THE JOURNAL OF PHYSICAL CHEMISTRY B

ARTICLE

pubs.acs.org/JPCB

Volumetric Characterization of Interactions of Glycine Betaine with Protein Groups

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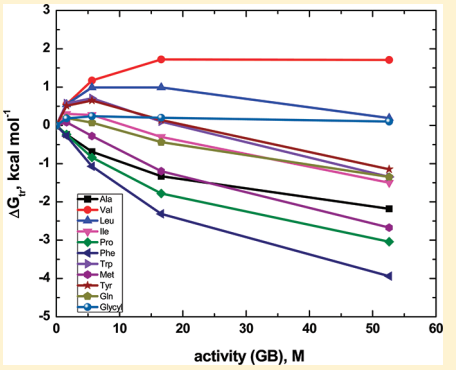
ABSTRACT: We report the partial molar volumes and adiabatic compressibilities of *N*-acetyl amino acid amides and oligoglycines at glycine betaine (GB) concentrations ranging from 0 to 4 M. We use these results to evaluate the volumetric contributions of amino acid side chains and the glycyl unit (−CH_2CONH−) as a function of GB concentration. We analyze the resulting GB dependences within the framework of a statistical thermodynamic model and evaluate the equilibrium constant for the reaction in which a GB molecule binds each of the functionalities under study replacing four water molecules. We calculate the free energy of the transfer of functional groups from water to concentrated GB solutions, ΔG_{tr}, as the sum of a change in the free energy of cavity formation, $\Delta\Delta G_C$, and the differential free energy of solute−solvent interactions, $\Delta\Delta G_I$, in a concentrated GB solution and water. Our results suggest that the transfer free energy, ΔG_{tr}, results from a fine balance between the large $\Delta\Delta G_C$ and $\Delta\Delta G_I$ contributions. The range of the magnitudes and the shape of the GB dependence of ΔG_{tr} depend on the identity of a specific solute group. The interplay between $\Delta\Delta G_C$ and $\Delta\Delta G_I$ results in pronounced maxima in the GB dependences of ΔG_{tr} for the Val, Leu, Ile, Trp, Tyr, and Gln side chains as well as the glycyl unit. This observation is in qualitative agreement with the experimental maxima in the T_M-versus-GB concentration plots reported for ribonuclease A and lysozyme.



INTRODUCTION

Water-miscible cosolvents may dramatically affect the stability and thermodynamic state of proteins and nucleic acids.[1−7] Small organic cosolvents are frequently referred to as osmolytes since their presence at high concentrations inside the cell is used by many organisms as protection against the osmotic loss of cellular water as well as other deleterious conditions such as elevated temperatures and pressures and desiccation.[1,8]

Cosolvents, in general, and osmolytes, in particular, are classified as protecting, neutral, or denaturing depending on their mode of action. The latter can be assessed based on the sign of a change in free energy, ΔG_{tr}, accompanying the transfer of a protein from water to a water-cosolvent mixture. One traditional way of evaluating ΔG_{tr} is based on measuring the differential solubility of a solute under study in a water-cosolvent mixture and water.[9] Such studies are, generally, conducted not on proteins per se but on relatively simple model compounds (such as amino acids) which mimic solvent-accessible protein groups.[9]

A change in free energy accompanying the transfer of a solute from the principal solvent (water) to a solvent-cosolvent mixture is the sum of the differential free energy of cavity formation, $\Delta\Delta G_C$, and the differential free energy of solute−solvent interactions, $\Delta\Delta G_I$, in a cosolvent solution and water[4,10,11]

$$\Delta G_{tr} = \Delta\Delta G_C + \Delta\Delta G_I \quad (1)$$

It is generally accepted that the cavity term, $\Delta\Delta G_C$, in eq 1 contributes unfavorably to the water-to-cosolvent transfer free energy, ΔG_{tr}, while the contribution of the interaction term, $\Delta\Delta G_I$, is favorable.[4,12] The interplay between the cavity and interaction contributions is poorly understood. Multifaceted approaches combining theoretical and experimental methods are needed to elucidate the balance of forces which renders a specific cosolvent denaturing, protecting, or neutral.

We have recently developed a volumetric technique in which the affinity of a cosolvent for a specific solute is evaluated based on the differential partial molar volume and compressibility measurements in solutions of model compounds mimicking a protein in water and water−cosolvent binary mixtures.[11,13] We have applied such measurements to characterizing the interactions between the denaturing osmolyte urea and the 20 naturally occurring amino acid residues.[11] In the present work, we extend these studies to the protective osmolyte glycine betaine (GB). To avoid the influence of the solute-induced shift in the ionization-neutralization equilibrium of GB on the measured volumetric observables, we limit our investigation to *N*-acetyl amino acid amides and oligoglycines lacking a net charge.

MATERIALS AND METHODS

Materials. GB, glycine, diglycine, triglycine, tetraglycine, *N*-acetyl−glycine amide, and *N*-acetyl tyrosine amide were

Received: June 20, 2011
Revised: August 15, 2011
Published: August 25, 2011

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purchased from Sigma-Aldrich Canada, Ltd. (Oakville, Ontario, Canada). *N*-acetyl alanine amide, *N*-acetyl valine amide, *N*-acetyl leucine amide, *N*-acetyl isoleucine amide, *N*-acetyl proline amide, *N*-acetyl phenylalanine amide, *N*-acetyl tryptophan amide, *N*-acetyl methionine amide, and *N*-acetyl glutamine amide were purchased from Bachem Bioscience, Inc. (King of Prussia, PA, USA). All amino acid derivatives were in L-stereoisomeric form. All of the reagents used in the studies reported here were of the highest purity commercially available and used without further purification.

Solution Preparation. We prepared aqueous solutions of GB with concentrations of 1, 2, 3, and 4 M by weighing 10−50 g of GB and adding pre-estimated amounts of water to achieve the desired molalities, m. The molar concentration, C, of a GB solution was determined from the molal value, m, using $C = [1/(m\rho_W) + \phi V/1000]^{-1}$, where ρ_W is the density of water and ϕV is the apparent molar volume of GB. The concentrated GB solutions were used as solvents for respective oligoglycines and amino acid derivatives. The concentrations of the samples were determined by weighing 10 to 20 mg of a solute material with a precision of ±0.02 mg and dissolving the sample in a known amount of solvent (GB solution). All chemicals were dried under vacuum in the presence of phosphorus pentoxide for 72 h prior to weighing.

