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The Structure of a Thermally Stable 3-Phosphoglycerate Kinase and a Comparison With Its Mesophilic Equivalent

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ABSTRACT **The structure of the phosphoglycerate kinase (PGK) from *Bacillus stearothermophilus*, a moderate thermophile, has been determined and compared with that of its mesophilic equivalent from yeast. The *Bacillus* enzyme structure was solved by molecular replacement and improved using constrained rigid-body, molecular dynamics and conventional refinement procedures. The refinement residual, calculated using all the measured data between 8 and 1.65 Å, is 0.18(1). The stereo chemical deviations of the final model from ideality are 0.01 Å for both bonds and planes.**

The mid-point temperatures of the *Bacillus* and yeast enzymes are 67 and 53°C, respectively. Differential scanning calorimetry indicates that the energy difference ($\Delta\Delta G$) between the mesophilic and thermophilic enzymes is of the order of 5 kcal mol^{-1} at room temperature. The structure comparison indicates that the features most likely to be responsible for the increased thermal stability of the *Bacillus* enzyme are the increased internal hydrophobicity, additional ion pairs, and better α-helix stability resulting from the removal of helix destablising residues and extra helix–dipole/helix side chain ionic interactions.

Key words: phosphoglycerate kinase, *Bacillus stearothermophilus*, crystallographic structure, thermal stability, increased hydrophobicity, helix stability

INTRODUCTION

Although some attempts have been made to relate the property of enhanced thermal stability to features inherent in the structures of homologous mesophilic/thermophilic enzymes, most of the published work in this area has been concerned with the comparison of amino acid sequences. Perutz and Raidt[1] reported that the stability of the ferredoxin structure was enhanced by the formation of extra salt-bridges and hydrogen bonds. Wonacott and colleagues[2] observed ion pair enhancement and also an increase in the number of hydrophobic interactions when comparing the structures of the enzyme glyceraldehyde-3-phosphate dehydrogenase derived from a mesophile (lobster) and a thermophilic organism (*Bacillus stearothermophilus*). Rossmann and colleagues[3] took these observations a step further by carrying out a statistical analysis of the differences in amino acid composition of various mesophilic and thermophilic dehydrogenases and ferredoxins whose structures were known. Their conclusions were that the enhancement of thermal stability is brought about by a combination of increasing the volume of hydrophobic residues involved in internal packing, decreasing the hydrophobic surface area, and increasing the proportion of "good" helix forming residues in α-helical regions of the structure.

As part of a multidisciplinary study of the thermal stability of proteins we have isolated phosphoglycerate kinase from a series of thermophilic organisms.[4–6] These different genes have been cloned and sequenced and suitable overexpression systems developed for subsequent work using site-directed mutagenesis. It is intended that the overexpressed native and mutant protein will be used in various biophysical experiments, including structure determination, aimed at understanding the reasons for the evolutionary changes required for the enzyme to function under different operating conditions. The validity of comparative deductions will be tested, genetically, by altering the mesophilic yeast enzyme to include the relevant features of the thermophilic proteins. It is argued that such a systematic, experimentally based, study will help define those factors used in vivo to enhance thermal stability. A similar, though less biophysically oriented, study has been undertaken on a group of proteases.[7] In that work the information explicit in the sequences of mesophilic and thermophilic enzymes has been used to

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direct protein engineering experiments intended to enhance the thermal stability of those proteins. In addition, Matthews and co-workers have demonstrated improvement in the stability of bacteriophage T4 lysozyme using specifically designed mutant proteins based on the X-ray structure of the protein.[8–10]

Phosphoglycerate kinase (PGK) is the enzyme responsible for the first substrate level phosphorylation in glycolysis. In view of its role in such an important biochemical pathway it is perhaps not surprising that its structure appears to have been highly conserved throughout evolution.[11] The tertiary structure is composed of two distinct domains joined by a waist region. It has been suggested by Blake and colleagues[12] that the two domains change their relative orientation during catalysis. Here we report on the structure determination of the PGK from the thermophilic bacterium *Bacillus stearothermophilus* from crystals grown in the presence of the nucleotide substrate. The structure of this thermophilic enzyme, which is stable at temperatures in excess of 65°C, is compared with that of the mesophilic yeast enzyme. Differences between the two structures, particularly those that appear to relate to thermal stability, are defined.

