

Substrate Binding Closes the Cleft between the Domains of Yeast Phosphoglycerate Kinase*

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Clifford A. Pickover,‡ David B. McKay,§ Donald M. Engelman, and Thomas A. Steitz

From the Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520

Using small angle x-ray scattering from solutions of yeast phosphoglycerate kinase, we have measured the radius of gyration of the enzyme both in the presence and in the absence of ligands. We find that the radius of gyration decreases by 1.09 ± 0.34 Å upon binding both substrates MgATP and 3-phosphoglycerate to form the ternary complex. Smaller decreases, at the limit of the precision of the measurement, were found for the separate binding of MgATP (0.58 ± 0.32 Å) and 3-phosphoglycerate (0.30 ± 0.50 Å). Using computer modeling, it has been estimated that a substrate-induced cleft closure in phosphoglycerate kinase resulting from one lobe rotating $8-14^\circ$ relative to the other lobe is consistent with this observed change in radius of gyration. We suggest, therefore, that the conformational change that results in the smaller radius of gyration for the ternary complex is a hinge motion of the two lobes which produces a closing of the cleft between the two lobes. The apparent similarity of the ligand-induced change in phosphoglycerate kinase to the cleft closure in hexokinase suggests that this kind of conformational change may prove to be a rather general kinase phenomenon (Bennett, W. S., and Steitz, T. A. (1978) *Proc. Natl. Acad. Sci. U. S. A.* 75, 4848-4852; Anderson, C. M., Zucker, F. H., and Steitz, T. A. (1979) *Science* 204, 375-380).

by 0.95 ± 0.24 Å, precisely as predicted from the crystallographic coordinates of the native and liganded enzyme (14).

The structures of both yeast (7) and horse (2) phosphoglycerate kinase, crystallized without the addition of substrates, contain a very deep cleft formed by two quite separate protein domains. The shape of the phosphoglycerate kinase cleft is rather similar to the hexokinase cleft (6). Phosphoglycerate kinase is a monomer of about $M_r = 40,000$ and shows no tendency to form dimers (18). The binding of ATP and ADP has been examined by diffusing these ligands into crystals (2, 7), however, the enzyme has not been crystallized as the ternary complex with ATP·Mg and 3-phosphoglycerate or with the separate addition of either of these substrates to the crystallization medium. Although there is as yet no direct crystallographic evidence, it has been suggested by analogy with hexokinase that upon addition of the appropriate substrates the cleft in phosphoglycerate kinase will close by a relative motion of the two domains (6).

In order to obtain experimental evidence to test the idea of such a conformational change in phosphoglycerate kinase, we have measured the radius of gyration of native yeast phosphoglycerate kinase and its complexes with 3-phosphoglycerate, ATP·Mg, and both substrates. We observe a 1 Å reduction in the radius of gyration of phosphoglycerate kinase upon formation of the ternary complex which we show is consistent with a substrate-induced hinged motion of the two lobes which results in a reduction in the size of the cleft.

MATERIALS AND METHODS

Yeast phosphoglycerate kinase was purchased from Sigma Chemical Co. (type 4; No. P-7634, Lot 126C-8055) as an ammonium sulfate precipitate and was used without further purification. The ammonium sulfate precipitate was pelleted and dissolved in double distilled water. The enzyme was dialyzed against a buffer containing 50 mM imidazole/HCl, pH 7.0, 10^{-4} M dithioerythritol, and 10^{-4} M NaN_3 . After dialysis, the enzyme solution was centrifuged at about $10,000 \times g$ just prior to each set of measurements in order to remove any residual solid material. The solutions were refrigerated and used within 2 days of preparation. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate indicated that the enzyme was at least 95% pure. Protein concentrations were computed from absorbance measurements using an $E_{280}^{1\text{mg/ml}}$ of 0.57 (1).

Experimental runs for four separate sets of ligands were conducted. To 0.9 ml of enzyme solution was added 0.1 ml of the same buffer solution containing: 1) 32 mM MgCl_2 , 2) 130 mM 3-phosphoglycerate (Sigma, No. P-0259, Lot 117C-5013) and 32 mM MgCl_2 , 3) 32 mM ATP (P-L Biochemicals, Inc., No. 100-A, Lot 11001) and 32 mM MgCl_2 , and 4) a solution containing both the MgATP and 3-phosphoglycerate at the above concentrations. These concentrations of substrate ligands were chosen to be 10 times their K_m values in the phosphoglycerate kinase reaction (18).

