

Sucrose Isomerases : 3D structures give insights into substrate recognition, binding and specificity

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Various diseases related to the over-consumption of sugar make a growing need for sugar substitutes. Since sucrose is an inexpensive and readily available D-glucose donor, the industrial potential for enzymatic synthesis of the sucrose isomers trehalulose and/or isomaltulose from sucrose is large. The product specificity of sucrose isomerases which catalyze this reaction depends essentially on the possibility for tautomerization of sucrose which is required for trehalulose formation. For optimal use of the enzyme, targeting controlled synthesis of these functional isomers, it is necessary to minimize the side reactions. This requires an extensive analysis of substrate binding modes and of the specificity-determining sites in the structure. We have recently cloned, purified, crystallized and solved the structure of two sucrose isomerases; MutB, from *Pseudomonas mesoacidophila* MX-45 which mainly produces trehalulose [1,3], and SmuA from *Protaminobacter rubrum* which mainly produces isomaltulose [2]. The 1.6 to 2.2 Å resolution three-dimensional structures of native and mutant complexes of a trehalulose synthase from *Pseudomonas mesoacidophila* MX-45 mimic successive states of the enzyme reaction. Combined with mutagenesis studies they give for the first time thorough insights into substrate recognition and processing, and reaction specificities of these enzymes. Amongst the important outcomes of this study is the revelation of an aromatic clamp defined by Phe256 and Phe280 playing an essential role in substrate recognition and in controlling the reaction specificity, which is further supported by mutagenesis studies. Furthermore, this study highlights essential residues for binding the glucosyl- and fructosyl-moieties. The introduction of subtle changes informed by comparative 3D structural data observed within our study can lead to fundamental modifications in the mode of action of sucrose isomerases, and hence provide a template for industrial catalysts.

[1] Ravaud S, Watzlawick H., Haser R., Mattes R., Aghajari N. *Acta Cryst F*, 2005, 61, 100-103.

[2] Ravaud S, Watzlawick H., Haser R., Mattes R., Aghajari N. *Acta Cryst F*, 2006, 62, 74-76.

[3] Ravaud S, Robert, X., Watzlawick H., Haser R., Mattes R., Aghajari N. *J. Biol. Chem.*, 2007, 282, 28126-28136.