METHODS

Protein Preparation and Crystallization

Bacillus stearothermophilus (NCA-1503) PGK was prepared initially from a large-scale multienzyme preparation. Subsequent preparations of the enzyme utilized a strain of *Escherichia coli* modified so as to overexpress *B. stearothermophilus* PGK.[6] Crystals were grown at 18°C by vapor-phase diffusion using the hanging-drop technique with 30–40% polyethylene glycol 600 (PEG 600) as the precipitant as described previously.[13] The protein concentration was 15 mg ml^{-1} in a solution buffered with 8 mM Tris-HCl (pH 7.0), with the addition of 1 mM MgATP, 0.1 mM NaN_3, and 10% (v/v) PEG 600.

X-Ray Data Collection

Three-dimensional data sets were collected on film at the Daresbury synchrotron and on the Hendrix-Lentfer image plate at the EMBL Hamburg outstation. The lower resolution (3.5 Å) film set, collected from one crystal, was recorded using short exposure times so as to ensure that the low order terms were not overloaded. Two crystals were required to complete the 1.65 Å resolution data set. The higher resolution data were collected using the EMBL beam line, X-11, and the imaging plate scanner. The images were processed using a modified version of the MOSFLM suite of programs (Imperial College, London) essentially as described by Dauter and colleagues.[14] Full details of the data collection and subsequent refinement of the PGK structure will be given elsewhere.

Molecular Replacement

The rotation parameters were determined using the MERLOT suite of programs.[15] The coordinates of the known PGK structure, either those derived for horse muscle[12] or for yeast[16] were placed in artificial P1 unit cells ($a = b = c = 100$ Å, $\alpha = \gamma = 90.0°$, $\beta = 99.8°$) and full space rotation space maps calculated using the fast rotation function.[17] The *R*-factor search program RSEARCH[18] was used to determine the translational parameters.

Refinement

The structure was initially treated as two rigid-bodies, corresponding to the N- and C-terminal domains, and the rotational and translational parameters refined using the program CORELS.[19] Seven cycles of refinement were carried out using data between 10.0 and 5.0 Å. Further molecular improvement was then achieved using the protein structure refinement programs X-PLOR[20] and PROLSQ.[21] Cycles of refinement were interspersed with manual rebuilding using the molecular graphics program FRODO.[22]

Calorimetry

The stability of the thermophilic *B. stearothermophilus* PGK was compared to yeast PGK by differential scanning calorimetry (DSC) using a Microcal MC2-D instrument, fitted with an EM Electronics model N2a nanovolt preamplifier, at a scan rate of 30 Deg hr^{-1}. Protein samples for DSC were dialyzed extensively against buffer (50 mM PIPES pH 7.0, containing 0.1 mM dithiothreitol and 0.05% Na azide) and centrifuged before use. Sample and reference buffer solutions were degassed under vacuum, with gentle stirring, before loading.

RESULTS

Structure Determination and Refinement

Using the seeding technique described by Davies and co-workers[13] single crystals of *B. stearothermophilus* PGK suitable for high resolution data collection (see Fig. 1) grew in 4–14 days. The native data set obtained using the image plate device, contained 123,309 observations relating to 44,754 unique reflections. Calculations show that this data set constitutes some 94.6% of the unique data between 10 and 1.65 Å. The resolution shell containing the reflections beyond 1.7 Å contained 93.6% of the data. The agreement factor (R_{merge}) was 0.056 for the complete data set and only 0.156 for the data beyond 1.7 Å resolution.

Both the yeast and horse model structures gave a single rotation function peak corresponding to an Eulerian rotation of $\alpha = 20°$, $\beta = 45°$, $\gamma = 5°$. The best result (an rms of 6.9 compared to 4.3 with the yeast structure) was obtained using the horse PGK as a model structure, presumably reflecting the im-

