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Glucose-induced conformational change in yeast hexokinase

(protein crystallography/induced fit/interdomain protein flexibility/hydrophobic effect)

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ABSTRACT **The A isozyme of yeast hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) crystallized as a complex with glucose has a conformation that is dramatically different from the conformation of the B isozyme crystallized in the absence of glucose. Comparison of the high-resolution structures shows that one lobe of the molecule is rotated by 12° relative to the other lobe, resulting in movements of as much as 8 Å in the polypeptide backbone and closing the cleft between the lobes into which glucose is bound. The conformational change is produced by the binding of glucose (R. C. McDonald, T. A. Steitz, and D. M. Engelman, unpublished data) and is essential for catalysis [Anderson, C. M., Stenkamp, R. E., McDonald, R. C. & Steitz, T. A. (1978) *J. Mol. Biol.* 123, 207–219] and thus provides an example of induced fit. The surface area of the hexokinase A·glucose complex exposed to solvent is smaller than that of native hexokinase B. By using the change in exposed surface area to estimate the hydrophobic contribution to the free energy changes upon glucose binding, we find that the hydrophobic effect alone favors the active conformation of hexokinase in the presence and absence of sugar. The observed stability of the inactive conformation of the enzyme in the absence of substrates may result from a deficiency of complementary interactions within the cavity that forms when the two lobes close together.**

The ability of hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) to discriminate against water as a substrate led Koshland (1) to suggest that this enzyme utilizes an "induced fit" mechanism of enzyme specificity. He proposed that sugar substrates can induce a protein conformational change that is essential for catalysis, whereas water cannot provide the specific interactions with the protein that are required to induce the change.

Considerable experimental support exists for induced fit in yeast hexokinase. Kaji and Colowick (2) detected a small ATPase activity in the absence of sugar and showed that the K_m for ATP in this reaction was 50 times higher than in the hexokinase reaction. DelaFuente *et al.* (3) showed that the ATPase activity could be stimulated by hexose analogs lacking the reactive 6-hydroxymethyl group, suggesting that the unreactive pyranose ring of these analogs could produce the required conformational change. More direct evidence that sugars actually induce some conformational change in the enzyme was provided by earlier crystallographic studies. High concentrations of glucose shatter all crystal forms of the B isozyme (W. F. Anderson and T. A. Steitz, unpublished observations; ref. 4) and, at lower concentrations, produce small structural changes throughout one lobe of the enzyme (5, 6). Other kinetic and spectroscopic evidence consistent with a glucose-induced conformational change has also been reported (7–9).

We present here the structure of a complex between yeast hexokinase A and glucose (HKA·G) at 3.5-Å resolution. Comparison of this structure with the 2.1-Å resolution structure of the native hexokinase B monomer (BIII; refs. 10 and 11) reveals a substantial difference in structure that has been identified with the glucose-induced conformational change shown to be essential for catalysis (12). Using these two structures, we have examined the roles of hydrophobic forces and structural complementarity in this ligand-induced conformational change.

EXPERIMENTAL

Crystallization of HKA·G Complex. Crystals of yeast HKA·G complex were grown at room temperature by a method similar to that of Womack *et al.* (13): protein (5 to 10 mg/ml) was dialyzed against 50–55% saturated ammonium sulfate/0.1 M potassium phosphate, pH 6.6/0.01 M glucose. Glucose is strictly required for crystal growth and preservation. Crystals are in space group $P2_12_12_1$ (a = 145.0 Å, b = 79.0 Å, c = 62.0 Å) with one monomer per asymmetric unit. All hexokinase A used in these studies was the generous gift of F. C. Womack and S. P. Colowick of Vanderbilt University.

Structure Determination. The HKA·G structure was independently solved at 4.5-Å resolution by the multiple isomorphous replacement method using two heavy atom derivatives, platinum(II) chloride and potassium osmiate. The mean figure of merit was 0.68. Data measurement and processing procedures were similar to those described by Anderson *et al.* (10).

