

Crystal structures of L-sorbose reductase from acetic acid bacterium, *Gluconobacter frateurii*, complexed with NADPH or L-sorbose

Keiko Kubota¹, Koji Nagata¹, Masahiro Okai¹, Ken-ichi Miyazono¹, Shintaro Maruoka¹, Jun Ohtsuka¹, Akihiro Yamamura¹, Hirohide Toyama^{2,3}, Kazunobu Matsushita², Masaru Tanokura^{1*}

¹ Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

² Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi-shi, Yamaguchi 753-8515, Japan

³ Department of Bioscience and Biotechnology, Faculty of Agriculture, University of The Ryukyus, 1 Senbaru, Nishihara-cho, Okinawa 903-0213, Japan

Introduction

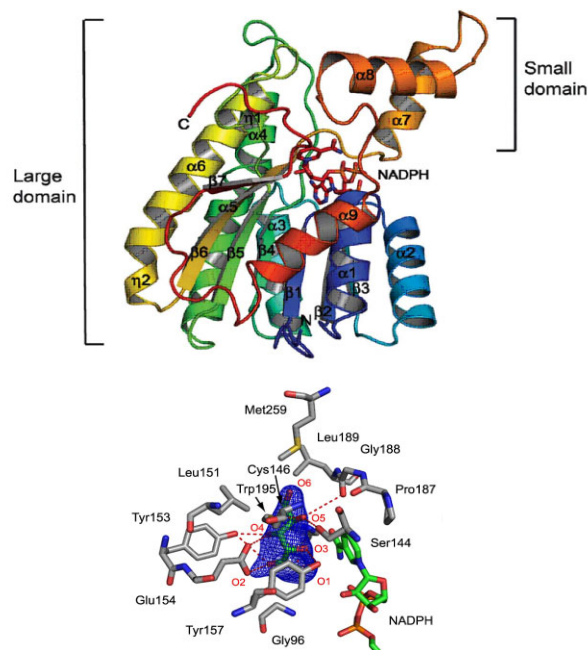
L-Sorbose reductase from acetic acid bacterium *Gluconobacter frateurii* (SR) is an NADPH-dependent oxido-reductase that belongs to the short-chain dehydrogenase/reductase (SDR) family. SR catalyzes the oxido-reduction between L-sorbose and D-sorbitol [1], and can utilize only these sugars as substrates with a high specificity. In contrast, the substrate specificities of the other SDR family proteins that are capable of utilizing L-sorbose are generally low. To understand the structural bases of the high substrate specificity and the catalytic mechanism of SR, we have determined the structures of SR complexed with NADPH and with L-sorbose at 1.90-Å and 2.38-Å resolutions, respectively, by X-ray crystallography.

Results and Discussion

The crystals of SR-L-sorbose complex and SR-NADPH complex were obtained with reservoir solutions containing PEG 2000 and PEG 400 as precipitants and diffracted X-rays to 2.38 and 1.90 Å resolutions, respectively. The crystals of SR-L-sorbose belonged to the space group $C222_1$ with the unit-cell parameters of $a = 124.2$, $b = 124.1$, and $c = 60.8$ Å. The crystals of SR-NADPH complex belonged to the space group $P2_1$ with the unit-cell parameters of $a = 124.3$, $b = 61.0$, and $c = 124.5$ Å, $\beta = 89.99^\circ$. The respective crystals contained 2 and 8 molecules in the asymmetric unit. [2]

SR belongs to the short chain dehydrogenase/reductase (SDR) family and consists of a large domain containing a Rossmann-fold and a small domain consisting of two α -helices. SR forms a tetrameric assembly in crystal and solution at optimal pH's, pH 6.2 for the conversion of L-sorbose to D-sorbitol and pH 9.0 for the conversion of D-sorbitol to L-sorbose. The NADPH is located in the deep cleft of the large domain. The conformation of NADPH when bound to SR was very similar to that bound to other SDR family members. The L-sorbose is observed in a pocket near the NADPH-binding site and recognized by $\eta 1$, $\alpha 4$, and $\alpha 7$ helices and loops.

Site-directed mutagenesis of the residues around the L-sorbose binding site showed that not only the substitution of the putative catalytic residues, H116, S144, Y157, and K161, but also the substitution of the residues which are used for the recognition of C4 and C5 hydroxyl groups of L-sorbose, Cys146, Tyr153, Glu154 and Gly188, resulted in the almost entire loss of the enzymatic activities. The recognitions of C4 and C5 hydroxyl groups of L-sorbose would be very important for the substrate specificity of SR. Our results indicate that these residues are crucial for the substrate recognition and specificity of SR.



References

- [1] Soemphol, W., Toyama, H., Moonmangmee, D., Adachi, O. & Matsushita, K. J. Bacteriol. 189, 4800 (2007).
 [2] Kubota, K., Nagata, K., Miyazono, K., Toyama, H., Matsushita, K. & Tanokura, M., Acta Crystallogr. F65, 562. (2009).

* amtanok@mail.ecc.u-tokyo.ac.jp