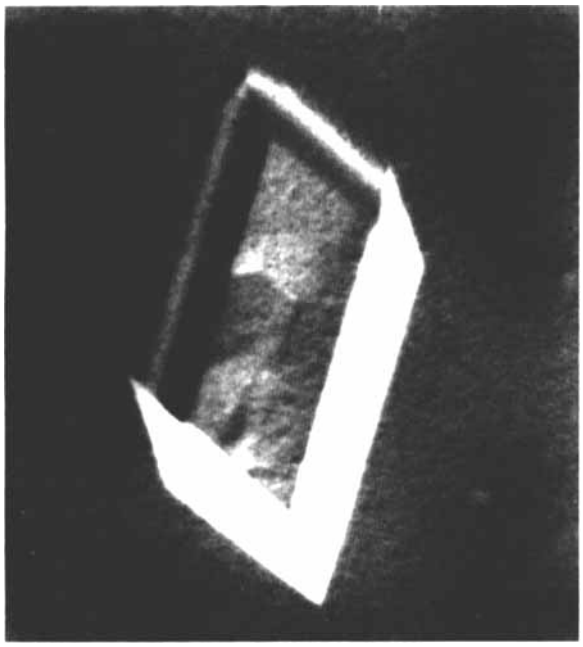

Fig. 1. Crystals of *B. stearothermophilus* PGK grown by vapor-phase diffusion, using the hanging-drop method, from 40% (v/v) PEG 600, 8 mM Tris-HCl pH 7.0, 0.05% (w/v) NaN_3 and in the presence of the nucleotide substrate MgATP. The crystal shown had dimensions close to 0.6 × 0.4 × 0.3 mm.

portance of using the most highly refined set of coordinates available for such calculations. The correctly oriented PGK coordinates gave a single peak in the *R*-factor search procedure resulting in a value (between 10 and 5 Å resolution) some 6% lower than at any other position in the unit cell.

The amino acid sequence of the *Bacillus* enzyme was built into the initial molecular replacement model and the resulting structure refined as two rigid bodies, corresponding to the N- and C-terminal domains using CORELS.[19] Seven cycles of refinement resulted in an *R*-factor drop of some 4% (data between 10 and 5 Å) with the N-terminal domain moving by approximately 4° relative to the C-terminal domain.

In order to check the validity of the molecular replacement solution a set of phases were calculated using the coordinates derived following the rigid-body refinement. These phases were used, together with the appropriate amplitude information, to calculate a difference Fourier map. The resulting difference Fourier map contained one major peak whose position agreed with the single site solution obtained from the corresponding mercury acetate difference Patterson map. This peak was some 4.5 Å from the position, predicted by the molecular replacement calculations, for the sulfur atom of the single cysteine of the *B. stearothermophilus* PGK.

The subsequent refinement of the structure derived using the molecular replacement technique produced relatively large movements of atoms located in a number of the loop regions but only small movements elsewhere. The current crystallographic *R*-factor, for all the data between 8 and 1.65 Å, is 0.18(1). This measure of the agreement between the observed and calculated structure amplitudes was obtained using a model which has rms deviations from stereochemical ideality of 0.010 Å for covalent bond distances and 0.037 Å (1–3 bonding distance) for bond angles between covalently linked atoms. A summary of the stereo chemistry of the final model is given Table I.

Thermodynamic Properties

The differential scanning calorimetry experiments show that both yeast and *B. stearothermophilus* PGK underdo endothermic transitions typical of the cooperative thermal unfolding of globular proteins. The transition mid-point temperature (T_m = 67°C) of the thermophilic enzyme is some 13–14 degrees higher than yeast PGK (Cooper and Whytelaw, unpublished results) under the same conditions. The overall integrated calorimetric enthalpies are of similar magnitude (ΔH_m 130–140 kcal mol^{-1}), but the relatively large uncertainties (±15 kcal mol^{-1}) prevent us from determining whether the major source of stabilization of the *B. stearothermophilus* PGK (amounting to a free energy difference, $\Delta\Delta G$, of the order of 5 kcal mol^{-1} at room temperature) is entropic or enthalpic.[23]

DISCUSSION

Domain Orientation

Low angle X-ray scattering experiments have indicated that yeast PGK becomes more compact on binding one, or both of its substrates.[24] Exhaustive attempts to crystallize the "closed" or substrate-bound form of the yeast enzyme have proved unsuccessful and have led to the suggestion that the bound sulphate ions may, in some way, prevent domain movement. Figure 2 shows the structures of the refined yeast (unpublished results) and *B. stearothermophilus* structures superimposed. The most striking feature of this comparison is that, despite considerable sequence diversity and different crystallization conditions, the structures are remarkably similar. There is, however, a small, but significant, difference in the orientation of the two domains. This may be considered as a "closing" of the two domains of the *B. stearothermophilus* PGK by approximately 4.2°. Although this does not correspond to the large change in domain orientation predicted from solution studies, it is nevertheless a significant finding. A similar small change in the relative domain orientation was found by Blake and his colleagues[25] when they compared the structure of the 3-PGA pig muscle PGK with that of the substate free enzyme. The *B. stearothermophilus* PGK crystal structure, with its bound nucleotide, sug-