Determination of Partial Molar Volumes and Adiabatic Compressibilities of Solutes. Solution densities were measured at 25 °C with a precision of $\pm 1.5 \times 10^{-4}$ % using a vibrating tube densimeter (DMA-5000, Anton Paar, Gratz, Austria). The apparent molar volumes, ϕV, of the solutes were evaluated from the relationship

$$\phi V = M/\rho - (\rho - \rho_0)/(\rho\rho_0 m) \quad (2)$$

where M is the molecular weight of the solute, m is the molal concentration of the solute, and ρ and ρ_0 are the densities of the solution and the solvent (water or a GB solution), respectively.

Solution sound velocities, U, were measured at 25 °C at a frequency of 7.2 MHz using the resonator method and a previously described differential technique.[14−17] The analysis of the frequency characteristics of the ultrasonic resonator cells required for sound velocity measurements was carried out by a Hewlett-Packard model E5100A network/spectrum analyzer (Mississauga, ON, Canada). For the type of ultrasonic resonators used in this work, the precision of sound velocity measurements is on the order of $\pm 1 \times 10^{-4}$%.[15,18,19] The acoustic characteristics of a solute which can be derived directly from ultrasonic measurements is the relative molar sound velocity increment, $[U] = (U - U_0)/(U_0 C)$, where C is the molar concentration of a solute, and U and U_0 are the sound velocities in the solution and the solvent, respectively.

The values of $[U]$ were used in conjunction with the ϕV values derived from densimetric measurements to calculate the apparent molar adiabatic compressibility, ϕK_S

$$\phi K_S = \beta_{S0}(2\phi V - 2[U] - M/\rho_0) \quad (3)$$

where $\beta_{S0} = \rho_0^{-1} U_0^{-2}$ is the coefficient of adiabatic compressibility of the solvent. The values of ρ_0, U_0, and β_{S0} were directly determined for each GB solution from our densimetric and acoustic measurements. For each evaluation of ϕV or ϕK_S, three to five independent measurements were carried out within a concentration range of 2−3 mg/mL. Our reported values of ϕV or ϕK_S represent the averages of these measurements, while the errors were calculated as standard deviations.

Table 1. Density of Solvent, ρ_0, Coefficient of Adiabatic Compressibility of Solvent, β_{S0}, Excess Partial Molar Volume of Water, ΔV°_1, and Excess Partial Molar Adiabatic Compressibility of Water, ΔK°_{S1}, as a Function of GB Concentration

[GB], M	ρ_0, g cm^{-3}	β_{S0}, 10^{-6} bar^{-1}	ΔV°_1, 10^{-3} cm^3 mol^{-1}	ΔK°_{S1}, 10^{-4} cm^3 mol^{-1} bar^{-1}
0	0.997047	44.773	0	0
1	1.016001	39.610	0.1 ± 0.6	−0.04 ± 0.01
2	1.035113	34.946	7.1 ± 2.2	−0.16 ± 0.01
3	1.054651	30.841	23.2 ± 0.8	−0.37 ± 0.01
4	1.074620	27.161	53.5 ± 2.2	−0.69 ± 0.01

Determination of Excess Partial Molar Volume and Adiabatic Compressibility of Water. The excess partial molar volume of water in a GB solution is given by $\Delta V^\circ_1 = V_1 - V^\circ_1$, where $V^\circ_1 = 18.07$ cm^3 mol^{-1} is the partial molar volume of pure water (in the absence of GB) and V_1 is the partial molar volume of water in solution at a given GB concentration. The excess partial molar adiabatic compressibility of water is given by $\Delta K^\circ_{S1} = K_{S1} - K^\circ_{S1}$, where $K^\circ_{S1} = 8.09 \times 10^{-4}$ cm^3 mol^{-1}bar^{-1} is the partial molar adiabatic compressibility of pure water and K_{S1} is the partial molar adiabatic compressibility of water at a given GB concentration. To determine the values of V_1 and K_{S1}, ∼0.1 g of water was added to ∼10 g of each GB solution (with concentrations of 1, 2, 3, and 4 M) with the resulting changes in density and sound velocity being measured. The values of V_1 and K_{S1} were computed from eqs 2 and 3.

RESULTS

Table 1 lists the values of ρ_0 (column 2) and β_{S0} (column 3) at 0, 1, 2, 3, and 4 M GB. The excess partial molar volume, ΔV°_1, and adiabatic compressibility, ΔK°_{S1}, of water measured at different GB concentrations are shown in Table 1 (columns 4 and 5, respectively). Tables 2 and 3 present, respectively, the apparent molar volumes, ϕV, and adiabatic compressibility, ϕK_S, of the solutes investigated in this study at 0, 1, 2, 3, and 4 M GB. To the best of our knowledge, no data of this kind have been reported. Therefore, our results cannot be compared with the literature.

The apparent molar volumes and adiabatic compressibilities of oligopeptides and N-acetyl amino acid amides in water do not strongly depend on concentration.[20−23] By extension, we assume that the concentration dependences of the volumetric properties of these solutes are insignificant also in concentrated solutions of GB, especially, given the diminutive solute concentrations used in this work. Consequently, we do not discriminate between the apparent molar volumetric characteristics the solutes studied in this work and their partial molar characteristics obtained by extrapolation to infinite dilution.

DISCUSSION

Volume and Compressibility Contributions of Amino Acid Side Chains and Glycyl Unit. Tables 4 and 5 list, respectively, the volume and adiabatic compressibility contributions for the 10 amino acid side chains studied here and the glycyl residue as a function of GB concentration. The volume or compressibility contribution of a specific amino acid side chain was computed as the

Table 2. Partial Molar Volumes, V° ($cm^3\ mol^{-1}$), of Solutes as a Function of GB Concentration

