Allosteric Mechanism of Pyruvate Kinase from *Leishmania mexicana* Uses a Rock and Lock Model*

Hugh P. Morgan¹, Iain W. McNae², Matthew W. Nowicki³, Véronique Hannart⁴, Paul A. M. Michels⁵, Linda A. Fothergill-Gilmore⁶, and Malcolm D. Walkinshaw⁷

From the Structural Biochemistry Group, Institute of Structural and Molecular Biology, University of Edinburgh, Michael Swann Building, King’s Buildings, Mayfield Road, Edinburgh EH9 3JH, Scotland, United Kingdom and the Research Unit for Tropical Diseases, de Duve Institute and Laboratory of Biochemistry, Université Catholique de Louvain, Avenue Hippocrate 74, B-1200 Brussels, Belgium

Allosteric regulation provides a rate management system for enzymes involved in many cellular processes. Ligand-controlled regulation is easily recognizable, but the underlying molecular mechanisms have remained elusive. We have obtained the first complete series of allosteric structures, in all possible ligated states, for the tetrameric enzyme, pyruvate kinase, from *Leishmania mexicana*. The transition between inactive T-state and active R-state is accompanied by a simple symmetrical 6° rigid body rocking motion of the A- and C-domain cores in each of the four subunits. However, formation of the R-state in this way is only part of the mechanism; eight essential salt bridge locks that form across the C-C interface provide tetramer rigidity with a coupled 7-fold increase in rate. The results presented here illustrate how conformational changes coupled with effector binding correlate with loss of flexibility and increase in thermal stability providing a general mechanism for allosteric control.

Allosteric regulation (“the second secret of life” quotation ascribed to Jacob Monod) (see Ref. 1) controls many important cellular processes, including signal transduction, transcription, and metabolism (2). It describes the effect of binding one ligand on the subsequent binding of a second ligand at a topographically distinct site. Human pyruvate kinase (hPYK)² provides a striking example of the significance of allosteric regulation: a splicing switch of the primary RNA transcript to yield the M1 or M2 isoenzymes is now known to be responsible for the Warburg effect in cancer (3, 4) and opens up the possibility of developing isofrom specific therapeutics (5). Additional mechanisms of activity regulation, including the binding of amino acids, phosphorylation, and the binding of oncoproteins, may provide further therapeutic approaches for targeting hPYK (6, 7).

Most allosterically regulated proteins are enzymes in which the binding of an activator or inhibitor to the effector site can affect the binding of a substrate at the active site. The Monod-Wyman-Changeux model of allostery (8) suggests that oligomeric enzymes undergo symmetrical transitions (classically between the T- and R-states)³ (36) that can be stabilized by ligand binding. However, there are now examples of allosteric control that do not show obvious conformational change (9), and a growing body of work exists to support the idea that flexible regions of molecules (which exist as an ensemble of conformers) may undergo allosteric regulation by changes in the conformer population (10). There are examples of allosteric enzymes in which the same protein has been captured in both a T-state (which has low affinity for substrate) and an R-state (which has higher affinity for substrate), and some structural insight has been obtained by the study of at least one of the allosteric states of >50 proteins (10–12). However, a full understanding of the allosteric effect requires structural information on each of four states: (i) apoenzyme, (ii) active site complex, (iii) effector site complex, and (iv) complex with filled active and effector sites. For allosterically regulated enzymes, no such data exist (see supplemental Table S1), and in many cases even when the T- and R-state structures are available, they have been examined under different conditions (of differing pH and salt concentrations) (12), all of which can affect structural conformation. This lack of a consistent structural series has to date precluded the full systematic study of allosteric mechanisms. Here, we present the structures and thermodynamic properties of the four allosteric states of LmPYK, the pyruvate kinase from *Leishmania mexicana*, a protozoan parasite belonging to the trypanosomatid family.

Pyruvate kinases are homotetrameric enzymes that catalyze the final reaction of glycolysis in which phosphoenolpyruvate and ADP are converted into pyruvate and ATP. PYK monomers (50–60 kDa depending on species) are composed of four
domains: the N-terminal, A-, B-, and C-domains (Fig. 1). The C-domain houses the effector site which is located 40 Å from the active site. Adjacent C-domains form the C-C or “small” interface, and bordering A-domains form the A-A or “large” interface. The B-domain forms a mobile lid at one end of the A-A interface, and the active site lies in the cavity between them.