X-rays produced by a Philips copper fine-focus tube were passed through a nickel filter and focused by reflection from a glass mirror, resulting in a beam consisting almost entirely of CuK_α radiation ($\lambda = 1.54$ Å). The beam was passed through a 1-mm glass capillary containing the solution, and scattered x-rays were recorded by a

The several kinases for which crystal structures are known consist of two lobes separated by a deep cleft in which substrates bind (1-9). Evidence from a variety of techniques, for example, magnetic resonance studies on arginine kinase (10, 11) and phosphoglycerate kinase (12), small angle x-ray scattering on pyruvate kinase (13) and hexokinase (14), kinetic studies of CH_3S -blocked creatine kinase (15), and changes in tryptophan fluorescence in hexokinase (16), suggests that many kinases undergo conformational changes in solution upon binding substrate ligands. Crystallographic studies on yeast hexokinase show that when glucose binds into the deep cleft, the two lobes move together in a hinged action, closing the cleft and burying the substrate (17). Small angle x-ray-scattering measurements on solutions of monomeric yeast hexokinase established that the formation of the glucose complex results in a reduction of the enzyme's radius of gyration

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Tennelec PSD 100 position-sensitive detector. The backscatter from the lead beamstop was used to monitor the transmitted beam intensity, and data were collected using the monitor count to time the experiment (1 to 4 h/sample). Background given by the scatter of buffer was subtracted to give the net scattering of the protein molecules as previously described (14).

X-ray irradiation produced no observable alteration in sodium dodecyl sulfate gel electrophoretic bands of the protein and remeasurement of samples showed no change in the radius of gyration. Therefore, x-ray damage was assumed to be minimal. The enzyme solutions were kept at 25°C for all experiments.

Data Analysis—For sufficiently small scattering angles, \ln (scattered intensity) versus the square of the reciprocal space coordinate is approximately linear; the apparent radius of gyration can be extracted from the slope of the linear region as (19):

$$R_g = \frac{\lambda\sqrt{3}}{2\pi} \left[\frac{\Delta \ln(I)}{\Delta(2\theta)^2} \right]^{1/2} \quad (1)$$

where R_g is the apparent radius of gyration, I is the net scattered intensity at angle 2θ , λ is the x-ray wavelength ($\lambda = 1.54 \text{ \AA}$ was used in these computations), and 2θ has been used to approximate $\sin(2\theta)$. The region of the scattering curve anticipated to be linear was estimated by computing, from the x-ray crystallographic coordinates, the scattering curve predicted for the phosphoglycerate kinase molecule (see below). For each experiment, a straight line was fit by the method of weighted least squares (20) to $\ln(N_S)$ versus $(2\theta)^2$, where the signal count N_S equals sample count N_T minus scaled background count N_B ; each datum $\ln(N_S)$ (corresponding to a single channel of the multichannel analyzer) was given a statistical weight equal to $(N_S^2/(N_T + N_B))$, the reciprocal of its variance.

For the set of experiments for each ligand, two methods were used to estimate $R_g(c=0)$, the radius of gyration at zero protein concentration, from the measurements of apparent R_g as a function of (non-zero) protein concentrations, c . In the first method, data for each ligand set was analyzed independently; a straight line was fit by the method of weighted least squares to the measured R_g versus c ; $R_g(c=0)$ and its standard deviation were then computed from the zero intercept and associated standard deviation of the straight line.

The second method consisted of least squares fitting straight lines to R_g versus c for all ligand sets simultaneously, allowing each line to have a unique intercept, but constraining all lines to have the same slope (which corresponds to assuming that there was no significant protein aggregation in the sample). Formally, using i to index a particular ligand data set, and j to index the individual datum within a set, and defining: n = number of ligand data sets; N_i = number of data within i th set; p_{ij} = statistical weight of ij th datum (taken to be $1/\sigma_{ij}^2$),

$$\epsilon_{ij} \equiv ac_{ij} + b_i - R_{gij} \quad (2)$$

and,

$$\overline{x_i^k y_i^l} \equiv \frac{\sum_{j=1}^{N_i} p_{ij} x_{ij}^k y_{ij}^l}{\sum_{j=1}^{N_i} p_{ij}} \quad (3)$$

$$\sigma^2(x_i) \equiv \frac{\sum_{j=1}^{N_i} p_{ij} (x_{ij} - \bar{x}_i)^2}{\sum_{j=1}^{N_i} p_{ij}} \quad (4)$$

