Volumetric and spectroscopic characterizations of glucose–hexokinase association

Rana Filfil, Tigran V. Chalikian*

Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, 19 Russell Street, Toronto, ON, Canada M5S 2S2

Received 5 September 2003; revised 8 October 2003; accepted 9 October 2003

First published online 21 October 2003

Edited by Thomas L. James

Abstract The binding of D-glucose to hexokinase PII at 25°C and pH 8.7 has been investigated by a combination of ultrasonic velocimetry, high precision densimetry, and fluorescence spectroscopy. The binding of glucose to the enzyme results in significant dehydration of the two interacting molecules, while the intrinsic coefficient of adiabatic compressibility of hexokinase slightly decreases. Glucose–hexokinase association is an entropy-driven process. The favorable change in entropy results from compensation between two large contributions. The binding-induced increase in hydrational entropy slightly prevails over the decrease in the configurational entropy of the enzyme. Taken together, our results emphasize the crucial role of water in modulating the energetics of protein recognition.

Key words: Glucose; Yeast hexokinase; Protein–ligand recognition; Thermodynamics; Hydration; Volumetric properties

1. Introduction

Hexokinase is the first enzyme in the glycolytic pathway, catalyzing the transfer of a phosphoryl group from ATP to glucose to form glucose-6-phosphate. From this viewpoint, the interaction of glucose with hexokinase is central to glucose metabolism. There are two known isoenzymes of yeast hexokinase, PI and PII, with an overall homology in their amino acid sequences of about 75% [1,2]. PII is the predominant form of hexokinase that participates in the catabolism of glucose [3]. At physiological pH, native yeast hexokinase PII mostly exists as a homodimer with a molecular weight of 104 kDa per dimer [4,5]. Upon an increase in pH and/or ionic strength, the dimer dissociates into two identical 52 kDa subunits [6]. The monomer consists of a single polypeptide chain of 461 amino acids that is distinctly folded into two unequal domains. The large (residues 2–58 and 187–458) and small (residues 59–186) domains are separated by a deep cleft which represents the glucose binding site [7,8]. Hexokinase PII consists of 14 α-helices and 13 β-strands, while, in overall shape, it resembles a kidney with approximate dimensions of $59\times78\times54$ Å^3 [9]. Upon its association with glucose, hexokinase PII undergoes a pronounced conformational change that results in closing the cleft between the two lobes [9–12]. This binding-induced transition of the enzyme brings about a significant decrease in solvent-accessible surface area thereby leading to dehydration of previously solvent-exposed atomic groups [8,11].

The energetics of the binding of D-glucose to yeast hexokinase PII was studied by Takahashi et al. [13] who employed to this end a combination isothermal flow and batch calorimetry, differential scanning calorimetry (DSC), and fluorometric titration measurements. Catanzano et al. [14] used DSC to characterize thermally–induced denaturation of hexokinase PII in the absence and presence of D-glucose. These two independent studies revealed that, at room temperature and high ionic strength, the association of the substrate with the enzyme is an entropy-driven process with an insignificant change in enthalpy [13,14]. It was suggested that the favorable change in entropy results from compensation between three main contributions: dehydration of the protein and the ligand, a tightening of the protein structure, and a loss of translational and rotational degrees of freedom of the interacting species upon their complex formation [13,14]. However, no attempt was made to quantify these entropic contributions and thereby evaluate the relative importance of each of the three factors.

On the other hand, osmotic stress measurements performed several years ago by Reid and Rand [15] revealed that more than 300 water molecules may become released to the bulk upon glucose association with hexokinase. Such extensive dehydration of interacting surfaces clearly should significantly influence the binding energetics. However, as mentioned above, the contribution of hydration as well as the contribution of other microscopic events to the energetics of glucose–hexokinase association has not been resolved as of yet. This deficiency is unfortunate and prevents one from identifying and evaluating the relative importance of molecular interactions that stabilize/destabilize the glucose–hexokinase complex and ultimately facilitate transfer of a phosphoryl group from ATP to glucose. To alleviate the situation, we combine volumetric and spectroscopic measurements to investigate the binding of glucose to hexokinase. We use these results in conjunction with previously reported calorimetric and structural data to characterize the binding-induced changes in hydration and intrinsic packing of the interacting molecules. We also estimate the energetic impact of these changes.

2. Materials and methods

2.1. Materials

Crude baker's yeast hexokinase was purchased from Sigma-Aldrich

*Corresponding author. Fax: (1)-416-978 8511.
E-mail address: chalikan@phm.utoronto.ca (T.V. Chalikian).

