METAL-CATION-BASED REGULATION OF ENZYME DYNAMICS

***Krzysztof Brzezinski* and Justyna Czyrko**
University of Bialystok, Ciolkowskiego 1K 15-245 Bialystok, Poland, k.brzezinski@uwb.edu.pl

Acknowledgments: Work supported by an OPUS grant No. 2013/09/B/NZ1/01880 from the Polish National Science Center. The BioNanoTechno Center at the Institute of Chemistry, University of Bialystok was founded by the European Funds for Regional Development and the National Funds of the Ministry of Science and Higher Education, as part of the Operational Programme Development of Eastern Poland 2007-2013, project POPW.01.03.00-20-034/09-00. The research leading to these results received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under BioStruct-X (grant agreement N 283570). We thank HZB for the allocation of synchrotron radiation beamtime.

S-adenosyl-L-homocysteine hydrolase (SAHase) controls a cellular concentration of *S*-adenosyl-L-homocysteine (SAH), a byproduct of methylation reactions that utilize *S*-adenosyl-L-methionine (SAM) as a methyl donor. SAH is a negative feedback inhibitor of SAM-dependent methyltransferases, therefore the enzyme serves as a key regulator of SAM-dependent biological methylation reactions.

Our research has been focused on bacterial SAHase from *Pseudomonas aeruginosa* (PaSAHase). The aim of this study has been (i) to investigate the role of monovalent cations in SAHase catalysis, with emphasis on K^+ ions, and (ii) to explain the mechanism of PaSAHase inhibition by zinc ions. By combining X-ray crystallography with enzyme kinetics assays and ITC studies, as well as with ^{23}Na NMR spectroscopy, we were able to elucidate the effect of the monovalent cations on ligand binding, and to explain why the enzyme is most efficient in the presence of potassium. PaSAHase preferentially binds K^+ ion at the monovalent cation coordination site of the protein hinge region. Enzymatic and ITC studies confirm that among the alkali cations, the K^+ ion stimulates the highest enzymatic activity and strongest ligand binding. K^+, but not other alkali cations, enables unique dynamic properties (domain movement) of the enzyme to ensure its maximum catalytic activity. The K^+ ion stabilizes the enzyme-substrate complex in the closed conformation for a time interval required to complete the catalytic cycle. Also, we confirmed the presence of zinc ions by X-ray fluorescence and explained structurally that the potent inhibitory effect of Zn^{2+} cations on PaSAHase activity is based on arresting the enzyme in the closed, inactive conformation.