$$f_i \equiv \frac{\sum_{j=1}^{N_i} p_{ij}}{\sum_{i=1}^n \sum_{j=1}^{N_i} p_{ij}} \quad (5)$$

where x and y may represent R_g , c , or ϵ , the common slope is given by:

$$a = \frac{\sum_{i=1}^n f_i [c_i \overline{R_{g_i}} - \overline{c_i R_{g_i}}]}{\sum_{i=1}^n f_i \sigma^2(c_i)} \quad (6)$$

and the K th intercept is given by:

$$b_K = \frac{\left\{ \overline{R_{g_K}} \sum_{i=1}^n f_i \sigma^2(c_i) - \overline{c_K} \sum_{i=1}^n f_i [c_i \overline{R_{g_i}} - \overline{c_i R_{g_i}}] \right\}}{\sum_{i=1}^n f_i \sigma^2(c_i)} \quad (7)$$

The variances are then:

$$\sigma^2(a) = \frac{\sum_{i=1}^n f_i \sigma^2(\epsilon_i)}{\left[\sum_{i=1}^n N_i - (n+1) \right] \sum_{i=1}^n f_i \sigma^2(c_i)} \quad (8)$$

$$\sigma^2(b_K) = \frac{\left[f_K (\overline{c_K})^2 + \sum_{i=1}^n f_i \sigma^2(c_i) \right] \sigma^2(\epsilon_K)}{(N_K - 2) \sum_{i=1}^n f_i \sigma^2(c_i)} \quad (9)$$

noting that the slope is adjusted with $\sum_{i=1}^n N_i$ data and $(n+1)$ degrees of freedom, but that the K th intercept is adjusted with only N_K data and 2 degrees of freedom.

It is readily apparent that for $n=1$, these equations reduce to the familiar result for least squares fitting of one line to a set of data:

$$a(n=1) = \frac{\overline{cR_g} - \overline{c} \overline{R_g}}{\sigma^2(c)} \quad (10)$$

$$b(n=1) = \frac{\overline{R_g c^2} - \overline{c} \overline{R_g} \cdot \overline{c}}{\sigma^2(c)} \quad (11)$$

$$\sigma^2(a(n=1)) = \frac{\overline{\epsilon^2}}{(N-2)\sigma^2(c)} \quad (12)$$

$$\sigma^2(b(n=1)) = \frac{\overline{c^2 \epsilon^2}}{(N-2)\sigma^2(c)} \quad (13)$$

Computer-simulated Cleft Closure—Computer modeling of the proposed cleft-closing conformational change was used to estimate how far one lobe of phosphoglycerate kinase must move relative to the other (and in what direction) in order to produce the observed radius of gyration change measured by solution scattering. Computer programs for a PDP-11/70 interfaced to a Versatec electrostatic plotter and an Evans and Sutherland Picture System II allowed visualization of the backbone atoms of phosphoglycerate kinase and a closing of its cleft by rotation of one lobe with respect to another. A view of the protein was selected which most completely revealed the cleft in projection, and a hinge point was then chosen for rotation. The rotation axis is perpendicular to the projected view. Rotations about residues 187 and 204 were explored, since these two points occur at each end of the α helix which connects the two lobes of the

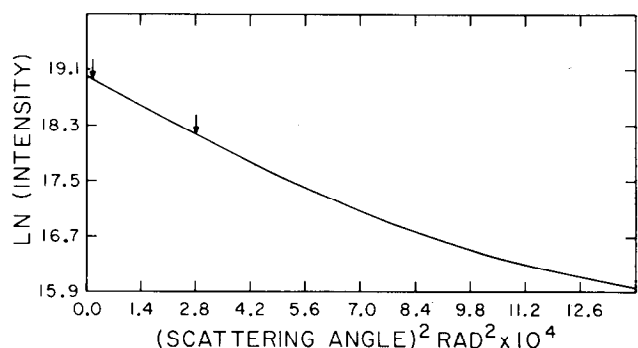


FIG. 1. Calculated scattering curve for phosphoglycerate kinase in solution. The scattering curve was calculated using the Debye (21) formula and plotted using the Guinier representation (19). The curve is linear over a portion of the curve near zero angle. The arrows indicate the region chosen for measurement in the experimental determination of radii of gyration in this paper.

