Design of new small molecular regulators (inhibitors, activators) of the catalytic activity of adenylate kinases. Molecular basis for the mechanism of the regulator action.

Adenylate kinases (AK) are phosphotransferases catalyzing the reversible transfer of high-energy phosphates between nucleotides. These enzymes participate in the control of many processes, such as cell differentiation and proliferation, inflammatory processes or adaptation to stress conditions. Consequently, adenylate kinases play an important role in the development of some pathological states and diseases. For these reasons, it is important to search for new compounds regulating their activity. Furthermore, so far only one efficient AK regulator (inhibitor) was identified. The aim of the project is to determine the molecular basis and mechanism of the AK activity regulation by the new groups of compounds: statins ,substrate analogues and new modulators synthesized basing on statins and analogues structures. Analysis of the literature reports and our preliminary research led us to the conclusion that depending on the phylogenetic classification, the tissue distribution and the AK structure, compounds similar to the AK substrates (substrate analogues) inhibit or activate adenylate kinases with different efficiency. It is also known that one of the important steps of the reaction catalyzed by AK is the change in position of one of AK domains called LID. Therefore, the **main hypothesis** in the project is that the mechanism and regulatory effect on the AK activity depends on the kinase structure, in particular on the length of the LID domain. For that, two adenylate kinases differing by the LID length have been chosen for the project research. Adenylate kinase is reported to participate in the control of the HDL endocytosis by the liver cells. In cardiology, the drugs used for lowering blood cholesterol levels are statins. These compounds have a common β-hydroxy acid moiety that might mimic the binding interactions of the AK substrate phosphates. Our preliminary studies revealed that rosuvastatin efficiently inhibits the activity of AK with the short LID domain, while not affecting the long type kinases significantly. Therefore the **additional hypothesis** is that statins are the AK inhibitors with the mechanism of action different from that of the substrate analogues, and their inhibitory effect also depends on the LID structure.

To verify both hypotheses, the effect of other statins, substrate analogues and newly synthetized compounds on the activity of both AK will be determined. The co-crystallization and structure determination of the best modulators with both adenylate kinases will be performed to determine the mechanisms of this regulation. The obtained results will constitute the basis for further rational modification of the regulator structure, and these new compounds will be synthesized and characterized in the project. Characterization of new AK modulators will also include the determination of their potential cytotoxic properties against human cells and their stability in the blood serum. The research includes the determination of regulatory effects of the most efficient statins and new modulators on the function of the extracellular AK in the regulation of the HDL endocytosis.

We expect that our results will explain the differences in the AK regulation by the same modulator, depending on the different LID structure. That will enable the future design of the modulators dedicated for a selected AK type (long or short form). Synthesis of new group of efficient AK inhibitors and activators might play an important role in developing therapies of the civilization-related diseases, such as atherosclerosis or myocardial infarction. Understanding of the AK regulation by various new compounds will enable to define the ligand database allowing to build a pharmacophore model concerning the spatial relations between atoms and functional groups necessary for the regulator interactions with AK. Our research will also reveal the existence of additional mechanisms of the pleiotropic action of statins (AK inhibition) and the necessity for further research on the significance of these compounds for regulation of other AK functions.