BL-5A, NW-12A, NE-3A/2006G165, 2011G129

Structural and mutational analyses of *Bacillus megaterium* glucose 1-dehydrogenase IV

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1 Introduction

NAD(P)$^+$-dependent glucose 1-dehydrogenase isozymes from *Bacillus megaterium* (BmGlcDHs) catalyze the oxidation of β-D-glucose to D-glucono-1,5-lactone, using NAD(P)$^+$ as a cofactor. BmGlcDH is a member of the short-chain dehydrogenase/reductase (SDR) superfamily, and has been used clinically to examine blood glucose levels [1]. Two other types of GlcDHs have been used for blood glucose detection. One is a pyrroloquinoline-quinone-containing GlcDH (PQQ-GlcDH), which is a homodimer with a subunit weight of approximately 50 kDa. Another one is an FAD-containing enzyme (FAD-GlcDH) that has been isolated from 2 organisms, namely, *Aspergillus oryzae* (*A. oryzae*) and *Burkholderia cepacia* (*B. cepacia*). The *B. cepacia* FAD-GlcDH is known to be self-sufficient and relatively more thermostable than the PQQ-GlcDHs. In contrast to these 2 types of GlcDHs, BmGlcDH requires the addition of NAD$^+$ to the assay mixture. However, it is currently applied for the clinical assay, because of its relatively narrow substrate specificity toward D-glucose. Here, we describe for the first time the X-ray structures of the substrate-free, NADH-bound, and D-glucose-bound forms of BmGlcDH-IV. These structures reveal that the C-terminal carboxyl group derived from a neighboring subunit directly interacts with D-glucose. Based on the structures, we have performed site-directed mutagenesis of BmGlcDH-IV, and have obtained a useful mutant that showed the improvement of D-glucose specificity with retaining similar thermostability to the wild-type enzyme.

2 Experiment

The recombinant BmGlcDH-IV and its mutants were obtained using *Escherichia coli* as an expression host. His-tagged BmGlcDH-IV was purified by Ni-affinity chromatography. All crystals were obtained by the hanging-drop vapor-diffusion method at 20°C. The structures were solved by molecular replacement with GlcDH from *B. megaterium* IWG3 [2] as a search model.

3 Results and Discussion

The structures of BmGlcDH-IV in ligand-free form, in complex with NADH, and in complex with D-glucose were determined to a resolution of 2.0 Å. The homotetramer having a *222*-point-group symmetry was generated from the dimer in the asymmetric unit with the crystallographic 2-fold symmetry. Clear electron density showed that the trapped D-glucose is in the β form with the C1-hydroxyl group in the equatorial configuration. These results are consistent with previous reports that BmGlcDH acts on β-D-glucose, but not on α-D-glucose. It is interesting to note that the C-terminal carboxyl group (Gly261) derived from a neighboring subunit is inserted into the active-site pocket and directly interacts via hydrogen bonds with C4- and C6-hydroxyl groups in the bound β-D-glucose (Fig. 1). The side-chain amino group of Lys199 is also located near the C-terminal carboxyl groups at a distance of less than 3.0 Å. These elaborate inter-atomic interactions probably play a critical role in stabilizing the conformation of the active-site and the bound β-D-glucose. A site-directed mutagenic study showed that destabilization of the BmGlcDH-IV C-terminal region by substitution with more bulky and hydrophobic amino acid residues greatly affects the activity of the enzyme, as well as its thermostability and substrate specificity. Of the five mutants created, the G259A variant exhibited the narrowest substrate specificity relative to the wild-type enzyme, while retaining comparable catalytic activity with D-glucose, and similar thermostability to the wild-type enzyme.

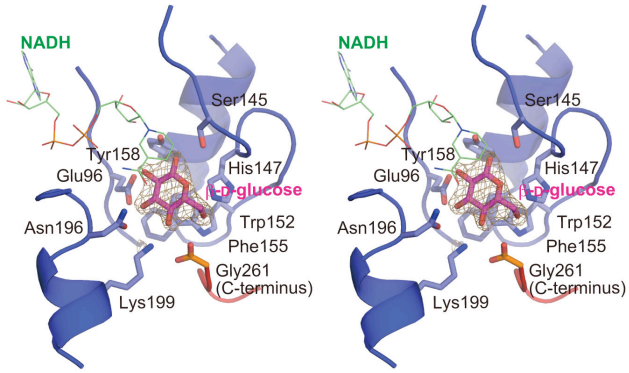

Fig. 1: Stereo view active site of BmGlcDH-IV. Fo-Fc omit map for the bound substrate is also shown.

References

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