The Association of Yeast Phosphoglycerate Kinase (EC 2.7.2.3)

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1. A mol.wt. of 40030 ± 830 has been estimated for phosphoglycerate kinase in concentrations less than $0.1 \text{ g}/100 \text{ cm}^3$, comparing favourably with expected values from X-ray diffraction measurements but 10% lower than the previously reported molecular weights made at higher concentrations. 2. The $s_{20,w}^0$ was estimated to be $3.12 (\pm 0.02) \times 10^{-13} \text{ s}$ and the coefficient had a low concentration dependency giving a g value (concentration-dependency) of $2.3 \pm 1.6 \text{ cm}^3 \cdot \text{g}^{-1}$. This agrees with previous qualitative observations. 3. By using fluctuation-intensity spectroscopy, the $D_{20,w}$ was estimated to be $7.4 (\pm 0.2) \times 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$, and this was indistinguishable from the $D_{20,w}$ calculated from ultracentrifuge results. The water of hydration was estimated to be 0.46 g/g of protein. 4. It is inferred from the estimates that phosphoglycerate kinase associates with an interaction coefficient at 20°C for monomer/dimer of between 10 and $12 \text{ cm}^3 \cdot \text{g}^{-1}$. 5. The ratio of molecular asymmetry (a/b) was estimated to be 2.5 ± 0.2 from the values for $D_{20,w}$ and water of hydration. This compares favourably with the ratio from the overall dimensions estimated from X-ray diffraction measurements.

Structural studies by Watson and his collaborators (Wendell *et al.*, 1972; Bryant *et al.*, 1974), have shown that the overall shape of yeast phosphoglycerate kinase is a prolate ellipsoid having dimensions $8.5 \times 5.5 \times 5.5 \text{ nm}$. More detailed analyses show that it is constructed from approximately equally sized lobes joined through a waist of diameter 2.0 nm . The measurements of X-ray diffraction suggest that the mol.wt. should lie in the range 39000 to 41000, yet previous estimates (summarized in Table 1 of Scopes, 1973) suggest the mol.wt. lies within the range 34000 to 50000 with a mean of 44200 ± 1740 (the standard error of the mean was calculated from the eight estimates given by Scopes, 1973). The methods of analysis include gel filtration (mean 42000, based on two estimates), ultracentrifuge (mean 43000, based on three estimates), amino acid analysis (mean 45000, based on two estimates) and sodium dodecyl sulphate/gel filtration (50000, one estimate). All analyses show that phosphoglycerate kinase is a single chain, but the differences between estimates from X-ray analyses using crystals and those from solutions are significant, making it possible that part of the chain was missed in the structural studies. We have repeated the estimates of the hydrodynamic parameters on relatively low concentrations of phosphoglycerate kinase in an attempt to resolve this uncertainty.

For this work samples of phosphoglycerate kinase were taken from the same preparations that were used in the X-ray analyses (kindly donated by Dr. H.

Watson, University of Bristol, Bristol, U.K.) and the molecular weight was calculated assuming a partial specific volume estimated from the amino acid analyses (Krietsch & Bücher, 1970). This assumption could introduce bias in the estimates of molecular weight; therefore we needed an independent estimate for a molecular parameter to show consistency between $s_{20,w}^0$, $D_{20,w}$, partial specific volume and molecular weight. We chose the independent procedure of intensity-fluctuation spectroscopy (Foord *et al.*, 1970; Cummins & Swinney, 1970; Jamieson & Moret, 1973; Cummins & Pike, 1974) and compared the result with the $D_{20,w}$ calculated back from the molecular weight from the ultracentrifuge. By combining this data with estimates of the hydration of phosphoglycerate kinase, we were able to compare the overall frictional ratios from hydrodynamic measurements with those expected from X-ray studies.

Methods

Samples of phosphoglycerate kinase

Crystalline samples of phosphoglycerate kinase prepared by the method of Scopes (1971) were kindly donated by Dr. H. Watson, University of Bristol. Approximately 2 mg samples were separated from the mother liquor $[3\text{-M}(\text{NH}_4)_2\text{SO}_4]$ by centrifuging at low speeds, and were then dissolved in 1 cm^3 of phosphate buffer, $\text{pH } 7.0 \pm 0.05$, containing phosphate

ion (0.05M), 0.05M-NaCl and 1mM-EDTA ($I = 0.1\text{mol/l}$). The solution was equilibrated with the phosphate buffer by elution through a column ($10\text{cm} \times 1\text{cm}$) of Sephadex G-100 previously equilibrated with the buffer. Fractions of about 0.6cm^3 were collected; the maximum of the peak had absorbances ranging between 0.8 and 2.0 at 280nm. Three fractions comprising the peak and those each side were combined and used for analysis.