compounds	0 M	1 M	2 M	3 M	4 M
N-Ac-Gly-NH_2	91.1 ± 0.1	90.5 ± 0.2	90.4 ± 0.2	90.5 ± 0.1	90.7 ± 0.3
N-Ac-Ala-NH_2	108.3 ± 0.2	107.3 ± 0.3	106.6 ± 0.2	106.1 ± 0.3	106.5 ± 0.4
N-Ac-Val-NH_2	138.5 ± 0.2	137.0 ± 0.5	136.1 ± 0.3	135.6 ± 0.5	135.4 ± 0.1
N-Ac-Leu-NH_2	156.4 ± 0.3	154.4 ± 0.1	153.5 ± 0.2	152.6 ± 0.2	153.1 ± 0.4
N-Ac-Ile-NH_2	153.8 ± 0.3	152.3 ± 0.2	151.4 ± 0.3	149.9 ± 0.4	149.2 ± 0.2
N-Ac-Pro-NH_2	126.1 ± 0.4	125.2 ± 0.5	124.4 ± 0.3	123.9 ± 0.2	123.5 ± 0.1
N-Ac-Phe-NH_2	170.7 ± 0.2	168.6 ± 0.1	166.7 ± 0.4	166.6 ± 0.3	168.0 ± 0.4
N-Ac-Trp-NH_2	192.9 ± 0.2	191.0 ± 0.4	189.8 ± 0.3	189.6 ± 0.1	190.3 ± 0.5
N-Ac-Met-NH_2	153.6 ± 0.1	152.9 ± 0.4	152.4 ± 0.3	152.1 ± 0.2	151.9 ± 0.3
N-Ac-Tyr-NH_2	172.9 ± 0.3	172.4 ± 0.2	172.3 ± 0.1	172.3 ± 0.1	172.3 ± 0.4
N-Ac-Gln-NH_2	141.2 ± 0.2	141.8 ± 0.1	142.2 ± 0.4	142.6 ± 0.5	142.9 ± 0.3
glycine	43.5 ± 0.2	44.3 ± 0.2	45.3 ± 0.2	46.4 ± 0.1	47.2 ± 0.3
diglycine	76.7 ± 0.2	77.9 ± 0.2	79.4 ± 0.1	80.5 ± 0.3	81.4 ± 0.2
triglycine	112.7 ± 0.3	113.9 ± 0.3	115.4 ± 0.1	117.5 ± 0.1	119.4 ± 0.4
tetraglycine	149.3 ± 0.4	152.0 ± 0.4	154.3 ± 0.3	156.4 ± 0.3	158.5 ± 0.2

Table 3. Partial Molar Adiabatic Compressibilities, K°_S ($10^{-4}\ cm^3\ mol^{-1}\ bar^{-1}$), of Solutes as a Function of GB Concentration

compounds	0 M	1 M	2 M	3 M	4 M
N-Ac-Gly-NH_2	−0.8 ± 0.2	3.7 ± 0.4	8.2 ± 0.4	11.9 ± 0.4	14.9 ± 0.3
N-Ac-Ala-NH_2	−0.2 ± 0.3	4.6 ± 0.4	9.0 ± 0.3	12.3 ± 0.8	15.9 ± 0.6
N-Ac-Val-NH_2	−0.7 ± 0.3	5.1 ± 0.7	10.9 ± 0.4	16.4 ± 0.6	20.7 ± 0.6
N-Ac-Leu-NH_2	−0.5 ± 0.4	6.8 ± 0.6	13.6 ± 0.3	20.1 ± 0.4	25.9 ± 0.6
N-Ac-Ile-NH_2	−2.7 ± 0.4	3.6 ± 0.4	9.6 ± 0.7	14.3 ± 0.5	17.9 ± 0.8
N-Ac-Pro-NH_2	−6.0 ± 0.5	0.2 ± 0.7	5.9 ± 0.6	10.1 ± 0.4	13.3 ± 0.6
N-Ac-Phe-NH_2	−0.2 ± 0.3	8.3 ± 0.3	15.7 ± 0.5	21.0 ± 0.9	24.4 ± 0.9
N-Ac-Trp-NH_2	2.6 ± 0.2	11.3 ± 0.7	19.8 ± 0.3	28.6 ± 0.6	33.0 ± 0.8
N-Ac-Met-NH_2	−2.2 ± 0.2	6.0 ± 0.4	13.8 ± 0.5	19.6 ± 0.6	24.7 ± 0.6
N-Ac-Tyr-NH_2	5.9 ± 0.4	13.4 ± 0.2	21.7 ± 0.2	28.2 ± 0.3	33.5 ± 0.6
N-Ac-Gln-NH_2	−2.6 ± 0.2	4.9 ± 0.3	11.8 ± 0.5	17.7 ± 0.5	22.2 ± 0.5
glycine	−26.6 ± 0.2	−19.2 ± 0.4	−12.7 ± 0.5	−6.7 ± 0.4	−2.3 ± 0.7
diglycine	−39.7 ± 0.3	−29.8 ± 0.4	−19.5 ± 0.2	−10.9 ± 0.6	−4.6 ± 0.6
triglycine	−43.9 ± 0.4	−31.1 ± 0.5	−18.7 ± 0.2	−8.6 ± 0.2	0.6 ± 0.4
tetraglycine	−46.0 ± 0.5	−30.8 ± 0.5	−16.5 ± 0.4	−4.9 ± 0.3	4.4 ± 0.3

Table 4. Partial Molar Volume Contributions of Amino Acid Side Chains and Glycyl Unit ($-CH_2CONH-$), V ($cm^3\ mol^{-1}$), as a Function of GB Concentration

compounds	0 M	1 M	2 M	3 M	4 M
Ala	17.3 ± 0.2	16.8 ± 0.3	16.1 ± 0.3	15.6 ± 0.4	15.9 ± 0.5
Val	47.2 ± 0.2	46.5 ± 0.5	45.7 ± 0.4	45.2 ± 0.5	44.8 ± 0.3
Leu	65.3 ± 0.3	63.9 ± 0.2	63.1 ± 0.2	62.2 ± 0.2	62.5 ± 0.5
Ile	62.7 ± 0.3	61.8 ± 0.3	61.0 ± 0.4	59.4 ± 0.4	58.6 ± 0.3
Pro	35.1 ± 0.4	34.7 ± 0.5	34.0 ± 0.4	33.4 ± 0.2	32.8 ± 0.3
Phe	79.6 ± 0.3	78.1 ± 0.2	76.2 ± 0.4	76.2 ± 0.4	77.4 ± 0.5
Trp	101.8 ± 0.1	100.5 ± 0.4	99.4 ± 0.3	99.2 ± 0.2	99.6 ± 0.5
Met	62.5 ± 0.2	62.4 ± 0.4	62.0 ± 0.3	61.7 ± 0.2	61.2 ± 0.4
Tyr	81.9 ± 0.3	81.9 ± 0.3	81.9 ± 0.2	81.8 ± 0.2	81.6 ± 0.5
Gln	50.2 ± 0.2	51.2 ± 0.2	51.8 ± 0.4	52.2 ± 0.5	52.3 ± 0.4
$-CH_2CONH-$	36.6 ± 0.5	38.1 ± 0.5	38.9 ± 0.3	39.0 ± 0.3	39.1 ± 0.4

difference in the partial molar volume, V°, or adiabatic compressibility, K°_S, between the corresponding *N*-acetyl amino acid amide and *N*-acetyl-glycine amide. The volumetric contributions of the glycyl residue ($-CH_2CONH-$) was obtained as the difference between the values corresponding to triglycine and tetraglycine.