0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)01187-6

Canada (Oakville, ON, Canada) as a mixture of isoenzymes. The hexokinase PII isoenzyme was isolated and purified from the mixture of isoenzymes following a procedure described by Kaji et al. [16]. When purifying hexokinase PII, we have used a Bio-Rad model Bio-Logic Duo-Flow chromatography system (Bio-Rad Laboratories (Canada), Mississauga, ON, Canada). The final protein sample was chromatographically and electrophoretically pure. D-Glucose was also acquired from Sigma-Aldrich Chemical (Oakville, ON, Canada) and used without further purification. All measurements were performed in a pH 8.7 buffer consisting of 20 mM diglycine and 200 mM NaCl. All buffer solutions were prepared using doubly distilled water. The concentration of hexokinase PII was determined spectrophotometrically using the previously reported extinction coefficient of $\varepsilon_{280} = 0.947 \pm 0.02$ l g^{-1} cm^{-1} [6].

2.2. Fluorescence

Fluorescence intensity measurements were performed in a 10 mm path length cuvette using an Aviv model ATF 105 spectrofluorometer (Aviv Associates, Lakewood, NJ, USA). Fluorescence titration profiles were measured by incrementally adding aliquots of glucose to a cell containing a known amount of hexokinase. The protein samples were excited at 295 nm and the intensity of emission light was recorded through a monochromator at 359 nm. For all fluorescence measurements, the protein concentration was ~4 μM. When calculating the relative fluorescence intensity of hexokinase, we have taken into account the change in the concentration of the protein upon each addition of the titrant (D-glucose).

2.3. Volumetric measurements

All solution density and sound velocity titration measurements were performed at 25°C as described previously [17]. The partial molar volume, $V°$, of hexokinase was calculated from density data using $V° = M/\rho_0 - (\rho - \rho_0)/(\rho_0 C)$, where ρ and ρ_0 are the densities of the protein solution and the neat solvent, respectively; M is the protein's molecular weight; C is the molar concentration of the protein. The relative molar sound velocity increment, $[U]$, of the protein was calculated from sound velocity data using $[U] = (U - U_0)/(U_0 C)$, where U and U_0 are the sound velocities in the protein solution and the neat solvent, respectively. The values of $[U]$ and $V°$ were combined to calculate the partial molar adiabatic compressibility, $K°_S$, of the protein using the following relationship [18,19]:

$$K°_S = \beta_{S0}(2V° - 2[U] - M/\rho_0) \quad (1)$$

where β_{S0} is the coefficient of adiabatic compressibility of the solvent.

For all densimetric and ultrasonic velocimetric titration measurements, the initial protein concentration was 10–15 μM, while the concentration of the added glucose solution was within the range of 27–68 mM. The volumetric measurements have been carried out at least three times with the average values of $[U]$ and $V°$ being used in Eq. 1.

2.4. Determination of intrinsic volumes and solvent-accessible surface areas

The atomic coordinates of free yeast hexokinase PII (open conformation) and the glucose–hexokinase complex (closed conformation) needed for calculating intrinsic molecular volumes and solvent-accessible surface areas were obtained from the Protein Data Bank (PDB) [20,21]: 2YHX for free hexokinase PII [10] and 1HKG for the complex [22]. We have calculated the solvent-accessible surface area, S_A, for each structure from the sum of the accessible surface areas of all atoms in the structure. The intrinsic volumes, V_M, of free hexokinase, the ligand, and the ligand–protein complex were calculated using the Voronoi polyhedra approach as described by Richards [23]. For S_A and V_M computations, we have used the most recent version of the original Lee and Richards algorithm [23,24].

3. Results

3.1. Fluorescence titration profile

Fig. 1 presents the relative fluorescence intensity for hexokinase in the presence and absence of glucose at various ligand to enzyme binding ratios, r = [glucose]/[hexokinase]. The dependence shown in Fig. 1 was fitted under the assumption

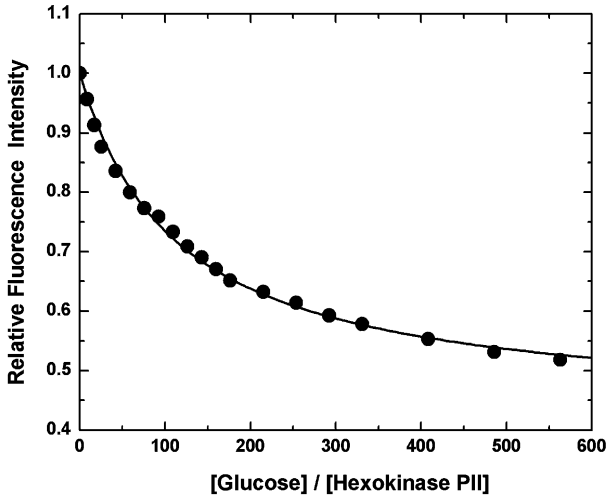

Fig. 1. Relative fluorescence intensity of a solution containing hexokinase plotted against the glucose–hexokinase molar ratio, r. The excitation and emission wavelengths are 295 and 359 nm, respectively. When calculating the relative fluorescence intensity of hexokinase, the change in the concentration of the protein upon each addition of the titrant (D-glucose) was taken into account. The initial concentration of hexokinase was 4 μM. The experimental points are fitted using Eq. 2 (solid lines).

