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Thermodynamic nonideality of enzyme solutions supplemented with inert solutes: yeast hexokinase revisited

Donald J. Winzor [a,*], Peter R. Wills [b]

[a] *Centre for Protein Structure, Function and Engineering, Department of Biochemistry, University of Queensland, Brisbane, QLD 4072, Australia*

[b] *Department of Physics, University of Auckland, Auckland, New Zealand*

Abstract

Published experimental results on the activating effect of polyethylene glycol on the interaction of yeast hexokinase with glucose (R.P. Rand, N.L. Fuller, P. Butko, G. Francis and P. Nicholls, Biochemistry, 32 (1993) 5925) are reinterpreted in statistical–mechanical terms of excluded volume. Of particular interest is the ability of this standard treatment of thermodynamic nonideality to accommodate the observed non-exponential dependence of the activation upon osmotic pressure of the polyethylene glycol solution – a dependence which is not predicted by analyses based on the concept of osmotic stress that was invoked originally to account for the results.

Keywords: Excluded volume; Enzyme isomerization; Hexokinase; Molecular crowding; Thermodynamic nonideality

1. Introduction

Attention was drawn recently to the potential use of high concentrations of an inert solute, polyethylene glycol (PEG), to perturb the extent of conformational changes in yeast hexokinase arising from interaction with substrate [1]. Such use of thermodynamic nonideality to probe protein isomerizations (pre-existing or ligand-induced) has been put into practice on several occasions during the past decade [2–11] by interpreting the thermodynamic nonideality in terms of excluded volume on the basis of a statistical–mechanical analysis. However, the concept of osmotic stress [12–14] has been invoked to account for the activating effect of PEG on the interaction of hexokinase with glucose [1]. The purpose of this communication is to establish that the observed activation of hexokinase by PEG is also amenable to rationalization in terms of the standard statistical mechanical concept of molecular crowding as the source of the thermodynamic nonideality.

2. Theory

2.1. Basic enzyme kinetic expressions

Consider the situation in which the binding of substrate (glucose), G, to hexokinase (enzyme), E, gives rise to the following reaction sequence [15,16]

$$E + G \overset{K}{\rightleftarrows} EG \overset{k}{\rightarrow} E + P \qquad (1)$$

in which k, the rate constant for breakdown of enzyme–substrate complex EG is considered to be

* Corresponding author.

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the rate-limiting step, thereby allowing the previous step effectively to be in a state of chemical equilibrium governed by thermodynamic dissociation constant K. Because investigations of the reaction either by initial velocity measurements in kinetic studies, or by spectrofluorimetry, are conducted at constant temperature and pressure (rather than constant chemical potential of solvent), we choose to define concentrations on the molal scale. Thus,

$$K = \phi y_G (m_E m_G / m_{EG}); \ \phi = y_E / y_{EG} \qquad (2)$$

where m_i denotes the molal concentration of species i, and ϕ is the ratio of the activity coefficients, y_i, of the two macromolecular species [2,6]: y_G, the activity coefficient of substrate, is a parameter that later will be considered separately. For either type of experiment the total enzyme concentration, $\overline{m}_E$, is related to its free concentration, m_E, by the expression

$$\overline{m}_E = m_E [1 + \phi y_G m_G / K] \qquad (3)$$

The total concentration of substrate, $\overline{m}_G$, may be substituted for its free concentration, m_G, in Eq. 3 on the grounds that $\overline{m}_G \gg \overline{m}_E$ in spectrofluorimetry as well as in determinations of the initial reaction velocity.

For hexokinase the quenching of fluorescence and the catalytic activity are both associated with the enzyme–substrate complex, EG [16], and accordingly theoretical expressions may be written in a form applicable to results obtained by using either technique. That K may be identified as either the dissociation constant or the Michaelis constant is supported by the reported identity of the Michaelis constant and the dissociation constant obtained by spectrofluorimetry [1]. We choose to develop those expressions from considerations of enzyme kinetic studies. On the basis of Eqs. 2 and 3, the initial velocity, v, for a reaction mixture is

$$v = k m_{EG} = k y_G \overline{m}_E \overline{m}_G \phi / [K + y_G \overline{m}_G \phi] \qquad (4)$$

whereupon v_{max}, the maximal initial velocity in the limit of infinite substrate concentration, is simply $k\overline{m}_E$. Expression of the initial velocity as a proportion of the maximal velocity thus gives rise to a dimensionless parameter, α, defined by the relationship

$$\alpha = v / v_{max} = y_G \overline{m}_G \phi / [K + y_G \overline{m}_G \phi] \qquad (5)$$