Allosteric enzyme behavior of PYKs may be manifested either through the binding of the effector molecule or through the binding of the substrate phosphoenolpyruvate (13, 14). In mammals and many other species fructose 1,6-bisphosphate (Fru-1,6-BP) acts as the effector, but trypanosomatid PYKs are allosterically activated by fructose 2,6-bisphosphate (15). PYK is particularly suitable for studying allosteric regulation because unlike many other allosteric enzymes, the product does not bind to the effector site and participate in allosteric regulation. The PYK allosteric mechanism has also been investigated using a large number of site point mutants (16–19). A general conclusion from the wealth of data is that intersubunit interactions on the A-A and C-C interfaces strongly influence the allosteric effect whereas mutations affecting the intrasubunit A-C interface are less sensitive (20).

EXPERIMENTAL PROCEDURES

Expression and Purification—LmPYK was overexpressed and purified by a modified version of the published protocol (21). Sulfate molecules are commonly observed bound to the effector and active sites of LmPYK structures (22), competing with the binding of the natural glycolytic ligands. Additional purification steps were introduced to remove contaminating sulfate molecules. Briefly, LmPYK-sulfate samples were concentrated and buffer exchanged (PD-10 column; Amersham Biosciences) into buffer A (50 mM triethanolamine-HCl (TEA) buffer (pH 7.2), 20 mM KCl, 20% glycerol) using standard protocols. LmPYK samples were loaded onto a (35-ml) DEAE-Sepharose ion-exchange column at 0.5 ml min⁻¹, preequilibrated in buffer A. The column was washed (3.0 ml min⁻¹) with 10 column volumes of buffer A. LmPYK was eluted over a 5-column volume elution gradient (0–60%) with buffer B (50 mM TEA buffer (pH 7.2), 200 mM KCl, 20% glycerol). Fractions containing LmPYK were pooled, concentrated, and buffer exchanged into buffer C (20 mM TEA buffer (pH 7.2) and 20% glycerol). LmPYK samples were concentrated to 30-mg ml⁻¹, and aliquots were stored at −20 °C for up to 3 months.

Site-directed Mutagenesis and Characterization—Site-directed mutagenesis of the L. mexicana PYK gene was performed on plasmid pET28a-LmPYK (17). For each mutation, two complementary oligonucleotides containing the desired mutation were synthesized. The total volume of amplification mixture was 50 µl containing 100 ng of plasmid, 0.5 µg of each primer, a 200 µM concentration of each of the four deoxynucleotides, and 2.5 units of Pfu polymerase. PCR was performed using the following program: first 1 min 95 °C; 16 cycles: 30 s
Pyruvate Kinase Allostery

95 °C, 1 min 55 °C and 14 min 72 °C; and a final incubation of 10 min at 72 °C. 10 units of the DpnI restriction enzyme was then added directly to each amplification reaction. The reaction mixtures were incubated at 37 °C for 1 h to digest the parental DNA and were used to transform Escherichia coli XL1-blue cells. The presence of the mutations and the absence of other changes in the gene were ascertained by sequencing. The mutated plasmids were introduced into E. coli BL21(DE3) for gene expression. The mutant LmPYKs were expressed and purified as described in Ref. 17 but using slightly modified conditions; after induction of expression by the addition of 1 mM isopropyl 1-thio-β-D-galactopyranoside, growth was continued overnight at 16 °C. Enzyme assays and kinetic studies were performed as described previously (17).

Crystallography and Data Collection—Purified LmPYK aliquots (30 mg ml⁻¹) were diluted to 15 mg ml⁻¹ using a buffer containing 20 mM TEA buffer (pH 7.2). For co-crystallization experiments, the appropriate ligand(s) at a final concentration of 3.3 mM were added to the protein sample. All reagents used were of the highest purity available. Both LmPYK and complex crystals were obtained at 4 °C (except ATP/oxalate crystals, which were obtained at 17 °C) by vapor diffusion using the hanging-drop technique (23). The drops were formed by mixing 1.5 μl of protein solution with 1.5 μl of a well solution, composed of 10−16% PEG 8000, 20 mM TEA buffer (pH 7.2), 50 mM MgCl₂, 100 mM KCl, and 10−15% glycerol. The drops were equilibrated against a reservoir filled with 0.5 ml of well solution. Crystals grew to maximum dimensions after 1 week.

