Excluded Volume in Solvation: Sensitivity of Scaled-Particle Theory to Solvent Size and Density

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ABSTRACT Changes in solvent environment greatly affect macromolecular structure and stability. To investigate the role of excluded volume in solvation, scaled-particle theory is often used to calculate ΔG_{tr}^{ev}, the excluded-volume portion of the solute transfer free energy, ΔG_{tr}. The inputs to SPT are the solvent radii and molarities. Real molecules are not spheres. Hence, molecular radii are not uniquely defined and vary for any given species. Since ΔG_{tr}^{ev} is extremely sensitive to solvent radii, uncertainty in these radii causes a large uncertainty in ΔG_{tr}^{ev}—several kcal/mol for amino acid solutes transferring from water to aqueous mixtures. This uncertainty is larger than the experimental ΔG_{tr} values. Also, ΔG_{tr}^{ev} can be either positive or negative. Adding neutral crowding molecules may not necessarily reduce solubility. Lastly, ΔG_{tr}^{ev} is very sensitive to solvent density, ρ. A few percent error in ρ may even cause qualitative deviations in ΔG_{tr}^{ev}. For example, if ρ is calculated by assuming the hard-sphere pressure to be constant, then ΔG_{tr}^{ev} values and uncertainties are now only tenths of a kcal/mol and are positive. Because ΔG_{tr}^{ev} values calculated by scaled-particle theory are strongly sensitive to solvent radii and densities, determining the excluded-volume contribution to transfer free energies using SPT may be problematic.

INTRODUCTION

Altering the solvent environment by adding large quantities of cosolvent can cause significant changes in the structure and stability of biological macromolecules. For example, several-molar concentrations of urea, guanidine HCl, or alcohol cause protein denaturation, whereas sucrose stabilizes protein native states; alcohols have long been used to promote DNA condensation. (Technically, molecules such as urea, guanidine HCl, and sucrose are cosolutes, being solid in their pure form. However, at typical concentrations, they make up a significant fraction of the solution—8 M urea is 43 wt% urea and 2 M sucrose is 55 wt% sucrose. These cosolute molecules bathe and solvate the macromolecular solute just as much as water does; they also solvate water molecules as water solvates them. In that sense, these cosolutes behave like solvent molecules. To emphasize this point, and to put cosolvents and cosolutes on an equal footing, we refer to all species which solvate as "cosolvent" molecules. Only in the Theory and Methods section discussion on obtaining molecular hard-sphere radii, where we need to emphasize the solid nature of the pure substances, do we use the term "cosolute.") Also, when a macromolecule changes structure, parts of it experience a change in solvent environment, e.g., when proteins denature and the protein interior moves from a primarily hydrophobic milieu to an aqueous one. Despite the importance of understanding solvation effects and much research effort along these lines, how (co)solvents interact with proteins and DNA is still not well understood.

To probe solvent-macromolecule interactions, one would like to measure the solvation free energy, G_{solv}, the free energy of interaction between solute and solvent. Since G_{solv} is difficult to obtain experimentally, one measures instead the free energy of transfer, ΔG_{tr}, of the macromolecule (or its constituent parts) from one solvent environment (denoted *A*) to another (*B*): $\Delta G_{tr} = G_{solv}(B) - G_{solv}(A)$. How does one interpret ΔG_{tr}? Let us first return to G_{solv} and dissect it into more meaningful parts—a part due to "soft" (e.g., dispersion, hydrogen-bonding, dipole, and electrostatic) interactions, denoted here as G_{solv}^{i}, and a part due to "excluded-volume" interactions (G_{solv}^{ev}). $G_{solv} = G_{solv}^{ev} + G_{solv}^{i}$. ($G_{solv}^{ev}$ and G_{solv}^{i} are defined more exactly in Theory, below) G_{solv}^{ev} describes the work of making room for the solute, i.e., of creating a hard cavity. Not only is G_{solv}^{i} dependent on the solvent environment, but so is G_{solv}^{ev}. The price of creating a fixed-size cavity depends on the amount of unoccupied or free volume. Creating a cavity in a dense environment is generally more difficult than in one that has a lot of free volume. Now the transfer free energy, ΔG_{tr}, can also be split into soft and excluded-volume parts: $\Delta G_{tr} = \Delta G_{tr}^{ev} + \Delta G_{tr}^{i}$, where $\Delta G_{tr}^{ev} = G_{solv}^{ev}(B) - G_{solv}^{ev}(A)$, and likewise for ΔG_{tr}^{i}. ΔG_{tr}^{ev}, which we call the "free energy of cavity transfer" from environment *A* to *B*, is the difference in free energy between creating a cavity in *B* versus in *A*. A positive (negative) value indicates that it is harder (easier) to create a cavity in environment *B*. $\Delta G_{tr}^{i} = G_{solv}^{i}(B) - G_{solv}^{i}(A)$ embodies the difference in soft interactions between the two environments.

