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Space-Filling Models of Kinase Clefts and Conformation Changes

Comparison of the surface structures of kinase enzymes implicates closing clefts in their mechanism.

C. M. Anderson, F. H. Zucker, T. A. Steitz

By finding similarities in the structures of different enzymes that perform the same function, one may discover general principles governing their catalytic mechanism. In this article we consider some of the structural similarities that exist among the kinase enzymes. For those kinases that have been studied crystallographically, the investigators in each case have reported a striking structural feature; the enzymes contain two lobes separated by a cleft (*1–6*). Evidence from various techniques suggests a second generalization; kinases undergo conformational changes in solution on binding substrate ligands. For example, magnetic resonance studies on arginine kinase (*7*), small angle scattering on pyruvate kinase (*8*) and hexokinase (*9*), kinetic studies of CH_3S-blocked creatine kinase (*10*), and changes in tryptophan fluorescence in hexokinase (*11*) all indicate substrate-induced conformational changes. It is clear from crystallographic studies that for hexokinase these two structural generalizations—a clefted shape and a conformational change—are functionally related (*9, 12*). The conformational change that occurs when glucose binds consists of a large relative motion of the two lobes resulting in a closing of the cleft.

A similarity has been noted in the secondary structure of several kinases (*2, 4, 6*), which is thought to be related to the binding of nucleotides. This structural

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motif is not related to the clefted nature of kinases since the overall tertiary structure of the five kinases described in this article is diverse, and the cleft that these proteins contain has been formed in different ways in each case. These kinases do not form a closely homologous family of proteins such as the pancreatic serine proteases do, suggesting that any common structural features must be related to kinase function, and not to a nonessential remnant of divergent evolution.

Summary. Space-filling models of yeast hexokinase, adenylate kinase, and phosphoglycerate kinase drawn by computer clearly portray the bilobal character of these phosphoryl transfer enzymes, and the deep cleft which is formed between the lobes. A dramatic conformational change occurs in hexokinase as glucose binds to the bottom of the cleft, which causes the two lobes of hexokinase to come together. A substrate-induced closing of the active site cleft is postulated to occur in other kinases as well. This change may provide a mechanism by which some of these enzymes reduce their inherent adenosine triphosphatase activity and could be a general requirement of the kinase reaction.

We present computer-generated drawings of space-filling models of hexokinase, phosphoglycerate kinase, and adenylate kinase, which clearly portray the deep active site cleft that these enzymes have in common. These drawings provide a more informative view of the shape and size of the cleft than either the standard α-carbon backbone or tube and arrow representations. The clefts appear more equivalent than is apparent from α-carbon drawings alone. The space-filling drawings show the nature and magnitude of the large conformational change observed in hexokinase upon binding glucose (*9*, *12*); the two lobes of the molecule move toward each other, engulfing the sugar. We suggest that in other kinases as well substrate binds in the deep cleft and that this cleft is closed in the ternary complex. We further suggest that this conformational change is important for the specificity of the enzymes and for the kinase mechanism.

Computer-Drawn Space-Filling Models

Although drawings of space-filling models have only recently been introduced to depict large molecular structures, their use is likely to become routine in the near future. Unlike standard ball and stick or α-carbon drawings, they portray the surface of a molecule and therefore reveal the true size and shape of ligand binding sites and their accessibility. Computer programs that calculate space-filling representations of macromolecules have been written by Feldmann (*13*), Cherry and Knowlton (*14*), and others. The computer program used to draw the figures presented was written at Yale and runs on a PDP-11/70 interfaced to a Versatec electrostatic plotter;