This rectangular hyperbolic dependence of α upon m_G must be a function of thermodynamic nonideality imposed by the presence of a high concentration of inert solute (polyethylene glycol), M, because the activity coefficients, y_i, of all species depend on the concentration of the inert solute. By adopting the reasonable assumption that thermodynamic ideality may be said to prevail (i.e., that an activity coefficient of unity applies to all species) in the absence of inert solute, the ratio of measured Michaelis constants in the absence and presence of inert solute (K and K_M respectively) is given by

$$K / K_M = y_G \phi \qquad (6)$$

Eq. 5 may also be applied to fluorescence measurements on the hexokinase system by defining α as the ratio of measured to maximal extents of fluorescence quenching ($\Delta F / \Delta F_{max}$). Such data may thus be subjected to the same analysis by substituting the ratio of measured dissociation constants for the left-hand side of Eq. 6.

2.2. Expressions for activity coefficient ratios

In general, the consideration of thermodynamic nonideality on a statistical–mechanical basis requires specification of the activity coefficient of any given species in terms of the concentrations of every species in the solution. However, the experiments on hexokinase [1] have been conducted under conditions such that the concentration of inert solute (polyethylene glycol, M) greatly exceeds that of enzyme ($m_M \gg \overline{m}_E$). Under that simplifying circumstance the effects of the inert solute dominate, and the required activity coefficient ratio may be written [17,18]

$$\phi = \exp[(A_{E,M} - A_{EG,M}) m_M + (A_{E,M,M} - A_{EG,M,M}) m_M^2 + \cdots] \qquad (7)$$

where $A_{E,M}$, $A_{EG,M}$ and $A_{E,M,M}$, $A_{EG,M,M}$ are second and third virial coefficients in the multinominal expansion of the chemical potential of solvent (μ_s)

in terms of all solute species (i or j) at constant temperature and pressure. Specifically, the decrease in the thermodynamic activity, a_s, of solvent due to the addition of inert solutes i, j, etc., is given by

$$-\ln a_s = -(1/RT)\left(\mu_s - \mu_s^0\right)_{T,P} \tag{8a}$$

$$= \sum_i m_i + \sum_{i,j \geq i} A_{i,j} m_i m_j + \sum_{i,j \geq i,k \geq j} A_{i,j,k} m_i m_j m_k + \cdots \tag{8b}$$

where μ_s^0 is the chemical potential of the solvent in the standard state (unit activity) at any temperature T and pressure P. Because aqueous solutions are essentially incompressible, the osmotic pressure may be related, to good approximation, directly to the chemical potential of solvent by the expression

$$\Pi = -\left(\mu_s - \mu_s^0\right)\rho_s \tag{9}$$

where ρ_s is the solvent density. Coefficients $B_{i,j}$ from the more familiar virial expansion of osmotic pressure at constant chemical potential of solvent in terms of molar concentration of solutes, C_i, namely,

$$(\Pi/RT)_{T,\mu_s} = \sum_i C_i + \sum_{i,j \geq i} B_{i,j} C_i C_j + \sum_{i,j \geq i,k \geq j} B_{i,j,k} C_i C_j C_k + \cdots \tag{10}$$

may be substituted into Eq. 8 to give

$$\phi = \exp\left[\left(B_{E,M} - B_{EG,M}\right) m_M \rho_s + \left(B_{E,M,M} - B_{EG,M,M}\right)\left(m_M \rho_s\right)^2 + \cdots\right] \tag{11}$$

The two sets of virial coefficients ($B_{i,j}$, $B_{i,j,k}$, etc.) have exact, simple statistical mechanical definitions in terms of intermolecular forces [19].