Because this electron density map supported our earlier conclusion (14) that the tertiary structures of the hexokinase isozymes were very similar except for a change in the relative orientation of two domains, we used the refined BIII model (11) to extend the phasing of HKA·G data to 3.5 Å resolution. The BIII model was positioned in the HKA·G unit cell (14), and the orientation of the small lobe was adjusted by hand to fit the HKA·G electron density. The orientation of each lobe was then refined independently to optimize its fit to the HKA·G electron density by the real-space procedure of Fletterick and Wyckoff (15). The resulting HKA·G model was subjected to several cycles of difference Fourier refinement alternated with idealization of the model, using procedures similar to those of Anderson *et al.* (11). Because the amino acid sequence of the A isozyme is not known, the sequence of the B isozyme obtained from the x-ray structure (11) was used. This sequence was not changed during the HKA·G refinement.

There are several indications that the HKA·G structure is essentially correct. Refinement of the HKA·G model was terminated when further progress would have required altering the x-ray-determined (11) amino acid sequence. The final ($F_o - F_c$) difference electron density map (in which F_o and F_c are the observed and calculated structure factors) showed fewer features than the initial difference maps and the conventional crystallographic R factor for the final model was 0.26 at 3.5-Å resolution. The root mean square (rms) deviation of bond

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Abbreviations: E and E′, inactive and active enzyme; G, glucose; HKA·G, hexokinase A complexed with glucose; BIII, crystal form BIII of hexokinase B; rms, root mean square; OTG, *o*-toluoyl-2-glucosamine.

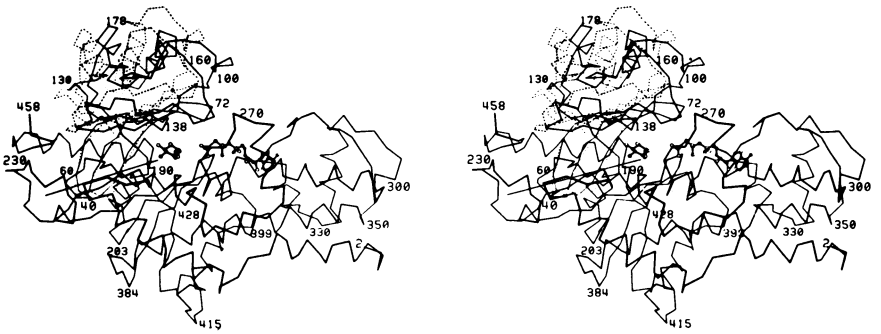

FIG. 1. Stereo drawing of the C_α backbone of the HKA·G and BIII models after the large lobes were superimposed as described in the legend to Fig. 2. HKA·G is represented with solid bonds between adjacent C_α atoms and the small lobe of BIII with dashed bonds. The small lobe as drawn contains 37% of the atoms of the molecule. Because the rest of the BIII structure is similar to that of HKA·G (Fig. 2), it is omitted for clarity. The atomic coordinates of glucose (11) and ATP (6) from binding experiments on the BIII crystal form are represented by open circles. The long line piercing the left end of the molecule indicates the position of the screw axis relating the two small lobes.

lengths from ideality was 0.1 Å. Heavy atom or substrate difference electron density maps calculated using phases derived from the model improved markedly during the refinement. Also, $(F_o - F_c)$ difference maps using phases and structure factors calculated from a model in which a portion of the refined protein was omitted clearly showed the density of the deleted region.*

We have estimated the rms coordinate error to be 0.45 Å by the method of Luzzati (16). Although this model may be inaccurate in some places, especially where side chains differ between isozymes, we have considerable confidence in the placement of the polypeptide backbone. Details of the structure determination will be described elsewhere.*

Solvent Accessibility Calculations. The areas of the HKA·G and BIII structures accessible to solvent were calculated by the procedure of Lee and Richards (17) by using computer programs written by T. J. Richmond of Yale University. To compare the magnitude of the hydrophobic effect resulting from changes in exposed surface area with the corresponding equilibria of hexokinase in solution, hydrophobic free energy changes for the glucose-induced conformational change were estimated from changes in accessible surface area by using the empirical relationship developed by Chothia (18). Because a hydrogen-bonded donor–acceptor pair behaves essentially as a nonpolar group in protein packing (19) and most of the hydroxyl groups of a glucose bound to hexokinase are extensively hydrogen bonded (12), we have used the same relationship between the accessible surface area of glucose and hydrophobic free energy in these estimates. The hydrogen bonds made by the bound glucose are assumed in our calculations to be equivalent to those made with water and to provide no net binding energy.

