Structural Studies on Subunit Interaction of Glucose Dehydrogenase

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Introduction

Glucose dehydrogenase from *Bacillus megaterium* is a tetrameric enzyme (Mr 112,800) with four identical subunits. GlcDH belongs to the short-chain dehydrogenases/reductases (SDR) family. The active form of the SDR enzyme is either a tetramer or a dimer, where each subunit is typically consists of about 250 amino acid residues.

Despite sharing low sequence identities, the three-dimensional structures of SDRs indicate striking similarities in overall folding and intersubunit contacts. To date, however, only GlcDH shows a reversible dissociation-association of subunits under moderate conditions [1]. In order to understand the remarkable distinct feature of GlcDH from other SDRs, information of the three-dimensional structure is indispensable. Here we report the refined crystal structure of glucose dehydrogenase from *B. megaterium* IWG3 complexed with NAD^+ at 1.7 Å resolution showing detailed subunit interactions.

Results and Discussion

Crystals of GlcDH belong to the monoclinic space group *C*2 and the unit-cell are a = 120.8(1) Å, b = 66.7(1) Å, c = 119.6(2) Å, and β = 93.25(3)°. High-resolution data to 1.7 Å were collected at 290 K using the synchrotron radiation source at the Photon Factory. 357,354 measured reflections reduced to 94,821 unique reflections with an overall R_{merge} of 3.3 %. This represented 91.9 % completeness at 1.7 Å resolution. [1]

The crystal structure was solved by molecular replacement with the program AMoRe. Coordinates of 3α, 20β-hydroxysteroid dehydrogenase having 35.0% sequence identity with GlcDH was used as a search model. Structure refinement was carried out initially with the program XPLOR of version 3.84 and at the later stages with the program CNS of version 0.9 The model obtained by the molecular replacement calculation was used as the initial model. Subsequently the molecular model was manually rebuilt in the maps with coefficients of sigma weighted $2F_o$-F_c and F_o-F_c maps using the program O of version 6.2.2. The final R-value was 17.9 % (R_{free} = 19.2 %) for the resolution range of 40 – 1.7 Å. The stereochemistry of the model was verified using the software package PROCHECK.

The tetramer structure as well as subunit structure of GlcDH resemble to those of SDRs of known structure. In the tetrameric SDR enzymes, the four subunits are related by three mutually perpendicular 2-fold axes designated as P, Q and R. Some SDRs such as 7α-hydroxysteroid dehydrogenase have no direct interactions among R-axis related subunits. Hence, the tetrameric structures of SDR enzymes, as well as that of GlcDH, are mainly maintained by the interactions through the P- and Q-axes interfaces.

The Q-axis interface of GlcDH comprises two long helices αE and αF of subunit A, which form a four-helix bundle together with those of helices of subunit B. Hydrophobic interactions especially of aromatic residues are predominant at the Q-axis interface of GlcDH. The hydrophobic interactions of GlcDH are, however, less extensive than those of other SDRs. Moreover, one pair of salt bridges further stabilizes the Q-axis interfaces of other SDRs, whereas no salt bridge is observed in GlcDH. In addition, the total number of hydrogen bonds and salt bridges in GlcDH between the Q-axis related subunit pair is one of the smallest among the SDR family. On the contrary, interactions between the P-axis-related subunits are not extensive compared to those of the Q-axis. The strength of the P-axis related interface of GlcDH has the same extent as the other SDRs.

Comparison of the subunit interactions reveals that the strength of the interactions of the Q-axis related interface, especially αE-αE contact, might be insufficient to maintain the tetrameric structure of GlcDH at pH9.0 [3].

References

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