Poster Presentation

MS29.P39

Structural studies of myo-inositol kinase

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The TK2285 protein from a hyperthermopilic archaeon Thermococcus kodakarensis is a myo-inositol kinase. Only two myo-inositol kinases have been identified so far. One is the TK2285 protein and the other is an enzyme from Zea mays. Both of them synthesize myo-inositol monophosphate that shows enantiomerism. Because it is too difficult to discriminate enantiomers by NMR or chromatography analysis, it has not been identified which of the six hydroxyls is phosphorylated by these enzymes. Also, little is known about the substrate recognition of myo-inositol kinase, since only the unliganded crystal structure of TK2285 has been reported. In order to reveal the substrate-binding mechanism of myo-inositol kinase and identify the phosphorylated hydroxyl group of the product, we determined the crystal structures of TK2285 as the substrate-complex and the product-complex. The substrate-complex of TK2285 was prepared by using the TK2285, myo-inositol and AMP-PCP, and the products-complex was prepared by incubating the TK2285 with myo-inositol and ATP. The substrate-complex structure showed that all of the six hydroxyls of myo-inositol interacted with TK2285. This coincides with the fact that the Km value for myo-inositol is 100-1000 fold lower than those for other sugars. Also 3-hydroxyl group of myo-inositol, which the gamma-phosphate of AMP-PCP was nearest to, was thought to be phosphorylated by this enzyme. This was proved by the product-complex structure that had ADP and myo-inositol 3-phosphate. Site-directed mutagenesis and structure comparison with TK2285 homologs also provided information about the substrate-binding mechanism of myo-inositol kinase.

Keywords: enzyme, ribokinase, myo-inositol