of one-to-one stoichiometric binding using the following equation:

$$X = X_0 + \alpha\,\Delta X \quad (2)$$

where X is a binding-dependent observable (in this case, X is the relative fluorescence intensity), X_0 is the initial value of X in the absence of the ligand, ΔX is the maximum change in X when the protein is saturated with the ligand; α is the fraction of hexokinase molecules associated with glucose; $\alpha = 0.5(r+1) + Y^{-1} - [0.25(r-1)^2 + (r+1)/Y + Y^{-1}]^{1/2}$; $[PL]$ is the concentration of the hexokinase–glucose complex; $[P]$ is the concentration of the free protein; $Y = 2K_b([P]+[PL])$; $K_b = [PI]/([P][L])$ is the binding constant; and $[L]$ is the concentration of the free ligand.

Using Eq. 2 and the binding profile shown in Fig. 1, we calculate a binding constant, K_b, of 3.1 ± 0.2 mM^{-1} for glucose association with yeast hexokinase PII at 25°C and pH 8.7. Our binding constant is in reasonable agreement with the previously reported values of 5.6 mM^{-1} [13] and 3.2 mM^{-1} [25]. Using $\Delta G_b = -RT\ln K_b$, we determine a binding free energy, ΔG_b, of -4.8 ± 0.5 kcal/mol.

3.2. Volumetric properties

Figs. 2 and 3 respectively depict changes in the relative molar sound velocity increment, $\Delta[U]$, and partial molar volume, ΔV, of hexokinase PII in the absence and presence of the substrate (D-glucose) at various glucose-to-hexokinase binding

Table 1
Summary of thermodynamic data on the binding of glucose to hexokinase at 25°C

ΔG_b, kcal/mol	-4.8 ± 0.5
ΔH_b, kcal/mol	-0.7 ± 0.9[a]
ΔS_b, cal/mol/K	13.7 ± 3.1
$\Delta[U]_b$, cm^3/mol	-197 ± 40
ΔV_b, cm^3/mol	76 ± 30
ΔK_{Sb}, 10^{-4} cm^3/mol/bar	244 ± 64

[a] From [13].

ratios, r. We have used Eq. 2 to fit the volumetric binding profiles presented in Figs. 2 and 3. From these fits, we have determined the changes in the relative molar sound velocity increment, $\Delta[U]_b$, and volume, ΔV_b, accompanying saturation of hexokinase PII with D-glucose. The change in adiabatic compressibility, ΔK_{Sb}, accompanying the substrate–enzyme binding can be calculated from the values of $\Delta[U]_b$ and ΔV_b by differentiating Eq. 1; $\Delta K_{Sb} = 2\beta_{S0}(\Delta V_b - \Delta[U]_b)$. Our determined values of $\Delta[U]_b$, ΔV_b, and ΔK_{Sb} are -197 ± 40 cm^3/mol, 76 ± 30 cm^3/mol, and $(244 \pm 64) \times 10^{-4}$ cm^3/mol/bar, respectively. These values are listed in Table 1.

When a ligand–protein association event is coupled with proton release or uptake, buffer ionization generally contributes to the observed changes in volume, ΔV_b, and adiabatic compressibility, ΔK_{Sb}. However, in our measurements, the buffer (diglycine) ionization component of ΔV_b and ΔK_{Sb} is small and can be neglected in the analysis. This notion is based on the changes in volume, ΔV_i, and compressibility, ΔK_{Si}, associated with ionization of the amino-terminus of diglycine. The ionization volume, ΔV_i, is equal to -25.7 cm^3/mol [26], while the ionization compressibility, ΔK_{Si} is $-(61.8 \pm 3) \times 10^{-4}$ cm^3/mol/bar. The latter can be estimated as the mean of the ionization compressibilities of the amino termini of glycine and triglycine [27,28]. Note that the values of ΔV_i and ΔK_{Si} are on the order of uncertainties of our measured net changes in volume, ΔV_b, and compressibility, ΔK_{Si}, associated with glucose–hexokinase binding. Thus, we ignore the buffer ionization contribution to ΔV_b and ΔK_{Sb}.