TABLE I. Observed Deviations of the Stereo Chemistry of the Model From Ideality for the Final *B. stearothermophilus* PGK Model*

		Target σ	Observed σ	Number of parameters
Distances (A)				
Bond lengths	(1–2 neighbors)	0.020	0.010	3093
Bond angles	(1–3 neighbors)	0.040	0.034	4174
Dihedral angles	(1–4 neighbors)	0.050	0.037	987
Planar groups		0.020	0.010	527
Chiral volumes (Å^3)		0.060	0.035	469
Nonbonded contacts (Å)				
Single torsion contacts		0.300	0.175	1029
Multiple torsion contacts		0.300	0.259	1180
Torsion angles (°)				
Peptide plane (ω)		5	2.5	413
Staggered		15	17	564
Orthonormal		20	41	33

*The model consists of 3,009 protein atoms, 27 nucleotide substrate atoms, one magnesium ion, and 265 water molecules. The coordinates from which this table was derived will be deposited with the Brookhaven Protein Data Bank.

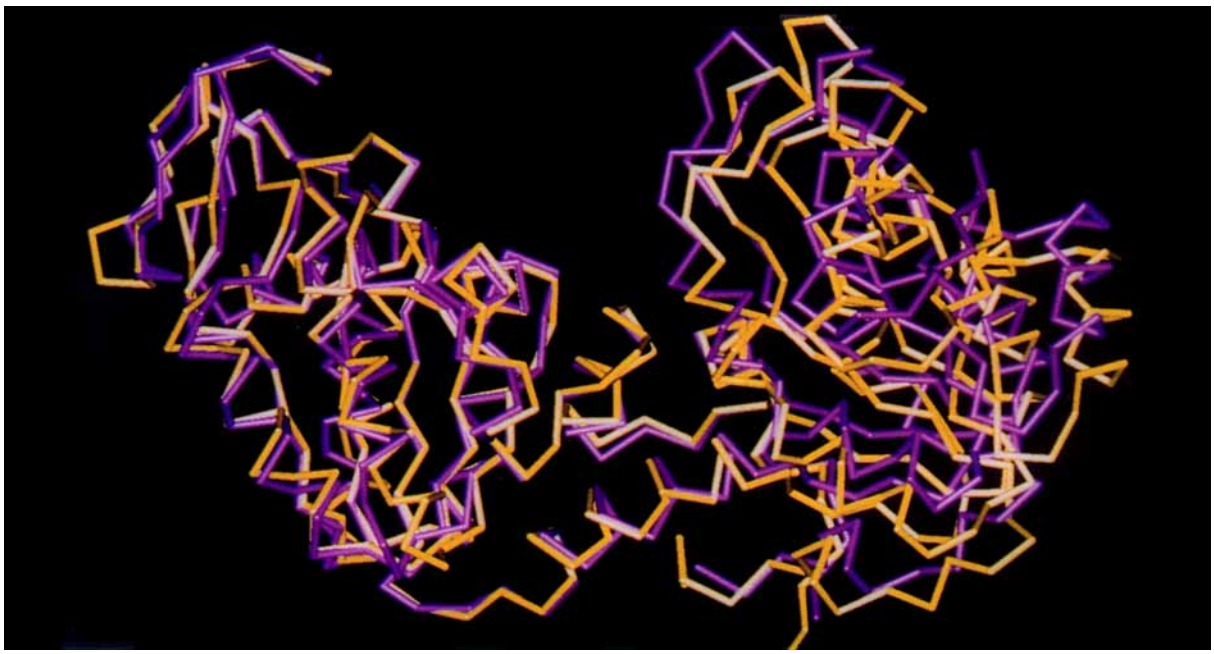

Fig. 2. The superimposed α-carbon backbones of the phosphoglycerate kinases from *Bacillus stearothermophilus* (mauve) and yeast (yellow). The α-carbon coordinates of the C-terminal amino acids were superimposed using the QUANTA program (Molecular Simulations Inc.).

gests that, although a conformational change could occur upon nucleotide binding, a major domain movement does not occur in the absence of the sugar substrate.

