SUMMARY

The scientific achievement, described therein, which constitutes the basis for being conferred a doctoral degree, concerns the biochemical and structural characterization of *S*-adenosyl-L-homocysteine hydrolases (SAHases) from various bacteria, such as *Thermotoga maritima*, *Pseudomonas aeruginosa* and *Cytophaga hutchinsonii*. SAHase is an essential element of cell metabolism, involved in the regulation of methylation reactions that utilize *S*-adenosyl-L-methionine (SAM) as a methyl group donor. SAM-Dependent methylation generates equimolar amounts of *S*-adenosyl-L-homocysteine (SAH), a potent inhibitor of SAM-dependent methylation processes. Therefore, cellular concentration of SAH has to be strictly controlled, and this function is fulfilled by SAHase. The enzyme is usually active as a homotetramer with a subunit folded into three domains. Each subunit binds one substrate and one nicotinamide adenine dinucleotide cofactor in its oxidized state (NAD^+). A presence of the cofactor is required for the enzyme activity. Two principal domains, involved in substrate and cofactor binding, are connected by a two-part hinge element and the enzyme oscillates between two conformational states: open (ligand-free) and closed (with ligand bound) during the catalytic cycle. The studies presented herein were performed to broaden a biochemical and structural knowledge about SAHases. The interdisciplinary nature of the research were of key importance for the success of the research.

In the course of research on hyperthermophilic SAHase from *T. maritima*, I revealed that a recombinant protein expressed and purified at room temperature is not active. A closer inspection of a crystallographic model of the protein showed, that individual subunits adopt two distinct and atypical conformations, which do not permit a protein folded in such manner to be enzymatically active. In addition, only two of the four subunits bind the cofactor, however, mainly in its reduced form (NADH). This fact also precludes the enzymatic degradation of SAH. I have developed a new assay that indicated that the protein could gain a full catalytic activity only at a high temperature in the presence of the oxidized form of the cofactor (NAD^+). Based on crystallographic models of both, active and inactive forms of SAHase from *T. maritima*, I elucidated a mechanism of thermoactivation of the enyzme that is based on a spatial rearrangement of properly folded domains.

While conducting research related to SAHase from *P. aeruginosa*, I noticed that the catalytic activity of the enzyme varies considerably, depending on the alkali metal ion present in the reaction mixture. Among tested cations, the K^+ ion stimulates the highest enzymatic activity. An explanation of this phenomenon is that K^+, but not other alkali cations, enables unique dynamic properties of the enzyme to ensure its maximum catalytic activity. The enzymatic activity can be influenced *via* regulation of protein dynamics, which depends on the type of coordinated cation. This mechanism can also be exploited for noncompetitive inhibition of the enzyme, as I observed for reactions performed in a presence of Rb^+ and Zn^{2+} cations.

The interdisciplinary research was also aimed at biochemical and structural characterization of SAHase from *C. hutchinsonii*. Within the study, I presented the crystal structure of recombinant enzyme in a ternary complex with NAD^+, a reaction product/substrate (adenosine). Additionally, a sodium cation was identified in close proximity of the active site. The crystal contains two translational NCS-related dimers in the asymmetric unit. Two complete tetrameric enzyme molecules are generated from these dimers within the crystal lattice through the operation of two separate crystallographic twofold axes.

To summarize, the results obtained are new discoveries, which allow a deeper understanding of numerous biochemical and structural aspects of *S*-adenosyl-L-homocysteine hydrolase, especially these related to previously unknown regulation mechanisms of the enzyme activity.

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