4. Discussion

4.1. Volumetric properties of glucose association with hexokinase

Changes in protein volume, ΔV_b, and adiabatic compressibility, ΔK_{Sb}, associated with a protein-binding event can be rationalized in terms of the intrinsic and hydration contributions [17,29–34]:

$$\Delta V_b = \Delta V_M + \Delta\Delta V_h \quad (3)$$

$$\Delta K_{Sb} = \Delta K_M + \Delta\Delta K_h \quad (4)$$

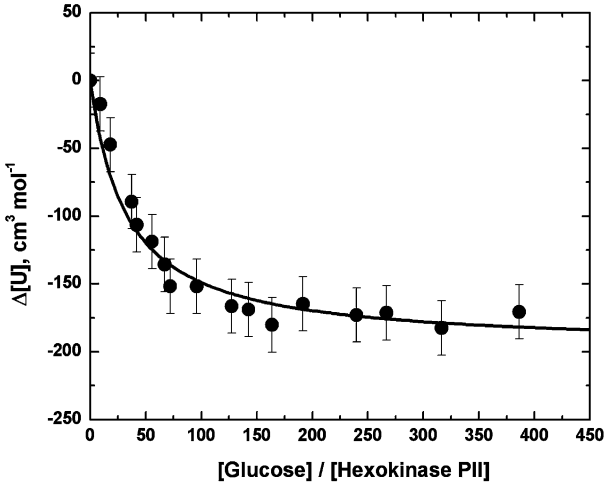

Fig. 2. Change in the relative molar sound velocity increment of hexokinase plotted against the glucose–hexokinase molar ratio, r. The initial concentration of hexokinase is 14 μM. The experimental points are fitted using Eq. 2 (solid lines).

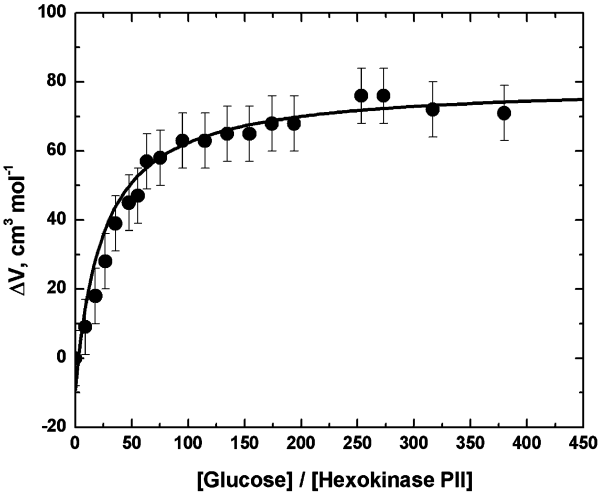

Fig. 3. Change in the partial molar volume of hexokinase plotted against the glucose–hexokinase molar ratio, r. The initial concentration of hexokinase is 14 μM. The experimental points are fitted using Eq. 2 (solid lines).

where ΔV_M is the change in V_M, the intrinsic volume of the protein and the ligand; $\Delta\Delta V_h$ is the change in ΔV_h, the hydration contribution to volume of the protein and the ligand; ΔK_M is the change in $K_M = \beta_M V_M$, the intrinsic compressibility of the protein and the ligand; β_M is the coefficient of adiabatic compressibility of the protein interior; and $\Delta\Delta K_h$ is the change in ΔK_h, the hydration contribution to compressibility of the protein and the ligand.

The value of $\Delta\Delta V_h$ in turn can be presented as the sum of two terms: $\Delta\Delta V_h = \Delta V_T + \Delta V_I$, where ΔV_T is the change in thermal volume, V_T, which originates from thermally activated mutual vibrational motions of solute and solvent molecules; and ΔV_I is the change in interaction volume, V_I, which represents solvent contraction in the vicinity of charged and polar groups of a solute [17,31,34–37]. Hence, Eq. 3 can be rearranged to an expanded form:

$$\Delta V_b = \Delta V_M + \Delta V_T + \Delta V_I \quad (5)$$

The binding-induced change in intrinsic volume, ΔV_M, can be calculated based on the knowledge of X-ray crystallographic structure of the glucose–hexokinase complex (closed conformation), the free ligand, and the free enzyme (open conformation). There are two conventional definitions of intrinsic volume both of which have been widely used in protein-related studies [23,38]; namely, Voronoi volume [23,39] and molecular volume [23,40,41]. We have previously discussed advantages and disadvantages of using the Voronoi and molecular definitions of intrinsic volume in analyses of the volumetric properties of proteins [34]. It is our opinion that both definitions are valid and can be used as long as distinctions between them are appreciated and taken into account [34]. In the analysis performed in this paper, we use the more frequently employed definition of Voronoi volume. Our calculated values of the intrinsic volume, V_M, of the complex, free hexokinase, and free glucose (using the PDB entries 1HKG and 2YHX for the complex and free hexokinase, respectively) are 57 045, 57 440, and 144 Å^3, respectively. Consequently, the change in intrinsic volume, ΔV_M, is negative and equal to -540 Å^3 ($57\,045 - 57\,440 - 144$) or -325 cm^3/mol.