Structural Features Relating to Thermal Stability

Alignment of the sequences of PGK from the thermophilic bacteria *Bacillus stearothermophilus* and *Thermus thermophilus* with that from yeast[6] reveals trends in amino acid substitutions consistent with the attainment of thermal stability (for reviews see refs. 3 and 26–28). The most significant features of the *B. stearothermophilus* sequence are (1) a decrease in the serine and threonine content, mainly accounted for by substitution to alanine and either aspartic or glutamic acids, and (2) a decrease in the content of lysine with a concomitant increase in arginine and glutamate residues.

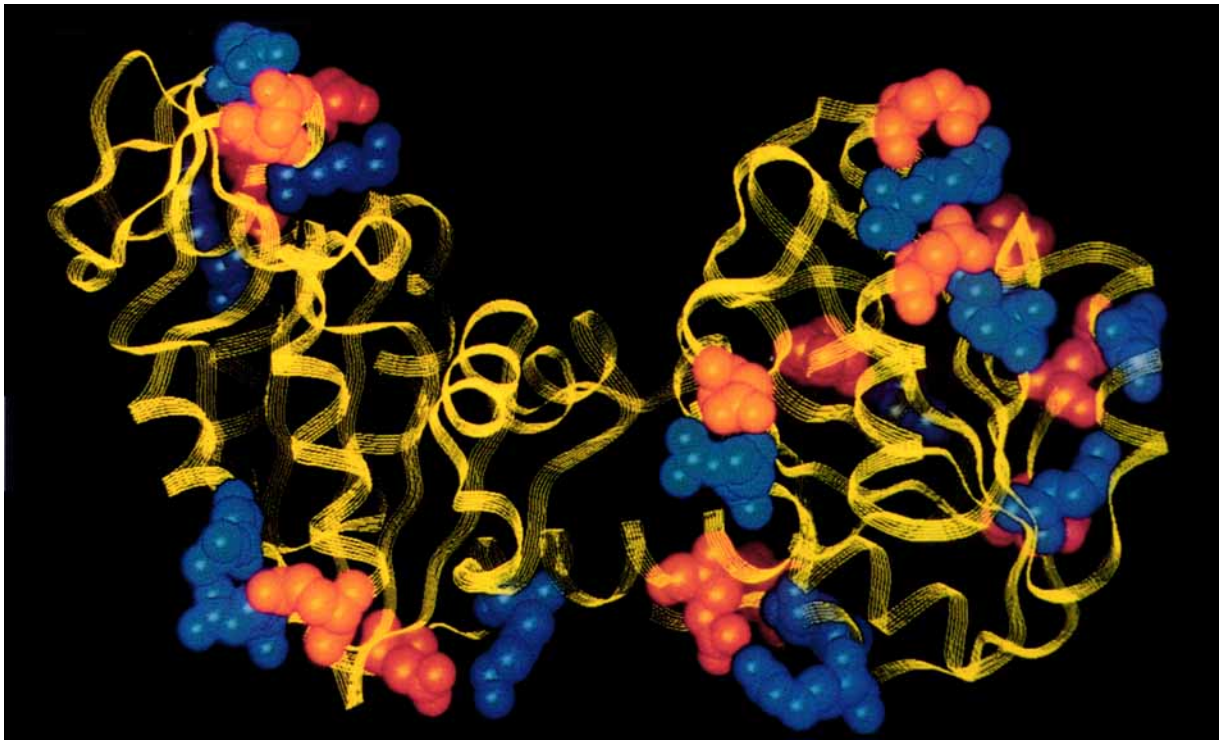

Fig. 3. A diagram of the main chain of *B. stearothermophilus* PGK displayed as a ribbon using the INSIGHT software package (Biosym Technologies, San Diego, CA). Ion pairs formed by residues, whose charged groups are less than 4 Å apart and which are present in the *B. stearothermophilus* PGK structure but not in the yeast structure, are indicated in space filling mode. Positively charged side chains are shown in blue and negatively charged side chains in red.

It was proposed by Rossmann and colleagues[3] that the decrease in serine and threonine residues found in thermally stable proteins occurs mainly in helical regions, these amino acids being substituted for those with a greater propensity for helix formation.[29] This observation has subsequently been refined[30] as a result of comparing some 70 mesophilic/thermophilc proteins from six different enzyme families. This more detailed comparison shows that serine to alanine substitutions preferentially occur in helices whereas threonine to alanine substitutions occur in regions of β-sheet. The *B. stearothermophilus* enzyme has some 20 fewer serine and threonines than the mesophilic yeast enzyme (similar trends are observed for any pairwise comparison with other PGK sequences from a mesophilic organism). Inspection of the structure presented here shows that three of the four threonines changed to alanine are located in β-sheet regions of the *Bacillus* structure whereas only one of the four serine to alanine substitutions occurs in α-helices.

