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Y.V. DUBROVSKAYA, T.N. MAKARIEVA,
L.K. SHUBINA, I.Y. BAKUNINA

Effect of pentacyclic guanidine alkaloids on activity of natural 1,3β-D-glucanases from marine hydrobionts

The effect of pentacyclic guanidine alkaloids monanchomycalin B, monanchocidin A and normonanchocidin A isolated from the marine sponge Monanchora pulchra was investigated towards the activity of exo-1,3-β-D-glucanase from the filamentous marine fungus Chaetomium indicum and endo-1,3-β-D-glucanase LIV from the marine bivalve mollusk Pseudocardium(Spisula) sachalinensis. All compounds were shown to be slow irreversible inhibitors of exo-1,3-β-D-glucanase and significantly activated endo-1,3-β-D-glucanase. The inhibitory capacities of alkaloids were shown to depend on the structure of the "anchor" part of the molecule of the compounds. Normonanchocidin A was the best inhibitor of exo-1,3-β-D-glucanase from fungus.

Keywords: sponges Monanchora pulchra, exo-1,3-β-D-glucanase, endo-1,3-β-D-glucanase, Chaetomium indicum, Pseudocardium sachalinensis, inhibitors, monanchomycalin B, monanchocidin A, and normonanchocidin A.

1,3-β-D-Glucanases are a large group of O-glycoside hydrolases that catalyze the hydrolysis of β-(1,3)-O-glycosidic bonds in β-(1,3)- and β-(1,3;1,6)-D-glucans. 1,3-β-D-Glucanases are devided into endo- and exo-type. Influence of inhibitors on these enzymes is the basis for regulation of their activity in the marine organism [3]. Many secondary metabolites of marine sponges are inhibitors of enzymes of different classes, including marine 1,3-β-D-glucanases [1-4]. The substances of unique structures have been isolated from the marine Far-Eastern sponge *Monanchora pulchra* [7].

The aim of this work is a comparative study of the effect of pentacyclic guanidine alkaloids – monanchomycalin B, monanchocidin A and normonanchocidin A, isolated from the marine sponge *Monanchora pulchra* –on the activity of 1,3-β-D-glucanases from the marine fungus *Chaetomium indicum* and the bivalve mollusk *Pseudocardium sachalinensis.*

To study the effect of sponge metabolites on the activity of O-glycoside hydrolases, two well-studied 1,3-β-D-glucanases –endo-1,3-β-D-glucanase L_{IV} from the marine bivalve mollusk *Pseudocardium sachalinensis* (*Ps*Lam_{IV}) [10] and exo-1,3-β-D-glucanase of the marine fungus *Chaetomium indicum* KMM 4631 (*Chin*Lam) were selected from the collection of the Laboratory of Enzyme Chemistry [5]. Samples of the sponge *Monanchora pulchra* are stored in the collection of marine invertebrates of the PIBOC FEB RAS.

The activity of 1,3-β-D-glucanases was determined by increasing the amount of reducing sugars measured by the Somogyi-Nelson method [8]. To study the effect of an aqueous solution

* DUBROVSKAYA Yuliya Valerievna – PhD, Senior Researcher, MAKARIEVA Tatyana Nikolaevna –DSc, Principal Researcher, SHUBINA Larisa Kimovna – PhD, Senior Researcher, BAKUNINA Irina Yurievna – DSc, Associate Professor, Leading Researcher (G.B. Elyakov Pacific Institute of Bioorganic Chemistry, FEB RAS, Vladivostok, Russia). *E-mail: burtseva@piboc.dvo.ru

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of alkaloids in concentrations (Table 1) on *Ps*Lam$_{IV}$ and *Chin*Lam, were added to 0.025 mL of the enzyme solution in 0.025 M Na^+ succinate buffer (pH 5.2).

The mixture was incubated for 30 minutes at 20°C, then 0.2 mL of laminaran (1 mg/mL) were added and incubated for 10 min at 37°C. The residual activity of 1,3-β-D-glucanases was determined as the ratio A/A_0, where A is the enzyme activity in the presence of compound under study, and A_0 is the enzyme activity in the it absence. The reversibility of the inhibition of *Chin*Lam activity, the monanchomycalin B solution was determined for 60 minutes. A sample of *Chin*Lam glucanase untreated by alkaloid was used as control.

The compounds isolated from the Far-Eastern sponge have the same "vessel" part and differ in the structure of the "anchor" part of the molecule (Fig. 1). So, the "anchor" part of the molecule is presented in compound **1** by the spermidine residue, in compound **2** by tetra-substituted morpholinone derivative, and in compound **3** by monosubstituted diaminopropane.

Table 1

Effect of compounds from the marine sponge *Monanchora pulchra* on the activity of marine 1,3-β-D-glucanases

Glucanase	Residual activity $A^*/A_{0^{**}}$, %			
	H_2O	monanchomycalin B (0.278 mM)	monanchocidin A (0.257 mM)	normonanchocidin A (0.287 mM)
*Ps*Lam$_{IV}$	100	275.5	364.1	466.7
*Chin*Lam	100	1.5	1.4	0.7

