

Analysis of protein structure and function of xylose isomerase from *Thermotoga neopolitana*: mutation on site 185

Oh-Hee Kwon, J. G. Zeikus¹, Pil Kim

Major in Biotechnology, The Catholic Univ. of Korea, Bucheon, Korea

¹Dept. of Biochemistry and Molecular Biology, Michigan State Univ., East Lansing, USA

The *Thermotoga neopolitana* xylose isomerase(TNXI) is extremely thermostable and optimally active at 95°C. It's derivative, TNXI Val185Thr(V185T), which acquired from directed evolution for high activity on glucose at low temperature and low pH, was analyzed based on the 3D protein structure. The change of Valine to Threonine enabled the enzyme 3-times more efficient toward glucose as substrate while little difference toward xylose. The reaction temperature used in the current industrial glucose isomerization process is limited to 60°C because of by-product and color formation that occur at high temperature and alkaline pH, and because the isomerases themselves are not highly thermostable. Thermostable Xis with neutral or slightly acidic pH optima have a potential for industrial applications. The reasons of enhancing catalytic activity by the mutation on site 185 are discussed, and another sites candidates for rational designed enzyme to achieve the above industrial demands are suggested.

References

1. Sripadundh D., C. Vieille, and J. G. Zeikus, "Molecular determination of xylose isomerase thermal stability and activity: analysis of thermozymes by site-directed mutagenesis", (2000)Protein Engineering 14:259-265
2. Sripadundh D., C. Vieille, and J. G. Zeikus, "Directed evolution of *Thermotoga neopolitana* xylose isomerase: high activity on glucose at low temperature and low pH", (2003)Protein Engineering 16:683-690
3. Kim P., "Current studies on biological tagatose production using L-arabinose isomerase: a review and future perspective" (2005) Appl. Microbiol Biotechnol 65: 243-249