Characterization and Molecular Modeling of a Thermophilic and Alkaliphilic Galactokinase from *Thermus caldophilus* GK24

Dooil Kim[a,b,*], Bo Hyun Park[a], Jungdon Bae[a], Suk-In Hong[b], Dae-Sil Lee[a,*]

[a]*Systems Microbiology Research Center, KRIBB, Daejeon 305-806, Korea*
[b]*Department of Biomicrosystems and Technology, Korea University, Seoul 136-701, Korea*

The gene encoding for a galactokinase (*Tca* GalK) from an extremely thermophilic *Thermus caldophilus* GK24 was cloned and the biochemical characteristics of the resulting recombinant protein as well as its substrate specificity were examined. The *Tca* galK was overexpressed from a pTrc99A-based plasmid under the control of the *trc* promoter in *E. coli*. The recombinant purified enzyme was optimally active in the range of 75-90°C, showing the high thermostability, particularly in the presence of ATP. And the *Tca* GalK enzyme had unique optimum pH range of 9.0 and 10.0 at 70°C. The *Tca* GalK phosphorylated not only D-galactose to D-galactose-1-phosphate in the presence of ATP-Mg^{2+}, but also did 2-deoxy-D-galactose, D-galactosamine, D-talose, L-glucose, galacturonic acid, and D-fucose as well. Moreover, the enzyme was able to efficiently utilize TTP, GTP, UTP, or CTP in the place of ATP as phosphate donor. In order to understand the sugar specificity at a molecular level, the enzyme-sugar binding models have constructed. It has been shown that the sugar specificity is probably dependent on the interaction energy occurred by the positional proximity of sugars bound in the active site of the enzyme.

Keywords: Galactokinase, Sugar kinase, Alkaliphilic enzyme, *Thermus caldophilus* GK24.