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Crystal structure of glucose-6-phosphate isomerase from *Thermus thermophilus* HB8, which exists in monomer-dimer equilibrium in solution.

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Glucose-6-phosphate isomerase (GPI) is a key enzyme at the second step of glycolytic pathway in the wide variety of organisms. This enzyme catalyzes isomerization reaction between glucose-6-phosphate and fructose-6-phosphate. Almost all of the other conventional GPIs have been reported to be dimer or tetramer in solution, and dimerization is necessary for its enzymatic activity.

The crystal structure of the glucose-6-phosphate isomerase from hyperthermophilic bacteria *Thermus thermophilus* HB8 (*Tt*GPI) has been determined at 1.95 Å resolution. The crystallographic asymmetric unit contains one *Tt*GPI dimer. The core fold of protomer and the relative arrangement between two protomers of the dimer are quite similar to those of *B.stearothermophilus* GPI (*Gs*GPI) and mammalian GPIs (mGPIs). Residues for GPI activity are strictly conserved in the putative active site in the cleft between the two protomers. These results suggest that *Tt*GPI is a member of the conventional GPI family. However, some residues on the dimer interface are not conserved: (i) The *Tt*GPI lacks N-terminal 50 residues and C-terminal 30 residues and have altered topology of intermediate 'hook' region, which build up the extensive interprotomer association in mGPI dimer; (ii) Arg347, Glu382, Asn185, Gln342, Thr224 and Arg417 conserved on the dimer interface of mGPIs and involving polar dimeric interactions are mutated to His256, Ala286, His100, Glu252, Val138 and Phe335 in *Tt*GPI, respectively. These results raise a question whether dimer association of *Tt*GPI is stable or not.

To investigate the oligomeric state of *Tt*GPI in solution, several biochemical and biophysical experiments were conducted. Gel filtration chromatography and dynamic light scattering experiment showed unusual hydrodynamic property corresponding to an intermediate molecular size between monomer and dimer. Glutaraldehyde cross-linking experiment showed that cross-linked origomer of *Tt*GPI was observed only after prolonged reaction time in contrast with the reported shorter reaction time of rabbit GPI dimer. Sedimentation-equilibrium data by analytical ultracentrifugation successfully fitted to a monomer-dimer equilibrium model.

Comparative analysis revealed that *Tt*GPI dimer might be less stable than *Gs*GPI and human GPI dimer because of the reduced polar and nonpolar dimeric interactions. Moreover, several factors potentially contributing to thermal stability of *Tt*GPI protomer were identified: (i) an increased number of ion pair; (ii) an increased number of proline content; (iii) a higher ratio of arginine to lysine; (iv) an entropic effect from amino acid composition; (v) decreased cavity volume.

Taken together, *Tt*GPI may exist in dynamic equilibrium between monomer and catalytically active dimer, indicating that the dimer association of *Tt*GPI is less stable than those of the other GPIs reported previously. Probably the *Tt*GPI protomer itself has intrinsically sufficient thermal stability and the dimerization is not necessary for the thermal stability.