The most striking feature of the *B. stearothermophilus* PGK sequence, when compared to its mesophilic counterparts, is the decrease in the number of lysine residues. These residues are mainly replaced by arginine and glutamate. Although the extra arginine residues do not appear to be located in special positions of the structure, six out of the eight lysine residues replaced by glutamate occur in α-helical regions. This observation is contrary to that made by Menendez-Arias and Argos[30] who reported that such changes occur in regions of protein joining elements of secondary structure. In support of the present finding is the fact that glutamate is known to be a better helix stabilizer than lysine.[29] This lysine to glutamate change may be thought, therefore, as contributing to the helix stability of the thermophilic protein. This could be brought about by the interaction of the carboxyl side chain with the helical dipole at the amino-end of the helix.[31] It appears as though 6 of the 13 helices in *Bacillus* PGK are stabilized relative to those of the yeast enzyme by the replacement of an uncharged residue with one that has a charge more suitable for its position within the helix.

An analysis of the *B. stearothermophilus* PGK structure for oppositely charged groups in a position to make ionic interactions reveals that it contains some 15 such interactions which do not occur in the yeast enzyme. Analysis of the locations of these ionic interactions shows that their positions are far from random. Only five of the these extra ionic interactions are unique to the C-terminal domain; the remainder are formed either in the N-terminal do-

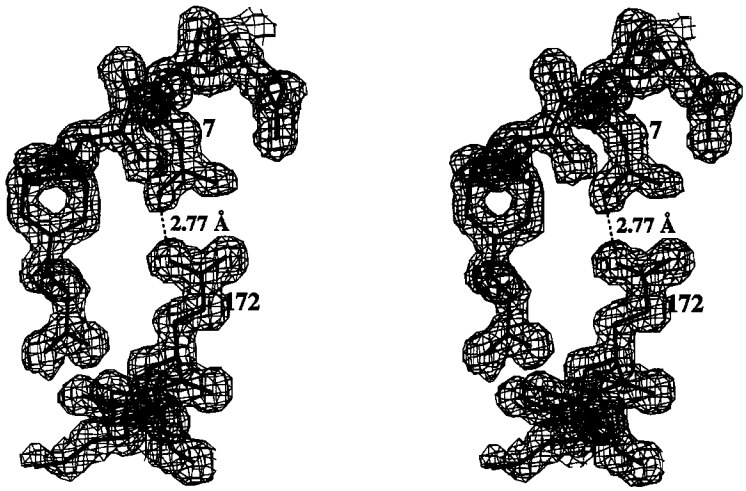

Fig. 4. A section of the $|2F_{obs} - F_{calc}|\ \alpha_{calc}$ Fourier map of *B. stearothermophilus* PGK at 1.65 Å showing a typical ionic interaction that is present in the *B. stearothermophilus* PGK structure but that does not occur in mesophilic PGKs. The map is contoured at 0.33 $eÅ^{-3}$. The stereo diagram shows the ion pair formed between side chain of aspartic acid residue 172 and arginine residue 7.

main or in the domain interface as is shown in Figure 3. In the mesophilic PGKs the N-terminal domain is known to be less stable than is the C-terminal counterpart[32] and it is tempting to speculate that these ionic interactions provide a means of increasing the stability of that domain. Closer inspection of the extra salt bridges that may be enhancing the stability of the N-terminal domain shows that they are shielded from the solvent where they invariably link secondary structural elements that are distant in the primary sequence, as is shown in Figure 4. Support for the hypothesis that these extra salt bridges are contributing to the enhanced thermal stability also comes from a structural analysis of the yeast and *T. thermophilus* sequences.[5] That comparison shows that the amino acid changes that give rise to oppositely charged groups in suitable positions for the formation of salt links in the *B. stearothermophilus* structure are also present in the *T. thermophilus* sequence.

CONCLUSIONS

There are some 52 possible ion pairs in the *B. stearothermophilus* PGK structure compared with only 37 in yeast. This feature of the mesophilic/thermophilic PGK comparison is therefore in agreement with the observations relating to the ferredoxin and dehydrogenase proteins. A critical role for the charged groups in stabilizing α-helices has been demonstrated by both Matthews[9] and by Shoemaker[33] and their colleagues. Presumably these stabilizing interactions are, by their nature, shielded from the solvent. Such interactions have been observed for *B. stearothermophilus* PGK and would appear to be responsible for part of the enzyme's enhanced thermal stability.