RESULTS

The Conformational Change. Comparison of the refined HKA·G and BIII models confirms that their tertiary structures are nearly identical except for a large change in the relative orientations of two lobes. To demonstrate this change, the C_α atoms of each of the lobes of the two models were superimposed by using a least-squares procedure, treating the lobes as rigid bodies. The large lobes (residues 2–58 and 187–458) of the HKA·G and BIII models superimpose with an rms residual of 1.0 Å between C_α atoms. The small lobes (residues 59–186) superimpose with an rms residual of 1.6 Å. Although there are small systematic deviations from the rigid-body fit near crystal packing contacts and in some regions of the small lobe that are brought into contact with glucose,* the low residuals of the superposition show that each lobe behaves predominantly as a rigid body in the conformational change between the native and glucose-complex structures.

Fig. 1 shows the positions of the small lobe in the two structures after superposition of the large lobes, and Fig. 2 shows the distance between equivalent C_α atoms with the structures aligned as in Fig. 1. Parts of the polypeptide backbone of the small lobe move as much as 8 Å towards the large lobe in the glucose complex, closing the cleft in which the glucose molecule is bound and bringing atoms of the small lobe into contact with both the large lobe and the substrates.

The conformational change may also be described by the rigid-body transformation needed to superimpose the small lobes once the large lobes are aligned as in Fig. 1. The resulting transform, expressed as a single screw operation by the method of Cox (21), corresponds to a rotation of 12° around and a translation of 0.9 Å along the screw axis shown in the figure.

Surface Area Changes. Table 1 presents the results of comparing accessible surface areas of the hexokinase species represented schematically in Fig. 3 and compares the equilibrium constants calculated from this hydrophobic effect alone

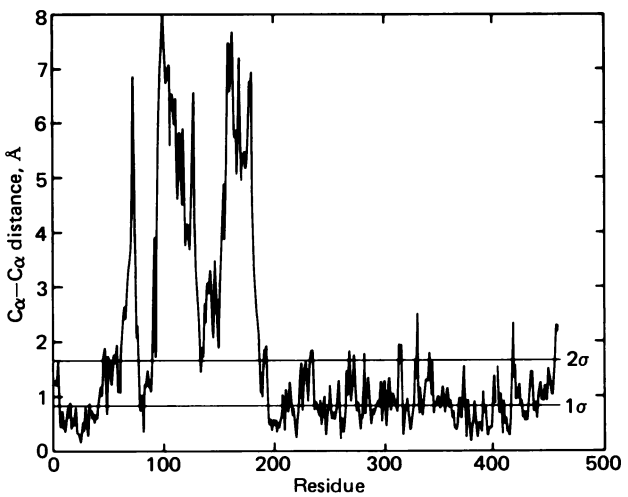

FIG. 2. Magnitude of the conformational change. The distance between equivalent C_α atoms is plotted after superposition of the large lobes of the HKA·G and BIII models by a rigid-body transformation giving the best least-squares fit of selected C_α atoms. Short lengths of polypeptide whose conformations are different in the two large lobes were systematically omitted from the least-squares procedure by criteria similar to those of Rossmann and Argos (20). These omissions amount to 24% of the C_α atoms of the large lobe and are mostly regions involved in crystal packing contacts. σ is the rms residual of 0.8 Å for the 258 C_α atoms used in the least-squares fit.

* W. S. Bennett and T. A. Steitz, unpublished.