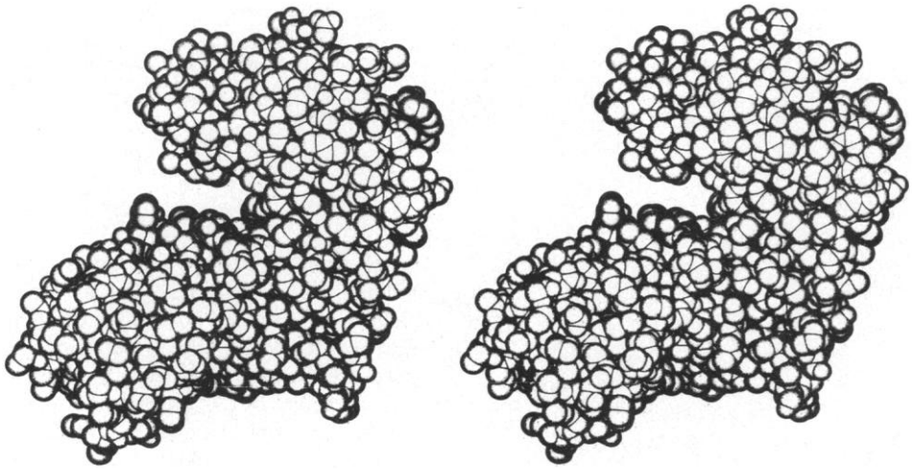

Fig. 1. A stereo drawing of a space-filling model of yeast hexokinase B in its unliganded, native conformation. The most prominent feature of this drawing is the cleft that divides the molecule into two lobes and is the binding site for glucose.

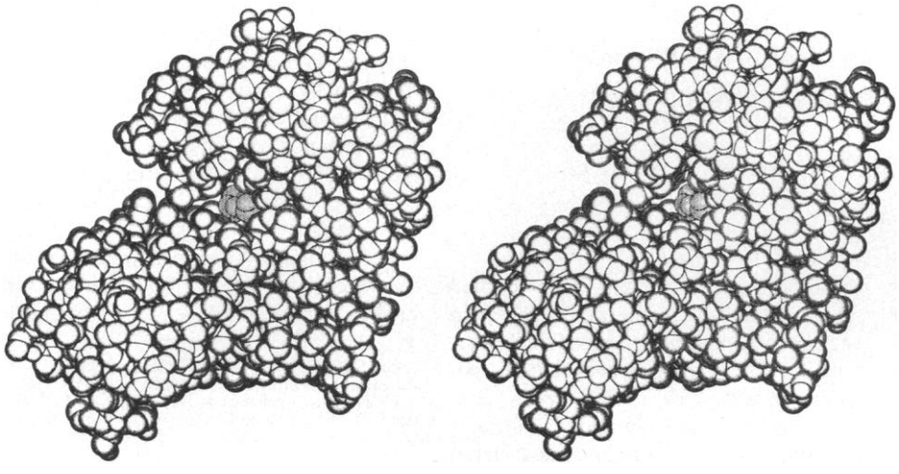

Fig. 2. A stereo drawing of yeast hexokinase A complexed with glucose. The bound glucose molecule is indicated by a zigzag pattern. The active site cleft is closed by a motion that is essentially a rotation of the upper lobe by 12° relative to the lower lobe (*12*).

the algorithm used is similar to that in Feldmann's program. Various classes of atoms or regions of a molecule can be drawn to have different surface patterns, allowing one to indicate binding sites, ligands, or other regions of the molecule of particular interest. Once the desired view of a molecule has been chosen (which is accomplished by rotating an α-carbon drawing on an Evans and Sutherland Picture System 2), it takes about 40 minutes to produce a 2000 by 2000 point raster drawing of a 3000-atom molecule.

Hydrogen atoms are not drawn separately, but their radii are incorporated into the radius of the atom type to which each is attached. This approximation is commonly used in accessibility and energy calculations, and makes the drawings simpler in appearance. The following van der Waals radii were used (*15*): trigonal carbon atoms, 1.76 Å; tetrahedral carbon atoms, 2.0 Å; carbonyl oxygen atoms, 1.4 Å; all other oxygen atoms, 1.6 Å; trigonal nitrogen atoms, 1.7 Å; tetrahedral nitrogen atoms, 2.0 Å; all sulfur atoms, 1.85 Å; and the phosphorus atom, 1.8 Å.