We note that Eq. 9 is established through the Gibbs–Duhem relationship, which justifies consideration of the effect in terms of either decreased solvent activity [12–14] or increased solute activity (the present approach).

3. Consideration of the hexokinase results

An important point to note is that all expressions up to and including Eq. 6 are thermodynamically based; and that the multinominal representation of thermodynamic nonideality, motivated by statistical–mechanical considerations, is only introduced in Eqs. 7 and 11 as a convenient means of specifying the composition dependence of ϕ, the activity coefficient ratio for macromolecular species. Before further pursuit of that line we note in Eq. 6 the requirement for specification of the effect of polyethylene glycol on the magnitude of the activity coefficient of glucose, y_G. This activity coefficient was taken as unity by Rand and coworkers [1] on the grounds that the osmotic pressures of polyethylene and glucose were additive. The validity of substituting glucose molalities for substrate activities has been confirmed by equilibrium dialysis of mixtures of glucose (2 mM) and PEG 20000 (4–15%), which yielded an activity coefficient of 0.99 ($\pm$0.04).

3.1. Effect of PEG concentration on hexokinase affinity for glucose

Having established that y_G may be taken as unity, we now proceed to use Eq. 11 to analyze the enzyme kinetic and spectrofluorimetric results for the hexokinase–glucose system that are reported in Fig. 2 of Ref. [1]. By substituting Eq. 11 for ϕ in Eq. 6, we obtain

$$\ln(K/K_M) \simeq \left(B_{E,M} - B_{EG,M}\right) m_M \rho_s + \left(B_{E,M,M} - B_{EG,M,M}\right)\left(m_M \rho_s\right)^2 + \cdots \tag{12}$$

where terms up to second order in $m_M \rho_s$ have been included in the exponent to emphasize that it is itself a series. A limiting linear dependence of the logarithm of the affinity ratio upon m_M is thus predicted for the range of PEG concentration in which second virial coefficients suffice to describe the thermodynamic nonideality.

Affinity ratios deduced from Fig. 2 of Ref. [1] are presented in Fig. 1 according to Eq. 12. We first note

that the data are very limited but that both sets of data may be interpreted reasonably as demonstrating a linear concentration dependence. Indeed, both sets of results attest to the fact that the second virial coefficient for PEG 2000 and the enzyme–substrate complex, $B_{EG,M}$, is smaller than $B_{E,M}$; but slightly different estimates of the difference $(B_{E,M} - B_{EG,M})$ are obtained from the kinetic (5.0 ± 0.2 l/mol) and fluorescence (6.2 ± 1.0 l/mol) results (broken lines in Fig. 1). Combining the two sets of results in Fig. 1 gives a best estimate of 5.7 (± 0.8) l/mol for the magnitude of $(B_{E,M} - B_{EG,M})$ — the solid line in Fig. 1.

3.2. Dependence of the osmotic pressure on polymer concentration

Provided that the concentration of inert polymer greatly exceeds that of enzyme, the osmotic pressure, Π, may be approximated as a simple polynomial in the molar concentration of polymer, C_M. Thus,

$$\Pi/RT \simeq C_M + B_{M,M}C_M^2 + B_{M,M,M}C_M^3 + \cdots \quad (13)$$

which on conversion to the molal concentration scale becomes

$$\Pi/RT\rho_s \simeq m_M + A_{M,M}m_M^2 + A_{M,M,M}m_M^3 + \cdots \quad (14)$$

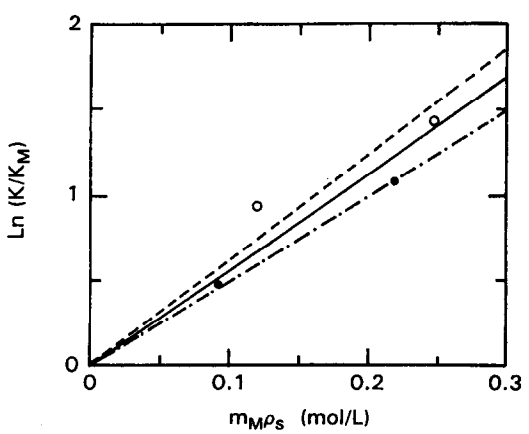

Fig. 1. Effect of PEG 2000 concentration on the relative Michaelis constant (●) and dissociation constant (○) for the interaction of glucose with yeast hexokinase, the results being taken from Fig. 2 of Ref. [1]. Broken lines describe the separate sets of results, whereas the solid line is obtained by subjecting the combined sets of results to linear regression analysis.