Table 1. Changes in accessible surface area

Step	Change in contact surface area,* Å²			Change in hydrophobic free energy,† kcal/mol		
	Protein	Sugar	Total	ΔG^H	$\Delta G°$ (calc.)	$\Delta G°$ (obs.)
E + G → E·G	−37.6	−64.8	−102.4	−8	−1	−2‡
E·G → E′·G	−46.4	−14.9	−61.3	−5	−5	−1 to −3 (ref. 9)
E + G → E′·G	−84.0	−79.7	−163.7	−13	−6	−5 (ref. 9)
E → E′	−36.8	—	−36.8	−3	−3	+6§
E + OTG → E·OTG	−75.2	−96.1	−171.3	−14	−7	−7¶

OTG, *o*-toluoyl-2-glucosamine.

* Area change is defined as final state contact surface less initial state surface in the direction given. The absolute magnitude of the *contact* surface area is about 3.5 times smaller than the *accessible* surface area (22) used by other workers (17, 18, 23). The contact surface was calculated for a solvent sphere of 1.4-Å radius; other van der Waals radii are from ref. 22. To ensure that only changes in accessibility due to the conformational change were considered, only atoms capable of participating in contacts between the lobes were included in the tabulated changes. An atom i was included in the "contact zone" if it was within $r_i + r_j + 2.8$ Å of any atom j on the opposite lobe, in which r_i and r_j are the van der Waals radii of the atoms. The contact zone contained 668 atoms. The change of accessibility of the 2630 atoms outside the contact zone was −28.7 Å², and the total contact surface area of the BIII structure is 5590 Å².

† Hydrophobic free energy, ΔG^H, is estimated from the change in contact surface area by using a proportionality constant of 80 cal/Å² (22) (1 cal = 4.184 J). $\Delta G°$ (calc.) is obtained from ΔG^H by including +7 kcal/mol to account for the loss of translational and rotational entropy of the free sugar, where appropriate. $\Delta G°$ (obs.) = $-RT \ln K$ for the appropriate equilibrium constant in solution, as given in the references cited.

‡ $K_1 \cong 50\ M^{-1}$ from crystal binding experiments (T. A. Steitz, unpublished).

§ $K_3 = 2.5 \times 10^{-5}$ estimated from the ratios of the maximal velocities of the ATPase and kinase reactions (3, 24).

¶ K_I (OTG) ≅ 10 μM (W. F. Anderson and T. A. Steitz, unpublished results; ref. 29).

with those measured or estimated experimentally. For these calculations we have used the HKA·G structure for E′, the active conformation, and the BIII structure for E, the inactive conformation. Although the two crystals contain different isozymes, our models both have the x-ray-determined sequence of the B isozyme (11). The area changes in the table must be interpreted with some caution, because they are sums of small area changes over large numbers of atoms. However, these area changes are substantially larger than the error of about 7 Å² estimated from atoms outside the "contact zone" (Table 1).

The total accessible surface area of hexokinase and glucose is reduced upon formation of the encounter complex, E·G, and is further reduced by the conformational change to E′·G; thus, hydrophobic forces may be expected to favor the active conformation in the presence of sugar, assuming that all hydrogen bond donors and acceptors are satisfied in E′·G. However, the surface area is also reduced when the active conformation is formed in the absence of sugar (E → E′). Because there is less than a factor of 2 difference between the changes in accessible surface for the E·G → E′·G and the E → E′ transitions, the hydrophobic effect cannot account for the large difference in the conformational equilibrium constants K_2 and K_3 in the presence and absence of sugar.

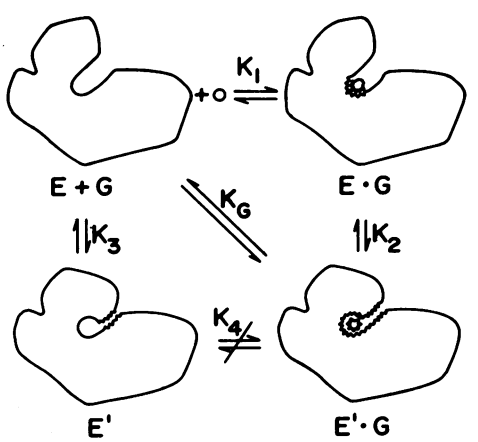

FIG. 3. Schematic representation of the hexose-induced fit. E and E′ are the inactive and active conformations of the enzyme, respectively. G is the sugar substrate. Regions of protein or substrate surface excluded from contact with solvent are indicated by a crinkled line. Note that K_G is a dissociation constant and the others are association constants.