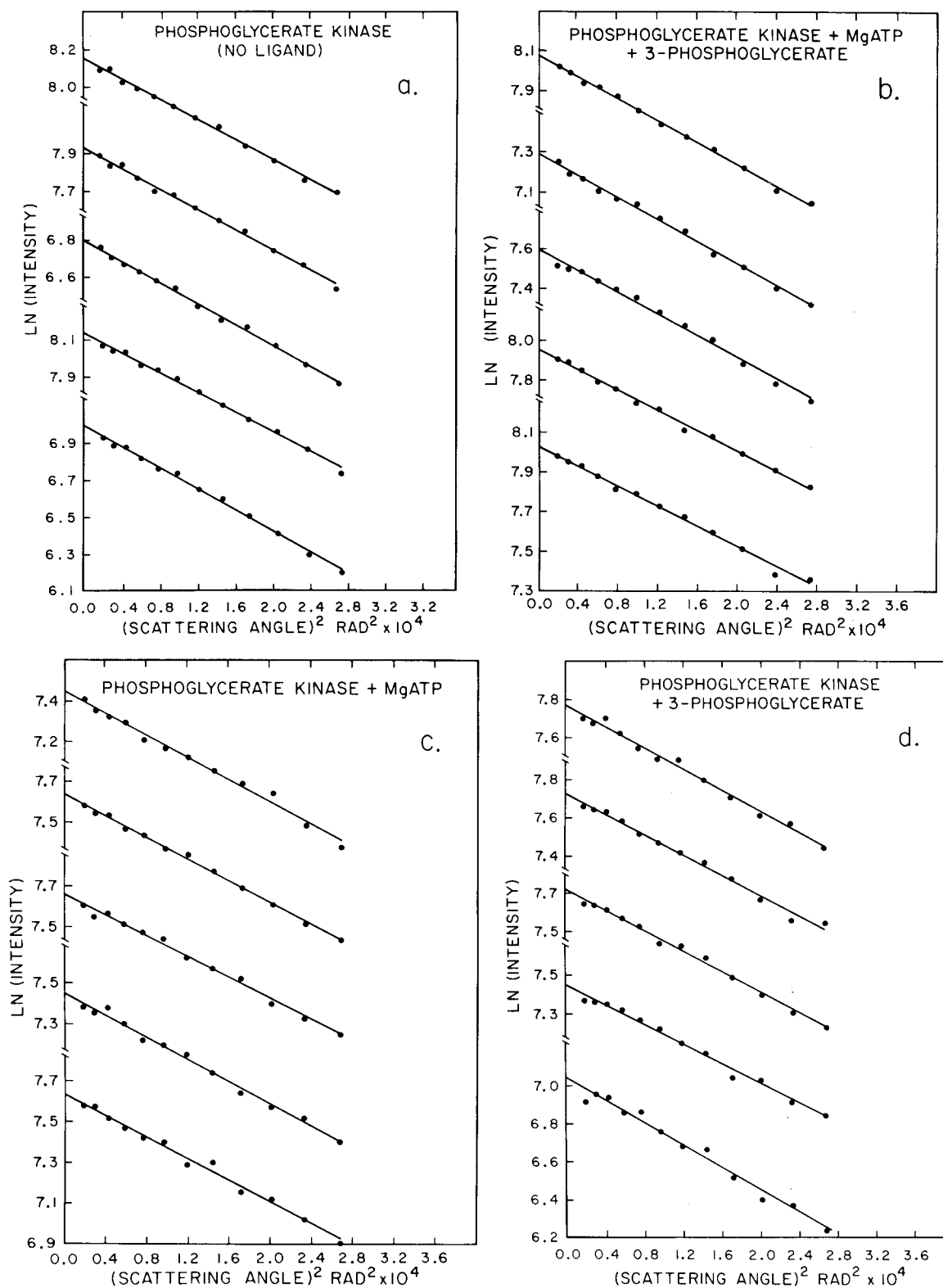


FIG. 2. Examples of data used in the determination of radii of gyration for different concentrations of phosphoglycerate kinase in the presence and absence of substrates. The region used for analysis corresponds to the indicated portion of the scattering curve in Fig. 1. The lines which are shown were fit using a variance-

weighted least squares procedure. Scattering curves of phosphoglycerate kinase in the absence of ligands are shown in *a*. Scattering curves for phosphoglycerate kinase in the presence of both MgATP and 3-phosphoglycerate (*b*), MgATP alone (*c*), and 3-phosphoglycerate alone (*d*) are also shown.