Prior to data collection, crystals were equilibrated for 24 h over a well solution composed of 12−18% PEG 8000 (2% above the reservoir concentration), 20 mM TEA buffer (pH 7.2), 50 mM MgCl₂, 100 mM KCl, and 25% glycerol, which greatly improved the resolution of diffraction and eliminated the appearance of ice rings. Intensity data were collected at the European synchrotron radiation facility in Grenoble, France, on beamlines 1-BM14, and also at the Diamond synchrotron radiation facility in Oxfordshire, United Kingdom, on beamline IO3 from single crystals flash frozen in liquid nitrogen at 100 K. Data were then processed with MOSFLM (24) and scaled with SCALA (25).

Structure Determination—Generally, LmPYK structures were solved by molecular replacement using the program PHASER (26). A monomer from the previously determined structure of LmPYK (Protein Data Bank code 1PKL (21) divided into two ensembles, ensemble 1 (residues Pro87−Pro187, a complete B-domain) and ensemble 2 (residues 1−86, 188−481, 489−498), served as search models. The resulting model was divided into three rigid body domains (B-domain, 87−187; A-domain, 1−86 and 187−356; and C-domain, 358−498) and subjected to 10 cycles of rigid body refinement using the program REFMAC (27). When appropriate, ligands and water molecules were added to the models. Models were then subjected to cycles of restrained refinement (except the LmPYK:Fru-2,6-BP (5 Å) model), with manual adjustments to ligands and side chains using the program COOT (28). Figures were generated using PyMOL (29). A more detailed description of the refine-ment processes for each of the different LmPYK crystal structures can be found in the supplemental text.

Protein Data Bank Accession Codes—The atomic coordinates of the apoenzyme (3HQN), ATP and oxalate (3HQO), effector Fru-2,6-BP plus ATP and oxalate (3HQP), and effector only (3HQQ) have been deposited in the Protein Data Bank (entry codes shown in parentheses).

Analysis of Model Geometry—The geometry of the model was assessed using MolProbity (30). Although electron density was well defined for Thr296 (a key active site residue), it commonly exhibits geometry outwith the Ramachandran plot in many PYK structures. This is primarily due to a restricted geometry, which facilitates interactions with active site ligands.

Structural Analysis—Superpositions of PYK structures were performed using both PyMOL and CCP4 superpose. The allosteric rigid body rotations were calculated by simultaneously superposing the A- and C-domains (AC cores, residues 18−86 and 188−480) of the T- and R-state tetramer structures (root mean square fit of the Ca atoms is 1.86 Å). The resulting coordinates were recorded for both structures. Using the new coordinates, CCP4 SUPERPOSE was used to superpose each individual chain of the inactive onto the active structure (the average root mean square fit of the Ca atoms for each chain is 0.58 Å), providing both the centroid and the rotation matrix. The rotation matrices were used to calculate the angle of rotation.

Thermal Shift Assay—Solutions of 25 μl of Sypro Orange (diluted 1/500 in 20 mM TEA buffer (pH 7.2); Molecular Probes), 1 μl of each ligand (ligands were dissolved in 100 mM TEA buffer (pH 7.2) to 100 μM), 5 μl of 10× metal solution (200 mM TEA buffer (pH 7.2), 500 mM MgCl₂, and 1 mM KCl), and 1 μl of 2.0 mg ml⁻¹ protein were added to the wells of a 96-well PCR plate (Bio-Rad), and the final volume was adjusted to 50 μl using 20 mM TEA buffer (pH 7.2). Buffer was added instead of test ligand/metals in the control samples. The plates were sealed with optical quality sealing tape (Bio-Rad) and heated in an i-Cycler iQ5 real-time PCR detection system (Bio-Rad) from 20 to 80 °C in increments of 1 °C. Fluorescence changes in the wells of the plate were monitored simultaneously with a charge-coupled (CCD) camera. The wavelengths for excitation and emission were 485 and 575 nm, respectively. The temperature midpoint for the protein unfolding transition, Tₘ, was calculated using the Bio-Rad iQ5 software.

