UDC 577.2 DOI: 10.25808/08697698.2018.202.6S.005

A.A. BELIK, K.M. TABAKMAKHER, T.N. MAKARIEVA,
T.N. ZVYAGINTSEVA, S.P. ERMAKOVA

Modes of action of sulfated steroids on recombinant endo-1,3-β-D-glucanase and alginate lyase from marine bacterium *Formosa algae* KMM 3553

Keywords: alginate lyase, endo-1,3-β-D-glucanase, Formosa algae, inhibitor, halistanol sulfate, topsentiasterol sulfate , chlorotopsentiasterol sulfate, GFA, ALFA3, enzyme.

There is lack of information about natural organic inhibitors of endo-1,3-β-D-glucanases (EC 3.2.1.39) and alginate lyases (EC 4.2.2.3) of marine origin. For endo-1,3-β-D-glucanases of marine mollusks, there was shown inhibition only by sulfated polyoxysteroids from marine sponges, starfishes and ophiuroids. Endo-1,3-β-D-glucanases of glycoside hydrolase family 16 (GH16) from *Pseudocardium sacchalinensis*, *Chlamys albidus* and *Patinopecten yessoensis* are irreversibly inhibited to 70% in the concentration of halistanol sulfate 1mkM (molecular ratio enzyme : substrate about 1:2.5).

There was studied action of halistanol sulfate, topsentiasterol sulfate D and chlorotopsentiasterol sulfate D from marine sponge *Halichondria* sp. on recombinant endo-1,3-β-D-glucanase GFA of GH16 family and alginate lyase ALFA3 of polysaccharide lyase PL7 family, both from marine bacterium *Formosa algae* KMM 3553.

In contrast to endo-1,3-β-D-glucanases of marine mollusks, the inhibiting action of sulfated steroids was relatively low. Halistanol sulfate caused 59% inhibition of GFA in molecular ratio 1:2665. The inhibition was reversible and competitive, constant of inhibition was 590 mkM. Topsentiasterol sulfate D appeared to be weaker agent and caused 38% inhibition of GFA in molecular ratio enzyme: substrate as 1:1262. Inhibition was reversible. Chlorotopsentiasterol sulfate D appeared to be the strongest one and caused 70% inhibition of GFA in ratio 1:2665 and 98% inhibition of GFA in ratio 1:2420, inhibition was irreversible. It's interesting to note that introducing of chlorine atom to the molecule changes the type of inhibition from reversible to irreversible.

Varying modes of action of substances with similar structure along with striking difference of action of halistanol sulfate on endo-1,3-β-D-glucanases of marine mollusks gives insight to the importance of surrounding of enzyme's active center with polypeptide chain fragments.

In case of alginate lyase (ALFA3) halistanol sulfate was the strongest agent and caused irreversible 100% inhibition in ratio 1:1530 and 59% inhibition in ratio 1:382. Topsentiasterol sulfate D was the weakest agent and caused reversible 59% inhibition in ratio 1:1455. Chlorotopsentiasterol sulfate D caused 88% inhibition in ratio 1:2793 and irreversible 56%

* BELIK Alexey Anatolievich – Junior Researcher, TABAKMAKHER Kseniya Mikhailovna – PhD, Junior Researcher, MAKARIEVA Tatyana Nikolaevna –DSc, Principal Researcher, ZVYAGINTSEVA Tatyana Nikolaevna – DSc, Principal Researcher, ERMAKOVA Svetlana Pavlovna – DSc, Associate Professor, The Head of The Laboratory (G.B. Elyakov Pacific Institute of Bioorganic Chemistry, FEB RAS, Vladivostok, Russia). *E-mail: belik_a_a@mail.ru

The work was supported by RFBR Grant № 18-04-00905 and RFBR Grant № 18-53-54002 V'iet_a.

inhibition in ratio 1:348. Thus, it can be concluded that chlorotopsentiasterol sulfate D is the strongest inhibitor of GFA among tested substances, while halistanol sulfate is the strongest inhibitor of ALFA3.This data supports the idea that inhibitors detect essential differences between enzymes, belonging to the same type of activity and even to the same structural family.