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EFFECT OF POLYHYDROXYSTEROIDS FROM STARFISH AND BRITTLE STARS ON THE ACTIVITY OF β-1,3-GLUCANASES

V. V. Sova, É. V. Levina, P. V. Andriyashchenko, S. N. Fedorov, and L. A. Elyakova

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The action of sulfated polyhydroxysteroids from starfish and brittle stars on the activity of β-1,3-glucanases from various sources has been investigated. All the substances activated the exoglucanase L_{II}, nine out of ten activated L_e, and seven out of ten of the sulfates activated the endoglucanase L_{III}. Other endo-β-1,3-glucanases from marine molluscs were specifically inhibited by the steroid sulfates investigated, and it was shown that the efficiency of inhibition depends on the structural features of the molecules of these inhibitors.

Systematic investigations of natural effectors of β-1,3-glucanases that we have begun have shown that marine invertebrates are a promising source of various compounds capable of inhibiting or accelerating the enzymatic reaction. Thus, the influence of extracts from a large number of marine invertebrates of the Indian and Pacific Oceans on the activity of β-1,3-glucanases has been studied [1, 2]. Individual compounds have been isolated and characterized, including a high-molecular-mass glycoprotein from a sponge that is an inhibitor of β-1,3-glucanases [2] and a macrolide that is an activator of β-1,3-glucanases from marine mollusks [3]. Among the marine natural compounds studied, the most effective inhibitors have proved to be steroid sulfates from sponges of the family Halihondriidae [1, 4].

In the present work we have investigated the action of a number of new natural polyhydroxysteroids from starfish and brittle stars on the activity of β-1,3-glucanases. The action of the polyhydroxysteroids was evaluated from their capacity for inhibiting or accelerating the hydrolysis of laminarin (a β-1,3-glucan) by the enzymes. Table 1 gives the values of the residual activities determined in the presence of 50 μg of the polyhydroxysteroid. The effects of higher concentrations could be connected with nonspecific interactions. In order to characterize the substances showing a high inhibiting capacity, we have determined the amount of inhibitor in the sample causing a 50% fall in the activity of glucanases (I_{50}). A broad set of β-1,3-glucanases with an established type of action was used, namely: endo-β-1,3-glucanases from the crystalline styles of the bivalve mollusks *Spisula sachalinensis* (L_{III} and L_{IV}) and *Chlamys albidus* (L_0), an endo-β-1,3-glucanase from the chiton *Acanthopleura sp.* (L_x), an endo-β-1,3-glucanase from Antarctic krill *Euphausia superba* (L_{kr}), endo-β-1,3-glucanases from potato leaves (K_1 and K_2), an exo-β-1,3-glucanase from the terrestrial mollusk *Eulota maakii* (L_{II}), and an exo-β-1,3-glucanase from the eggs of the sea urchin *Strongylocentrotus intermedius* (L_e).

It is known that glucanases are divided into two types according to the method of cleaving the substrate. Endoglucanases cleave internal glycosidic bonds remote from the end of the polymeric chain, while exoglucanases split out glucose from the nonreducing end of the glucan molecule. In addition, the exo- and endo-enzymes have considerable differences in molecular mass, charge, and other characteristics of the protein molecule. At the same time, the endo-enzymes under investigation differ from one another only by certain details of the mechanism of their action and have close molecular masses and isoelectric points and identical catalytic groups. On the other hand, certain structural differences have been noted between endo-β-1,3-glucanases L_{IV} and L_0. Thus, the glucanase L_{IV} molecule has five disulfide bridges, and glucanase L_0 only two, and in L_{IV} there is a higher carbohydrate content, the amino acid sequences from the N-ends of the molecules of these enzymes do not coincide, and peptide maps of tryptic hydrolysates do not have analogous peptides [5].