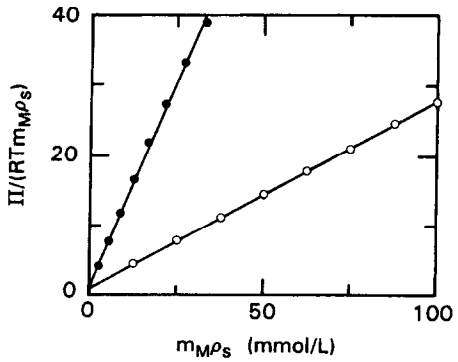

Fig. 2. Evaluation of second virial coefficients, $A_{M,M}$, from the concentration dependence of osmotic pressure for PEG 20000 (●) and PEG 8000 (○). The data, plotted in accordance with Eq. 14, have been inferred from relationships reported in Refs. [14] and [20,21] for PEG 20000 and PEG 8000 respectively.

where $A_{M,M}$, $A_{M,M,M}$, etc., the alternative second and third virial coefficients for self-interaction of M when concentrations are expressed on the molal scale, are defined by Eq. 8. Reported dependencies of $\Pi/(RTm_M\rho_s)$ upon $m_M\rho_s$ for PEG 20000 (●) and PEG 8000 (○) are summarized in Fig. 2. The former is based on a relationship between Π and polymer concentration for PEG 20000 at 30°C [14], whilst that for PEG 8000 has been obtained by substituting a temperature of 30°C into the expression reported by Michel [20,21]. These plots are essentially linear and hence osmotic pressures may be represented with good accuracy by Eq. 14 truncated at the quadratic term. Convergence of the polynomial series is evidently rapid. Values of $A_{M,M}/\rho_s = (B_{M,M} - M_M\bar{v}_M)$ may be obtained from the slopes of these plots, yielding values of 1200 l/mol for PEG 20000 and 260 l/mol for PEG 8000.

3.3. Dependence of the activation upon osmotic pressure

The actual evidence for a linear relationship between ln (K/K_M) and $m_M\rho_s$ over the concentration range of Fig. 1 is minimal; and we therefore attempt to employ the more extensive measurements of ln (K/K_M) as a function of osmotic pressure (Fig. 3 of Ref. [1]) to analyze further the effect of molecular crowding on the hexokinase–glucose system. The data (Figs. 1 and 2) indicate that, in the relevant

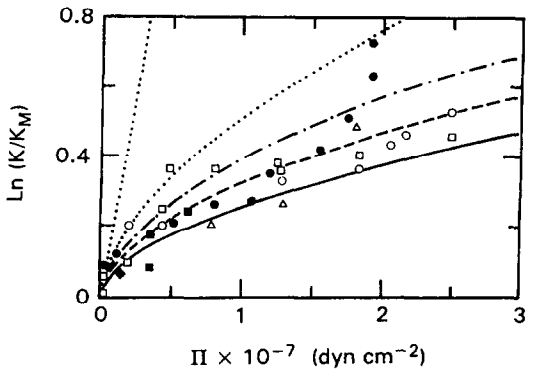

Fig. 3. Dependence of the logarithm of the affinity ratio, either in terms of Michaelis constant (solid symbols) or dissociation constant (open symbols), in the absence and presence of PEG 2000 (●,○), PEG 4000 (■,□), PEG 8000 (△) and PEG 20000 (◆) upon the osmotic pressure of the polymer solution, the results being taken from Fig. 3 of Ref. [1]. The dotted straight line describes the theoretical limiting tangent to these results on the basis of a value of 5.7 l/mol for $(B_{E,M} - B_{EG,M})$, the best-fit covolume difference deduced from Fig. 1 for PEG 2000, whereas the dotted curve is the quadratic form of the dependence predicted by eq. 15 with the term in $(B_{E,M,M} - B_{EG,M,M})$ neglected. Corresponding curves for PEG 4000 (-· ·· -), PEG 8000 (- - -) and PEG 20000 (—) are also shown.

