

Poster Presentation

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Crystal structure of an alleged mannosylphosphate transferase Ktr6p at 3.06 Å

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Ktr6p is an alleged mannosylphosphate transferase from yeast Golgi. It has been implicated in decorating both O-linked and N-linked glycans with mannosylphosphate *in vivo*. However, based on sequence similarity, Ktr6p belongs to GT15 family of α -1,2-mannosyltransferases. To address this disagreement, the soluble portion of Ktr6p was expressed in *P. pastoris* and purified by liquid chromatography. The purified protein, GDP-mannose and various acceptors were used in a number of direct and indirect activity assays, however, neither mannosyltransferase nor mannosylphosphate transferase activity was detected. Ktr6p was crystallized in a number of PEG-containing conditions, but the crystals resisted all attempts at cryoprotection. Three crystals were used to collect a 3.06 Å resolution dataset on a home source at room temperature. The crystals belong to P 21 21 21 spacegroup with 2 molecules per asymmetric unit. The structure was solved by molecular replacement using a structure of Kre2p, a close homolog from GT15 family (40% sequence identity). The structure was refined to R/Rfree 16.1%/21.2%. The overall structure of Ktr6p is very similar to the structure of Kre2p having less than 2 Å overall backbone RMSD. However even at 3 Å resolution the difference in the active site is striking. The guanine moiety binding pocket is occluded by a well-ordered loop making GDP-mannose binding impossible in this conformation. Several amino acid substitutions in the Mn²⁺ coordinating environment suggest that Ktr6 does not depend on manganese for its postulated activity. These observations indicate that Ktr6p functions quite differently from Kre2p.

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