As a first approximation, the thermal volume, V_T, of a globular protein is proportional to its solvent-accessible surface area, S_A, with the proportionality coefficient, δ, of $\sim$1 Å [34,42]. The value of ΔV_T can be calculated by multiplying δ by the binding-induced change in solvent-accessible surface area of the ligand and the enzyme, ΔS_A. Our calculated values of S_A for the complex, free hexokinase, and free glucose are 18 148, 18 304, and 170 Å^2, respectively. Hence, the change in solvent-accessible surface area, ΔS_A, equals −326 Å^2 (18 148−18 304−170), while the change in V_T ($\Delta V_T = \delta \Delta S_A$), equals −326 Å^3 or −196 cm^3/mol. Armed with the values of ΔV_M and ΔV_T, we now use Eq. 5 to evaluate $\Delta V_I = \Delta V_b - \Delta V_M - \Delta V_T$, which equals 597 ± 60 cm^3/mol (76+196+325).

The observed increase in V_I results from dehydration of the interacting surfaces of the protein and the ligand upon their complex formation with release of water molecules to the bulk. The value of ΔV_I equals the product $-\Delta n_h(V_h - V_0)$, where Δn_h is the number of water molecules released to the bulk, and $(V_h - V_0)$ is the average difference in the partial molar volume between water of solutes' (hexokinase and glucose) hydration and bulk water. Using a two-state structural model of liquid water, we have recently analyzed the hydration properties of a large number of biologically relevant solutes including but not limited to globular proteins [43]. Based on this analysis, the average value of $(V_h - V_0)$ for a globular protein is −1.8 cm^3/mol ($\sim$10% of the partial molar volume of bulk water) [43]. A similar value was computed for glucose [43].

The number of water molecules released to the bulk, Δn_h, upon formation of the glucose–hexokinase complex can be evaluated as the ratio of ΔV_I to $-(V_h - V_0)$; $\Delta n_h = 332 \pm 20$ (597/1.8). Our determined value of Δn_h is in close agreement with 326, the value previously reported by Reid and Rand based on their osmotic stress measurements [15]. The observed agreement between the two evaluations that are based on completely independent experimental techniques (volumetric versus osmotic stress) is quite remarkable.

It should be noted, however, that volumetric and osmotic stress results can be compared directly only if the two techniques sample the same population of water molecules. This may or may not be true. Volumetric observables, such as volume and compressibility, sense only those waters of hydration that exhibit altered density and compressibility relative to the bulk solvent. In contrast, 'osmotic stress' detects all waters around the solute that are not accessible to the osmolyte due to its size and/or the nature of solute–osmolyte interactions [44–46]. The question of whether or not waters that are not accessible to osmolyte molecules exhibit altered volumetric parameters has not been addressed as of yet. One empirical way to study this question is to systematically parallel the volumetric and osmotic stress techniques to evaluate changes in hydration associated with various processes involving proteins. The present work represents one such investigation. In the absence of fortuitous compensations, the observed agreement between the volumetric and osmotic stress results lends support to the notion that the two techniques may sample the same population of water molecules. However, further systematic studies are required to prove or refute the veracity and/or generality of this initial observation.

Reid and Rand [15] pointed out that 326 water molecules are far too many to be accounted for by the amount of water expelled from the binding cleft of hexokinase. Nor was it possible to rationalize this number based on the X-ray crystallographic structure of the protein even assuming that its hydration shell involves three layers of water molecules [15]. In view of this inconsistency, Reid and Rand proposed that crystal and solution structures of hexokinase might be significantly distinct, in particular, with respect to conformational flexibility [15]. As an alternative possibility, we propose that the extensive dehydration of hexokinase, detected by both osmotic stress and volumetric approaches, is related to the fact that the hydration shell of a globular protein is not uniform but rather heterogeneous with respect to its thickness [42]. Specifically, our previous volumetric results suggested that polar domains on the protein surface may be solvated by up to three layers of water molecules while there is a single layer of waters in the hydration shells of charged and nonpolar protein groups [42]. Enhanced solvation of polar protein domains reflects cooperative formation of networks of water molecules adjacent to the rigid matrix of closely located polar groups, with these networks involving waters from the second and third coordination spheres. In line with this notion, one may suggest that the glucose binding-induced conformational transition of hexokinase rearranges relative positions of polar groups at loci that are distant from the binding cleft. Such a rearrangement that is not necessarily accompanied by changes in solvent-accessible surface area of the protein may, nevertheless, modulate the thickness of the solvation shell at the affected loci by partially disrupting water networks. Consequently, considerable changes in protein hydration may accompany hexokinase–glucose association, with these changes not being reflected in hexokinase structure.