Analytical Treatment of Volumetric Data. We analyze the GB dependences of the volume and compressibility contributions of the amino acid side chains and the glycyl unit within the framework of a recently developed statistical thermodynamic model.[11,13] In this model, the binding of a cosolvent molecule to a hydrated solute is accompanied by a release of r water molecules from the hydration shell of a solute to the bulk. If there are n binding sites for the principal solvent (water), the maximum number of cosolvent binding sites is n/r. Under the simplification of n/r identical and independent cosolvent binding sites, a change in volume associated with the transfer of a solute from water to a concentrated cosolvent solution is given by the relationship[11]

$$\Delta V^\circ = \Delta V_C - \gamma_1 n \Delta V^\circ_1 + \Delta V(n/r)(a_3/a_1{}^r)k/[1 + (a_3/a_1{}^r)k] \quad (4)$$

where ΔV_C is the differential volume of a cavity enclosing a solute

dx.doi.org/10.1021/jp205777a |J. Phys. Chem. B 2011, 115, 11481–11489

Table 5. Partial Molar Adiabatic Compressibility Contributions of Amino Acid Side Chains and Glycyl Unit ($-CH_2CONH-$), K_S (10^{-4} cm^3 mol^{-1} bar^{-1}), as a Function of GB Concentration

compounds	0 M	1 M	2 M	3 M	4 M
Ala	0.7 ± 0.4	0.9 ± 0.6	0.7 ± 0.5	0.4 ± 0.9	1.0 ± 0.6
Val	0.1 ± 0.3	1.4 ± 0.8	2.6 ± 0.5	4.4 ± 0.8	5.8 ± 0.7
Leu	0.3 ± 0.5	3.1 ± 0.8	5.3 ± 0.5	8.2 ± 0.6	11.1 ± 0.7
Ile	−1.9 ± 0.4	0.0 ± 0.5	1.4 ± 0.8	2.4 ± 0.7	3.0 ± 0.9
Pro	−5.2 ± 0.5	−3.5 ± 0.8	−2.3 ± 0.7	−1.8 ± 0.6	−1.6 ± 0.6
Phe	0.7 ± 0.4	4.7 ± 0.5	7.5 ± 0.6	9.1 ± 1.0	9.5 ± 0.9
Trp	3.4 ± 0.3	2.0 ± 0.8	11.6 ± 0.5	16.6 ± 0.7	18.1 ± 0.8
Met	−1.3 ± 0.3	2.3 ± 0.6	5.5 ± 0.6	7.7 ± 0.8	9.8 ± 0.7
Tyr	6.7 ± 0.4	9.7 ± 0.5	13.4 ± 0.4	16.3 ± 0.6	18.6 ± 0.7
Gln	−1.7 ± 0.3	1.2 ± 0.5	3.5 ± 0.6	5.8 ± 0.6	7.3 ± 0.6
$-CH_2CONH-$	−2.2 ± 0.6	0.3 ± 0.7	2.2 ± 0.4	3.7 ± 0.4	3.8 ± 0.5

in a concentrated cosolvent solution and water; k is the effective equilibrium constant for the reaction in which a cosolvent molecule replaces r water molecules by binding to each of the n/r binding sites; a_1 and a_3 are the activities of water and cosolvent, respectively; $\Delta V = \Delta V_0 + \gamma_1 r\, \Delta V^\circ_1 - \gamma_3 \Delta V^\circ_3$ is the change in volume associated with replacement of water with cosolvent normalized per binding site in a concentrated cosolvent solution; ΔV_0 is the solvent exchange volume in an ideal solution; ΔV°_1 and ΔV°_3 are the excess partial molar volumes of water and cosolvent in a concentrated solution, respectively; and γ_1 and γ_3 are the correction factors reflecting the influence of the bulk solvent on the properties of solvating water and cosolvent, respectively. The values of γ_1 and γ_3 may change from 0 (the properties of the solvation shell change in parallel with those of the bulk) to 1 (the properties of the solvation shell are independent of those of the bulk).[11,13]

A change in isothermal compressibility accompanying the water-to-cosolvent transfer of a solute is described by the relationship[11]

$$\Delta K^\circ_T = \Delta K_{TC} - \gamma_1 n \Delta K^\circ_{T1} + \Delta K_T (n/r)(a_3/a_1{}^r)k/[1 + (a_3/a_1{}^r)k] + \Delta V^2 (n/r)(a_3/a_1{}^r)k/RT[1 + (a_3/a_1{}^r)k]^2 \quad (5)$$

where $\Delta K_{TC} = -(\partial \Delta V_C/\partial P)_T$ is the differential compressibility of the cavity enclosing a solute in water and a concentrated cosolvent solution; $\Delta K_T = \Delta K_{T0} + \gamma_1 r \Delta K^\circ_{T1} - \gamma_3 \Delta K^\circ_{T3}$ is the change in compressibility associated with replacement of water with cosolvent normalized per binding site in a concentrated cosolvent solution; ΔK°_{T1} and ΔK°_{T3} are the excess partial molar isothermal compressibilities of water and cosolvent in a concentrated cosolvent solution, respectively; and $\Delta K_{T0} = -(\partial \Delta V_0/\partial P)_T$ is the change in compressibility associated with the solvent replacement in an ideal solution.

Scaled particle theory (SPT)-based calculations have revealed that, for solutes with hard-sphere diameters smaller than ~6 Å (corresponding to the size of *N*-acetyl amino acid amides), the differential cavity volume, ΔV_C, in eq 4 does not exceed ~1 cm^3 mol^{-1} as the GB concentration changes from 0 to 4 M.[24] Consequently, the ΔV_C term in eq 4 and, by extension, the ΔK_{TC} term in eq 5 can be neglected in the subsequent analysis.

In eqs 4 and 5, the number of water binding sites, n, for each solute can be taken equal to the number of water molecules in direct contact with a solute (confined within the first coordination layer). The latter can be calculated as the ratio of the solvent-accessible surface area of a solute to 9 Å^2, the effective cross-section of water molecule. The values of n for individual amino acid side chains and the glycyl unit are presented in our previous publication.[11]

The number of water molecules, r, replaced by GB in the vicinity of a solute can be estimated as the ratio of n to the maximum number of GB molecules that can be in simultaneous contact with a solute. The latter is equal to the number of GB molecule within the first solute-cosolvent coordination sphere. For a spherical solute with a radius r_S, the number of water and GB molecules within their respective (solute-water and solute-cosolvent) first coordination spheres are equal to $(r_S + r_W)^2/r_W{}^2$ and $(r_S + r_{GB})^2/r_{GB}{}^2$, respectively ($r_W$ and r_{GB} are the hard sphere radii of water and GB, respectively). Hence, the value of r is given by $[(r_S/r_W + 1)/(r_S/r_{GB} + 1)]^2$, where $r_W = 1.4$ Å and $r_{GB} = 3.04$ Å are the hard sphere radii of water and GB. The latter was calculated based on the spherical approximation of a GB molecule as $r_{GB} = (3V_{GB}/4\pi)^{1/3}$, where V_{GB} is the van der Waals volume of GB.[24] For solutes with radii, r_S, corresponding to the protein range (above ~5 Å), the number of water molecules, r, released to the bulk upon GB binding is ~4. Hence, in our analysis below we use the value of r equal to 4.

