Saturday, March 24, Session VIII

L30

MOLECULAR BASIS OF THE 14-3-3 PROTEIN-DEPENDENT ACTIVATION OF YEAST NEUTRAL TREHALASE Nth1

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The 14-3-3 proteins, a family of highly conserved scaffolding proteins ubiquitously expressed in all eukaryotic cells, interact with and regulate the function of several hundreds of partner proteins. Yeast neutral trehalases (Nth), enzymes responsible for the hydrolysis of trehalose to glucose, compared with trehalases from other organisms, possess distinct structure and regulation involving phosphorylation at multiple sites followed by binding to the 14-3-3 protein [1].

Here we report the crystal structures of yeast Nth1 and its complex with Bmh1 (yeast 14-3-3 isoform), which, together with mutational, hydrogen/deuterium exchange coupled to mass spectrometry and fluorescence studies, indicate that the binding of Nth1 by 14-3-3 triggers Nth1's activity by enabling the proper 3D configuration of Nth1's catalytic and calcium-binding domains relative to each other, thus stabilizing the flexible part of the active site required for catalysis [2-4]. The presented structure of the Bmh1:Nth1 complex highlights the ability of 14-3-3 to modulate the structure of a multidomain binding partner and to function as an allosteric effector. Furthermore, comparison of the Bmh1:Nth1 complex structure with those of other 14-3-3 protein complexes revealed similarities in the 3D structures of bound partner proteins, suggesting the highly conserved nature of 14-3-3 affects the structures of many client proteins.

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STRUCTURAL CHARACTERIZATION OF PROTEIN KINASE ASK1 AND ITS INTERACTION WITH THIOREDOXIN

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Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase (MAPK) cascade, an essential part of the cell defence system against stressors. The function of ASK1 is associated with the activation of apoptosis in various cells, the regulation of ASK1 depends on several stimuli, including oxidative stress (presence of ROS) and therefore, ASK1 plays a key role in the pathogenesis of many diseases including cancer, neurodegeneration and cardiovascular diseases. The activity of ASK1 is regulated by its interaction with multiple proteins; current research is focused on two physiological inhibitors, mammalian thioredoxin (TRX) and the 14-3-3 protein [1]. ASK1 is under normal conditions in an inactive complex with bound TRX and 14-3-3. As a response to oxidative stress condition, TRX and 14-3-3 dissociate and ASK1 become active. However, the molecular mechanism of the ASK1 activation is still not fully understood, as there are almost no structural data available. Therefore, the aim of this study was the structural characterization of the TRX-binding domain of ASK1 (ASK1-TBD) and ASK1-TBD: TRX complex formation.

We have previously shown that ASK1-TBD forms with TRX well defined and stable complex under reducing conditions. Site-directed mutagenesis revealed that formation of disulfide bond between Cys32 and Cys35 in a TRX molecule is the main factor responsible for complex dissociation under oxidative stress. ASK1-TBD contains seven cysteine residues with the residue Cys250 being the only

cysteine which is both solvent exposed and essential for TRX binding in reducing conditions. The oxidative stress also induces intramolecular disulfide bonds formation within ASK1-TBD and affects its structure in regions important for TRX1 binding [2,3].

In this study we present structural characterization of the regulation of ASK1 via structural model of ASK1-TBD in both reduced and oxidized conditions and ASK1-TBD: TRX complex based on sparse NMR data, crosslinking mass spectrometry and small-angle x-ray scattering (SAXS) data.

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This work was supported by the Grant Agency of the Charles University in Prague (No. 368216).

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STRUCTURAL ANALYSIS OF PHOSPHATIDYLINOSITOL 4-KINASE III (PI4KB) PROTEIN COMPLEXES WITH 14-3-3, ACBD3 AND VIRAL PROTEINS

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Phosphatidylinositol 4-kinase III (PI4KB) is responsible for the synthesis of the Golgi and trans-Golgi network (TGN) pool of phosphatidylinositol 4-phospahte (PI4P). PI4P is the defining lipid hallmark of Golgi and TGN and also serves as a signaling lipid and as a precursor for higher phosphoinositides. In addition, PI4KB is hijacked by many single stranded plus RNA (+RNA) viruses to generate PI4P-rich membranes that serve as viral replication organelles. Given the importance of this enzyme in cells, it has to be regulated. 14-3-3 proteins bind PI4KB upon its phosphorylation by protein kinase D, however, the structural basis of PI4KB recognition by 14-3-3 proteins is unknown. We recently characterized the PI4KB:14-3-3 protein complex biophysically and structurally. We discovered that the PI4KB:14-3-3 protein complex is tight and is formed with 2:2 stoichiometry. Surprisingly, the enzymatic activity of PI4KB is not directly modulated by 14-3-3 proteins. However, 14-3-3 proteins protect PI4KB from proteolytic degradation in vitro. Our structural analysis revealed that the PI4KB:14-3-3 protein complex is flexible but mostly within the disordered regions connecting the 14-3-3 binding site of the PI4KB with the rest of the PI4KB enzyme. It also predicted no direct modulation of PI4KB enzymatic activity by 14-3-3 proteins and that 14-3-3 binding will not interfere with PI4KB recruitment to the membrane by the ACBD3 protein. In addition, the structural analysis explains the observed protection from degradation; it revealed that several disordered regions of PI4KB become protected from proteolytical degradation upon 14-3-3 binding.

Recently we also structurally characterized PI4KB: ACBD3 and PI4KB:ACBD3:3A protein complexes that revealed surprisingly large flexibility of these protein assemblies.

This project was supported by Project InterBioMed LO1302 from Ministry of Education of the Czech Republic, the support of the Academy of Sciences of the Czech Republic (RVO: 61388963) is also acknowledged.