Conformational Change in Hexokinase

The structure of the 51,000-dalton monomer of yeast hexokinase has been independently determined from two crystal forms, one grown in the absence of glucose and the other as the glucose complex. Comparison of these two structures established the existence of a large conformational change in the enzyme (*12*). The structure of the B isozyme crystallized without glucose has been refined at 2.1-Å resolution to a crystallographic R factor of 0.25 (*16*). Since the amino acid sequence has not been chemically determined, and some side chains are disordered in our electron density maps, about 300 of the 3600 atoms other than hydrogen are missing in the current model. Figure 1 shows a stereo space-filling model drawn with the use of these refined coordinates. Approximately 1000 atoms can be seen in this view. The shape and size of the deep cleft dividing this enzyme into two lobes is clearly portrayed.

The structure of the A isozyme crystallized as a complex with glucose has been independently solved at 4.5-Å resolution and refined at 3.5-Å resolution by Bennett and Steitz (*12*). Comparison of the two structures shows a striking difference in the protein conformation; one lobe, constituting about 40 percent of the molecule, rotates by 12° relative to the other lobe, resulting in atomic movements of the polypeptide backbone of as much as 8 Å (*12*).

Several lines of evidence establish that this conformational change is produced by the binding of glucose and is not the result of an isozyme difference or crystal packing forces. Most conclusive is the fact that the radius of gyration of the B isozyme in solution, measured by x-ray scattering, is reduced by 0.95 ± 0.24 Å when glucose is bound (*9*). This value is accurately predicted by the difference of 0.9 Å in the radius of gyration calculated between the refined coordinates of the native B isozyme and the A isozyme-glucose complex. Also, the disintegration of crystals of the A isozyme when glucose is removed, and of B isozyme crystals when high concentrations of glucose are introduced, suggest that glucose produces the change. Finally, low concentrations of glucose do not cause the B isozyme crystals to disintegrate, but rather induces an alteration in the BIII structure which is qualitatively similar but, because of lattice forces, quantitatively smaller than the change observed between the two crystal forms. In both crystal forms it appears that the sugar-induced conformational change is sufficiently large to disrupt the crystal lattice if allowed to go to completion.

The structure of the glucose complex with the A isozyme is shown in Fig. 2 and from the other side in Fig. 4a. The cleft has closed so that only the 6-hydroxymethyl group of the glucose is accessible to solvent (*12*). Atoms from the smaller lobe come into contact with atoms of the larger lobe, the glucose 6-hydroxyl, and possibly with the phosphates of adenosine triphosphate (ATP) (Fig. 3). Glucose is sufficiently surrounded by the enzyme in this closed conformation that it cannot enter or leave its binding site, which provides an explanation for the observation (*17*) that glucose dissociates very slowly (dissociation constant, 58 sec^{-1}) from its bi-