* A - the enzyme activity in the presence of compound under study

** A_0 - the enzyme activity in the absence of compound

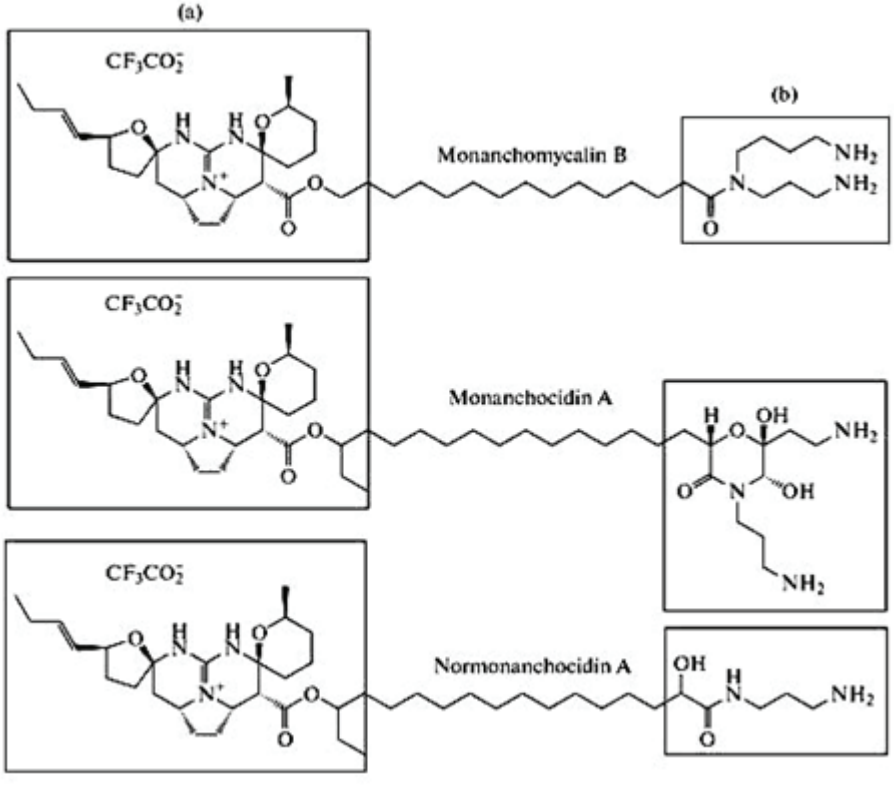

Figure. 1. Structural formulas of pentacyclic guanidine alkaloids. (a) – the "vessel" part and (b) – the "anchor" part of the molecule.

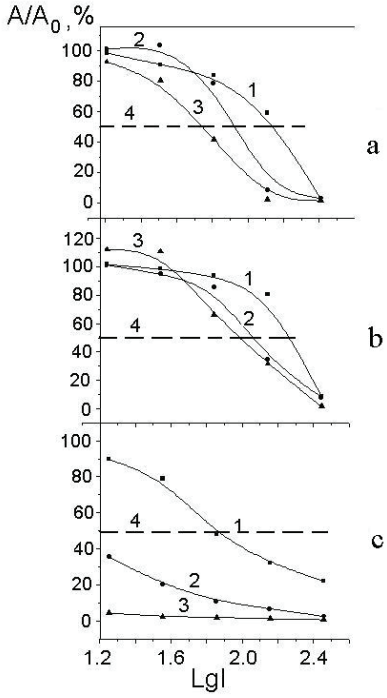

Figure. 2. The dependence of the residual activity of the *Chin*Lam (A/A_0) - on the concentration of pentacyclic guanidine alkaloids (LgI) at different incubation times: *1* - 1.5 min, *2* - 15 min, and *3* - 30 min; the dashed line cuts off the IC_{50}-concentration at which 50% inhibition is achieved. a - monanchomycalin B; b - monanchocidin A; c –normonanchocidin A.

It have been shown that all three compounds significantly activate *Ps*Lam$_{IV}$ of the mollusk and completely inhibit *Chin*Lamof the marine fungus (Table 1).

It was previously shown that sulfated steroids isolated from the sponges *Halichondria* sp. and topsentiasterol sulfates from the sea sponge *Topsentia* sp. served as inhibitors of endo-1,3-β-D-glucanases of marine mollusks [3, 5]. For exo-1,3-β-D-glucanase from the terrestrial mollusk *Karaftohelix maackii* [3, 5], as well as for exo-1,3-β-D-glucanases from the marine fungi *Ch. indicum* and *Trichoderma aureviride* [5], these compounds were reported as activators and did not affect the enzyme activity.

We have proved with the example of monanchomycalin B that pentacyclic guanidine alkaloids irreversibly inhibit the *Chin*Lam. The activity of the enzyme did not recover after dialysis against the buffer for 72 hours. The study of the dependence of the residual activity (A/A_o) of *Chin*Lam glucanase on different concentrations of monanchomycalin B, monanchocidin A and normonanhocidin A at different retention times with the inhibitor showed that 50% inhibition of the enzyme (IC_{50}) decreased with an increase in the retention time of *Chin*Lam glucanase with an inhibitor (Figure 2: *1, 2* and *3,* respectively). Inhibition develops relatively slowly, within a few minutes under these experimental conditions.

The molecules of pentacyclic guanidine alkaloid compounds consist of polar nitrogen-containing residues connected by hydrophobic polymethylene chains. In this case, the “anchor” part of the molecule is very mobile. We assume that the binding of the “vessel” part of the molecule directs and promotes an increase in the affinity of the “anchor” part, so the binding of the latter occurs more slowly and leads to a loss of enzyme activity. The inhibitory properties towards *Chin*Lam 1,3-β-D-glucanase are determined by the structure and the volume of the “anchor” part.

Thus, among the metabolites of sea sponges, we have for the first time found compounds that inhibit exo-type glucanase and activate endo-type glucanase. Monanchomycalin B, monanchocidin A and normonanchocidin A are slow irreversible inhibitors of exo-1,3-β-D-glucanase from the marine microscopic fungus and activators of endo-1,3-β-D-glucanase from the marine mollusk. It can be assumed that normonanchocidin A is the most effective inhibitor of exo-1,3-β-D-glucanase from marine fungus, and the enzyme as a model for studying the mechanism of action of pentacyclic guanidine alkaloid.

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