The mole fraction activity of water, a_1, and the molar activity of GB, a_3, have been reported by Felitsky and Record.[25] The values of a_1 are 1.000, 0.976, 0.939, 0.880, and 0.782, whereas the values of a_3 are 0, 1.62, 5.65, 16.59, and 52.61 at 0, 1, 2, 3, and 4 M, respectively.[25] We use these values in eqs 4 and 5 when analyzing our data below. The relationship between a_1 and a_3 is well approximated by the exponential function $a_1 = 0.76 + 0.24 \exp(-a_3/24.11)$.

Equation 5 has been derived for partial molar isothermal compressibility data.[11,13] However, its use for treating the partial molar adiabatic compressibility data presented in Table 4 is warranted given the small difference between the partial molar adiabatic and isothermal compressibilities of solutes in aqueous solutions due to the large heat capacity and small expansibility of water.[26,27] When applied to analyzing cosolvent-induced changes in the partial molar adiabatic compressibility of a solute, ΔK°_S, the isothermal quantities ΔK°_{T1}, ΔK_T, and ΔK_{T0} in eq 5 should be replaced by their adiabatic analogs.

The experimental values of ΔV°_1 and ΔK°_{S1} (shown in Table 1) can be related to the activity of GB, a_3, via the functions; ΔV°_1(cm^3 mol^{-1}) = $1.65 \times 10^{-3} a_3 - 1.15 \times 10^{-5} a_3{}^2$ and

dx.doi.org/10.1021/jp205777a |J. Phys. Chem. B 2011, 115, 11481–11489

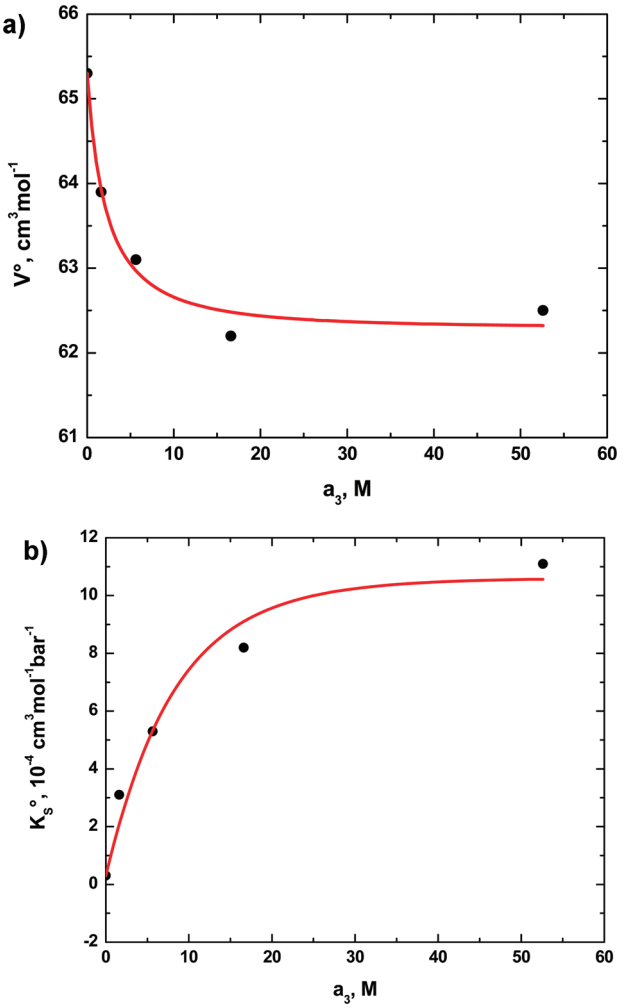

Figure 1. Partial molar volume (panel A) and adiabatic compressibility (panel B) contributions of the leucine side chain as a function of GB activity. The experimental points were fitted using eqs 4 (panel A) and 5 (panel B).

$\Delta K°_{S1}(10^{-4}\,\mathrm{cm^3\,mol^{-1}\,bar^{-1}}) = -0.79 + 0.79\exp(-a_3/25.44)$. In eqs 4 and 5, the correction factor γ_3 can be taken to be 0, and γ_1 can be taken equal to the nonpolar fraction of the solvent accessible surface area of a solute or an individual atomic group.[11,13] The specific values of γ_1 for the amino acid side chains and the glycyl unit have been estimated in a previous work.[11]

We used eqs 4 and 5 to fit the GB dependences of the volume and compressibility contributions of the amino acid side chains and the glycyl unit presented in Tables 4 and 5 and to determine the effective binding constants, k, and changes in volume, ΔV_0, and adiabatic compressibility, ΔK_{S0}, associated with replacement of water with cosolvent at the binding site of a solute in an ideal solution. Figure 1 shows the representative dependences of the volume (panel A) and adiabatic compressibility (panel B) contributions of the leucine side chain on the activity of GB, a_3, approximated by eqs 4 and 5, respectively. Table 6 lists our determined values of k (columns 6 and 7), ΔV_0 (column 4), and ΔK_{S0} (column 5) for the 10 amino acid side chains studied here, the glycyl residue, and the zwitterionic amino acid glycine as an example of a highly charged solute.

Within the context of the analysis presented below, it is pertinent to note that the binding of GB to a solute may be accompanied by disruption of GB-water and GB-GB interactions. Clearly, in this case, the values of k, ΔV_0, and ΔK_{S0} will be affected by dehydration of GB and disruption of GB-GB contacts, although the binding-induced disruption of pre-existing GB interactions is not formally included in the model.

Effective Binding Constants. Inspection of Table 6 reveals that the effective binding constants, k, determined from the volume (column 6) and compressibility (column 7) data may significantly differ from each other. Given the larger GB-induced changes in compressibility relative to error of measurements compared to those in volume, it is reasonable to expect that the values of k determined from the compressibility data are more reliable than those determined from the volume data. In fact, the ratio of the net compressibility change-to-error ratio is, on average, four times as large as the volume change-to-error ratio. Therefore, in our analysis below, we use compressibility-based estimates of the binding constants with the exception of Ala. For the Ala side chain, the GB-induced changes in compressibility are small which makes them unsuitable for any reliable estimate of k. For Ala, we use, therefore, the value of k derived from the partial molar volume measurements.