Scaled-particle theory (SPT) is commonly used to calculate ΔG_{tr}^{ev}. Since SPT was designed to capture the packing interactions of a hard-sphere solute in a fluid of hard spheres (there are no soft interactions), it would seem to be an ideal theory for calculating G_{solv}^{ev} and ΔG_{tr}^{ev}. The only input parameters necessary are the solute radius and the concentrations and radii of the solvent species.

Received for publication 2 December 1999 and in final form 7 June 2000.

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0006-3495/00/11/2222/13 $2.00

However, there are indications that using SPT for making (semi)quantitative calculations of $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ may be problematic:

1. It has been shown that solvation energies ($G_{\mathrm{solv}}^{\mathrm{ev}}$ values), as calculated by SPT (Morel-Desrosiers and Morel, 1981; Wilhelm and Battino, 1972; Lucas, 1976; Pierotti, 1976; Crovetto et al., 1982; Postma et al., 1982; Ben-Naim et al., 1989; Madan and Lee, 1994; Prévost et al., 1996) and by more realistic models (Postma et al., 1982; Pohorille and Pratt, 1990; Madan and Lee, 1994; Prévost et al., 1996; Floris et al., 1997), are strongly dependent on the solute radius (Morel-Desrosiers and Morel, 1981; Lucas, 1976; Pierotti, 1976; Crovetto et al., 1982; Postma et al., 1982; Ben-Naim et al., 1989; Pohorille and Pratt, 1990; Madan and Lee, 1994; Prévost et al., 1996; Floris et al., 1997) and especially on the solvent radius (Morel-Desrosiers and Morel, 1981; Wilhelm and Battino, 1972; Lucas, 1976; Pohorille and Pratt, 1990; Madan and Lee, 1994). A change of 2% in solvent radius results in a change of ~15% in $G_{\mathrm{solv}}^{\mathrm{ev}}$ (Wilhelm and Battino, 1972). Preliminary results (Lucas, 1976) and evidence from heat capacities of transfer (Desrosiers and Desnoyers, 1976) and partition coefficients (Watarai et al., 1982) suggest that the transfer free energy, $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$, is also sensitive to solvent size. Unfortunately, determining the radii of real molecules, which are not spherical, is somewhat ambiguous. Different experimental and theoretical methods yield different values (see, e.g., Gogonea et al., 1998). These two facts—the sensitivity of $G_{\mathrm{solv}}^{\mathrm{ev}}$ to solvent size and the ambiguity in obtaining these sizes—suggest that calculating actual numbers for $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values using SPT might be problematic.

2. One of the contributions to $G_{\mathrm{solv}}^{\mathrm{ev}}$ is the mechanical pressure-volume (pV) work of displacing solvent or the atmosphere around it. However, which pressure value to use, the hard-sphere pressure (p_{hs}) needed to maintain the system of hard spheres at the experimental fluid density or atmospheric pressure (p_{atm}) is not yet clear (Shimizu et al., 1999). The choice presumably depends on how G_{solv} is dissected and on which interactions are being apportioned to the excluded-volume part of the free energy ($G_{\mathrm{solv}}^{\mathrm{ev}}$). (Note that even if p_{hs} is used, soft interactions are still included implicitly in $G_{\mathrm{solv}}^{\mathrm{ev}}$. Soft interactions determine the experimental solvent densities, which are then used as input parameters in SPT calculations.) Unfortunately, which pressure value is used does make a significant difference in $G_{\mathrm{solv}}^{\mathrm{ev}}$ (Pierotti, 1976), and possibly in $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$, since at fluid densities p_{hs} is typically orders of magnitude greater than p_{atm} (Pierotti, 1976).

