 24 cm) of DEAE-cellulose equilibrated with the same buffer. The column was washed with the buffer (1 liter), and then with a buffer having a linearly increasing concentrations of NaCl from 0 to 2 M and, finally, with 0.5 M NaOH, with the collection of 6-ml fractions which were analyzed for the presence of carbohydrates and protein. The fractions were dialyzed against distilled water and were lyophilized.

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