BL-5A/2011G002, 2011G502

Insight into thermostability of a UDP-glucose dehydrogenase from the hyperthermophilic archaeon *Pyrobaculum islandicum*

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1 Introduction

UDP-glucose dehydrogenase (UDP-GDH) (EC 1.1.1.22) catalyzes a two-step NAD-dependent oxidation of UDP-glucose (UDP-Glc) to produce UDP-glucuronic acid (UDP-GlcA). In many strains of pathogenic bacteria, the UDP-GlcA is known to be necessary for construction of the antiphagocytic capsular polysaccharide. We recently demonstrated for the first time the presence of UDP-GDH in Archaea, the third domain of life. On the basis of genome information, we identified a gene (Pisl_1505) encoding a UDP-GDH homologue in the anaerobic hyperthermophilic archaeon, *Pyrobaculum islandicum* and confirmed that the gene product exhibits a high level of UDP-GDH activity [1]. The enzyme was found to be the most thermostable UDP-GDH so far described, with a half-life of 10 min at 90°C. Up to now, there is no information about either the structure of archaeal UDP-GDH or the structural features underlying thermostability of hyperthermophilic UDP-GDH. In the present study, we succeeded in determining crystal structure of the *P. islandicum* UDP-GDH [2]. Through comparison with the structure of the mesophilic UDP-GDHs, we evaluated the structural features responsible for the high thermostability of *P. islandicum* UDP-GDH.

2 Experiment

Diffraction data were collected at 2.0 Å resolution (λ=1.0 Å) on the beamline 5A at the Photon Factory. The initial phases for the structure were determined by molecular replacement.

3 Results and Discussion

The overall fold of the *P. islandicum* UDP-GDH was comprised of an N-terminal NAD^+ dinucleotide binding domain and a C-terminal UDP-sugar binding domain connected by a long α-helix (α9) (Fig. 1), and the main-chain coordinates of the enzyme were similar to those of the previously studied mesophilic UDP-GDHs, including the enzymes from *Burkholderia cepacia*, *Streptococcus pyrogenes* and *Klebsiella pneumoniae*.

Structural studies of hyperthermophilic proteins have suggested that the greater numbers of hydrophobic interactions and ion pairs are responsible to their high thermostability. We therefore compared the numbers of hydrophobic interactions and ion pairs within the structures of *B. cepacia*, *S. pyrogenes*, *K. pneumoniae* and *P. islandicum* UDP-GDHs (Table 1). With regard to the number of ion pairs within the monomeric structure, we found the numbers did not vary much among the *B. cepacia*, *K. pneumoniae* and *P. islandicum* enzymes (52-56), though the *S. pyrogenes* enzyme has actually fewer ion pairs (40) than the other UDP-GDHs. In addition, we identified a total of 4 intersubunit ion pairs in *P. islandicum* UDP-GDH, which is fewer than in *K. pneumoniae* enzyme (6). These results suggest that ion pair interactions do not mainly contribute to the higher thermostability of *P. islandicum* UDP-GDH.

On the other hand, when we counted the hydrophobic interactions, we found that the number of the intersubunit interactions in the *P. islandicum* UDP-GDH is markedly larger than those in the other enzymes; the 199 interactions found in *P. islandicum* UDP-GDH was 1.5 to 2.4 times larger than the numbers in the UDP-GDHs from the other sources (Table 1). Moreover, five Phe residues (Phe214, Phe221, Phe254, Phe272 and Phe275) were found to form intersubunit-aromatic pair network. Though the 136 intersubunit interactions were found in the *B. cepacia* UDP-GDH, there was no aromatic pair in the dimer interfaces of the enzyme. These observations suggest that the presence of extensive intersubunit hydrophobic interactions, as well as formation of intersubunit-aromatic pair network, is likely the main factor contributing the hyperthermostability of *P. islandicum* UDP-GDH.

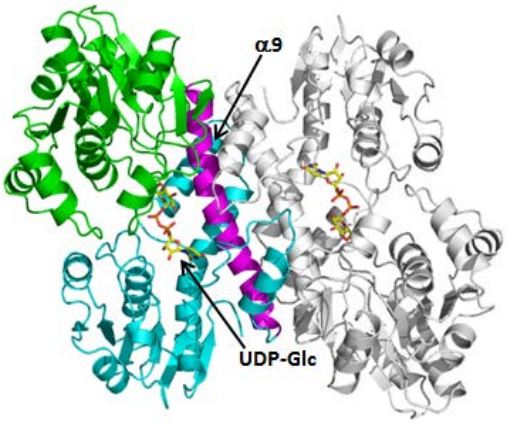

Fig 1. Dimer structure of *P. islandicum* UDP-GDH. The N-terminal NAD^+ dinucleotide binding and C-terminal UDP-sugar binding domains are shown in green and cyan, respectively.

Table 1: Comparison of the structural features of *P. islandicum* (Pis) UDP-GDH with those of *S. pyrogenes* (Spy), *B. cepacia* (Bce) and *K. pneumoniae* (Kpn) UDP-GDHs.

UDP-GDHs	Pis	Spy	Bce	Kpn
(PDB entry)	(3vtf)	(1dli)	(2y0e)	(3pln)
No. of ion pairs				
monomer	54	40	56	52
interface	4	0	0	6
No. of hydrophobic interactions				
monomer	552	526	627	508
interface	199	93	136	83

Acknowledgement

We thank Drs. Matsugaki, Igarashi and Wakatsuki for their kind assistance with the data collection.

References

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