3. The last potential difficulty regards obtaining the water molarity in an aqueous mixed solvent ($n_{\mathrm{w}}^{\mathrm{mix}}$). For a specific solvent one can get $n_{\mathrm{w}}^{\mathrm{mix}}$ from the experimental solution density (ρ) plus the cosolvent molarity ($n_{\mathrm{c}}^{\mathrm{mix}}$). However, for making calculations on generic cosolvents, $n_{\mathrm{w}}^{\mathrm{mix}}$ must be obtained theoretically. Some researchers (Berg, 1990, Guttman et al., 1995, Saunders et al., 2000) use the approximation of applying the Gibbs-Duhem relation at constant temperature and pressure to the SPT portion of the equation of state; this is thermodynamically equivalent to holding p_{hs} fixed (Guttman et al., 1995) to the value of pure water, $p_{\mathrm{hs}}^{\mathrm{wat}}$. (The Gibbs-Duhem relation, of course, applies to the entire equation of state, but not necessarily to a subset of it.) How good is this approximation for the purpose of calculating $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$?

In this work, we determined the uncertainties in $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ due to ambiguities in SPT input parameters. Are these uncertainties small enough such that ΔG_{tr} can be usefully separated into excluded-volume and soft-interaction terms? We performed calculations and comparisons for the transfer of amino acid solutes from water to aqueous solutions of ethanol, ethylene glycol, sucrose, and urea to compare with the experimental results of Nozaki and Tanford (1971, 1965) and Bolen and colleagues (Liu and Bolen, 1995; Wang and Bolen, 1997). We have addressed the above three particular concerns as follows:

1. To determine the degree of uncertainty in $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ caused by uncertainties in molecular radii, we varied the input solvent radii within the range of representative solvent radii from the literature and looked at the spread in the $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values.
2. To see how choice of pressure affects $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$, we calculated $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ using both p_{atm} and p_{hs}.
3. To check the approximation of fixing the hard-sphere pressure at $p_{\mathrm{hs}}^{\mathrm{wat}}$ to determine $n_{\mathrm{w}}^{\mathrm{mix}}$, we compared the predicted solvent densities with the experimental values as well as the $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values calculated with the predicted versus the experimentally determined $n_{\mathrm{w}}^{\mathrm{mix}}$ values.

In addition, we discuss why the work of formation of a hard cavity is so dependent on solvent size.

THEORY AND METHODS

Theory

First, let us more carefully define $G_{\mathrm{solv}}^{\mathrm{ev}}$. The solute-insertion process can be separated into three steps. In step 1 all soft interactions are turned off; only hard interactions remain. However, the solvent density is kept fixed at the fluid density. In step 2, a hard cavity in which to place the solute is created within the solvent of hard particles. In step 3, the soft interactions, both solvent-solvent and solute-solvent, are turned back on. The free energy associated with step 2 is $G_{\mathrm{solv}}^{\mathrm{ev}}$; that associated with both steps 1 and 3 is $G_{\mathrm{solv}}^{\mathrm{i}}$. $G_{\mathrm{solv}} = G_{\mathrm{solv}}^{\mathrm{ev}} + G_{\mathrm{solv}}^{\mathrm{i}}$. Since step 2 involves no explicit soft interactions, any hard-particle theory of fluids can be used to calculate $G_{\mathrm{solv}}^{\mathrm{ev}}$.

(Note, there is another common separation of G_{solv} into a cavity and a soft-interaction term: $G_{\mathrm{solv}} = G_{\mathrm{solv}}^{\mathrm{cavity}} + G_{\mathrm{solv}}^{\mathrm{interaction}}$. $G_{\mathrm{solv}}^{\mathrm{cavity}}$ is the work of creating a hard cavity in a solvent whose solvent-solvent interactions are on; $G_{\mathrm{solv}}^{\mathrm{interaction}}$ is the conditional free energy of turning on the solute-solvent soft interactions, once the cavity has been created. One of the advantages of this dissection of $G_{\mathrm{solv}}^{\mathrm{ev}}$ is that one can easily write analytic formulas for $G_{\mathrm{solv}}^{\mathrm{cavity}}$ and $G_{\mathrm{solv}}^{\mathrm{interaction}}$ in terms of ensemble averages. For more details, see section 3.5 of Ben-Naim (1987). However, because the solvent-solvent soft interactions are always on, there are solvent reorganization and redistribution terms in $G_{\mathrm{solv}}^{\mathrm{cavity}}$ which are not present in $G_{\mathrm{solv}}^{\mathrm{ev}}$ and which are, unfortunately, hard to ascertain. Hence, there is an enthalpic component to

G^{cavity}_{solv}, whereas G^{ev}_{solv} is purely entropic. To examine only solvent-size effects, it would seem more useful to determine G^{ev}_{solv}.)