Sedimentation-velocity experiments involving concentrations greater than $2\text{mg} \cdot \text{cm}^{-3}$ were made with crystalline samples of phosphoglycerate kinase purchased from Sigma (London) Ltd., London S.W.6, U.K. These were equilibrated by dialysing against 1 litre of buffer for 14h at 4°C .

Ultracentrifuge experiments

Apparatus. Records were collected from experiments using both a Spinco Model E and an MSE analytical ultracentrifuge. Both instruments were fitted with temperature controllers for the rotors and in general there was no significant difference between estimates made from different machines.

Estimation of $s_{20,w}^0$. Sedimentation coefficients were calculated from records collected from samples with concentrations ranging from 0.5 to $9.6\text{mg} \cdot \text{cm}^{-3}$ by using double-sector cells fitted with either 10mm or 20mm centre pieces. The results from schlieren optics were recorded on film, which was subsequently measured to the nearest 10^{-3}mm by using a Nikon measuring microscope. The sedimentation coefficients (uncorrected for density and viscosity) were estimated by linear regression analysis. The runs were carried out at 20°C and the measured coefficients were corrected for viscosity and density by assuming a constant partial specific volume of $0.745\text{cm}^3 \cdot \text{g}^{-1}$.

Centrifugal-equilibrium experiments. Small columns of liquid (between 1 and 2mm long) were run at speeds ranging between 13500 and 15000 rev./min. The solutions were allowed to equilibrate for 17h before records were collected. The temperature was controlled at $20^\circ\text{C} \pm 0.05^\circ\text{C}$ for the MSE centrifuge, but a larger error occurred with the Model E. Absorbances were recorded by an automatic collection system employing a Nova 1220 computer (Data General Ltd., Greenford, Mddx. UB6 9BH, U.K.) for the MSE system, and a PDP8 (Digital Equipment Corporation, Reading RG1 7QN, U.K.) for the Model E. Both systems measured absorbances at discrete intervals along the column, and errors in the absorbances were decreased by averaging the transmissions at each position (Spragg, 1967; Spragg & Goodman, 1969; Spragg *et al.*, 1975). These data were stored by the computer and analysed immediately by regression analysis in which statistical tests were made for non-linearity of the relationship

between logarithm of concentration and the square of the radius. Molecular weights were calculated from the slopes of the linear relationships, assuming that the partial specific volume of phosphoglycerate kinase was $0.745\text{cm}^3 \cdot \text{g}^{-1}$ as calculated from the amino acid composition (Krietsch & Bücher, 1970) and the partial specific volume of the amino acids (Cohn & Edsall, 1943).

Measurement of translational diffusion coefficient

Diffusion coefficients were calculated from records of intensity-fluctuation spectroscopy (Foord *et al.*, 1970; Ford, 1972). Here the broadening of the Rayleigh line by scattering of laser light was measured with a digital autocorrelator (Precision Devices Ltd., Malvern, Worcs., U.K.). A focused beam from a 50mW Ar-ion laser (Coherent Radiation, Palo Alto, Calif., U.S.A.) was scattered by the solution and the scattering observed at 90°C to the incident beam. The normalized second-order correlation function [$g^2(\tau)$] is related to the translational diffusion coefficient by the relationship

$$g^2(\tau) = 1 + A \cdot e^{-2K^2 D_T \tau} \quad (1)$$

where A = constant depending upon temporal and spatial coherence, K = scattering vector, D_T = diffusion coefficient, τ is the delay time [$=rt$, where r = channel number (1 to 24) and t is a suitably chosen sample period]. Hence, plotting $g^2(\tau) - 1$ on a logarithmic scale against rt gives a straight line whose slope $= -2K^2 D_T$. This slope was estimated by linear regression and significant curvature tested at the 95% probability level. The refractive index of the solution was measured at 20°C with a Pulfrich refractometer.

Gas bubbles and dust were removed by the centrifugation method of King *et al.* (1973) before examination in 10mm fluorimeter cuvettes maintained at a temperature of $20 \pm 0.1^\circ\text{C}$. The diffusion coefficients were corrected to give $D_{20,w}$, by using the relative viscosity of the solution compared with water at 20°C .