The binding constants, k, range from 0.02 to 0.32 M^{-1}. These values are comparable to those we have determined for the binding of urea, a denaturing cosolvent.[11] This observation is significant and suggests that protein-GB interactions are not negligible. Thus, the stabilizing action of GB results not from the weakness of its interactions with protein groups but rather from the highly unfavorable free energy of cavity formation in a concentrated GB solution relative to that in water (see below).

Interaction, Cavity, and Transfer Free Energies. The differential free energy of cavity formation, $\Delta\Delta G_C$, in eq 1 can be evaluated within the framework of scaled particle theory (SPT).[12,28,29] The free energy of cavity formation, ΔG_C, in a binary mixture is given by the relationship

$$\Delta G_C = RT[-\ln(1-\xi_3) + 3\xi_2 d_S/(1-\xi_3) + 3\xi_1 {d_S}^2/(1-\xi_3) + 4.5{\xi_2}^2 {d_S}^2/(1-\xi_3)^2 + N_A\pi P {d_S}^3/(6RT)] \quad (6)$$

where d_S is the diameter of a solute molecule; the hard-sphere pressure, P, is computed from $\pi P/RT = 6\xi_0/(1-\xi_3) + 18\xi_1\xi_2/(1-\xi_3)^2 + 18{\xi_2}^3/(1-\xi_3)^3$; N_A is Avogadro's number, $\xi_k = (\pi N_A/6)\sum_{i=1}^{m} C_i {d_i}^k$; k has the values of 0, 1, 2, and 3; m is the number of solvent components; C_i and d_i are respectively the molar concentration and the molecular diameter of the ith solvent component.[12,28,30]

We used eq 6 to calculate the free energies of cavity formation, ΔG_C, for our solutes in water and concentrated GB solutions. In these calculations, we used hard sphere diameters of water and GB of 2.76 and 6.08 Å, respectively. The hard sphere diameter of each solute was calculated from its van der Waals volume, V_W, using the spherical approximation; $d_S = (6V_W/\pi)^{1/3}$. The van der Waals volume, V_W, of each solute under study was calculated additively based on its chemical structure and the group contributions reported by Bondi.[31] The ΔG_C contribution for a specific side chain was computed as the difference between the values of ΔG_C corresponding to the respective N-acetyl amino acid amide and N-acetyl glycine amide. The free energy of cavity formation of the glycyl unit was evaluated as the difference between the value of ΔG_C for diglycine and glycine. Table 7 presents the values of ΔG_C we evaluated for the amino acid side chains and the glycyl unit.

Table 6. Correction Factor, γ_1, the Number of Binding Sites for Water, n, Equilibrium Constants, k, and Changes in Volume, ΔV_0, and Adiabatic Compressibility, ΔK_{S0}, Accompanying the Binding of GB to Amino Acid Side Chains and the Glycyl Unit in an Ideal Solution

SC	γ_1[a]	n[a]	ΔV_0, cm^3 mol^{-1}	$\Delta K_{S0} \times 10^4$, cm^3 mol^{-1} bar^{-1}	k,[b] M	k,[c] M
Ala	1.0	7	−0.93 ± 0.09	N/A	0.33 ± 0.15	N/A
Val	1.0	13	−0.79 ± 0.02	1.24 ± 0.34	0.27 ± 0.03	0.02 ± 0.02
Leu	1.0	16	−0.75 ± 0.04	2.53 ± 0.21	0.46 ± 0.13	0.06 ± 0.03
Ile	1.0	16	−1.09 ± 0.05	1.00 ± 0.09	0.10 ± 0.02	0.13 ± 0.06
Pro	1.0	12	−0.79 ± 0.03	1.11 ± 0.03	0.10 ± 0.02	0.29 ± 0.05
Phe	1.0	19	−0.64 ± 0.11	1.80 ± 0.03	0.90 ± 1.04	0.32 ± 0.03
Trp	0.9	23	−0.44 ± 0.03	2.54 ± 0.08	0.74 ± 0.36	0.13 ± 0.02
Met	0.7	17	−0.32 ± 0.02	2.50 ± 0.12	0.04 ± 0.01	0.19 ± 0.05
Tyr	0.8	20	0	2.30 ± 0.09	N/A	0.12 ± 0.03
Gln	0.4	13	0.66 ± 0.01	2.73 ± 0.11	0.48 ± 0.03	0.18 ± 0.03
−CH_2CONH−	0.4	6	1.70 ± 0.04	4.10 ± 0.08	0.88 ± 0.12	0.26 ± 0.03
glycine	1.0	15	1.04 ± 0.03	6.29 ± 0.27	0.13 ± 0.01	0.17 ± 0.03

[a] From ref 11. [b] Calculated from volume data with eq 4. [c] Calculated from compressibility data with eq 5.

Table 7. Change in Free Energy of Cavity Formation, $\Delta\Delta G_C$ (kcal mol^{-1}), of Amino Acid Side Chains and Glycyl Unit (−CH_2CONH−), Calculated as a Function of GB Concentration Using eq 6

SC	0	1	2	3	4
Ala	0	0.14	0.31	0.53	0.80
Val	0	0.42	0.94	1.58	2.39
Leu	0	0.57	1.25	2.10	3.18
Ile	0	0.57	1.25	2.10	3.18
Pro	0	0.32	0.72	1.22	1.84
Phe	0	0.73	1.60	2.69	4.07
Trp	0	0.95	2.11	3.55	5.37
Met	0	0.57	1.26	2.13	3.22
Tyr	0	0.80	1.76	2.96	4.48
Gln	0	0.54	1.20	2.02	3.05
−CH_2CONH−	0	0.43	0.96	1.61	2.44

Table 8. Differential Free Energy of Interaction, $\Delta\Delta G_I$ (kcal mol^{-1}), of Amino Acid Side Chains and Glycyl Unit (−CH_2CONH−), Calculated as a Function of GB Concentration Using eq 8

SC	0	1	2	3	4
Ala	0	−0.38	−1.01	−1.87	−2.99
Val	0	0.12	0.22	0.14	−0.68
Leu	0	−0.01	−0.26	−1.11	−2.99
Ile	0	−0.26	−0.98	−2.40	−4.68
Pro	0	−0.57	−1.57	−3.00	−4.88
Phe	0	−1.00	−2.67	−5.00	−8.00
Trp	0	−0.38	−1.41	−3.45	−6.73
Met	0	−0.49	−1.55	−3.33	−5.89
Tyr	0	−0.29	−1.11	−2.82	−5.63
Gln	0	−0.35	−1.13	−2.46	−4.40
−CH_2CONH−	0	−0.25	−0.72	−1.41	−2.35