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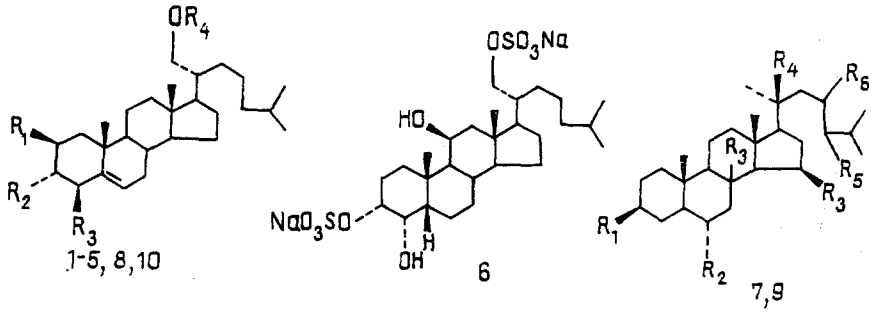
TABLE 1. Influence of Natural Polyhydroxysteroids from Starfish and Brittle Stars on β-1,3-Glucanases

Steroid	I_{50}	μg/residual activity, % (amount of inh. 50 μg).							
	L_{IV}	L_{III}	L_{II}	L_c	L_x	L_0	K_1	K_2	L_{kr}
1	26/	/118	/115	/120	/68	3/	/73	/80	/100
2	60/	/100	/103	/110	—/—	5-6/	/105	/103	/100
3	10/	/80	/105	/97	8/	2.5/	40/	/76	/100
4	6/	/52	/107	/110	12/	2/	/71	50/	/100
5	2.5/	/110	/120	/105	15/	0.5/	40/	/57	/100
6	20/	/86	/112	/106	/58	/2-3	50/	/64	/100
7	20/	/140	/113	/107	/83	8/	/77	/87	/100
8	20/	/110	/107	/111	/82	19/	45/	/79	/100
9	16/	/126	/122	/125	/70	5/	/64	/75	/100
10	0.5-1/	/170	/120	/128	45/	0.5/	/74	/79	/100

The investigation of the influence of natural sulfated polysteroids (1-10) on the activities of the β-1,3-glucanases showed that the natures of the action of these substances on the exo- and endo-enzymes were different. All the substances (see Table 1) caused activation of exoglucanases L_{II} and L_e, while they showed an inhibiting action on all the endoglucanases (L_{IV}, L_x, K_1, and K_2). An exception was the endo-β-1,3-glucanase L_{III}, which was slightly inhibited only by compounds (3), (4), and (6), while the other substances exerted an activating action on it. It is interesting to note a considerable difference in the effect of the polyhydroxysteroids on the activities of two isoenzymes - L_{IV} and L_{III} - isolated from the crystalline style of the marine mollusk *S. sachalinensis* and having only slight differences in their action mechanisms [6].



1. R_1=OSO_3Na, R_2=OH, R_3=H, R_4=SO_3Na
2. R_1=H, R_2=OSO_3Na, R_3=OH, R_4=SO_3Na, $\Delta^{25(26)}$
3. R_1=H, R_2=OSO_3Na, R_3=OH, R_4=SO_3Na
4. R_1=R_2=OSO_3Na, R_3=H, R_4=SO_3Na
5. R_1=R_3=H, R_2=OSO_3Na, R_4=SO_3Na, Δ^0
7. R_1=R_3=R_2=OH, R_4=R_6=H, R_5=OSO_3Na
8. R_1=R_2=R_3=OH, R_4=R_6=H, R_5=OSO_3Na
9. R_1=OSO_3Na, R_2=OH, R_3=R_5=H, R_4=OH, R_6=O, $\Delta^{9(11)}$
10. R_1=H, R_2=OSO_3Na, R_3=OH, R_4=H

In the series of β-1,3-glucanases investigated, glucanase L_0 was most readily inhibited by all the compounds. It is probable that features of the structure of this enzyme — low content of carbohydrates and of disulfide bridges — makes the molecule less resistant to various actions. A 50% fall in the activity of the endo-β-1,3-glucanase from the chiton (L_x) was brought about by steroids (3), (4), and (5), and (10) in concentrations of 8, 12, 15, and 45 μg, respectively, while the other compounds had practically no effect on its activity. Not one of the polyhydroxysteroids investigated inhibited the endo-β-1,3-glucanase from the Antarctic krill. The endo-β-1,3-glucanases from potato leaves was also fairly resistant to the action of the polyhydroxysteroids, although some differences were observed between two isoenzymes (K_1 and K_2), namely: K_1 was less resistant to the action of the substances than K_2 (Table 1). The polyhydroxysteroids are probably more specific to the glucanases of marine mollusks. A similar conclusion has been drawn previously in an investigation of the inhibiting capacity of polyhydroxysteroids from sponges [1].