Armed with the value of Δn_h, we now proceed to evaluate the change in the hydration contribution to compressibility, $\Delta\Delta K_h$, in Eq. 4. The value of $\Delta\Delta K_h$ equals $-\Delta n_h(K_{Sh} - K_{S0})$, where $(K_{Sh} - K_{S0})$ is the average difference in the partial molar adiabatic compressibility between water of solutes' (the protein and the ligand) hydration and bulk water. For an average globular protein, the value of $(K_{Sh} - K_{S0})$ is -1.3×10^{-4} cm^3/mol/bar ($\sim$20% of the partial molar adiabatic compressibility of bulk water) [43]. A similar value has been obtained for glucose [43]. Thus, we calculate $\Delta\Delta K_h$ to be 0.043 ± 0.002 cm^3/mol/bar ($332 \times 1.3 \times 10^{-4}$). The change in the intrinsic compressibility of the protein, ΔK_M, can be estimated from Eq. 4 as $\Delta K_M = \Delta K_{Sb} - \Delta\Delta K_h = -0.019 \pm 0.006$ cm^3/mol/bar (0.024–0.043). By differentiating $K_M = \beta_M V_M$, one obtains the following relationship:

$$\Delta K_M = \beta_M \Delta V_M + \Delta\beta_M V_M \quad (6)$$

For globular proteins, the intrinsic coefficient of adiabatic compressibility, β_M, is $\sim 25 \times 10^{-6}$ bar^{-1} [29,33,34,42]. Using this value and Eq. 6, we calculate the binding-induced change in the intrinsic coefficient of adiabatic compressibility of hexokinase, $\Delta\beta_M$, to be $-(0.3 \pm 0.1) \times 10^{-6}$ bar^{-1}. This value corresponds to a $\sim$1% decrease in the intrinsic coefficient of adiabatic compressibility which suggests a slight rigidification of the protein's interior upon the binding of the substrate.

4.2. *Resolving entropy data in terms of configurational and hydration contributions*

The binding entropy, ΔS_b, for ligand–enzyme association can be determined from the binding free energy, ΔG_b, and enthalpy, ΔH_b:

$$\Delta S_b = (\Delta H_b - \Delta G_b)/T \quad (7)$$

Recall that ΔG_b of glucose–hexokinase association equals -4.7 ± 0.5 kcal/mol. Under the experimental conditions of our work, the van 't Hoff enthalpy, ΔH_b, of glucose–hexokinase binding is equal to -0.7 ± 0.9 kcal/mol [13]. From ΔG_b and ΔH_b, we calculate a binding entropy, ΔS_b, of 90 ± 2 cal/mol/K. The energetic parameters of glucose–hexokinase association (ΔG_b, ΔH_b, and ΔS_b) are tabulated in Table 1.

A change in entropy, ΔS_b, accompanying a protein association event can be presented as a sum of the intrinsic (configurational), ΔS_{conf}, hydrational, ΔS_h, and rotational and translational, ΔS_{trans}, terms [47]:

$$\Delta S_b = \Delta S_{conf} + \Delta S_h + \Delta S_{trans} \quad (8)$$

For a 1:1 stoichiometric binding, the change in entropy due to a decrease in the translational and rotational degrees of freedom of the reactants, ΔS_{trans}, is unfavorable and equals -8 cal/K/mol [47]. The hydrational change in entropy, ΔS_h, associated with the binding of glucose to hexokinase can be estimated by multiplying the number of water molecules released to the bulk, Δn_h, by $-(S_h - S_0)$, the average difference in the partial molar entropy between water of protein hydration and bulk water. The data on entropy of hydration of different amino acid residues presented by Makhatadze and Privalov [48] suggest that, at 25°C, the value $-(S_h - S_0)$ is essentially independent of the chemical nature of a solvent-exposed group and, on average, equals 1.3 ± 0.3 cal/mol/K. With this value, we evaluate the hydration contribution to the binding entropy, ΔS_h, in Eq. 8 to be favorable and equal to 431 ± 22 cal/mol/K (1.3×332). The change in configurational entropy, ΔS_{conf}, can be calculated from Eq. 8 by subtracting ΔS_h and ΔS_{trans} from ΔS_b. In contrast to ΔS_h, ΔS_{conf} is unfavorable and equals -410 ± 20 cal/mol/K (13+8−431). The value of ΔS_{conf} is the sum of the changes in configurational entropy of the ligand, $\Delta S_{conf}(L)$, and the protein, $\Delta S_{conf}(P)$. The value of $\Delta S_{conf}(L)$ for a small non-peptide ligand can be calculated based on the knowledge of the number of its atoms (N_{atoms}) and rotatable covalent bonds (N_{rb}) [47]:

$$\Delta S_{conf}(L) = -1.76 N_{rb} + 0.414 N_{atoms} \quad (9)$$

Using this relationship, we estimate the value of $\Delta S_{conf}(L)$ for D-glucose to be equal to -30 cal/K/mol. With this value, the change in the configurational entropy of hexokinase, $\Delta S_{conf}(P)$, equals -380 ± 20 cal/K/mol (−410+30). In a previous work [17], we have analyzed the data on configurational entropies of globular proteins, S_{conf}, presented by Makhatadze and Privalov [48]. At 25°C, the value of S_{conf} (kcal/mol/K) correlates with the molecular weight of a protein (kDa) according to $S_{conf} = -0.54 + 0.17M - 0.0014M^2$ (with a correlation coefficient of 0.98). Thus, the configurational entropy of free hexokinase (with a molecular weight, M, of 52 kDa), S_{conf}, is 4.51 kcal/mol/K. Hence, ΔS_{conf} constitutes $\sim 8\%$ (−0.38/4.51) of the initial value of S_{conf} of free hexokinase. In other words, the binding of glucose to hexokinase results in a $\sim 8\%$ reduction in the conformational dynamics of the enzyme. This entropy-detected decrease in protein dynamics is in qualitative agreement with our observed rigidification of the protein's interior which was manifested in a $\sim 1\%$ diminution in the intrinsic coefficient of adiabatic compressibility, β_M.