Estimation of bound water in phosphoglycerate kinase

The proton-nuclear-magnetic-resonance method described by Kuntz and co-workers (Kuntz *et al.*, 1969; Kuntz, 1971), was used to estimate water bound to the polypeptide chain. An approximately 2% (w/v) solution of phosphoglycerate kinase contained in a 2mm spectrometer tube was frozen rapidly in liquid N_2 and the protein spectrum recorded at -30°C , by scanning over a range of 5000Hz in 250s with a Varian XL100 100MHz nuclear magnetic spectrometer. The spectrometer was calibrated at -30°C by using a solution containing $1.25\text{M} \cdot \text{H}_2\text{O}$, $4.5\text{M} \cdot \text{LiCl}$ and $0.01\text{M} \cdot \text{MnCl}_2$ in 5cm^3 of $^2\text{H}_2\text{O}$. The areas of the two peaks (protein

and water) were estimated by weighing to give by proportionation the weight of 'unfrozen' water associated with phosphoglycerate kinase.

Estimation of concentrations of phosphoglycerate kinase

Approximate concentrations were estimated from the absorbance at 280nm by using $E_{10\text{mm}}^{1\%} = 4.9 \pm 0.2$. The extinction coefficient was calculated by using the concentration estimated from the area of a schlieren profile formed by a solution of phosphoglycerate kinase having an absorbance of 1.08 at 280nm. The area was converted to concentration by using a constant for the magnification of the analytical ultracentrifuge. This constant was calculated from the mean of several estimates made with a solution of sucrose ($1\text{mg}\cdot\text{cm}^{-3}$). The error for the extinction coefficient was calculated by compounding the error in this constant with that incurred in estimating the area of the schlieren peak. The value for the extinction coefficient in the present study compares favourably with that quoted by Bücher (1955) of 5.0, but is higher than that calculated by Larsson-Raźnikiewicz (1970) from the amino acid composition (4.4).

The concentrations of solutions used in estimating $s_{20,w}^0$ were found from the schlieren records collected during the experiment, and those for bound water were taken from areas of schlieren peaks formed by an artificial boundary in the ultracentrifuge.

Estimation of density and viscosity

Conventional pycnometers and viscometers were used and measurements were made in a water bath with temperatures controlled to $\pm 0.02^\circ\text{C}$.

Spectrophotometric measurements

A Unicam SP.500 spectrophotometer with 10mm cuvettes was used for measuring absorbance.

Results

Ultracentrifuge

The estimated molecular weights for three solutions of phosphoglycerate kinase collected in one equilibrium experiment are summarized in Table 1. The range of molecular weights shown in Table 1 were typical of ranges found in subsequent experiments, and any dependency of molecular weights on concentration at these relatively low concentrations was masked by the errors. A total of eight concentrations in the range $0.2\text{--}0.03\text{g}/100\text{cm}^3$ were examined by using samples from two preparations of phosphoglycerate kinase. The mean mol.wt. from

these experiments was 40030 ± 820 (S.E.M. on eight estimates). In no case did the mol.wt. exceed 43000, and all the concentrations from each solution fitted a straight line better than a quadratic function. By using five concentrations in the range $0.05\text{--}1.0\text{g}/100\text{cm}^3$, the $s_{20,w}^0$ was estimated to be $3.12(\pm 0.02) \times 10^{-3}\text{s}$. The slope from the linear regression was $-0.72(\pm 0.31) \times 10^{-3}\text{s}\cdot\text{cm}^3\cdot\text{g}^{-1}$ giving a concentration dependency (g , eqn. 2 where c = concentration in $\text{g}\cdot\text{cm}^{-3}$) of $2.3 \pm 1.6\text{cm}^3\cdot\text{g}^{-1}$.

$$s_{20,w} = s_{20,w}^0(1 - gc) \quad (2)$$

Intensity-fluctuation spectroscopy

Many of the previous estimates of translational diffusion coefficients by intensity-fluctuation spectroscopy were made with concentrations of about $0.01\text{g}/\text{cm}^3$ (for example, bovine serum albumin; Raj and Flygare, 1974), but since the molecular weights were measured at concentrations considerably less than this, we wished to measure diffusion coefficients in similar dilute solutions. Preliminary experiments with bovine serum albumin showed that with a 25mW He/Ne laser (Scientifica Cook Ltd., London W3 8AU, U.K.), it was possible to obtain reproducible estimates of diffusion coefficients at concentrations of $0.2\text{g}/100\text{cm}^3$, but more precise results were obtained with a 50mW Ar-ion laser. These experiments also showed that records could not be used to detect heterogeneity in a sample by regression analysis using eqn. (1) and introducing two exponential terms to allow for two diffusion coefficients. The results shown in Fig. 1 illustrate this problem for two samples of bovine serum albumin which were contaminated with dimer. The quantity of dimer in the sample was estimated by measuring the $s_{20,w}$ by using second-moment analysis to locate