concentration range, the series represented in Eqs. 12 and 14 are quite rapidly convergent. Under these circumstances the two equations can be combined to predict the empirical relationship between the activation of the reaction, $\ln(K/K_M)$, and the osmotic pressure, Π:

$$\ln(K/K_M) = (B_{E,M} - B_{EG,M})(\Pi/RT) + [(B_{E,M,M} - B_{EG,M,M}) - (B_{M,M} - M_M\bar{v}_M) \times (B_{E,M} - B_{EG,M})\rho_s](\Pi/RT)^2 + \cdots \quad (15)$$

Note that we have used the relationship $A_{M,M} = (B_{M,M} - M_M\bar{v}_M)\rho_s$, where $M_M\bar{v}_M$ is the partial molar volume of M, this being implicit Eqs. 13 and 14; and that empirical estimates of all relevant quantities in Eq. 15 are available except for $(B_{E,M,M} - B_{EG,M,M})$.

From Fig. 3, which summarizes the activation data from Fig. 3 of Ref. [1] for PEG 2000, PEG 4000, PEG 8000 and PEG 20000, it is evident that the dependence of $\ln(K/K_m)$ upon Π is decidedly curvilinear; and that the expected limiting tangent (· · ·) for PEG 2000 (based on Fig. 1) would have been extremely difficult to infer from the experimental results. It is significant that the analysis of these results based on the concept of osmotic stress demands a linear relationship between $\ln(K/K_M)$ and Π across the entire range of polymer concentrations. The parabolic curves drawn in Fig. 3 represent applications of Eq. 15 based on a mixture of empirically and theoretically guided estimates of the various virial coefficients, $B_{E,M}$, $B_{EG,M}$, $B_{M,M}$, etc.

4. Interpretation of virial coefficients

According to the theory of McMillan and Mayer [19] the virial coefficients $B_{i,j,k,\ldots}$ appearing in Eq. 10 may be given a rigorous statistical mechanical interpretation. For example, the osmotic second virial coefficients are given in terms of the energy of interaction $u(r)$ between two molecules by

$$B_{i,j} = -4\pi N\int_0^\infty \{\exp[u(r)/kT] - 1\}r^2\,dr; \quad i \neq j \quad (16)$$

where r is the distance between the centres of mass of solute molecules i and j, N is Avogadro's number, k is Boltzmann's constant, and T is the absolute temperature: for i = j, the initial factor of 4 in Eq. 16 is replaced by a factor of 2. The integrand in Eq. 16 must be averaged over all configurations and orientations of the polymer and protein at each value of r. For rigid globular protein species (i = P) and in the absence of nett charge on the polymer (j = M), the energy of interaction, $u(r)$, may be taken to be infinite if the polymer chain overlaps with the protein domain (and the exponential term in the integrand of Eq. 16 is zero), or zero otherwise (in which case the exponential term is unity and there is no contribution to the integral). Thus $B_{P,M}$ is a measure of the covolume, $U_{P,M}$; that is, the statistically averaged volume which the centres of mass of molecules of protein and polymer mutually exclude. In a recent study [22] it has been found that the covolume of a spherical macromolecule with radius r_P and a ran-

dom-flight polymer with root-mean-square end-to-end distance, ℓ_M, is given by

$$U_{P,M}/N = (2\pi/3)\ell_M^2 r_P + 4(2\pi/3)^{1/2}\ell_M r_P^2 + (4\pi/3) r_P^3 \quad (17)$$

This formula describes with surprising accuracy the covolume of PEG 20000 with a wide range of proteins if r_P is taken as the Stokes radius, a rather crude measure of the average size of a protein [23].