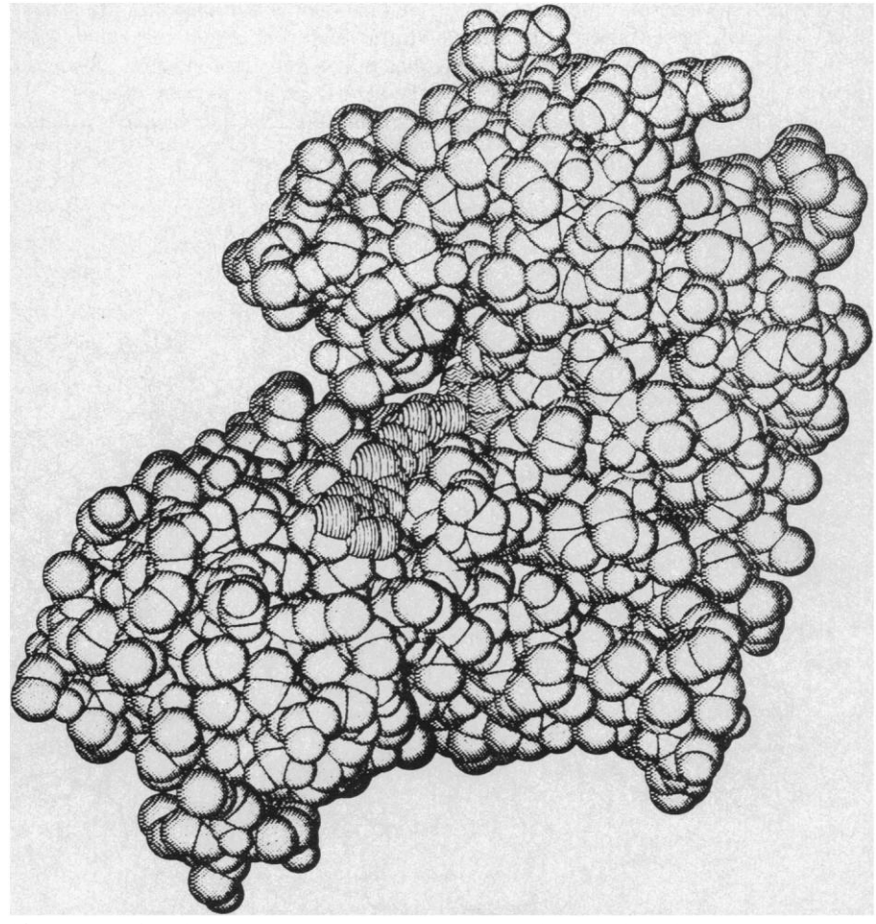

Fig. 3. A drawing of the glucose (zigzag pattern) complex with hexokinase in which the probable position of ATP (indicated by a striped ring pattern) is also shown. The ATP does not bind to either of the crystal forms of hexokinase, but AMP does bind to the B isozyme crystals, and from the known position of the adenosine moiety, the phosphate groups may be built into the position shown. This prediction for the position of ATP puts the γ-phosphate group 5.5 Å from the 6-hydroxyl, and superimposes it on a binding site for a sulfate ion in the crystal (*19*). Residues from the smaller, upper lobe are in contact (or nearly so) with the larger lobe and the phosphates of ATP in this closed conformation.

nary complex with hexokinase. Probably, the rate of glucose dissociation is limited by the rate of the enzyme conformational change from the closed to the open form.

The Conformational Change Is Essential for Catalysis

Analogs of glucose containing bulky substituents on the 2-carbon are competitive inhibitors of glucose binding but are not substrates (*18*). Crystallographic studies of *o*-toluoyl-2-glucosamine, one such analog, show that the sugar moiety of this inhibitor binds to the B isozyme in precisely the same orientation as the substrate glucose (*19*). However, the binding of *o*-toluoyl-2-glucosamine to the enzyme in solution does not produce the same conformational change, since the B isozyme can be crystallized isomorphously either in the presence or absence of this inhibitor (*6*). Thus, the conformational change induced in hexokinase by glucose is essential for catalysis.

If *o*-toluoyl-2-glucosamine binds exactly as glucose does, why does it not produce the same conformational change? The answer is illustrated in Fig. 4. The bulky toluoyl substituent lies in the cleft, which closes during the glucose-induced conformational change. Its presence sterically prevents the two lobes from coming together.

Other Kinases

One might ask whether this form of substrate-induced conformational change —a cleft which closes when substrate binds—is unique to hexokinase or applies to kinases in general. Crystallographic studies of four other kinases permit comparisons of the substrate binding sites to be made. The structures of these other kinases have been solved either in their native, unliganded state or as a substrate complex; in none of these enzymes has both the unliganded and liganded structure been crystallized and solved. Nevertheless, since solution studies indicate that many of these enzymes undergo a substrate-induced conformational change, and since they have clefts at their active sites into which substrates bind, one might reasonably predict that a substrate-induced closing of the cleft is general to many kinases.