RESULTS AND DISCUSSION

The T- to R-state Transition: Chain Rotations around a Single Pivot Point—The different LmPYK conformers observed in the four new crystal structures (see supplemental Table S2 for data collection and refinement statistics) were crystallized under near identical (physiological) conditions (pH 7.2, 50 mM MgCl₂, 100 mM KCl, 10−16% PEG 8000). The structure of the apoenzyme (LmPYK in the T-state with no ligands bound; see supplemental text T1) is shown in diagram form in Fig. 2a; the complex with active site ligands (LmPYK-ATP-OX with Mg²⁺ ATP and Mg²⁺ oxalate) in Fig. 2b; the R-state conformer with effector site plus active site ligands (LmPYK-ATP-OX-Fru-2,6-BP, supplemental text T2) in Fig. 2c; and the complex with effector ligand (LmPYK-Fru-2,6-BP) in Fig. 2d. The transition from the inactive (T) to active (R) state involves a rigid body rotation of the A- and C-domains (AC core, residues 18−86
**FIGURE 2. LmPYK crystal structures reveal the allosteric mechanism.**

*a*, schematic representation of the inactive T-state LmPYK structure. The relative positions of the AC core, pivot point (gray circle), active site, effector site, Arg310 (R310), Aα6, and Aα7 helices are shown for the LmPYK tetramer. 

*b*, binding of ATP and oxalate (red lozenges) to the apoenzyme, causing Arg310 to move into the R-state position, forming hydrogen bonds (shown as dashed red lines) with the active site Aα6 helix (for details, see supplemental Fig. S7) of the adjacent chain and a stabilizing bridge at the A-A interface, stabilizing the AC core in an R-conformation. The LmPYK-ATP-OX tetramer consists of two different conformers; conformer 1 has a partially closed B-domain (yellow), and conformer 2 has a fully closed B-domain (red). 

*c*, active R-state LmPYK-ATP-OX-Fru-2,6-BP structure. All monomers have rotated AC cores, fully closed B domains, Fru-2,6-BP-induced salt bridges across the C-C interface, and stabilizing Arg310-Aα6 hydrogen bonds across the A-A interface. 

*d*, binding of Fru-2,6-BP (green rectangles) to the apoenzyme, resulting in the formation of four pairs of stabilizing salt bridges (shown as red lines) formed across each C-C interface, locking the AC core in an R-conformation. The LmPYK-Fru-2,6-BP structure has fully open B-domains. 

*e*, superposition of the inactive T-state AC core structure onto the R-state AC core structure (all B-domains have been removed). The 6° rigid body rotations of the AC cores occurring on the T- to R-state transition have been highlighted using arrows to show the direction of movement.
Pyruvate Kinase Allostery

(a) Structural diagram showing the interaction sites labeled with residues E498, K484, R493, D482, D482, R493, and E498.

(b) Graph showing temperature (celsius) vs. change in fluorescence intensity (dRFU/M). The graph includes key stages such as K\(^2\)Mg\(^2\)\(^+\), Mg\(^2\)ATP:Mg\(^2\)OxK\(^-\), F-2,6-BP:Mg\(^2\)\(^+\), Mg\(^2\)ATP:Mg\(^2\)OxK\(^-\)F-2,6-BP.

(c) Diagrams illustrating T-state, R-state stabilized by R310, R-state locked by F-2,6-BP, and R-state locked by C-domain. Tm values are given for each state.
and 188 – 480) of 6° around a pivot point that lies at the base of the αβ-barrel of domain A (Fig. 2e, supplemental Fig. S2, and supplemental text T2 and animated gif file).

LmPYK Co-crystallized with Substrates Alone Is Stabilized in the R-state—LmPYK with active-site ligands alone adopts an R-state AC core conformation nearly identical to that of the fully ligated complex (as shown by the low (0.39 Å) root mean square fit of the AC cores for LmPYK-ATP-OX and LmPYK-ATP-OX-Fru-2,6-BP), although the B-domains are in different conformations (Fig. 2b and supplemental text T3). The bound oxalate molecule (an analogue of the enol pyruvate moiety of phosphoenolpyruvate and pyruvate) (31), mimics the major interactions of phosphoenolpyruvate and binds the short unstable (22) Aα6° helix in the active site. The 6° rigid body rocking motion (Fig. 2e) of LmPYK moves a crucially important Arg310 side chain (mutation of the equivalent Arg in E. coli PYK results in total inactivity (32), and a mutation to either Lys or Trp results in PYK deficiency in humans (19)) into the vicinity of the active site of the adjacent subunit (Fig. 2b), where it forms two stabilizing hydrogen bonds with the backbone carbonyls (residues Arg262 and Gly263) belonging to the oxalate (phosphoenolpyruvate)-stabilized Aα6° helix. This series of inter-subunit hydrogen bonds formed along the large A-A interface stabilizes an R-conformation (Fig. 2b). Thus, the 6° rigid body T- to R-state transition is shown to be required for enzyme activity; however, it does not explain how effector binding increases $k_{cat}/S_0.5$ by a factor of 7 (supplemental Table S4).