4.1. Consideration of hexokinase activation results

In order to interpret the magnitude of $B_{E,M} - B_{EG,M}$ in terms of a substrate-induced conformational change, we need an estimate of $B_{E,M}$ for hexokinase and PEG 2000. The Stokes radius, r_E, for hexokinase may be obtained from the sedimentation coefficient ($s_{20,w}$) of 3.4 S, the molar mass of 51000 g/mol and the partial specific volume ($\bar{v}$) of 0.74 ml/g reported by Hoggett and Kellett [15], whence we obtain $r_E = 3.4$ nm. On the basis of the reported dependence of the root-mean-square end-to-end distance of polyethylene glycol upon molar mass [24], we obtain $\ell_M^2 = 12$ nm^2 for PEG 2000. Combination of these values for r_E and ℓ_M^2 in Eq. 17 gives $B_{E,M} = U_{E,M} = 290$ l/mol.

From the viewpoint of interpreting the change in covolume in terms of a corresponding change in the volume of the hydrated protein ($4\pi N r_E^3/3 = 99$ l/mol), we see from Eq. 17 that this volume is only one term in the expression for covolume. On the basis that $U_{EG,M} = (290 - 5.7) = 284.3$ l/mol, a value of 3.37 nm (cf., 3.40 nm) is obtained for the effective radius of the EG complex. This corresponds to an effective spherical volume of 96.5 l/mol, which implies a 2.5% decrease in the apparent solvated volume. Such a change in the effective spherical volume of the hydrated protein may simply reflect a shape change, and therefore may not be identified unequivocally as a reduction in the actual size of solvated protein molecules. Both effects would contribute to $B_{E,M} - B_{EG,M}$.

On the basis of this effective radius of 3.37 nm for r_{EG} (cf, 3.40 nm for E), $(B_{E,M} - B_{EG,M})$ is calculated from Eq. 17 to be 15 l/mol for PEG 20000 as M; 9 l/mol for PEG 8000 as M; and 7 l/mol for PEG 4000 as M (cf., 5.7 l/mol for PEG 2000).

4.2. Osmotic pressure results

Theoretical relationships predicted by eqs. 12 and 14 for PEG 2000 ($\cdots$), PEG 4000 ($-\cdot-\cdot-$), PEG 8000 ($---$) and PEG 20000 (——) are compared with the ln (K/K_M) versus Π data [1] in Fig. 3. These curves have been generated by using the estimates of $(B_{E,M} - B_{EG,M})$ noted above and the values of $B_{M,M}$ for PEG 20000 and 8000 obtained from Fig. 2. Values of $B_{M,M}$ for PEG 2000 and PEG 4000 have been taken as 36 and 105 l/mol on the basis of the expected proportionality between $B_{M,M}$ and $M_M^{3/2}$, which follows from the fact that $B_{M,M} \propto \ell_M^3$ and $\ell_M^2 \propto M_M$ [24]: the partial specific volume of polyethylene glycol has been taken as 0.83 ml/g [25] in the interconversions of $A_{M,M}$ and $B_{M,M}$. The effect of interactions among molecule triplets (E, M, M and EG, M, M) has been neglected on the reasonable grounds (i) that the limited data in Fig. 1 showed no detectable curvature in the concentration range of interest and therefore no significant contribution from the $(B_{E,M,M} - B_{EG,M,M})$ term in Eq. 12, and (ii) that the quadratic coefficient in Eq. 15 is expected to be dominated by the large value of $B_{M,M}$. This approximation is not expected to have any effect on our general conclusion.

The first point to note in Fig. 3 is that the curvilinear form of all predicted dependencies is reasonably consistent with the experimental results, which are clearly incompatible with their previous interpretation in terms of a mandatory linear relationship [1]. Secondly, although the calculated limiting slopes (as $\Pi \to 0$) increase progressively with size of the inert polymer (2.5-fold greater for PEG 20000 than the dotted tangent for PEG 2000), ln (K/K_M) becomes progressively less sensitive to Π at higher pressures when larger and larger polymers are used to raise the osmotic pressure. Consequently, although some dependence of the curvilinear ln (K/K_M) versus Π relationship upon size of the polyethylene glycol is predicted, this cross-over of dependencies would almost certainly obscure any effect of polymer size, particularly in view of the extent of scatter exhibited by the experimental results (Fig. 3). Very much more precise data are needed to settle the issue

of whether the ln (K/K_M) versus Π plot shows any dependence on molecular weight of the polymer responsible for the osmotic pressure. Clearly, although measurement of the effect of osmotic pressure is a convenient method of detecting a substrate-induced conformational change, it does not seem to be a particularly rewarding means of quantifying $(B_{E,M} - B_{EG,M})$, the change in the polymer-dependent second virial coefficient associated with formation of EG from E.

