

Crystal structure of Pyruvate kinase from *Bacillus stearothermophilus*

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Introduction

Pyruvate kinase (PK) plays a major role in the regulation of glycolysis. The enzyme pyruvate kinase from the moderate thermophile *Bacillus stearothermophilus* (BstPK) was allosterically activated by AMP and ribose 5-phosphate but not by fructose 1,6-bisphosphate (FBP). However, it is known that almost all PKs will receive activation by FBP.

Experimental

Crystals were obtained at 12°C by the hanging drop vapor diffusion method, from solutions containing 2.1 M ammonium sulfate and 0.1M MES at pH 6.5. Crystal grew in 1 or 2 weeks. The space group was determined to be P6222, with cell dimensions $a, b = 145.9$

, $c = 118.0$ Å, and one protein molecule per asymmetric unit. Somehow sufficient reflection data with native protein was not obtained, so the crystals of mutant C9S/C268S were used for further data collection and analysis. The crystal structure was solved by the molecular replacement method using the structure of *E.coli* PKI (PDB entry 1PKY) as the search model..

Conclusions

The structure of BstPK A and B domains is very similar to those of *E.coli* PKI. The C terminal extra sequence of BstPK form a new domain and we named C' domain. Compared to YeastPK FBP binding site¹, BstPK putative effector binding site structure is closed. This may explain why FBP does not activate BstPK.

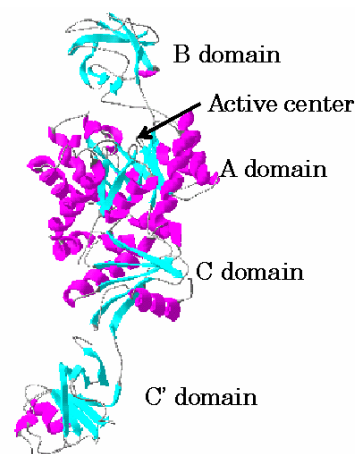


Figure 1 Ribbon diagram of BstPK C9S/C268S mutant.

References

[1] Melissa S Jurica et al., Structure Vol 6 No2 195-210(1998)

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