Pig muscle adenylate kinase is a monomer of about 22,000 daltons whose structure has been examined in two crystal forms (*20, 21*). This enzyme is smaller than hexokinase and has a different secondary structure, yet it does contain a cleft at the active site (*4*) (Fig. 5). Adenosine monophosphate (AMP) and ATP bind at opposite ends of this cleft, with their phosphates extending toward each other and in the center of the cleft. In the B crystal form, the cleft has narrowed by a few angstroms when compared with the cleft in the A crystal form. This motion is particularly large in a region proposed to be the AMP site where a helix moves to close the adenosine binding pocket. By examining the association of substrates to the crystal forms, Schulz and his colleagues have concluded that the open form is the native conformation, and that AMP causes the molecule to switch to the structure in which the cleft is more closed (*22*). At the resolution of the structural analysis presented (*23*), it is not possible to conclude with certainty whether the conformational change results from the relative motion of the two lobes or whether it is restricted to the changes observed in a few loops of polypeptide chain. A drawing of a space-filling model of adenylate kinase

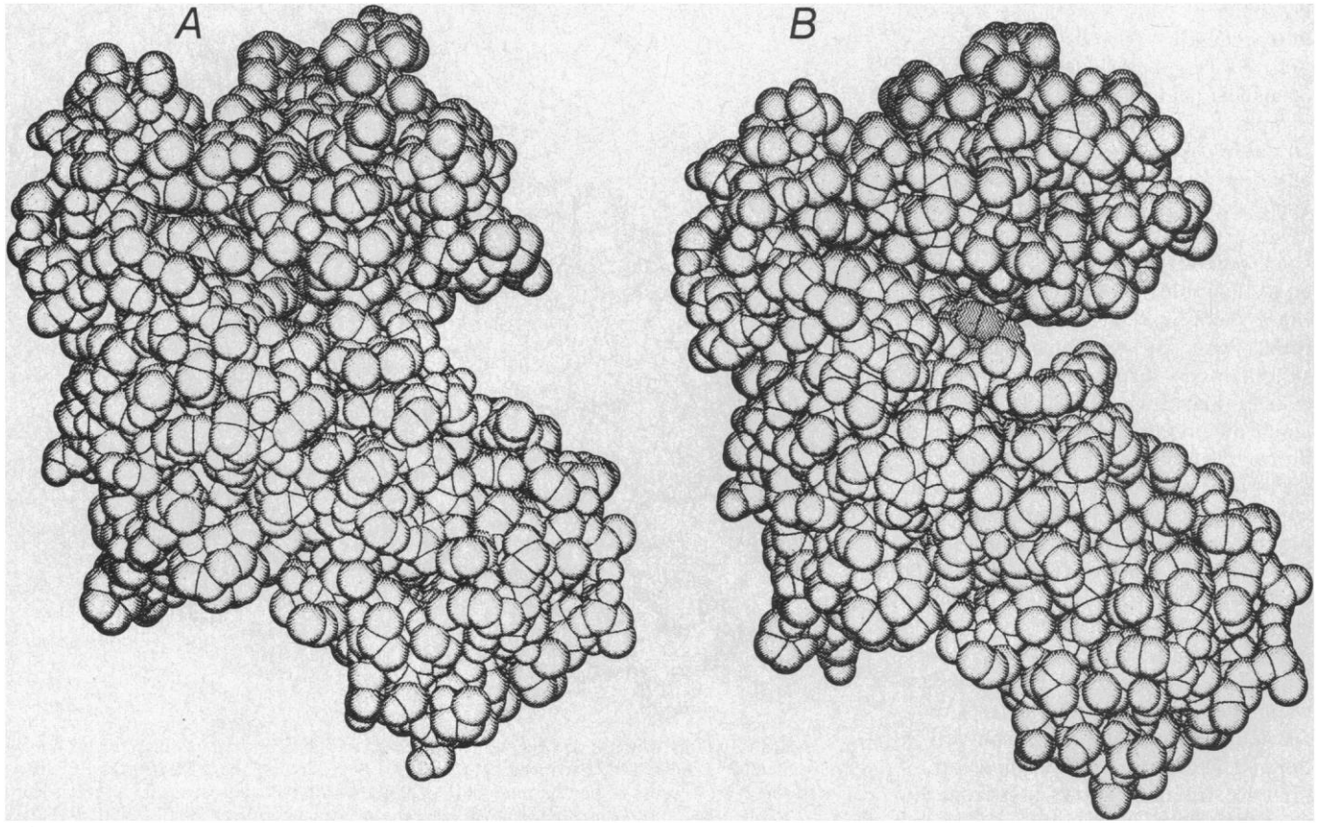

Fig. 4. (A) A view of the A isozyme of hexokinase complexed with glucose from the side of the enzyme opposite to the view in Fig. 2. Since the cleft is closed in this complex, the bound glucose molecule cannot be seen from this side. (B) The B isozyme of hexokinase, also viewed from the side opposite to Figs. 1 and 2. The zigzag patterned atoms belong to the toluoyl group of *o*-toluoyl-2-glucosamine. It is apparent that this group is making van der Waals contact with atoms from both lobes, thereby preventing them from coming together.

in the more closed conformation is shown in Fig. 5 constructed from coordinates obtained from the Protein Data Bank (*23*). Coordinates for the molecule in the open configuration and for the substrates are not yet available.

Since neither of these crystal forms was obtained in the presence of substrate, it is possible that the conformational change which adenylate kinase undergoes during catalysis is larger than the one observed in the previously reported crystal studies. A further closing of the cleft is likely in view of the fact that crystals crack in the presence of AMP, and neither crystal form will properly bind the inhibitor diadenosine pentaphosphate, A-5′-ppppp-5′-A (Ap_5A), which has a very high affinity for the enzyme in solution (*22, 24*). The possibility that