Within the framework of the assumption of n/r identical and independent cosolvent binding sites on the solute, $\Delta\Delta G_I$ is linked to the effective binding constant, k, via the relationship

$$\Delta\Delta G_I = -(n/r)RT\ln[(a_1/a_{10})^r + k(a_3/a_{10}{}^r)] \quad (7)$$

where a_{10} is the activity of water in the absence of cosolvent.[11] At low concentrations of a solute, when $a_{10} \approx 1$, eq 7 simplifies to the form

$$\Delta\Delta G_I = -(n/r)RT\ln(a_1{}^r + ka_3) \quad (8)$$

Table 8 presents our calculated values of $\Delta\Delta G_I$ for the amino acid side chains and the glycyl unit at different GB concentrations. We used these results in conjunction with the data on the cavity contribution, $\Delta\Delta G_C$, listed in Table 7 to compute the transfer free energies, ΔG_{tr}, from eq 1. The results of these computations are tabulated in Table 9 and plotted in Figure 2. Inspection of Table 9 and Figure 2 reveals that the aromatic side chain of Phe displays the most favorable interactions with GB. This observation is in excellent agreement with the results of a recent work from the Record laboratory.[32] Based on the determined GB-surface interaction potentials for various functional groups, these authors have concluded that the aromatic carbon surface of the benzyl group of benzoate is characterized by the most favorable interactions with GB.[32] Furthermore, our calculated moderately positive values of ΔG_{tr} for the glycyl unit are consistent with the prediction of moderate exclusion of GB from the vicinity of the peptide backbone made by Capp et al.[32]

It is instructive to compare the experimental transfer free energies, ΔG_{tr}, with those calculated from eqs 1, 6, and 8. Experimental data exist only for the changes in free energy accompanying the transfer of some amino acid side chains and the glycyl unit from water to a 1 M GB solution.[6,33,34] Table 10 compares the calculated and experimental transfer free energies, ΔG_{tr}, at 1 M GB. Although of the same order, there are some quantitative and qualitative disagreements between the two data sets. Several possible explanations can be put forward to account for the discrepancies. First, SPT-based computations of $\Delta\Delta G_C$ may be unreliable due to their critical dependence on the assumed hard sphere diameter of the cosolvent molecule.[12] The latter is not easy to determine given the necessity to approximate a nonrigid, nonspherical molecule by a rigid sphere.[12] In fact, seemingly insignificant

Table 9. Changes in Free Energies, ΔG_{tr}(calc) (kcal mol^{-1}), Calculated for the Water-to-GB Transfer of Amino Acid Side Chains and the Glycyl Unit (−CH_2CONH−) Using eq 1

SC	0	1	2	3	4
Ala	0	−0.24	−0.69	−1.33	−2.18
Val	0	0.55	1.17	1.72	1.71
Leu	0	0.55	0.99	0.99	0.19
Ile	0	0.30	0.27	−0.30	−1.50
Pro	0	−0.24	−0.84	−1.78	−3.04
Phe	0	−0.28	−1.07	−2.31	−3.94
Trp	0	0.57	0.71	0.10	−1.35
Met	0	0.08	−0.28	−1.20	−2.67
Tyr	0	0.51	0.65	0.14	−1.15
Gln	0	0.19	0.07	−0.44	−1.35
−CH_2CONH−	0	0.18	0.24	0.20	0.10

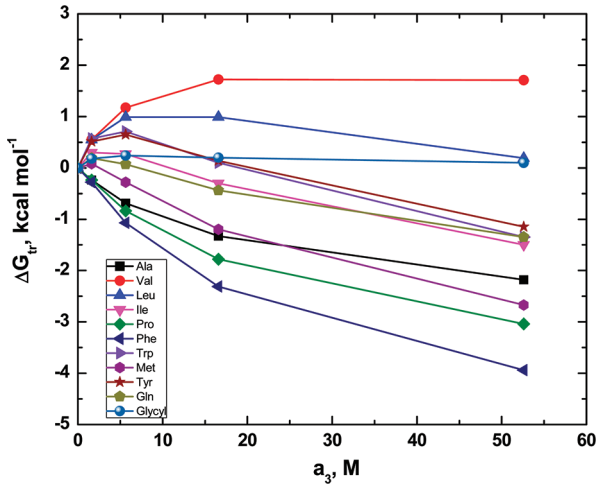

Figure 2. Water-to-GB transfer free energies of the amino acid side chains and the glycyl unit plotted as a function of GB activity.

changes in the hard sphere diameters of the solvent and cosolvent molecules may substantially alter the magnitude and even the sign of $\Delta\Delta G_C$.[12] Second, the absolute magnitude of the interaction free energy, $\Delta\Delta G_I$, may have been under- or overestimated since the number of GB binding sites in our model was taken equal to the maximum number of GB molecules that can be in contact with a solute. However, the actual number of GB binding sites may well be smaller than the maximum value given by n/r. The approximation of identical and independent GB-binding sites with a single k, while providing an adequate fit for the volume and compressibility data, may compromise quantitative evaluation of $\Delta\Delta G_I$. A more complete equation for $\Delta\Delta G_I$ is based on the assumption of independent but not identical binding sites:

$$\Delta\Delta G_I = -RT \sum_{i=1}^{n/r} \ln(a_1^{\,r} + k_i a_3) \qquad (9)$$

where k_i is the binding constant for the ith binding site. Determination of individual binding constants, k_i, from experimental GB dependences of volume and compressibility is not possible. Clearly, a further level of complexity is to introduce cooperativity to the binding by allowing the sites to interact with one another.

Table 10. Calculated, ΔG_{tr}(calc) (cal mol^{-1}), and Experimental, ΔG_{tr}(exp) (cal mol^{-1}), Free Energies for the Transfer of the Amino Acid Side Chains and the Glycyl Unit (−CH_2CONH−) from Water to 1 M GB

SC	ΔG_{tr}(calc)	ΔG_{tr}(exp)
Ala	−235	183.25[a]
Val	545	158.85[a]
Leu	553	160.75[a]
Ile	300	177.21[a]
Pro	−241	53.32[a]
Phe	−276	65.55[a]
Trp	574	
Met	82	164.32[a]
Tyr	508	
Gln	193	186.05[a]
−CH_2CONH−	183	65 ± 3[b]

[a] From ref 6. [b] From ref 34.