enzyme (2, 7). One lobe could be rotated relative to the other interactively on the Picture System. To obtain the exact angles of rotation, a computer-generated protractor was drawn on the screen which contained a pointer that moved with the rotating lobe. Radii of gyration were calculated from the relation:

$$R_g = \left[\frac{\sum_i Z_i R_i^2}{\sum_i Z_i} \right]^{1/2} \quad (14)$$

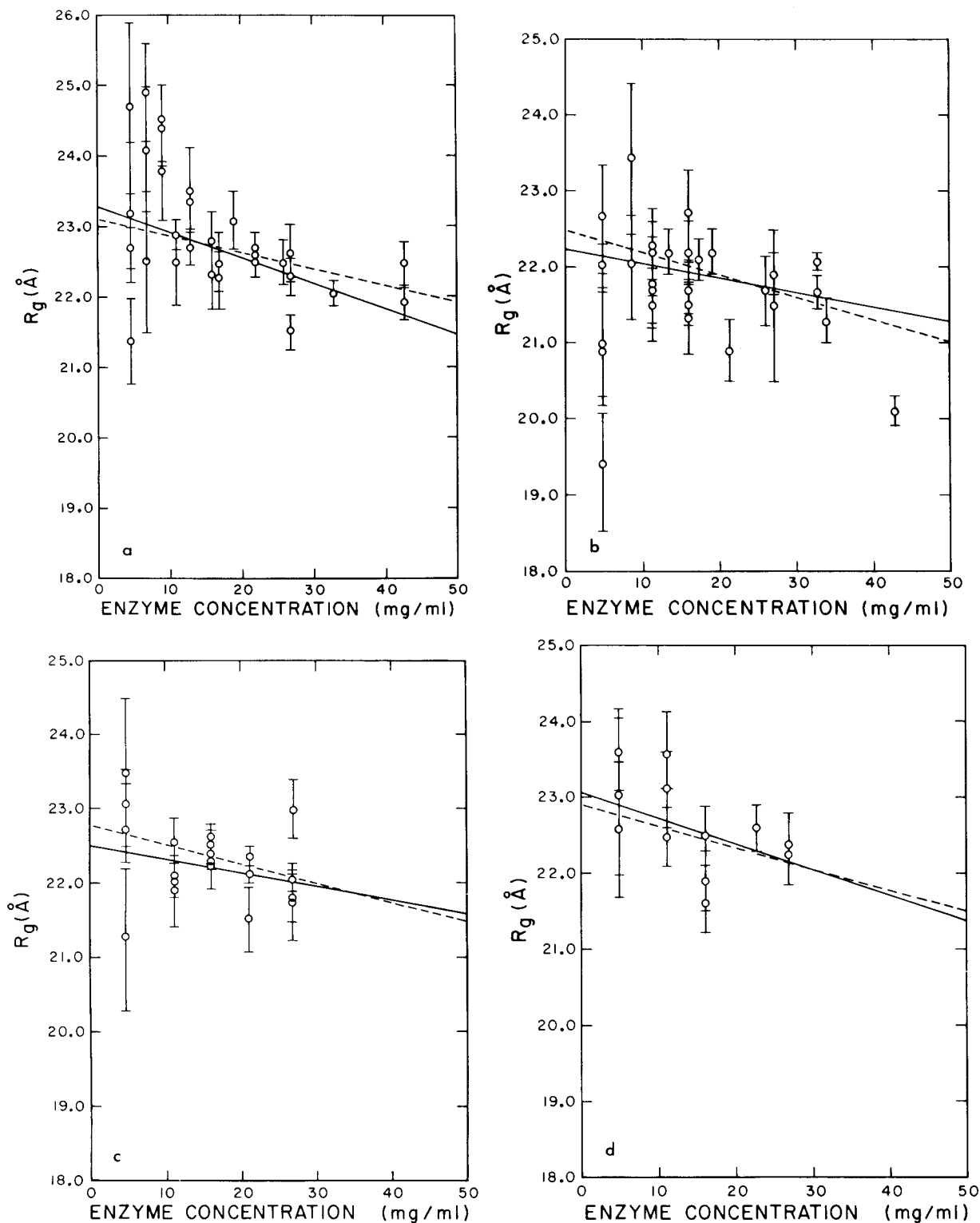


FIG. 3. Protein concentration dependence of the measured radii of gyration. The measured radii of gyration as a function of protein concentration are plotted for (a) native phosphoglycerate kinase and 3.2 mM MgCl_2 ; (b) in the presence of both 3.2 mM MgATP and 13 mM 3-phosphoglycerate; (c) in the presence of 3.2 mM MgATP ; and (d) in the presence of 13 mM 3-phosphoglycerate and 3.2 mM