Effect of Substrates on Stabilization of the LmPYK Tetramer in Solution—To separate the allosteric effects of active site ligands from those of effector site ligands, it is important to examine the individual complexes. The structure of LmPYK-Fru-2,6-BP (supplemental text T4) provides an answer to the controversial question of how binding of the effector molecule can influence enzyme activity at a site >40 Å away. LmPYK-Fru-2,6-BP was determined at 5 Å resolution and contained 24 monomers (six complete tetramers)/asymmetric unit. The LmPYK-Fru-2,6-BP crystals diffracted beyond 3 Å, but to obtain adequate separation of reflections the resolution was limited to 5 Å. Despite the poor resolution of the x-ray data, noncrystallographic averaging of the six tetramers resulted in high quality electron density maps (supplemental Fig. S6a), and Fru-2,6-BP molecules were clearly identified at the effector site of all monomers in the asymmetric unit (supplemental Fig. S6c). In the LmPYK-ATP-OX structure, the AC cores of the LmPYK-Fru-2,6-BP tetramer adopt an R-state conformation (Fig. 2d), with a root mean square fit between these two tetramers of 0.48 Å. The major structural difference, however, is that Fru-2,6-BP binding results in ordered electron density for the effector loop (supplemental Fig. S6d), which is normally disordered in the absence of bound Fru-2,6-BP. This ordered effector loop (residues 481 – 487) pushes outward across the small C-C interface toward the adjacent chain in a conformation identical to that observed for the LmPYK-ATP-OX-Fru-2,6-BP structure. The stabilization of the effector loop results in the formation of four pairs of strongly stabilizing salt bridge interactions (Asp482-Arg493 and Lys484-Glu498) (Fig. 3a and supplemental Fig. S6e), as observed in the LmPYK-ATP-OX-Fru-2,6-BP structure. These interactions link the adjacent C-domains and stabilize the small C-C interface, locking the LmPYK tetramer in the R-state, preprogrammed for highly efficient phosho transfer (Fig. 2d).

Movement of the Flexible Lid-like B-domain Is Not AllostERICALLY Regulated—Comparisons of previously determined inactive and active PYK structures (supplemental Table S3) show the lid-like B-domain hinged back in a range of open and closed conformations (33). The two B-domain conformations observed in the LmPYK-ATP-OX structure provide further clear examples that the degree of closure is related to the presence, type, and position of active site ligands, suggesting that the movement of the B-domain is not related to the allosteric mechanism.

Removal of the Salt Bridge Locking Mechanism by Site-directed Mutagenesis Results in a Nonallosteric but Active Enzyme—To test whether the enhanced activity stems from salt bridge formation consequent on effector binding, we examined a number of mutants (supplemental Table S4) including K484A, D482A, R349A, and E498A. The double mutant K484A/D482A should be sufficient to prevent formation of all eight salt bridges observed to form across the C-C interface upon effector binding. Using phosphoenolpyruvate as substrate (without effector), this mutant has a $k_{cat}/S_0.5$ value of 1.5 × 10^5 M⁻¹ s⁻¹; addition of effector made no significant difference to the enzyme efficiency ($k_{cat}/S_0.5 = 1.4 × 10^5$ M⁻¹ s⁻¹). In contrast, addition of effector to wild-type enzyme enhanced the $k_{cat}/S_0.5$ value ∼7-fold. This result strongly supports the conclusion that the allosteric effect of Fru-2,6-BP is from stabilization of the C-C interface.

Binding of Fru-2,6-BP Plays the Most Important Role in Stabilizing the LmPYK Tetramer in Solution—The stabilizing effects of ligands were also analyzed using a thermal shift assay (34). Addition of effector molecule (Fru-2,6-BP) to the apoenzyme dramatically increases the melting temperature ($T_m$) from 40 °C up to 59 °C (Fig. 3b and c). Addition of the active site ligands oxalate and ATP has a small additional stabilizing effect and increases the $T_m$ by 3 °C to 62 °C. Comparison of the x-ray structures shows that the only major differences observed between the LmPYK-ATP-OX-Fru-2,6-BP complex (Fig. 2c) ($T_m = 62 °C$) and the LmPYK-ATP-OX complex (Fig. 2b) ($T_m =$
Pyruvate Kinase Allostery

