

X-Ray Structure of the Yeast t-SNARE Sso1p

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Beamline(s): X12C

Introduction: The SNARE protein Sso1p, a homolog of the neuronal protein syntaxin, is required for exocytosis in yeast. Its well-ordered cytoplasmic domain forms sizable orthorhombic crystals. These crystals diffract x-rays to no better than 3.1Å at "home," using an R-Axis IIC mounted on a Rigaku R-200HB generator and fitted with double focusing mirrors. To obtain higher-resolution x-ray diffraction data, and because of the availability of selenomethionine-substituted crystals for multiwavelength anomalous diffraction (MAD) experiments, we opted to collect data at the NSLS.

Methods and Materials: All x-ray diffraction data were collected at 100 K. Native and four MAD data sets were collected using the Brandeis CCD detector on beamline X12C. Data were processed using Denzo/Scalepack. Three of the five selenium sites were found using SOLVE. Heavy atom site refinement (MLPHARE) and density modification (DM) resulted in a readily interpretable electron density map at 2.6Å resolution. Model building (O) and refinement (CNS) were used to produce the final model.

Results: The resulting model of Sso1p was refined to 2.1Å with a working R factor of 0.216 and a free R factor of 0.263. This model exhibits excellent geometry, with all residues within the most favored and additional allowed regions of the Ramachandran plot.

Conclusions: Intracellular trafficking requires the docking and fusion of vesicles, a process that in turn depends upon the assembly of SNARE protein complexes. This structure of Sso1p, the first of an uncomplexed, essentially intact SNARE protein, provides novel insight into the mechanism of SNARE assembly and its intra- and intermolecular regulation (Munson et al., 2000).

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References: Munson, M., Chen, X., Cocina, A.E., Schultz, S.M., and Hughson, F.M. (2000) Interactions within the yeast t-SNARE Sso1p that control SNARE complex assembly. *Nature Struct. Biol.*, in press.

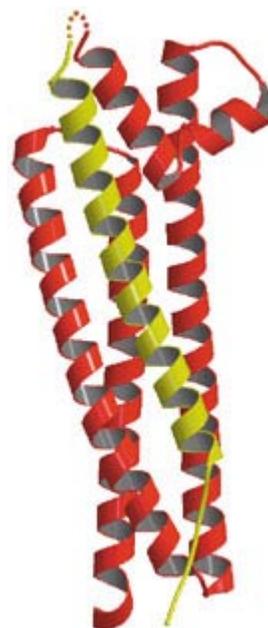


Figure 1. Ribbon diagram of Sso1p, prepared using Molscript and Bobscript.