crystal lattice forces are restricting the conformational change is analogous to the observation that the binding of glucose to crystals of native hexokinase causes a slight narrowing of the cleft while the complex formed in solution shows complete closing of the cleft. Recently the complex between Ap_5A and human adenylate kinase has been crystallized (*22*). The determination of this structure should establish the extent to which the cleft closes during catalysis.

The crystal structure of phosphoglycerate kinase from horse muscle has been obtained by Blake and Evans (*1, 3*), and that from yeast by Brant, Watson, and Wendell (*2*). The two homologous proteins are nearly identical to each other, but not similar in overall secondary structure to the other kinases depicted here. Until recently, no sequence data were available for either of the proteins, and so these investigators have reported only the coordinates of polypeptide backbone atoms. In preparing the space-filling drawing in Fig. 6, we placed a β-carbon on each residue of the horse muscle enzyme to provide a more accurate view of the surface of the molecule than is provided by polyglycine. Although the enzyme's secondary structure is not related to hexokinase, its general shape shows a superficial resemblance to hexokinase, particularly in the deep cleft separating the molecule into two lobes (compare Figs. 1 and 6).

Blake and Evans have located the binding site for ATP on the enzyme (*3*). As in hexokinase, the ATP associates with one of the lobes, with its phosphate groups extending toward the interlobe cleft. It appears from their drawings that the γ-phosphate is situated directly over the cleft. Efforts to locate the binding position of phosphoglycerate have not yet been successful since the crystals dissolve in the presence of high concentrations (70 m*M*) of this substrate (*1*). This could be due to a conformational change induced by the binding of phosphoglycerate or to some less specific effect such as binding between molecules in the crystal.

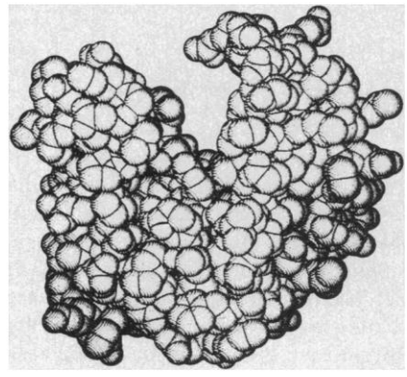

Fig. 5. A drawing of the space-filling model of the B crystal form of adenylate kinase showing the deep cleft. Both AMP and ATP bind at opposite ends of this cleft, with their phosphate groups lying in the center and nearly surrounded by the protein (*4, 22*).

There is as yet no crystallographic evidence that the cleft in the enzyme closes, since the enzyme has not been crystallized in the presence of substrates. Nevertheless, we suggest that, as in the case of hexokinase, the cleft in phosphoglycerate kinase will close in the ternary complex.