To compare the magnitude of the hydrophobic effect directly to equilibrium constants measured in solution, the changes in accessible surface area were converted to changes in hydrophobic free energy. We have used Chothia's empirical conversion factor (18) to estimate changes in hydrophobic free energy, ΔG^H, from the changes in surface areas of both the protein and the sugar. For equilibria involving sugar binding, it is also necessary to account for the loss of translational and rotational entropy of the free sugar. We have empirically estimated this entropic contribution to the overall free energy, $\Delta G°$, from the reduction of surface area upon binding *o*-toluoyl-2-glucosamine (OTG), a competitive inhibitor of glucose that cannot produce the conformational change (12), to the native B isozyme (line 5, Table 1). Because the observed dissociation constant of 10 μM accounts for 7 kcal/mol of the hydrophobic free energy, the remaining 7 kcal/mol is assumed to represent the entropy loss of immobilizing OTG or glucose on the enzyme surface.

A bound glucose molecule fits snugly between the two domains of the protein in the E′·G complex. The surface area of glucose accessible to solvent is reduced by 94% upon formation of the E′·G complex, and over half of the remaining 6% is associated with the 6-hydroxymethyl group.* That is, in the glucose complex, the glucose molecule is buried with only its 6-hydroxylmethyl group accessible to solvent or for reaction. Removing the glucose molecule from the model of the E′·G complex leaves a large cavity between the lobes of the protein. It does not appear to be possible for a glucose molecule to enter or to leave the hexose cavity of the E′ conformation.

DISCUSSION

Size and Nature of the Conformational Change. Our comparison of HKA·G complex and unliganded hexokinase B shows that the two lobes of hexokinase have moved together, closing the cleft into which the glucose is bound. A compact domain consisting of over a third of the polypeptide chain ro-

tates essentially as a rigid body by 12° relative to the rest of the molecule, causing movements of as much as 8 Å in the polypeptide backbone. This conformational change is comparable in both nature and magnitude to the change in quaternary structure seen in the allosteric transition of hemoglobin, in which the two $\alpha\beta$ dimers rotate 14° relative to one another as rigid units (21, 25). Intrasubunit flexibility involving entire domains has also been observed in tomato bushy stunt virus coat protein (26) and in immunoglobulins (27). Thus, the domains of relatively large, single polypeptide chains may show conformational changes between domains completely analogous to the subunit rearrangements exhibited by allosteric, oligomeric proteins.

Large changes in subunit conformation such as we find in hexokinase may be more common than current crystal-structure data suggest, because structural changes of this magnitude are often too large to be accommodated within one crystal lattice. Except for the virus coat protein, several crystal structures have been required to determine the extent of intrasubunit flexibility for these proteins. Our experience with hexokinase emphasizes that attempts to infer the extent of ligand-induced structural changes from crystals grown in only one ligation state can be misleading; the magnitude of the glucose-induced conformational change was not at all obvious from the glucose binding experiments on the B isozymes (5, 6).

Glucose Induces the Conformational Change. Because we have so far been unable to grow crystals of hexokinase A in the absence of glucose or of hexokinase B in the presence of glucose, one may ask whether the conformations of the enzyme observed in the HKA·G and BIII crystal forms are the result of the amino acid sequence differences of the two isozymes (28) or are caused by glucose binding. Several lines of evidence suggest that this conformational change is produced by the binding of glucose. The most compelling evidence is provided by the small angle x-ray scattering experiments of R. C. McDonald, T. A. Steitz, and D. M. Engelman (unpublished), which show that the radius of gyration of the hexokinase monomer measured in solution is reduced upon binding of glucose by the amount calculated from the atomic coordinates of BIII and HKA·G. Also, the disintegration of HKA·G crystals when glucose is removed and of B isozyme crystals in the presence of high concentrations of glucose suggests that glucose produces the change. Finally, low concentrations of glucose induce an alteration in the BIII structure that is qualitatively similar to but, due to lattice forces, quantitatively smaller than the change described here.