Inspection of the relative values of ΔS_b, ΔS_{conf}, ΔS_h, and ΔS_{trans} reveals that the hydrational and configurational terms represent the major contributors to the binding entropy. The highly favorable change in hydrational entropy, ΔS_h, prevails over the unfavorable change in configurational entropy, ΔS_{conf}. Based on this observation, we propose that hydration represents a major force driving the binding of glucose to hexokinase. We arrived at a similar conclusion when studying the binding of 2′-CMP and 3′-CMP to ribonuclease A [31] and association of turkey ovomucoid third domain (OMTKY3) with α-chymotrypsin [17].

The binding of glucose to hexokinase is an entropy-driven process. As discussed above, an increase in the hydrational entropy, ΔS_h, resulting from the binding-induced release of hydration water to the bulk is the only favorable entropic contribution. Comparison of the net binding free energy, ΔG_b (−4.8 kcal/mol), with $-T\Delta S_h$ (−129 kcal/mol) reveals that ΔG_b constitutes $\sim 4\%$ of $-T\Delta S_h$. This comparison underscores the dominant role of hydration in the energetics of glucose association with hexokinase. Simply speaking, release to the bulk of water molecules from the hydration shells of hexokinase and glucose is the main reason why the binding is a thermodynamically favorable process and, therefore, occurs spontaneously.

5. Concluding remarks

We report changes in spectroscopic and volumetric properties accompanying the binding of glucose to yeast hexokinase PII at pH 8.7 and 25°C. The binding of the ligand to the enzyme is accompanied by increases in volume, ΔV_b, and compressibility, ΔK_{Sb}, of 76 ± 30 cm^3/mol and $(244 \pm 50) \times 10^{-4}$ cm^3/mol/bar, respectively. We interpret these changes in conjunction with three-dimensional structures of the complex and the free protein in terms of the binding-induced changes in hydration and intrinsic packing; 332 ± 20 water molecules become released to the bulk upon the binding of glucose to hexokinase, while the coefficient of adiabatic compressibility of the protein's interior decreases by 1%.

The binding of glucose to hexokinase is an entropy-driven process. The favorable change in entropy is mainly determined by compensation between the changes in the hydrational, ΔS_h, and configurational, ΔS_{conf}, contributions. The values of ΔS_h and ΔS_{conf} are 431 ± 22 and -410 ± 21 cal/mol/K, respectively; a highly favorable change in hydrational entropy, ΔS_h, prevails over an unfavorable change in configurational entropy, ΔS_{conf}, thereby providing the thermodynamic impetus for glucose–hexokinase association. The relative magnitudes of ΔG_b and $T\Delta S_h$ suggest that, if only 4% fewer water molecules were released to the bulk, the binding of glucose to hexokinase would not occur.

Acknowledgements: We would like to thank Dr. R. Peter Rand for initiating this work and many stimulating discussions and Mr. Arno G. Siraki for his careful reading of the manuscript. This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (to T.V.C.) and the Canadian Institutes of Health Research (to T.V.C.).

References

[1] Kopetzki, E., Entian, K.D. and Mecke, D. (1985) Gene 39, 95–101.
[2] Frohlich, K.U., Entian, K.D. and Mecke, D. (1985) Gene 36, 105–111.