42/51 °C, phosphoenolpyruvate $T_m = 42/53 °C$ are the interactions formed by the effector loop. As discussed previously, these result in eight additional salt bridges across the C-C interface and correlate with a ~7-fold increase in $k_{cat}/S_{0.5}$ upon addition of effector ($k_{cat}/S_{0.5} 3.2 \times 10^9 M^{-1} s^{-1}$ to $k_{cat}/S_{0.5} 2.4 \times 10^6 M^{-1} s^{-1}$; supplemental Table S4). Because there are no additional structural changes near the active site on addition of Fru-2,6-BP, this increase in enzyme activity must be related to the enhanced stability and rigidity of the protein upon effector binding.

The Allosteric Mechanism of LmPYK Requires an Extension of the Monod, Wyman, and Changeux Model, Which Incorporates Changes in Flexibility—Rigid domain movements and local flexibility are both important in the allosteric regulation of LmPYK. The principal tenet of the Monod-Wyman-Changeux model is that “oligomers undergo reversible transitions between discrete conformations which primarily affect the quaternary organization, preserve its symmetry and are accessible in the absence of ligand” (35). The proposed allosteric mechanism for LmPYK based on analysis of the AC cores of the x-ray structures presented in this paper (Fig. 2) fits this model: the transition between the T- and R-states is characterized by the symmetrical rocking motion of the AC cores, a movement that governs the all-important regulatory switch. However, careful experimental dissection of the allosteric mechanism using the structural and thermodynamic results presented here clearly shows that the T- to R-state transition is not solely responsible for controlling enzyme activity: two completely independent ligand binding events stabilize an R-state tetramer but only effector-binding enhances enzyme activity. We have shown that the rate increase is due to a reduction in enzyme flexibility. Ligand binding to the active site in the absence of effector stabilizes a less active ($k_{cat}/S_{0.5}$ value of $3.2 \times 10^9 M^{-1} s^{-1}$) and less robust (ATP-OX complex, $T_m = 42/51 °C$) R-state. However effector binding locks via salt bridges a highly active ($k_{cat}/S_{0.5}$ value of $2.4 \times 10^6 M^{-1} s^{-1}$) rigidified R-state ($T_m = 59 °C$).

The different binding events neatly explain how the effector molecule apparently primes the active site, situated 40 Å away, without the need for a message to be transmitted through the chain: when the tetramer is in a conformation stabilized by the effector molecule, it is also in the optimal conformation (for each of the four subunits) to bind substrate (Fig. 2e). The structural, kinetic, and thermodynamic data for the different ligated states of LmPYK explain its allosteric mechanism and provide a number of general principles that are likely to apply in many other allosteric systems. We would, for example, expect that effector-bound complexes are typically less flexible, have higher thermal stability, and have greater enzymatic activity; a generalization that is indeed consistent with disparate available published structural and enzymatic data from different allosteric proteins. In the particular case of PYK, these observations open the door to the development of “allosteric drugs” to tackle parasitic diseases and cancer.

Acknowledgments—We thank Dr. J. Dornan for excellent advice and practical help. We also thank the staff at the Synchrotron facilities at European synchrotron radiation facility, Grenoble, France, and Diamond, Oxfordshire, United Kingdom.