Table 1. *Estimates by ultracentrifuge equilibrium of the molecular weight of phosphoglycerate kinase calculated by using a partial specific volume of $0.745\text{cm}^3\cdot\text{g}^{-1}$*

Between 20 and 30 values of concentration at equilibrium were fitted to the linear regression of $\ln(c)$ against the square of radius for each estimate of the slope. The relative precision was between $\pm 4\%$ and $\pm 6\%$ of the estimate. The means for each solution were calculated from three scans of the cell and the standard error of the mean quoted. The three solutions were measured in one ultracentrifuge experiment.

Concentration ($\text{g}/100\text{cm}^3$)	Mean mol.wt.
0.05	41 170 ± 1230
0.04	39 270 ± 1570
0.03	40 700 ± 850

Fig. 1. Logarithm of the correlation function $[g^2(r)]$ plotted against channel number of autocorrelate (r)

Results are from light-scattering experiments (see the text for details). $\circ$, Partially purified bovine serum albumin; $\bullet$, crude bovine serum albumin (both approximately $0.2\text{ g}/100\text{ cm}^3$); $\square$, phosphoglycerate kinase at approximately $0.16\text{ g}/100\text{ cm}^3$.

the centre of gravity of the boundary and comparing this estimate with similar estimates made on purified bovine serum albumin. In the case where contamination was low (less than 15% w/w) the relationship $\ln[g^2(r)]$ and channel number showed no significant curvature in regression analysis at the 5% probability level. The estimated $D_{20,w}$ was $5.78(\pm 0.1) \times 10^{-11} \text{ m}^2 \cdot \text{sec}^{-1}$. In the second case, crude bovine serum albumin was examined and here the relationship was not linear (Fig. 1). The average D value was estimated by forcing a straight line to fit the record (equivalent to drawing a chord across the curved record) to give a $D_{20,w}$ value of $5.47(\pm 0.1) \times 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$. For the same solutions the weight average $s_{20,w}$ was $4.60 \pm (0.05) \times 10^{-13} \text{ s}$ for the first and $4.83(\pm 0.07) \times 10^{-13} \text{ s}$ for the second. From these estimates and the corresponding $s_{20,w}$ for pure bovine serum albumin at $0.2\text{ g}/100\text{ cm}^3$ [$4.20 \times 10^{-13} \text{ s}$ (Baldwin, 1957) and confirmed by our experiments], the proportions of monomer to dimer were calculated to be 19 and 25% respectively. Similar estimates from the diffusion coefficients by using the averaged value of $6.0 \times 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$ for pure bovine serum albumin from Raj & Flygare (1974) gave 17% and 32% dimer. This consistency in estimates of contamination by two independent methods showed that although contamination of small quantities of

polymer would not be detected by internal testing of an autocorrelation record, it should be possible to detect heterogeneity in phosphoglycerate kinase by inconsistency between the measured diffusion coefficient compared with the value calculated from ultracentrifuge experiments.

Measurements were made on a solution of phosphoglycerate kinase (Fig. 1) at an absorbance of 0.8 at 280 nm (approximately $0.16\text{ g}/100\text{ cm}^3$). Eight records from the autocorrelator were collected and analysed separately by regression analysis to give a mean $D_{20,w}$ (with standard error) of $7.45(\pm 0.21) \times 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$.

Water by hydration

Hydration measured by the method of Kuntz *et al.* (1969) relies on the inability of tightly bound water to freeze at -30° , making it possible to detect proton magnetic resonance in this unfrozen water. A 1 cm^3 sample of phosphoglycerate kinase in 1 mM-KCl (pH 6.0) contained $2.16 \pm 0.06\text{ g}$ of protein/ 100 cm^3 and gave a relative area under the peak compared with the calibration of 0.43 ± 0.01 . This is equivalent to $0.46 \pm 0.03\text{ g}$ of water/g of protein. A similar experiment carried out with bovine serum albumin gave $0.41 \pm 0.03\text{ g}$ of water/g of protein, which is not significantly different from that found by Kuntz *et al.* (1969) of 0.42 g of water/g of protein.