A fourth kinase for which there is structural information is pyruvate kinase from cat muscle, which has been the work of Stammers and Muirhead and their co-workers (*25–27*). The enzyme differs from the other kinases in that it forms a binary complex with a divalent cation, such as manganese, and this cation is thought to interact with the transferring phosphate group (*28*). Pyruvate kinase has a folding topology which is strikingly different from that of hexokinase and folds into three quite separate domains. Since no coordinates are available yet for this enzyme, a space-filling drawing cannot be made. However, inspection of the substrate positions in low resolution difference electron density maps reveals binding behavior similar to that of the other kinases. Phosphoenolpyruvate is found in a cleft between two of the domains, a few angstroms away from the Mn^{2+} position (*26*). These two domains are connected by only two polypeptide chains, which might allow the domains to move flexibly relative to each other in solution. In fact, Stammers and Muirhead have suggested that such a

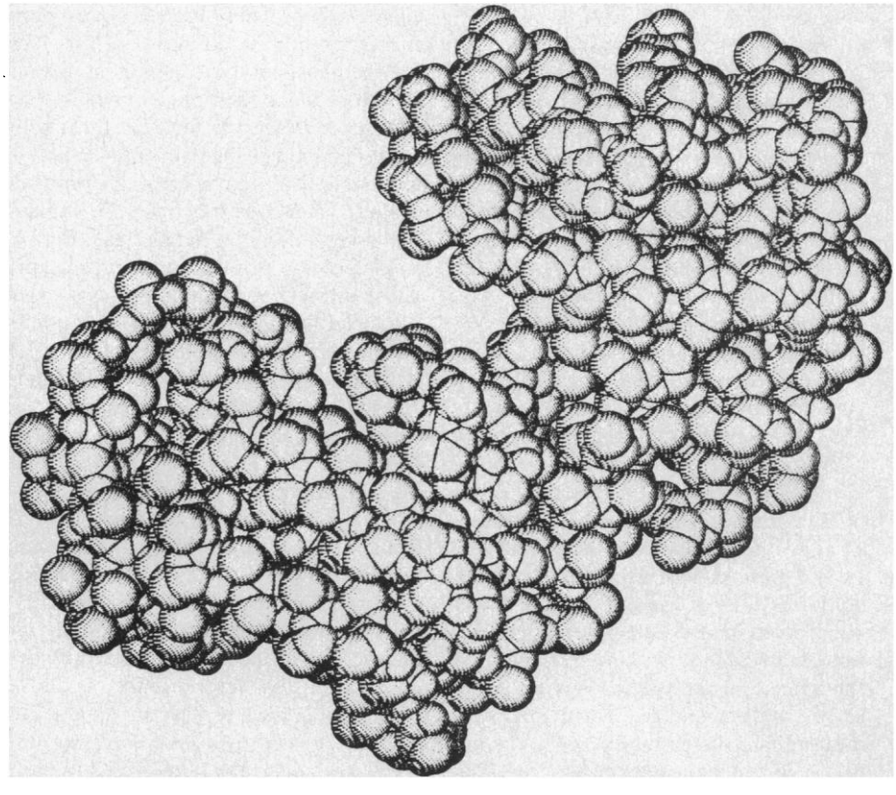

Fig. 6. A drawing of horse phosphoglycerate kinase (*3*). Since the amino acid sequence of this enzyme is unknown, the structure portrayed here is polyalanine. The secondary structure of this enzyme is not the same as hexokinase, but both enzymes have a deep cleft which divides them into two lobes and makes them appear similar in their surface structure.

motion could account for the lower electron density observed for one of the domains (*26*). Furthermore, there is evidence for a conformational change during catalysis; nuclear magnetic resonance studies by Mildvan and Cohn (*29*) of pyruvate kinase in the presence and absence of manganese and phosphoenolpyruvate are consistent with a conformational change in the protein at the active site.