REFERENCES
1. Fenton, A. W. (2008) Trends Biochem. Sci. 33, 420–425
2. Lindsey, J. E., and Rutter, J. (2006) Proc. Natl. Acad. Sci. U.S.A. 103, 10533–10535
3. Christofk, H. R., Vander Heiden, M. G., Harris, M. H., Ramanathan, A., Geiszten, R. E., Welt, R., Fleming, M. D., Schreiber, S. L., and Cantley, L. C. (2008) Nature 452, 230–233
4. Vander Heiden, M. G., Cantley, L. C., and Thompson, C. B. (2009) Science 324, 1029–1033
5. Steták, A., Verres, R., Ovádi, I., Csermely, P., Kéri, G., and Ulrrich, A. (2007) Cancer Res. 67, 1602–1608
6. Christofk, H. R., Vander Heiden, M. G., Wu, N., Asara, J. M., and Cantley, L. C. (2008) Nature 452, 181–186
7. Eigenbrodt, E., Reinacher, M., Scheefers-Borchel, U., Scheefers, H., and Friis, R. (1992) Crit. Rev. Oncog. 3, 91–115
8. Monod, J., Wyman, J., and Changeux, J. P. (1965) J. Mol. Biol. 12, 88–118
9. Tsai, C., del Sol, A., and Nussinov, R. (2008) J. Mol. Biol. 378, 1–11
10. Laskowski, R. A., Gerick, F., and Thornton, J. M. (2009) FEBS Lett. 583, 1692–1698
11. Jardetzky, O. (1996) Proc. Biophys. Mol. Biol. 65, 171–219
12. Daily, M. D., and Gray, J. J. 2007) Proteins 67, 385–399
13. Consler, T. G., Uberbacher, E. C., Bunick, G. J., Liebman, M. N., and Lee, J. C. (1988) J. Biol. Chem. 263, 2794–2801
14. Lee, J. C. (2008) Acta Biochim. Biophys. Sin. 40, 663–669
15. Ernst, I., Callens, M., Oppedrees, F. R., and Michels, P. A. (1994) Mol. Biochem. Parasitol. 64, 43–54
16. Collins, R. A., Kelly, S. M., Price, N. C., Fothergill-Gilmore, L. A., and Muirhead, H. (1996) Protein Eng. 9, 1203–1210
17. Hanneaert, V., Vernaux, C., Rigden, D. J., Fothergill-Gilmore, L. A., Oppedrees, F. R., and Michels, P. A. M. (2002) FEBS Lett. 514, 255–259
18. Boda, Y., Tanaka, T., and Noguchi, T. (1997) J. Biol. Chem. 272, 20495–20501
19. Pendergrass, D. C., Williams, R., Blair, J. B., and Fenton, A. W. (2006) IUBMB Life 58, 31–38
20. Valentini, G., Chiarelli, L. R., Fortin, R., Dolzan, M., Galizzi, A., Abraham, D. I., Wang, C., Bianchi, P., Zanella, A., and Mattevi, A. (2002) J. Biol. Chem. 277, 23807–23814
21. Rigden, D. J., Phillips, S. E. V., Michels, P. A. M., and Fothergill-Gilmore, L. A. (1999) J. Biol. Mol. Biol. 291, 615–635
22. Tulloch, L. B., Morgan, H. P., Hanneaert, V., Michels, P. A. M., Fothergill-Gilmore, L. A., and Walkinshaw, M. D. (2008) J. Mol. Biol. 383, 615–626
23. Blundell, T., and Johnson, L. (1976) Protein Crystallography, Academic Press, pp. 75–77, Orlando, FL
24. Pottorfer, E., Briggs, P., Turkenburg, M., and Dodson, E. (2003) Acta Crystallogr. D Biol. Crystallogr. 59, 1131–1137
25. Evans, P. (2005) Acta Crystallogr. D Biol. Crystallogr. 62, 72–82
26. McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C., and Read, R. J. (2007) J. Appl. Crystallogr. 40, 658–674
27. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Acta Crystallogr. D Biol. Crystallogr. 53, 240–255
28. Emsley, P., and Cowtan, K. (2004) Acta Crystallogr. Sect. D Biol. Crystallogr. 60, 2126–2132
29. DeLano, W. L. (2002) PyMOL v1.2, DeLano Scientific, San Carlos, CA
30. Davis, I. W., Leaver-Fay, A., Chen, V. B., Block, J. N., Kapral, G. J., Wang, X., Murray, L. W., Bryan Arendall III, W., Snoeyink, J., and Richardson, J. S. (2007) Nucleic Acids Res. W375–W83
31. Dombrăcuks, D. J., Santarsiero, B. D., and Mesecar, A. D. (2005) Biochemistry 44, 9417–9429
32. Valenti, G., Chiarelli, L., Fortin, R., Speranza, M. L., Galizzi, A., and Mattevi, A. (2000) J. Biol. Chem. 275, 18145–18152
33. Mattevi, A., Valenti, G., Rizzi, M., Speranza, M. L., Bolognesi, M., and Coda, A. (1995) Structure 3, 729–741
34. Ericsson, U. B., Hallberg, B. M., DeTitta, G. T., Dekker, N., and Nordlund, P. (2006) Anal. Biochem. 357, 289–298
35. Changeux, J. P., and Edelstein, S. J. (2005) Science 308, 1424–1428
36. Perutz, M. F. (1989) Q. Rev. Biophys. 22, 139–237