The band width of the protein peak was 524 Hz for phosphoglycerate kinase and 470 Hz for bovine serum albumin, suggesting the bound water had similar structural interaction with the peptide chain in both molecules.

Discussion

Our estimates of the molecular weight of phosphoglycerate kinase (40030 ± 820) at concentrations less than $0.1\text{ g}/100\text{ cm}^3$ are approximately 10% lower than those reported by previous workers using more concentrated solutions [Larsson-Raźnikiewicz (1970) obtained a mol.wt. of 44600 ± 1600 at about $0.7\text{ g}/100\text{ cm}^3$], but are very similar to the values from the X-ray diffraction studies (Bryant *et al.*, 1974) and experiments using sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (40900; Kulbe *et al.*, 1975). In contrast with the differing estimates of molecular weight, estimates of $s_{20,w}$ from our coefficients for the relationship between $s_{20,w}$ and concentration agreed closely with the earlier estimate made by Larsson-Raźnikiewicz (1970) at about $0.7\text{ g}/100\text{ cm}^3$. These inconsistencies would be resolved if phosphoglycerate kinase reversibly associated in solution. This can be the only explanation for the low dependency on concentration of $s_{20,w}$ over the range of concentrations studied [see Gilbert & Gilbert (1973) for details of interpreting results from the

ultracentrifuge when association occurs], since g for non-interacting proteins such as bovine serum albumin is about $0.054 \times 10^2 \text{ cm}^3 \cdot \text{g}^{-1}$ (Baldwin, 1957). Contamination of preparations with small quantities of polymeric material in the earlier preparations cannot be excluded. We found that gels of phosphoglycerate kinase could not be reconstituted after they had formed at the bottom of the ultracentrifuge cells during velocity experiments, and small quantities of these polymers in purified solutions of phosphoglycerate kinase would increase estimates of molecular weight. The experimenter would be unaware of their presence because a statistically significant linear relationship between $\ln c$ and the square of radius does not prove conclusively that the protein was homogeneous. Tests made with bovine serum albumin showed that as little as 5% (w/w) contamination would raise the weight-average molecular weight in equilibrium experiments by between 5 and 10%, and yet a straight line adequately described the ultracentrifuge results.

By using similar reasoning, it is possible to argue that our samples of phosphoglycerate kinase could be contaminated by material of small molecular weight, so lowering the estimated weight-average molecular weight. Fortunately the independent estimates of the diffusion coefficient showed this to be unlikely. Combining the $s_{20,w}^0$ of $3.12(\pm 0.02) \times 10^{-13} \text{ s}$ with the molecular weight and partial specific volume of $0.745 \text{ cm}^3 \cdot \text{g}^{-1}$ gives a $D_{20,w}$ of $7.43(\pm 0.16) \times 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$ (the errors quoted in this and subsequent parameters were calculated from the combination of variances assuming the co-variances were zero; Davies, 1961). This is indistinguishable from the value measured by intensity-fluctuation spectroscopy [$7.45(\pm 0.21) \times 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$], demonstrating that contamination of samples with low-molecular-weight material must be negligible.

It now remains to quantify the association of phosphoglycerate kinase by estimating an interaction constant (L) by using both the coefficients for concentration dependency of $s_{20,w}$ and molecular weights at low and higher concentrations. The close agreement between our calculated $s_{20,w}$ value ($3.09 \times 10^{-13} \text{ s}$) and that made by Larsson-Raźnikiewicz (1970) at $0.7 \text{ g}/100 \text{ cm}^3$ showed that our estimate for g (eqn. 2) of $0.024(\pm 0.016) \times 10^2 \text{ cm}^3 \cdot \text{g}^{-1}$ was accurate even though the internal precision was low. A low dependency of $s_{20,w}$ on concentration was observed by Larsson-Raźnikiewicz (1970) but only two concentrations were examined. Hence, assuming (a) the reaction is predominantly monomer-dimer, and (b) the concentration coefficient (g_0) was the same for monomer and dimer in the absence of interaction, L can be calculated by using the procedure described and illustrated by Gilbert & Gilbert (1973). All that is necessary here is to outline the strategy used in the calculations. The $s_{20,w}^0$ of the dimer was