MgCl_2 . To extrapolate these radii of gyration to zero protein concentration, straight lines were fit to these data in two ways: 1) all data were fit simultaneously to give one slope (---) using the method outlined here and 2) the data for each complex were fit separately (—).

where Z_i is the atomic number for atom i , and R_i is the atom's distance from the centroid of the atomic distribution in the molecule. Using only the β carbon atoms, radii of gyration were calculated at 3° intervals of rotation.

The calculations were based only on the β carbon atoms in order to reduce the computation time required. To establish that use of

only the β carbon atoms is a fair representation of the radius of gyration, the R_g was calculated for the yeast hexokinase A using 1) only the β carbon atoms (23.08 Å), 2) only the main chain atoms (22.98 Å), and 3) all of the atoms (22.82 Å). It appears that the β carbon atoms provide a reasonable approximation to the calculated radius of gyration. Although radii of gyration calculated in this way

will not predict the exact values observed in solution, the differences between them will be insensitive to solvent effects within the available accuracy of measurement (14).

Calculated Scattering Curve—The continuous scattering curve for phosphoglycerate kinase was calculated using the Debye relationship:

$$I(s) = \sum_{i=1}^N f_i^2 + 2 \sum_i \sum_{j>i} f_i f_j \frac{\sin 2\pi s R_{ij}}{2\pi s R_{ij}} \quad (15)$$

where $s = 2\sin\theta/\lambda$ and R_{ij} is the distance between atoms i and j in the molecule (21). The scattering curve, $I(S)$, was plotted using the Guinier representation (19) in order to define the linear region to be used for analysis.

RESULTS

A scattering curve was calculated using only the atomic coordinates of the backbone and β carbon atoms obtained from the crystal structure of horse phosphoglycerate kinase (2); the side chain coordinates are currently unavailable. From the Guinier plot of the small angle region of this calculated scattering curve, we chose the range (indicated by the arrows in Fig. 1) over which the curve is linear, and this region was used in the analysis of the experimental scattering data.

Examples of the Guinier plots for the ligand series are shown in Fig. 2. The data shown cover the angular range defined in Fig. 1. The straight line fits shown were obtained using the variance weighted least squares procedure described above.

Radii of gyration were calculated from these experimental data using Equation 1 and are shown as a function of enzyme concentration in Fig. 3. For each ligand two methods of extrapolating the radius of gyration to zero enzyme concentration are shown. The variance weighted least squares analyses of each of the ligand complexes calculated separately are shown as a smooth line in Fig. 3 while the fit of a straight line assuming a common slope but different intercepts is shown as a dashed line. Table I presents the values of the radius of gyration extrapolated to zero protein concentration obtained by the two methods of data analysis. Within the statistical error, the two methods of analysis give the same result: formation of a ternary complex with 3-phosphoglycerate and MgATP reduces the radius of gyration of phosphoglycerate kinase by about 1 Å. Addition of either MgATP or 3-phosphoglycerate separately reduces the radius of gyration by a smaller and not statistically significant amount.

Fig. 4 shows the relationship between the calculated radius of gyration and the extent of cleft-closure (computer-simulated) produced by rotation of one lobe relative to the other about residue 187 (squares) and residue 204 (circles). The radii of gyration were calculated after successive 3° rotations