The efficacy of the inhibition of β-1,3-glucanases depends both on the nature of the enzymes (source of isolation, type of action) and on the structural features of the compounds under investigation. Analysis of the structures and activities of the

polyhydroxysteroids that we isolated from brittle stars and starfish has permitted us to make some hypotheses on the values of particular structural elements for the efficacy of their action on β-1,3-glucanases.

As can be seen from Table 1, the greatest inhibiting effect in relation to the endo-β-1,3-glucanases L_0 and L_{IV} was possessed by compounds (5) and (10), and to L_x by compound (3). When a double bond was introduced into the cyclic part of the steroid molecule (5) (compare (5) and (8)), the efficacy of the inhibition of L_0 and L_{IV} decreased by an order of magnitude. A similar effect was produced by the presence of a double bond in the side-chain [(3) and (2)]. Increasing the hydrophilicity of the polycyclic part of the steroid through hydroxylation and sulfation in ring A again led to highly active derivatives [compare (8) and (3), (8) and (4), and (1) and (4)].

The same effect was produced by some increase in the hydrophobicity of the side-chain of the steroid through desulfatation. Thus, the inhibiting activity of steroid (10) in relation to the endoglucanases L_0 and L_{IV} was an order of magnitude higher than that of steroid (3). Compounds (6), (7), and (9), differing by the presence of additional hydroxy groups in rings *B* and *C*, revealed approximately the same inhibiting activity on L_0 and L_{IV} as compounds (1-4) and (8), having no such hydroxy groups.

It had been shown previously that the inhibiting properties of sulfated steroidal polyols depend substantially on the ratio of the hydrophilic and hydrophobic parts of the molecule [1]. Our results confirm this hypothesis and permit the conclusion that the high inhibiting activity of sulfates (5) and (10) is due to the optimum biphilicity — the ratio of hydrophilic and hydrophobic properties — of their molecules.

The mechanism of the action of a polyhydroxysteroid — halistanol sulfate from a tropical sponge — on β-1,3-glucanases L_{IV} and (L_0) has been investigated previously by spectral methods [4]. It was shown that halistanol sulfate causes conformational changes in the tertiary and secondary structures of glucanases. Under the action of halistanol sulfate, the polypeptide chain of L_{IV} folds into an α-helix almost completely. It is likely that the polyhydroxysteroids from starfish and brittle stars also cause conformational transitions in the enzyme molecules leading to changes in their activity.

EXPERIMENTAL

Enzymes. In this work we used glucanases from the collection of the enzyme chemistry laboratory of TIBOKh DVO RAN. The description of the enzymes and the methods of their isolation have been given in a review [7].

Inhibitors. The polyhydroxysteroid sulfates investigated were isolated from brittle stars collected on the expedition voyages Nos. 2, 7, and 10 of the Scientific Research Vessel Akademik Oparin. The isolation and structural identification of compounds have been described in the literature [8-11]. The steroid sulfate (1) was isolated from the brittle star *Astrocladus exiguus* by a known procedure [8, 9] and was purified with the aid of HPLC on a Zorbax-CN column with, as eluent, 40% methanol in H_2O at a rate of flow of 0.8 ml/min.

Sulfates (7) and (9) were isolated from the Far-Eastern starfish *Distrolasterias elegans* by known procedures [12] and were purified with the aid of HPLC on Zorbax-ODS with, as eluent, 75% methanol in H_2O at a rate of flow of 1 ml/min.

Inhibition Procedure. To 50 μl of a solution of the enzyme in 0.05 M succinate buffer was added 50 μl of an aqueous solution of the polyhydroxysteroid. The mixture was kept at 25°C for 15 min, and then 400 μl of a solution of laminarin (1 mg/ml) was added and incubation was carried out at 37°C for 15 min. The residual activity was determined from the rise in the amount of reducing sugars by Nelson's method [13].

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