Third, the number of waters released to the bulk upon GB binding, r, may differ from our estimate of 4. Finally, the experimental transfer free energy data may suffer from the concentration-dependent effects as has been discussed in a previous paper.[11]

Given the large disparities between the calculated and experimental data on ΔG_{tr}, we focus below on the qualitative inferences that can be drawn from our data. Inspection of Figure 2 in conjunction with the data presented in Tables 7 and 8 reveals several important observations. First, the transfer free energy, ΔG_{tr}, results from a fine balance between the large $\Delta\Delta G_C$ and $\Delta\Delta G_I$ contributions. Second, the range of the magnitudes and the shape of the GB dependence of ΔG_{tr} depend on the identity of a specific solute group. The Ala, Pro, and Phe side chains display favorable values of ΔG_{tr} within the entire GB concentration range. The other side side chains and the glycyl unit exhibit unfavorable ΔG_{tr} at low GB and favorable ΔG_{tr} at high GB. The interplay between $\Delta\Delta G_C$ and $\Delta\Delta G_I$ results in pronounced maxima in the GB dependences of ΔG_{tr} for the Val, Leu, Ile, Trp, Tyr, and Gln side chains as well as the glycyl unit. This observation is in qualitative agreement with the maxima in the T_M-versus-GB concentration plots observed by Bolen and coworkers for ribonuclease A and lysozyme within the 3 to 4 M GB range.[35] In addition, this observation is consistent with the analysis of the behavior of GB in the vicinity of lacI HTH DNA binding domain presented by Felitsky and Record which predicts a reversal of the stabilizing effect of GB at high concentrations.[25] These authors have determined that the local-bulk partition coefficient, K_P, of GB near the protein is smaller than 1 between 0 and ~4 M GB but intersects the unity line and becomes larger than 1 at higher cosolvent concentrations.[25]

Volumetric Parameters. As is seen from Table 6, the values of ΔV_0 are negative for predominantly nonpolar amino acid side chains, but show a tendency to become positive for the polar and charged entities, the glutamine side chain, the glycyl residue, and the zwitterionic amino acid glycine. Recall that ΔV_0 is the change in volume accompanying the binding of a cosolvent (urea or GB) to a particular solute or an atomic group in an ideal solution. As such, ΔV_0 is contributed by changes in thermal, ΔV_T, and interaction, ΔV_I, volumes.[36,37] The thermal volume, V_T, which mainly results from thermally induced mutual solute−solvent vibrations, represents the effective void volume around a solute.

Note that V_T is proportional to the number of solute−water and cosolvent−water contacts both of which decreases upon the association of a solute and cosolvent molecules.[37] Consequently, a change in thermal volume, ΔV_T, associated with solute-cosolvent binding event is invariably negative. The interaction volume, V_I, which reflects solvent contraction in the vicinity of polar and charged groups, may increase or remain the same upon solute-cosolvent binding events which are accompanied by release of waters of solute and cosolvent hydration to the bulk.[36] The values of ΔV_I will be large and positive if the associating species interact via polar and charged groups, while being near zero if the contacting groups are predominantly nonpolar.[36] The observation that, for charged and polar groups, the values of ΔV_0 are positive is consistent with a picture in which ΔV_I prevails over ΔV_T.

On other hand, the highly negative values of ΔV_0 observed for GB association with nonpolar groups suggest that, for these groups, ΔV_T prevails over ΔV_I. This observation can be accounted for by proposing that the contacts between GB and nonpolar groups of a solute are mainly implemented via the amino methyl groups of the former. Given the bulkiness of the amino terminus of GB, $-N(CH_3)^+$, and, hence, a low charge density around it, waters solvating the amino methyl groups are likely to display structural, volumetric, and thermodynamic properties similar to those displayed by waters solvating nonpolar substances. Therefore, we propose that release of waters solvating the amino methyl groups of GB to the bulk should have a near zero contribution to ΔV_I. In this scenario, ΔV_0 is dominated by a negative change in V_T, in agreement with the observed tendency.

Changes in compressibility, ΔK_{S0}, accompanying the binding of GB to the molecular entities studied in this work are all positive. This observation is in line with the fact that, since at 25 °C, water molecules solvating nonpolar, charged, and most of polar atomic groups are characterized by a lower partial molar compressibility relative to bulk water.[26,38] Consequently, release of hydration water to the bulk upon the association of GB with solute groups should result in positive changes in compressibility.

In the aggregate, individual values of ΔV_0 and ΔK_{S0} determined for water−cosolvent exchange reflect a host of molecular events including a decrease in the number of water−cosolvent and water−solute contacts, release of waters of solute and GB hydration to the bulk, and formation of specific solute-cosolvent contacts. In addition, each of these events may have a relaxation contribution to ΔK_{S0}.[39] Quantitative interpretation of and differentiation between the volumetric effects of these events is not a simple matter. However, independent of the "success" or "failure" of such interpretations or their veracity, the values of ΔV_0 and ΔK_{S0} represent a volumetric signature of solvent−cosolvent exchange in the vicinity of various solute groups and can be used as such for identification and characterization of the differential solvation of atomic groups in water and water−cosolvent mixtures.

CONCLUDING REMARKS

We measured the partial molar volumes and adiabatic compressibilities of *N*-acetyl amino acid amides and oligoglycines at GB concentrations ranging from 0 to 4 M. We used the resulting data to evaluate the volumetric contributions of the amino acid side chains and the glycyl unit ($-CH_2CONH-$) as a function of GB concentration. The resulting data were analyzed within the framework of a statistical thermodynamic model to evaluate the equilibrium constant for the reaction in which a GB molecule binds each of the studied functionalities replacing four water molecules. We calculated the free energy of the transfer of functional groups from water to concentrated GB solutions, ΔG_{tr}, as the sum of a change in the free energy of cavity formation, $\Delta\Delta G_C$, and the differential free energy of solute−solvent interactions, $\Delta\Delta G_I$, in a concentrated GB solution and water. Our data suggest that the transfer free energy, ΔG_{tr}, results from a fine balance between the large $\Delta\Delta G_C$ and $\Delta\Delta G_I$ contributions. The range of the magnitudes and the shape of the GB dependence of ΔG_{tr} depend on the identity of a specific solute group. In particular, the interplay between $\Delta\Delta G_C$ and $\Delta\Delta G_I$ results in pronounced maxima in the GB dependences of ΔG_{tr} for the Val, Leu, Ile, Trp, Tyr, and Gln side chains as well as the glycyl unit. This observation is in qualitative agreement with the maxima in the T_M-versus-GB concentration plots observed for ribonuclease A and lysozyme within the 3−4 M GB range.[35]

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ACKNOWLEDGMENT

This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada to T.V.C. Y.L.S. gratefully acknowledges his graduate support from the CIHR Protein Folding Training Program.

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