Biological Science

5A, 6A, 17A, NW12A/2006G158

Crystallographic analysis of sugar phosphorylases belonging to novel families

Masafumi Hidaka[1,2], Shinya FUSHINOBU*[1], Motomitsu KITAOKA[2], Takayoshi WAKAGI[1], Hirofumi SHOUN[1]
[1]Dept. of Biotechnology, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
[2]National Food Research Institute, 2-1-12, Kannondai, Tsukuba, Ibaraki, 305-8642, Japan

Introduction

Enzymes involved in the formation or cleavage of glycosyl linkages are mainly categorized into Glycoside Hydrolase (GH) or Glycosyl Transferase (GT) class (CAZy website at http://afmb.cnrs-mrs.fr/CAZY/), and each class comprises dozens of families classified on the basis of amino acid sequence similarity. Phosphorylases catalyze cleavage of glycosidic bonds by adding inorganic phosphate to generate glycosyl-phosphates (phosphorolysis). Since the energy of the glycosyl-phosphate bond is not as high as that of a glycosyl-nucleotide, their reactions are reversible. Therefore, phosphorylases can be employed for both the synthesis and degradation of sugar chains. Phosphorylases have been assigned EC numbers of glycosyltransferases (2.4.1.-) according to the apparent reaction scheme. However, they occupy a peculiar position in the CAZy database, as they are classified across the GH and GT classes. Cellobiose phosphorylase (CBP) and chitobiose phosphorylase (ChBP) were classified as belonging to a GT family, GT36, since none showed hydrolytic activity. We determined the ternary complex structure of ChBP from *Vibrio proteolyticus* with N-acetylglucosamine and sulfate (phosphate analog) [1]. The similarities of overall structures and catalytic mechanisms between ChBP and inverting GH enzymes led to a significant reorganization of the CAZy database; family GT36 was deleted, and then reclassified into a novel GH family, namely GH94. This type of reclassification, in which a family travels across two functionally distinct classes, was unprecedented. Such structural and functional similarity between inverting GHs and inverting phosphorylases suggests their possible evolutionary relationship [2, 3]. In this study, we focused on the structural analysis of this sugar phosphorylase classified in this novel family, as well as a new phosphorylase that is not classified yet.

Results and Discussion

Application of CBP for practical oligosaccharide synthesis has been established, so the 3D structure of CBP will aid in technical development in the production of new functional oligosaccharides. We have determined the glucose-sulfate and glucose-phosphate complex structures of CBP from *Cellvibrio gilvus* at maximal resolution of 2.0 Å [4]. The phosphate ion is strongly held through several hydrogen bonds, and the configuration appears to be suitable for direct nucleophilic attack to an anomeric centre. Structural features around the sugar-donor and sugar-acceptor sites were consistent with the results of kinetic studies. When we compared this structure with that of homologous ChBP, we identified key residues for substrate discrimination between glucose and N-acetylglucosamine in both the sugar-donor and sugar-acceptor sites. The active site pocket of cellobiose phosphorylase was covered by an additional loop, indicating that some conformational change is required upon substrate binding. Information on the three-dimensional structure of CBP enabled detailed computational analyses of its reaction itinerary [5], and will facilitate engineering of this enzyme. We have also determined two new sugar phosphorylase structures [6, 7]. Manuscripts are currently in preparation.

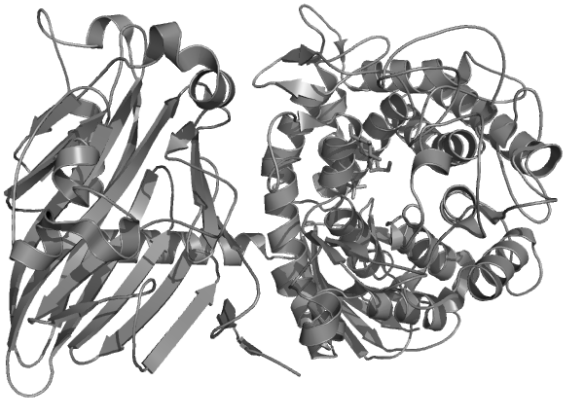

Figure 1 The crystal structure of CBP.

References

[1] M. Hidaka et al., #22 2004 Part A, Highlights, pp 43.
[2] S. Fushinobu et al., J. Appl. Glycosci. 54, 95 (2007).
[3] S. Fushinobu et al., Kagaku To Seibutsu 46, 308 (2008)
[4] M. Hidaka et al., Biochem. J. 398, 37 (2006).
[5] S. Fushinobu et al., Carbohydr. Res. 343, 1023 (2008).
[6] M. Hidaka et al., ISDSB2007 Symposium Booklet, 129 (2007).
[7] S. Fushinobu et al., Symposium on JSBBA Annual Meeting, 4SY16-3 (2008).

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