Biological Science

5A, NE3A/2009G040

Carbohydrate-protein interactions in the active site of sugar processing enzyme galactose mutarotase.

Akihiro KOBE, Shota KUDO, Jose M.M. CAAVEIRO, Kouhei TSUMOTO*
Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo 108-8639

Introduction

Galactose mutarotase (GalM) (aldose-1-epimerase) is an enzyme that catalyzes the equilibration of α- and β-anomeric forms of D-galactopyranose and other monosaccharides (Scheme 1) [1].

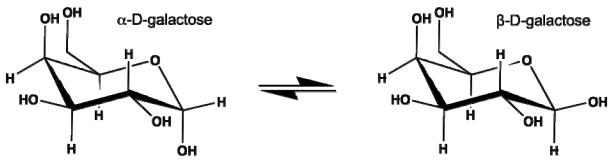

Figure 1. Reaction catalyzed by GalM.

We previously solved the crystal structure of GalM in complex with substrate D-galactose. The structure showed an elaborate network of hydrophilic and hydrophobic interactions in the active site of the enzyme.

In this report we sought to address two questions relevant for the field of carbohydrate-protein interactions: (i) to characterize the energetic sources of affinity between substrate and enzyme, and (ii) to investigate the physicochemical principles of substrate selectivity.

Experimental

GalM from *Escherichia coli* was expressed in BL21 (DE3) cells. GalM construct was furnished with a hexahistidine tag in the N-terminal side. Purification included Ni^{2+} affinity chromatography, treatment with protease TEV, and gel filtration. Crystals were grown by vapor diffusion method (hanging drop) in solutions containing PEG 8,000. Diffraction data of crystals of wild type protein with glucose bound, or variants of GalM containing point mutations, were collected at beamlines BL5A, NW-12 and NE3A of the Photon Factory. Three-dimensional structures were determined by the method of molecular replacement with PHASER. Models were refined with REFMAC5 and COOT.

In addition, characterization of GalM/sugar interaction included various biophysical techniques such as isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC), or circular dichroism (CD), as well as biochemical assays to determine enzymatic activity.

Results and Discussion

GalM shows strong preference for D-galactose versus D-glucose. To understand the physicochemical basis of these differences we first determined the crystal structure of GalM in complex with glucose at 1.5 Å resolution (Figure 2). Detailed examination of molecular contacts between each monosaccharide and GalM did not reveal any major difference in conformation, number, or type of interactions to which attribute the observed enzymatic selectivity.

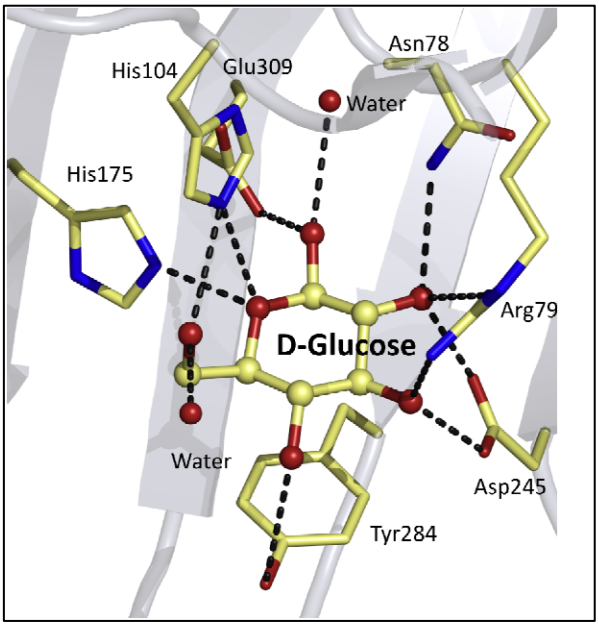

Figure 2. Crystal structure of GalM with D-glucose bound. Dashed lines correspond to H-bonds between sugar and protein (distance cut off = 3.2 Å).

We hypothesized that dynamic and energetic properties, not obvious from the static picture of a crystal structure, could explain selectivity preference. Biophysical techniques such as ITC and DSC were employed to fill-up the structure-activity gap. The data revealed that GalM-monosaccharide interaction is driven by a large and favorable enthalpy term and opposed by an almost equally large entropy component, resulting in affinities in the mM range. However, when comparing binding affinities with each sugar, we discovered that natural substrate D-galactose binds approximately 5-fold stronger than D-glucose. This observation satisfactorily explained the enzymatic preference of GalM.

We are currently expanding this approach to various muteins of GalM with different substrates and analogs to characterize, in more detail, the nature of these forces.

References

[1] J.A. Beebe et al., *Biochemistry* 37:14989 (2003).

* tsumoto@ims.u-tokyo.ac.jp