Abstract

Phosphatases of the haloacid dehalogenase superfamily are one of the cell's tools for dephosphorylation of many diverse endogenous and exogenous compounds. This work is aimed at enzymes Tt82 and cytosolic purine 5'-nucleotidase II (cN-II), two members of this large enzyme superfamily.

The Tt82 originates in the hyperthermophilic archaeon *Thermococcus thioreducens*. Up to date, there is only a small amount of knowledge about properties and biological function of this enzyme. Based on its sequence and structure, it was predicted that the Tt82 should possess a phosphatase catalytic activity. Consequently, potential substrates of the Tt82 were proposed by the molecular docking. In this work, the phosphatase activity of the Tt82 was confirmed together with several of its substrates: AMP, D-glucose 1-phosphate, D-glucose 6-phosphate and *p*-nitrophenyl phosphate (*p*NPP). Activity towards AMP and *p*NPP was then characterized by steady-state kinetics at 37 °C and 60 °C. In consistence with its thermophilic origin, the Tt82 showed markedly higher activity towards both substrates at 60 °C. Nonetheless, the effectivity of the Tt82 catalytic activity towards these substrates was actually very low. This leads to assumption, that the identified substrates are probably not biologically relevant. On the other hand, it is quite likely that the substrate specificity of the Tt82 might be much broader. Taken together with its thermal stability, the Tt82 could have a potential use in biotechnology.

The cN-II is a human allosteric enzyme. It catalyses the first step in the degradation pathway of the purine nucleotide metabolism, where it holds a key regulatory function. Aberrations in the regulation of the cN-II activity are caused by activating mutations in the NT5C2 gene and are associated with several serious diseases, including the acute lymphoblastic leukaemia. Hyperactive mutant cN-II variants are responsible for the development of resistance of the leukemic cells to the antitumor therapy with thiopurines. Therefore, the cN-II is an important therapeutic target. One of the possible strategies to overcome the cN-II hyperactivity is the development of effective inhibitors. In relation to that, compounds that clearly interact with the cN-II were sought in this work. Two C-terminally truncated cN-II variants were successfully prepared for this purpose. Using the saturation transfer difference NMR method, 68 compounds (fragments) from 1000 tested was found to show interaction with the cN-II. Finally, based on the measure of the interaction, 50 best scoring fragments were selected for further experiments. These results provide a solid basis for further development of the future inhibitors targeted against the hyperactive mutant cN-II variants.

Keywords: phosphatases from HAD superfamily, cytosolic purine 5'-nucleotidase II, fragment-based drug discovery, Tt82 phosphatase, characterization of catalytic activity