UDC 577.2 DOI: 10.25808/08697698.2018.202.6S.006

A.A. BELIK, A.S. SILCHENKO, O.S. MALYARENKO,
A.B. RASIN, M.I. KUSAYKIN

Properties and substrate specificities of alginate lyases from marine bacterium *Formosa algae* KMM 3553

Keywords: alginate lyase, Formosa algae, enzyme, bacterium, ^{1}H NMR, ALFA3.

Alginate lyases show antioxidant properties *in vitro*, inhibit growth and differentiation of adipocytes and absorption of saturated fatty acids, exhibit antiallergic properties by suppressing IgE and stimulate immune system by increasing of production of cytokines (G-CSF, TNF-α, interleukins) in murine macrophages, inhibit growth of osteosarcoma MG-63 cells by 60-70%. Alginate oligosaccharides have advances due to their low toxicity, availability of raw materials, environmentally safe production. Biological activity of alginates strongly depends on monosaccharide composition and polymerization degree that increases the importance of screening of suitable raw materials and main instruments of their modification.

We expressed recombinant forms of five alginate lyases of marine bacterium *Formosa algae* KMM 3553. By using the method of assaying of reducing sugars it was shown that these enzymes are active against polymannuronic, polyguluronic and mixed type of alginic acids. Five enzymes were classified as polymannuronate lyases (EC 4.2.2.3) and three as polyguluronate lyases (EC 4.2.2.11). Recombinant alginate lyase ALFA3 had been put under deeper research.

Amino acid sequence of recombinant alginate lyase ALFA3 from polysaccharide-degrading marine bacterium *Formosa algae* KMM 3553 appeared to be 288 residues, molecular weight – 33.8 kDa. Enzyme was equally able to digest three types of sodium alginates (M-enriched, G-enriched and MG-mixed) with equally effectiveness according to reducing sugars assay: 21.5 U/mg for M- and MG- and 18.7 U/mg for G-. pH optimum was 6.0 for all types of substrates. Temperature optimum was estimated at 35 °C, half-inactivation happened at 42 °C for 30 min. Ions of Na^+ in at least 0.1 M were essential for reaction proceeding, ions of K^+ and Ca^{2+} in concentrations up to 0.5 M did not influence the activity. Km was calculated as 0.12 mM. While sodium polymannuronate was completely digested to oligosaccharides in 24 hours, G-enriched substrate remained significant high-molecular fraction and MG-substrate remained slight high-molecular fraction. These fractions had tendency not to disappear even after exhaustive digestion. ^{1}H NMR analysis of products showed that in the case of sodium polyM there appeared only H-4 (ΔM) signals (5.5 ppm) and H-1 (M) signal intensity decreased (4.7 ppm). In the case of polyG-enriched substrate the intensity of H-4 (ΔG) (5.8 ppm) signal was only slightly higher than of H-4 (ΔM) (5.5 ppm), there was visible accumulation of M-red and G-red reducing ends as well, but not exhausting of G1 and G5 signals. This gives the evidence that in all substrates ALFA3 is able to hydrolyze MM, MG and GM bonds, but not GG. Products of polymannuronate digestion in

* BELIK Alexey Anatolievich – Junior Researcher, SILCHENKO Artem Sergeevich – PhD, Researcher, MALYARENKO Olesya Sergeevna – PhD, Researcher, RASIN Anton Borisovich - Graduate student, KUSAYKIN Mihail Igorevich – DSc, Deputy Director (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.

concentrations 200 μg/ml inhibited anchorage-independent colony formation of human melanoma cells SK-MEL-5, SK-MEL-28, RPMI-7951 up to 17% stronger in compare with native polymannuronate. This fact supports previous data and proposes the potential of mannuronate oligosaccharides to take part in tumor synergic therapy.