Conformational Change Essential. Anderson *et al.* (12) have concluded that the glucose-induced conformational change of hexokinase is required for catalysis. They find that the glucosyl moiety of the inhibitor OTG binds to the enzyme in the same orientation as glucose. Because the 6-hydroxyl group is in an identical position in both the glucose and OTG complexes with BIII crystals and because the toluoyl group cannot interfere directly with phosphate transfer, the observation that *N*-acyl-2-glucosamines are not substrates for hexokinase (29) has been explained by the fact that OTG does not produce the large conformational change (12). The conformational change is blocked by the toluoyl group, which lies in the slit between the two lobes. Although several residues of the small lobe are brought into the active site by the conformational change (Fig. 1), we cannot identify any likely catalytic groups at this stage of our analysis.

Energetic Aspects of the Conformational Change. It is evident from the changes in accessible surface area given in Table 1 that while hydrophobic forces alone may account for the formation of E′·G from E and free glucose, they cannot account for the stability of E relative to E′ in the absence of sugar. A more direct comparison of the accessibility data with equilibrium constants measured in solution requires conversion of changes in accessible area to changes in free energy. Chothia (18) has shown that accessible surface can be empirically related to the free energy of transfer of amino acid side chains from an organic phase to water, and that the hydrophobic free energy estimated by this procedure can account for the binding energy of protein complexes (23). Our extension of Chothia's method to the carbohydrate ligands of hexokinase is an approximation required by the absence of a measured free energy of transfer of hydrogen-bonded polar groups from an organic phase to water. In view of these uncertainties, the hydrophobic effect accounts remarkably well for the observed binding energy of glucose and the stabilization of the E′·G structure.

However, a major question posed by the solvent accessibility data is why the enzyme does not remain in the active, E′, conformation in the absence of ligands, because the hydrophobic effect alone predicts that the E′ structure should be more stable. We suggest that the answer may lie in the fact that the active structure in the absence of glucose contains a cavity in which water molecules are essentially buried in a protein interior. Although hydrogen bonds and van der Waals contacts normally contribute little to the stability of protein–protein and protein–ligand complexes, a failure to obtain complementary interactions within this cavity would result in unfavorable enthalpies due to loss of hydrogen bonds or van der Waals contacts relative to those made in the open-cleft structure (23). There may also be some loss of translational entropy upon trapping a small number of water molecules in the cavity. Presumably, water itself destabilizes the active structure by making favorable interactions only with the open, inactive structure. Only the correct substrate can provide the necessary van der Waals contacts and hydrogen bonds that permit the active structure to form.

Function of the Conformational Change. There are at least two possible functions for the glucose-induced conformational change: to allow an "embracing mechanism" (30) or to provide specificity (1, 24). The stereochemical mechanism of hexokinase may require the enzyme to surround or "embrace" its substrates such that the only way in which the substrates can enter and leave the active site is for the enzyme to open and close (30). The mechanism of hexokinase remains to be established. Alternatively or additionally, flexibility provides hexokinase with the ability to discriminate against water as a substrate. Hexokinase is a poor ATPase because it is not in the active conformation in the absence of the appropriate sugar, even though water may be properly bound to the binding site for the 6-hydroxyl group.

Fersht (31) has concluded that a flexible enzyme utilizing induced fit can be no more specific than the corresponding rigid enzyme by assuming that the free energy required to produce the conformational change is the same for all substrates. However, in the case of hexokinase, the free energy required to form the active conformation appears to be higher when water is the substrate, because water cannot provide the complementary interactions with the hexose cavity that glucose provides. Because the binding of glucose to the active, E′, structure is sterically blocked, flexibility appears to play a central and essential role in the specificity of hexokinase.

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