Apart from the ion pair observation described above, it is also reasonably clear from this study that the role of the decreased number of serine and threonine residues in enhancing protein thermal stability is not merely to improve helix stability as reported by Rossmann and colleagues. Other observations appear significant but they require confirmation either from comparisons with other thermophilic PGKs or by direct experimental observation. For example, several of the loop regions connecting the elements of secondary structure are "shorter" in the thermophilic PGKs. These shorter loop regions are characterized by additional proline and/or glycine residues. It has been argued that additional proline residues contribute toward protein stability by reducing the entropy of the unfolded state.[8] The role of the glycine residues in providing additional protein stability, if there is one, is currently being tested by replacing the loop regions in yeast with their thermophilic equivalents.

In this paper we have presented the structure of a thermally stable 3-phosphoglycerate kinase and have concentrated on its significance with respect to the understanding of protein thermal stability. It should not be overlooked, however, that this is the first reported structure of a prokaryotic PGK. It is also the first reported structure of a PGK crystallized in the absence of high-salt and together with its nucleotide substrate. As such, it has provided valuable information regarding the conformational change which has been postulated to occur during catalysis.

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REFERENCES

1. Perutz, M.F., Raidt, H. Stereo chemical basis of heat stability in bacterial ferredoxins and haemoglobin A2. Nature (London) 255:256–258, 1975.
2. Walker, J.E., Wonacott, A.J., Harris, J.I. Heat Stability of a tetrameric enzyme, D-glyceraldehyde-3-phosphate dehydrogenase. Eur. J. Biochem. 108:581–586, 1980.
3. Argos, P., Rossmann, M.G., Grau, U.M., Suborn, H., Frank, G., Tratschin, J.D. Thermal stability and protein structure. Biochemistry 18:5698–5703, 1979.
4. Littlechild, J.A., Davies, G.J., Gamblin, S.J., Watson, H.C. Phosphoglycerate kinase from the extreme thermophile *Thermus thermophilus*. FEBS Lett. 225:123–126, 1987.
5. Bowen, D., Littlechild, J.A., Fothergill, J.E., Watson, H.C., Hall, L. Nucleotide sequence of the phosphoglycerate kinase gene from the extreme thermophile *Thermus thermophilus*. Biochem. J. 254:509–517, 1988.
6. Davies, G.J., Littlechild, J.A., Watson, H.C., Hall, L. Sequence and expression of the gene encoding 3-phosphoglycerate kinase from *Bacillus stearothermophilus*. Gene 109: 39–45, 1991.
7. Imanaka, T., Shibazaki, M., Takagi, M. A new way of enhancing the thermostability of proteases. Nature (London) 324:695–697, 1986.
8. Matthews, B.W., Nicholson, H., Becktel, W.J. Enhanced protein thermostability from site directed mutants that decrease the entropy of unfolding. Proc. Natl. Acad. Sci. U.S.A. 84:6663–6667, 1987.
9. Nicholson, H., Becktel, W.J., Matthews, B.W. Enhanced protein thermostability from designed mutations that interact with α-helix dipoles. Nature (London) 336:651–656, 1988.
10. Matsumara, M., Wozniak, J.A., Dao-pin, S., Matthews, B.W. Structural studies of mutants of T4 lysozyme that alter hydrophobic stabilization. J. Biol. Chem. 264:16059–16066, 1989.
11. Watson, H.C., Littlechild, J.A. Isoenzymes of phosphoglycerate kinase: Evolutionary conservation of the structure of this glycolytic enzyme. Biochem. Soc. Transact. 18:187–190, 1990.
12. Banks, R.D., Blake, C.C.F., Evans, P.R., Haser, R., Rice, D.W., Hardy, G.W., Merrett, M., Phillips, A.W. Sequence, structure and activity of phosphoglycerate kinase: a possible hinge-bending enzyme. Nature (London) 279:773–777, 1979.