[3] Rose, M., Albig, W. and Entian, K.D. (1991) Eur. J. Biochem. 199, 511–518.
[4] Lazarus, N.R., Derechin, M. and Barnard, E.A. (1968) Biochemistry 7, 2390–2400.
[5] Derechin, M., Rustum, Y.M. and Barnard, E.A. (1972) Biochemistry 11, 1793–1797.
[6] Schmidt, J.J. and Colowick, S.P. (1973) Arch. Biochem. Biophys. 158, 458–470.
[7] Colowick, S.P. and Womack, F.C. (1969) J. Biol. Chem. 244, 774–777.
[8] Bennett Jr., W.S. and Steitz, T.A. (1980) J. Mol. Biol. 140, 211–230.
[9] Kuser, P.R., Krauchenco, S., Antunes, O.A. and Polikarpov, I. (2000) J. Biol. Chem. 275, 20814–20821.
[10] Anderson, C.M., Stenkamp, R.E. and Steitz, T.A. (1978) J. Mol. Biol. 123, 15–33.
[11] Bennett Jr., W.S. and Steitz, T.A. (1978) Proc. Natl. Acad. Sci. USA 75, 4848–4852.
[12] McDonald, R.C., Steitz, T.A. and Engelman, D.M. (1979) Biochemistry 18, 338–342.
[13] Takahashi, K., Casey, J.L. and Sturtevant, J.M. (1981) Biochemistry 20, 4693–4697.
[14] Catanzano, F., Gambuti, A., Graziano, G. and Barone, G. (1997) J. Biochem. (Tokyo) 121, 568–577.
[15] Reid, C. and Rand, R.P. (1997) Biophys. J. 72, 1022–1030.
[16] Kaji, A., Colowick, S.P. and Trayser, K.A. (1961) Ann. NY Acad. Sci. 94, 798–811.
[17] Filfil, R. and Chalikian, T.V. (2003) J. Mol. Biol. 326, 1271–1288.
[18] Barnatt, S. (1952) J. Chem. Phys. 20, 278–279.
[19] Owen, B.B. and Simons, H.L. (1957) J. Phys. Chem. 61, 479–482.
[20] Berstein, F.C., Koetzle, T.F., William, G.J.B., Meyer, E.F., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, M. (1977) J. Mol. Biol. 112, 535–542.
[21] Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N. and Bourne, P.E. (2000) Nucleic Acids Res. 28, 235–242.
[22] Steitz, T.A., Shoham, M. and Bennett Jr., W.S. (1981) Phil. Trans. R. Soc. Lond. B Biol. Sci. 293, 43–52.
[23] Richards, F.M. (1977) Annu. Rev. Biophys. Bioeng. 6, 151–176.
[24] Lee, B.K. and Richards, F.M. (1971) J. Mol. Biol. 55, 397–400.
[25] Feldman, I. and Kramp, D.C. (1978) Biochemistry 17, 1541–1547.
[26] Kauzmann, W., Bodanszky, A. and Rasper, J. (1962) J. Am. Chem. Soc. 84, 1771–1788.
[27] Chalikian, T.V., Kharakoz, D.P., Sarvazyan, A.P., Cain, C.A., Mcgough, R.J., Pogosova, I.V. and Gareginian, T.N. (1992) J. Phys. Chem. 96, 876–883.
[28] Chalikian, T.V., Gindikin, V.S. and Breslauer, K.J. (1998) Biophys. Chem. 75, 57–71.
[29] Chalikian, T.V. and Breslauer, K.J. (1996) Biopolymers 39, 619–626.
[30] Filfil, R. and Chalikian, T.V. (2000) J. Mol. Biol. 299, 827–842.
[31] Dubins, D.N., Filfil, R., Macgregor Jr., R.B. and Chalikian, T.V. (2000) J. Phys. Chem. B 104, 390–401.
[32] Kamiyama, T. and Gekko, K. (2000) Biochim. Biophys. Acta 1478, 257–266.
[33] Taulier, N. and Chalikian, T.V. (2002) Biochim. Biophys. Acta 1595, 48–70.
[34] Chalikian, T.V. (2003) Annu. Rev. Biophys. Biomol. Struct. 32, 207–235.
[35] Kharakoz, D.P. (1992) J. Solut. Chem. 21, 569–595.
[36] Chalikian, T.V., Sarvazyan, A.P. and Breslauer, T.V. (1994) Biophys. Chem. 51, 89–109.
[37] Chalikian, T.V. and Breslauer, K.J. (1998) Curr. Opin. Struct. Biol. 8, 657–664.
[38] Liang, J., Edelsbrunner, H., Fu, P., Sudhakar, P.V. and Subramaniam, S. (1998) Proteins Struct. Funct. Genet. 33, 1–17.
[39] Richards, F.M. (1985) Methods Enzymol. 115, 440–464.
[40] Connolly, M.L. (1983) J. Appl. Crystallogr. 16, 548–558.
[41] Connolly, M.L. (1983) Science 221, 709–713.
[42] Chalikian, T.V., Totrov, M., Abagyan, R. and Breslauer, K.J. (1996) J. Mol. Biol. 260, 588–603.
[43] Chalikian, T.V. (2001) J. Phys. Chem. B 105, 12566–12578.
[44] Parsegian, V.A., Rand, R.P. and Rau, D.C. (1995) Methods Enzymol. 259, 43–94.
[45] LiCata, V.A. and Allewell, N.M. (1998) Methods Enzymol. 295, 42–62.
[46] Rand, R.P., Parsegian, V.A. and Rau, D.C. (2000) Cell. Mol. Life Sci. 57, 1018–1032.
[47] Luque, I. and Freire, E. (1998) Methods Enzymol. 295, 100–127.
[48] Makhatadze, G.I. and Privalov, P.L. (1995) Adv. Protein Chem. 47, 307–425.