An α-carbon backbone model for phosphofructokinase isolated from *B. stearothermophilus* has been obtained from a 2.4-Å resolution map by Evans and Hudson (*30*). This is a 130,000-dalton tetramer of four identical subunits and has been crystallized as a complex with one of its substrates, fructose 6-phosphate. The active site is described as consisting of a cleft between two folding domains. Removal of both fructose 6-phosphate and phosphate (which binds at the sugar site) from these crystals causes the crystals to disintegrate (*30*). Again, it is possible that the binding of fructose 6-phosphate or of phosphate produces a conformational change in the enzyme, and this change may involve the relative orientation of the two domains.

Conclusions

The kinases whose crystal structures are known all possess a deep cleft. Space-filling drawings show that the clefts of hexokinase, phosphoglycerate kinase, and adenylate kinase are roughly equal in size and shape. For all five kinases described here substrates bind in the cleft, and in the case of both adenylate kinase (*22*) and hexokinase (*12*) the cleft is known to narrow or close upon binding the appropriate substrate. We suggest that a substrate-induced closing of a cleft between two lobes will be a general feature of kinase enzymes.

Experimental proof for this kind of conformational change is difficult to obtain by crystallographic structure determination, since it appears that in the cases cited the motion is so large that one crystal form cannot accommodate both conformations of the enzyme. At lower concentrations the substrate either does not bind or binds without inducing the full structural change, as has been observed with hexokinase when glucose is diffused into the native crystals. The only way in which the existence of this kind of interdomain flexibility can be established crystallographically is by producing crystals of the native and liganded enzyme separately (*12*).

Another approach which could establish the existence of such large changes in tertiary structure is to measure by means of small angle x-ray scattering the radius of gyration of these enzymes with and without substrates. This technique has established the existence of a large ligand-induced change in hexokinase and is being applied to other kinases (*31*). In fact, just recently, small angle x-ray scattering measurements have shown that the radius of gyration of phosphoglycerate kinase is reduced by about 1 Å upon formation of the ternary complex, consistent with a cleft closing structural change in this kinase (*31*).

At least two reasons can be suggested to explain why kinases would possess a deep cleft that closes upon substrate binding (*12, 32, 33*). One possibility is that the kinase mechanism may require the enzyme to surround, or embrace (*33*), its substrates either to orient catalytic groups or perhaps to exclude solvent. For example, a low dielectric environment would help to promote the nucleophilic attack of the phosphoryl acceptor on the phosphoryl group to be transferred. In the case of hexokinase (*19*), adenylate kinase (*22*), and phosphofructokinase (*30*), the carboxylate group of an aspartic acid residue (Asp) is hydrogen-bonded to the phosphoryl acceptor and probably functions as a general base catalyst. Removal of this Asp and the phosphoryl acceptor from solvent should enhance their nucleophilicity.

A second possible function for a substrate-induced change in conformation is to enable the enzyme to discriminate against water as a substrate. To explain the observation that hexokinase, for example, is not an efficient adenosine triphosphatase, Koshland proposed his "induced fit" hypothesis (*32*). He pointed out that, if hexokinase had a rigid structure, a water molecule at the binding site of the 6-hydroxyl group of the sugar could nucleophilically attack the γ-phosphate of ATP in the same way that the 6-hydroxyl does. If, however, hexokinase is normally in an inactive conformation and if the binding of glucose is required to put the enzyme in the active conformation, then its adenosine triphosphatase activity would be greatly reduced. This proposal is supported by the observation that the presence of xylose (which is identical in structure to glucose but lacks a 6-hydroxymethyl group) greatly enhances the rate of ATP hydrolysis (*34*). Like hexokinase, phosphofructokinase and other kinases are faced with analogous specificity problems. Thus, a large substrate-induced conformational change, which is necessary for the specificity and mechanism of kinases, may be a general feature of these enzymes.

While several other enzymes whose structure is known, such as lysozyme, have a deep groove at their active sites and some other proteins exhibit different relative orientations of two domains, the kinase system provides the clearest and best established example of a functionally important closing of an active site cleft in response to substrate binding. Nevertheless, this ligand-induced closing of a cleft may well occur in numerous other protein and enzyme systems besides kinases.

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