13. Davies, G.J., Gamblin, S.J., Littlechild, J.A., Watson, H.C. Purification, crystallisation and preliminary X-ray analysis of the 3-phosphoglycerate kinase from *Bacillus stearothermophilus*. J. Mol. Biol. 227:1263–1264, 1992.
14. Dauter, Z., Terry, H., Witzel, H., Wilson, K.S. Refinement of glucose isomerase from *Streptomyces albus* at 1.65Å with data from an imaging plate. Acta Crystallogr. B46: 833–841, 1990.
15. Fitzgerald, P.M. MERLOT, an integrated package of computer programs for the determination of crystal structures by molecular replacement. J. Appl. Crystallogr. 21:274–278, 1988.
16. Watson, H.C., Walker, N.P.C., Shaw, P.J., Bryant, T.N., Wendell, P.L., Fothergill, L.A., Perkins, R.E., Conroy, S.C., Dobson, M.J., Tuite, M.F., Kingsman, A.J., Kingsman, S.M. Sequence and structure of yeast phosphoglycerate kinase. EMBO J. 1:1635–1640, 1982.
17. Crowther, R.A. The Fast Rotation Function. In "The Molecular Replacement Method: A Collection of Papers on the Use of Non-crystallographic Symmetry." Rossmann, M.G., ed. New York: Gordon and Breach, 1972.
18. CCP4. The SERC (U.K.) Collaborative Computing Project No. 4, a Suite of Programs for Protein Crystallography, distributed from the Daresbury Laboratory, Warrington, WA4 4AD, U.K., 1979.
19. Sussman, J.L., Holbrook, S.R., Church, G.M., Kim, S-H. A structure-factor least-squares refinement procedure for macromolecular structure using constrained and restrained parameters. Acta Crystallogr. A33:800–804, 1977.
20. Brünger, A.T. Crystallographic refinement by simulated annealing. J. Mol. Biol. 203:803–816, 1988.
21. Konnert, J.H., Hendrickson, W.A. Stereochemically restrained crystallographic least-squares refinement of macromolecular structures. In: "Biomolecular Structure, Conformation, Function and Evolution," Vol. I. Srinivasen, R., ed. Oxford: Pergamon Press, 1981: pp. 43–57.
22. Jones, T.A. Interactive Computer Graphics: FRODO. Methods Enzymol. 115:157–171, 1985.
23. Johnson, C.M., Cooper, A., Brown, A.J.P. A comparison of the reactivity and stability of wild type and His388→Gln mutant phosphoglycerate kinase from yeast. Eur. J. Biochem. 202:1157–1164, 1991.
24. Ptitsyn, O.B., Pavlov, M. Yu., Sinev, M.A., Timchenko, A.A. "Multidomain Proteins," Patthy, L., Friedrich, P. eds. Budapest: Akadémiai Kiadó, 1986:2–18.
25. Harlos, K., Vas, M., Blake, C.F. Crystal structure of the binary complex of pig muscle phosphoglycerate kinase and its substrate 3-phospho-D-glycerate. Proteins 12:133–144, 1992.
26. Mozhaev, V.V., Martinek, K. Structure-stability relationships in proteins: new approaches to stabilising enzymes. Enzyme Microb. Technol. 6:50–59, 1984.
27. Goldenberg, D.P. Genetic studies of protein stability and mechanisms of folding. Annu. Rev. Biophys. Chem. 17: 481–507, 1988.
28. Mozhaev, V.V., Berezin, I.V., Martinek, K. Structure stability relationship in proteins: Fundamental tasks and strategy for the development of stabilized enzyme catalysts for biotechnology. CRC Crit. Rev. Biochem. 23:235–281, 1988.
29. Chou, P.Y., Fasman, G.D. Conformational parameters for amino acids in helical, sheet, and random coil regions calculated from proteins. Biochemistry 13:211–222, 1974.
30. Menendez-Arias, L., Argos, P. Engineering protein thermal stability: Sequence statistics point to residue substitution in α-helices. J. Mol. Biol. 206:397–406, 1989.
31. Hol, W.G.J., van Duijnen, P.T., Berendsen, H.J.C. The α-helix dipole and the properties of proteins. Nature (London) 273:443–446, 1978.
32. Fairbrother, W.J., Minard, P., Hall, L., Betton, J-M., Missiakas, D., Yon, J.M., Williams, R.J.P. Nuclear magnetic resonance studies of isolated structural domains of yeast phosphoglycerate kinase. Protein Eng. 3:5–11, 1989.
33. Shoemaker, K.R., Kim, P.S., York, E.J., Stewart, J.M., Baldwin, R.L. Tests of the helix dipole model for stabilization of α-helices. Nature (London) 326:563–567, 1987.