O-10 **Wed. 18. 06., 15^{00}-15^{20}**

Complex formation of pyrophosphatase with protein-partners investigated by small-angle X-ray scattering in solution

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Keywords: synchrotron radiation, small-angle X-ray scattering, proteins interaction

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Every living cell is a complicated adaptive system in which proteins and their molecular interactions are key factors. Taking into consideration the dynamic character of the system, it is important to understand how cells work in general, and to know functional relations between individual players participated in endless variations of responses to outer signals. For that it is necessary to study processes of cell metabolic regulation and interaction of different cellular components at functional level [1]. Inorganic pyrophosphatase (PPase) plays an important role in cell viability because its support of endergonic metabolic processes including biosynthesis of proteins and nucleic acids [2]. Therefore, PPase should be considered as a vital component of multiprotein complexes. Recently it has been shown [1] that PPase specifically interacts with such proteins expressed in E. coli as new type of fructose-1,6-bisphosphate aldolase (FbaB), cytoplasmic enzyme, which plays a crucial role in metabolic regulation [3]; 5-keto 4-deoxyuronate isomerase (KduI) involved in pectin degradation [4]; and glutamate decarboxylase (GadA) catalyzing the decarboxylation of glutamate to γ-aminobutyrate [5]. Study of interactions of these proteins could reveal new evidences on the PPase role in cell life cycle. In the present work for the first time PPase, its partner-proteins and their complexes in solutions at various concentrations were studied and characterized by small-angle X-ray scattering (SAXS) using synchrotron radiation on the P12 beamline at the PETRA III storage ring (DESY, Hamburg). On the basis of the experimental SAXS data, we calculated important structural parameters describing solution behavior of the specimens studied, and 3D models describing quaternary structure of the proteins and of their complexes in solution were offered. It was found that interactions took place in the presence of PPase, and complexes formed in solution comprised only some portion of the total amount of the proteins, being thus constantly in a dynamic equilibrium. A role of PPase in protein-protein interactions may consist in stabilization of its protein-partners preventing unfolding and consequent proteolytic degradation. Analysis of properties of the PPase partners shows that most of them are involved in stress response [1]. Thus, the results of our work provide new information on crucial role of PPase in metabolic regulation during processes like stress adaptation or “quorum sensing”.

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