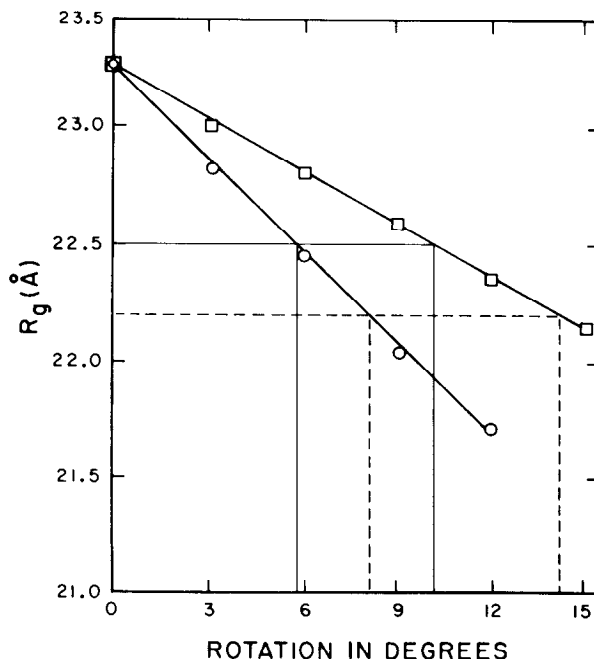


FIG. 4. Computer-simulated cleft closure. One lobe of phosphoglycerate kinase was rotated with respect to the other lobe on an Evans and Sutherland Picture System resulting in closure of the cleft between the lobes. Radii of gyration were calculated for the structures resulting from each 3° rotation of one lobe using only the β carbon atoms and Equation 2. A rotation axis was chosen which is perpendicular to the projected view showing the cleft most clearly. Rotations were performed about residues 187 (□—□) and 204 (○—○), since these two points occur at either end of an α helix which connected the two lobes of the enzyme. The relative rotations of the two lobes predicted by a 0.7-Å reduction (---) and a 1.1-Å reduction (—) in radius of gyration are shown.

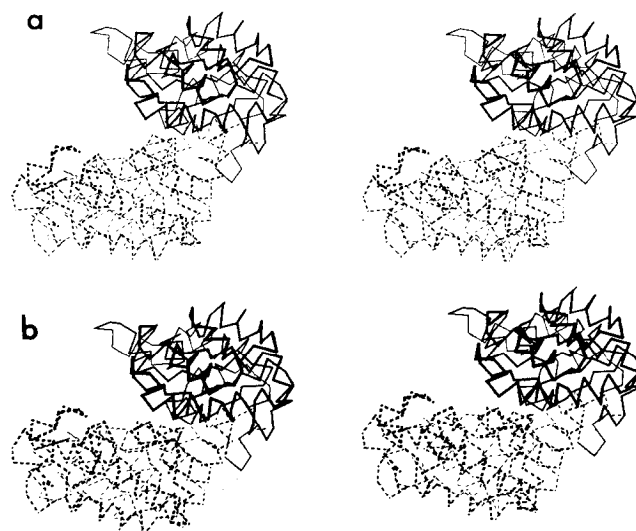


FIG. 5. Stereo drawing of phosphoglycerate kinase before (a) and after (b) a 12° rotation about residue 187. The drawings show only the β carbon atoms which were used to calculate the radius of gyration of the various modified structures. One lobe is represented in smooth lines while the other lobe is represented by dashed lines.

of one lobe using Equation 14 and including only the β carbon atoms (see "Materials and Methods"). Stereo drawings of native phosphoglycerate kinase and the enzyme after rotation of one lobe by 12° about residue 187 are shown in Fig. 5. It is assumed that the contribution of the ATP or 3-phosphoglycerate atoms to the radius of gyration is negligible, since radii

TABLE I

Influence of ligands on the radius of gyration of phosphoglycerate kinase

Ligand	Measured R_g	Decrease due to ligands
Å		
I. Variance-weighted least squares analysis, individual slopes		
No ligand	23.34 ± 0.22	
MgATP + 3PG ^a	22.25 ± 0.26	1.09 ± 0.34
MgATP	22.76 ± 0.24	0.58 ± 0.32
3PG	23.04 ± 0.45	0.30 ± 0.50
II. Variance-weighted least squares analysis, common slope		
No ligand	23.15 ± 0.16	
MgATP + 3PG	22.45 ± 0.18	0.70 ± 0.24
MgATP	22.84 ± 0.10	0.31 ± 0.19
3PG	22.96 ± 0.18	0.19 ± 0.24

^a 3PG, 3-phosphoglycerate.

of gyration calculated for the open hexokinase B structure with and without glucose are identical.¹ Because the coordinates of the substrate ligands bound to phosphoglycerate kinase are not available, they are not included in these calculations.

DISCUSSION

The radius of gyration measurements presented here show that phosphoglycerate kinase undergoes a large conformational change upon binding MgATP and 3-phosphoglycerate to form the ternary complex. The most straightforward interpretation of this result is that the conformational change consists of a closure of the large cleft observed (1, 2, 7) in this enzyme's structure. Such a change would be similar to that exhibited by hexokinase upon binding glucose or glucose 6-phosphate. Indeed, the reduction in the radius of gyration of hexokinase produced by the binding of glucose (0.95 ± 0.24 Å) is identical within error to the reduction observed with phosphoglycerate kinase upon formation of the ternary complex (1.09 ± 0.34 Å).

