

Crystal Structure of Lactose Synthase: A Complex Between β -1,4-galactosyltransferase and α -actalbumin with its Acceptor and Donor Substrates

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β -1,4-galactosyltransferase (β -1,4-Gal-T1) in the presence of manganese transfers galactose from uridine-diphospho-galactose (UDP-Gal) to the acceptor sugar N-acetylglucosamine (GlcNAc). In the mammary gland, during lactation, the acceptor sugar specificity of β -1,4-Gal-T1 is modulated by alpha-lactalbumin in a way that it transfers galactose to glucose (Glc) but not to GlcNAc. Although the crystal structures of alpha-lactalbumin and beta-1,4-Gal-T1 are known separately, still the molecular interactions between these two protein molecules are not clearly understood. This is mainly because the sugar acceptor binding site in the β -1,4-Gal-T1 is not known from its crystal structure. We have crystallized a 1:1 complex of the catalytic domain of β -1,4Gal-T1 with alpha-lactalbumin, also known as the lactose synthase complex. The crystals belong to monoclinic system, space group $P2_1$, with the cell dimensions $a = 55.2$, $b = 99.0$ and $c = 102.4$ Å and $\beta = 103.9$. The crystal structure of the complex has been solved by MAD methods using the lactose synthetase crystals grown with β -1,4-Gal-T1 containing seleno-methionine. The structure of lactose synthase demonstrates that the β -1,4-Gal-T1 molecule undergoes a large conformational change in the region between residues 345 to 365 upon substrate binding. Such conformational changes are required not only for the acceptor to bind but also for alpha-lactalbumin to bind to β -1,4-Gal-T1. Furthermore, the lactose synthase crystal structure has been solved in the presence of its acceptor substrates such as GlcNAc, Glc and N-butyl-GlcNAc and donor substrates such as UDP.Mn²⁺, UDPGal.Mn²⁺, and UDPGlc.Mn²⁺. These crystal structures have been solved and refined to 2.0 Å resolution. The structural information of the bound substrates to β -1,4-Gal-T1 rationalizes the substrate specificity of the enzyme which arises from its molecular interactions with the substrates. Also from the present structural information, together with the information of its native structure, enzyme kinetics and catalytic mechanism can be rationalized.