5. Discussion

The above considerations of the activating effect of polyethylene glycol on the interaction between hexokinase and glucose serve to re-emphasize that simple, accurate measurements of thermodynamic quantities can provide useful information about molecular events and mechanisms of biological interest [2–11]. In that regard, the conclusion from Figs. 1 and 3 that the second virial coefficient for enzyme–polymer interaction is decreased by substrate binding $(B_{EG,M} < B_{E,M})$ is clearly reflecting the substrate-induced change in enzyme conformation that is deduced by X-ray crystallography [26]. Closure of the active-site cleft as the result of glucose binding is manifested as a change in the covolume $(U_{P,M})$ from which the centres of mass of the inert solute (rather than solvent) molecules are on average excluded (Fig. 4). The difference between $B_{E,M}$ and $B_{EG,M}$ could be interpreted as a decrease in the extent of protein solvation [25,27,28] if M were a molecule comparable in size with a water molecule rather than a large extended polymer. However, inspection of the development of the protein solvation concept [17,18,25,29] reveals that the term 'preferential solvation' has been invoked merely to describe the solvent (water) molecules that occupy the volume shell corresponding to the difference between the covolume and the volume of the unsolvated protein. Consequently, adoption of the protein solvation approach also leads to the conclusion that the parameter being quantified in the hexokinase–glucose experiments [1] is the change in covolume, $U_{E,M} - U_{EG,M}$, which can be equated with a change in the hydrated volume of the protein (V_P) only when M is small and $U_{P,M} \simeq V_P$ [17,18]. Inasmuch as this covolume change was originally interpreted as the substrate-induced change in hydrated volume of the enzyme molecule [1], there is clearly conflict between interpretations based on the osmotic stress approach [12–14] and those based on standard thermodynamic and statistical–mechanical treatments of the same experimental results. It must be acknowledged, of course, that a change in the solvated volume of the protein will cause a change in the measured covolume, all else being equal.

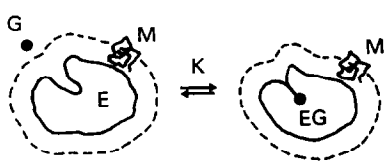

Fig. 4. Illustration of the change in covolume, $U_{P,M}$, between protein (P) and polymer (M) molecules as a result of ligand binding. The polymer molecule is shown in a relatively compact configuration, whereas the definition of covolume requires that an average be taken over all possible configurations and orientations of the polymer, and that the positions of molecules relative to one another be defined in terms of their centres of mass.

Thus, our interpretation on measured values of $B_{E,M}-B_{EG,M}$ based on Eq. 16 and the model approximations inherent in Eq. 17 is relatively robust, irrespective of any interpretation of the variation of osmotic pressure with concentration for extended polymers. We have made use of the empirical relationships for Π versus $m_M \rho_s$ without recourse to any interpretation of $B_{M,M}$ or higher-order virial coefficients for polymer–polymer interaction in terms of McMillan–Mayer theory, whose range of validity may be limited in the case of highly extended polymers such as polyethylene glycol.

As noted elsewhere [17,18], our preference for the statistical mechanical interpretation in terms of second (and higher) virial coefficients is its access to the magnitudes of activity coefficients and hence its potential for predicting quantitatively the displacement of macromolecular equilibria by inert solutes. The same approach also seems to have advantages in accounting for phenomena previously attributed to the consequences of osmotic stress. Although the predicted dependencies of the affinity ratio for the hexokinase–glucose system upon osmotic pressure cannot yet be regarded as very precise quantitatively,

because of their failure to take into account thermodynamic nonideality other than that arising from second virial coefficient effects, they do at least emulate the curvilinear form of the experimental results. What this investigation has thus shown is that the full form of the published results on the activation of the hexokinase–glucose interaction by polyethylene glycol [1] finds ready explanation in terms of thermodynamic nonideality arising from molecular crowding.

Acknowledgements

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