In developing a molecular explanation for this observed change in the radius of gyration of phosphoglycerate kinase upon binding substrates, two facts are particularly useful. First, the structure of the substrate-free phosphoglycerate kinase is known to consist of two quite separate but compact globular domains and, second, that its radius of gyration is reduced upon binding substrates. As observed in the hexokinase structures (17), and demonstrated by the model calculations on phosphoglycerate kinase, a 1-Å reduction in the radius of gyration can only be generated by a significant fraction of the molecule moving by several Angstroms. The observed reduction in the radius of gyration requires a decrease in the axial ratio of phosphoglycerate kinase. A major refolding of either domain is an unlikely cause of this decrease since no such structural change has yet been observed with other proteins, and a decrease in the volume occupied by the domains is not possible. Thus, we are led to conclude that a relative movement of the two domains into the cleft that separates them is the most likely cause of the observed ligand-induced reduction in radius of gyration. A hinged motion of the two domains would be analogous to the structural alterations observed in crystal structural studies of hexokinase (17), immunoglobulins (22), and tomato bushy stunt virus (23).

The relative movement of the two lobes of phosphoglycerate kinase that is required to produce the observed reduction in radius of gyration is very similar to that observed in the case of hexokinase. High resolution crystallographic studies have shown that one of the lobes of hexokinase rotates by 12° relative to the other lobe upon binding glucose (17). The data in Fig. 4 suggest that a 1.1-Å reduction in the radius of gyration of phosphoglycerate kinase could be generated by an 8 to 14° rotation of one of its lobes relative to the other. Clearly, we have no way of guessing the precise location of the rotation axis or whether the change also has a translational component; therefore, the correspondence between the observed change in radius of gyration and extent of rotation of the lobes must be considered approximate at best, indicating only the magnitude and direction of the conformational change. Nevertheless, these radii of gyration data are consistent with the hypothesis (6) that the nature and magnitude of ligand-induced conformational change are the same in hexokinase and phosphoglycerate kinase. A cleft-closing conformational change which brings the two lobes of this enzyme together is also consistent with the observation that MgATP binds to one lobe of phosphoglycerate kinase and the postulate

that 3-phosphoglycerate binds to the other lobe, some 12 Å from the ATP in the native structure; these substrate locations have led Blake and co-workers to also propose a hinge motion in phosphoglycerate kinase (24).

The reduction in radius of gyration of phosphoglycerate kinase upon binding either MgATP or 3-phosphoglycerate alone is small and at the limit of statistical significance. It appears that there is a synergistic interaction of these two substrates with this enzyme since the presence of both is required to shift equilibrium between the "open" and "closed" forms of the enzyme toward the closed form. It is possible that binding either of the substrates independently puts only a small fraction of these enzyme molecules in the closed form or produces only a partial closing of the cleft.

A large, substrate-induced conformational change consisting of a closing of a deep cleft may be general to many of the kinase enzymes (6). Since only hexokinase has been crystallized and its structure determined both in the presence and absence of substrates, there is currently little crystallographic evidence for this generalization. Adenylate kinase in the absence of substrates (3, 25) has a deep cleft which closes somewhat when they are diffused into the crystals. Phosphofructokinase, crystallized in the presence of the substrate, fructose 6-phosphate, is in a "closed" conformation with the sugar lying between the two lobes of the enzyme (9). It remains to be seen whether the two lobes are open in the absence of this sugar substrate.

It has been suggested that a cleft-closing conformational change may play an important role in catalysis and control of kinases (6). The use of small angle x-ray scattering on enzyme solutions both in the presence and absence of ligands appears to be a relatively rapid way to establish an experimental basis for the plausibility of such a generalization.

Furthermore, since numerous proteins consist of two or more lobes and many are known to undergo ligand-induced conformational changes, it may be true that many proteins in addition to kinases undergo cleft-closing conformational change. For example, completely analogous radius of gyration measurements on arabinose-binding protein done at Yale recently by Newcomer *et al.*² show a 1-Å reduction in this protein's radius of gyration upon binding arabinose. A hinged motion of the two domains of arabinose-binding protein analogous to the change in hexokinase is again suggested by these experiments.

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¹ R. C. McDonald, T. A. Steitz, and D. M. Engelman, unpublished observation.

² M. Newcomer, B. Lewis, and F. Quijcho, unpublished.

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