calculated to be $5.511 \times 10^{-13} \text{ s}$, assuming it equalled $[s_{20,w(m)}^0]^{3/2}$. From these two values a family of curves was constructed for a guessed value of g_0 relating $s_{20,w}$ to concentration for a range of values L . From this family was chosen the L which gave a value for $s_{20,w}$ at $0.7 \text{ g}/100 \text{ cm}^3$, approximately equal to the $s_{20,w}$ measured experimentally. By using this L , a weight-average molecular weight was calculated at $0.7 \text{ g}/100 \text{ cm}^3$ to compare with Larsson-Raźnikiewicz's result, and if there was disagreement, g_0 was increased and a further family of curves calculated. Iterating between $s_{20,w}$ and molecular weights, adjusting g_0 at each cycle until reasonable consistency was achieved, gave a value for L lying in the range $10\text{--}12 \text{ cm}^3 \cdot \text{g}^{-1}$ for $g_0 \approx 10 \text{ cm}^3 \cdot \text{g}^{-1}$ ($s_{20,w}$ at $0.7 \text{ g}/100 \text{ cm}^3$ was calculated to be $3.06 \times 10^{-13} \text{ s}$ and weight-average mol.wt. = 42280; with the same g_0 but $L = 12 \text{ cm}^3 \cdot \text{g}^{-1}$, then $s_{20,w} = 3.091 \times 10^{-13} \text{ s}$ and weight-average mol.wt. = 42920). L is relatively small compared with the association constants of molecules like haemoglobin ($200 \times 10^2 \text{ cm}^3 \cdot \text{g}^{-1}$; Kellett, 1971) and a concentration of approximately 20% (w/v) of phosphoglycerate kinase would be required before 50% of the molecules are associated.

Whether this self-association has biological significance for phosphoglycerate kinase or affects the rate of reaction is uncertain, but it has not gone unnoticed that the subunits of lactate dehydrogenase are structurally similar to phosphoglycerate kinase (Adams *et al.*, 1969) and this molecule is a strongly associated tetramer. At this stage, where the estimates of the association constant only indicate the order of magnitude of the association, it would be impossible to speculate quantitatively on the energetics of the association.

The consistency between the estimates of the diffusion coefficient at relatively low concentrations gave us confidence that when all allowances were made for hydration of phosphoglycerate kinase, a value for the molecular asymmetry could be calculated (Tanford, 1961). A sphere of mass 40030 g and partial specific volume $0.745 \text{ cm}^3 \cdot \text{g}^{-1}$ would have $D_{20,w}$ at infinite dilution of $9.41(\pm 0.03) \times 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$; the $D_{20,w}$ of phosphoglycerate kinase compared with this value for a sphere of the same mass, indicates a frictional ratio of 1.264 ± 0.09 . This frictional ratio can be divided into two multiplicative components, one equated with solvation and the other molecular asymmetry (Onckley, 1941). The contribution from solvation was calculated to be 1.174 ± 0.01 , assuming the solvating liquid had the same density as the bulk fluid. Hence the frictional ratio ascribed to asymmetry was 1.077 ± 0.034 . From this value the a/b ratio for a prolate ellipsoid was calculated by using the Perrin equation (Perrin, 1936) and found to be 2.5 ± 0.2 (the error in this last parameter is not Gaussian in distribution but is the range expected when allowing for the error

on the asymmetrical frictional ratio). Wendell *et al.* (1972) gave the overall dimensions for phosphoglycerate kinase ($8.5 \times 5.5 \times 5.5$ nm) but since it has two lobes joined by a waist of 2.0 nm diameter, these overall dimensions would underestimate the ratio in comparison with that calculated from hydrodynamic measurements. An average diameter (b) is required which we have calculated to be 3.5 nm by using relative volumes of three spheres, two having diameters of 5.5 nm and one of 2.0 nm. This value compared favourably with the equivalent Stokes radius of 3.0 nm for a sphere having $D_{20,w} = 7.45 \times 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$. From these dimensions an a/b ratio of 2.5 was calculated. It seems, therefore, that the structure of phosphoglycerate kinase is not appreciably different in dilute solutions from that found by X-ray diffraction measurements. Flexing of the molecule about the waist is possible, as was suggested by Wendell *et al.* (1972) and if this does occur, then the amount of movement must be within the error of the present results. These errors are comparatively large and amount to 8% of the a/b ratio, which would be equivalent to decreasing the length to approximately 7.5 nm. This is sufficient to decrease appreciably the distance between the two lobes.

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