SPT has commonly been employed to calculate G^{ev}_{solv}. The fundamental idea behind the theory was described by Reiss (1966) this way: "[T]he most important problem in the theory of liquids is concerned with the packing of hard cores. . . . In this model, the soft intermolecular potential (or the non-hard-core part of the potential) acts primarily to establish the overall density of the fluid, while the internal structure is determined by the packing of the hard cores. Thus it might be said that the soft potential determines the *volume* of a container which in turn is filled with a hard sphere fluid. . . . [S]caled particle theory . . . is geometric in nature and deals in a rigorous manner with the problem of the packing in a sufficiently dense fluid of molecular hard cores."

The derivation of SPT involves finding the probability, $P(R)$, of inserting a hard spherical cavity of radius R with its center at an arbitrary (fixed) location in a fluid whose m species have hard cores of radii R_i. $P(R)$ is simply related to the work of inserting the same cavity, $G^{ev}_{solv}(R)$ (Tolman, 1938):

$$P(R) = \exp(-G^{ev}_{solv}(R)/kT) \tag{1}$$

where k is Boltzmann's constant and T is the absolute temperature. There are several exact conditions on $P(R)$ and $G^{ev}_{solv}(R)$ for very small cavities and macroscopic cavities (Reiss, 1966). Combined with conditions on the smoothness of derivatives one obtains the following result for $G^{ev}_{solv}(R)$ (Lebowitz et al., 1965):

$$\frac{G^{ev}_{solv}(R)}{kT} = -\ln(1-\xi_3) \tag{2}$$

$$+ [6\xi_2/(1-\xi_3)]R \tag{3}$$

$$+ [12\xi_1/(1-\xi_3) + 18\xi_2^2/(1-\xi_3)^2]R^2 \tag{4}$$

$$+ \frac{p}{kT}\frac{4\pi}{3}R^3 \tag{5}$$

where

$$\xi_j = \frac{1}{6}\pi \sum_{i=1}^{m} n_i(2R_i)^j, \tag{6}$$

n_i is the number density of species i, and p is the pressure. Note that ξ_3 has a physical meaning. $\xi_3 = \Sigma_{i=1}^{m} n_i \frac{4}{3}\pi R_i^3$ = (fractional volume occupancy) ≡ (packing fraction) and $1 - \xi_3$ = (fractional free volume). We see in $G^{ev}_{solv}(R)$ the familiar pV term, the work of creating a macroscopic cavity of volume V (Eq. 5), as well as a surface-tension term $\propto R^2$ (Eq. 4) with a curvature correction $\propto R^1$ (Eq. 3).

The appropriate pressure to use in Eq. 5 is not yet clear (Shimizu et al., 1999 and references therein). Both p_{atm} and p_{hs} have been used, yielding very different values for $G^{ev}_{solv}(R)$ (Pierotti, 1976). The functional form of p_{hs} is given by Lebowitz et al. (1965):

$$\pi\frac{p_{hs}}{kT} = 6[\xi_0/(1-\xi_3)] + 18[\xi_1\xi_2/(1-\xi_3)^2] + 18[\xi_2^3/(1-\xi_3)^3]. \tag{7}$$

In general, p_{hs} is very high at fluid densities (Pierotti, 1976). For example, pure water's hard-sphere pressure, p^{wat}_{hs}, is 8000 atm at 25°C (obtained by using Eqs. 6 with one species and 7 with $n_w = n^{wat}_w = 55.342$ M and $R_w = 1.38$ Å). To cover both possibilities in this work, we calculate $G^{ev}_{solv}(R)$ with p set to both p_{hs} and p_{atm}.

Lastly, we point out that $G^{ev}_{solv}(R)$ is the work of inserting a hard cavity at a fixed site in the solvent. Translational and liberational entropies are not included. In comparing to experimental transfer data, the corresponding value is the difference in standard state chemical potential of the solute, $\Delta\mu° = \mu°(B) - \mu°(A)$, on the number-density (molarity) scale (Ben-Naim, 1978). (The translational entropy present in $\mu°$ cancels in transfer processes.) We have converted experimental data reported on the mole-fraction scale to molarity scale.

Molecular hard-sphere radii are not well defined

To calculate the work of cavity formation, SPT requires only the water and cosolvent number densities and their hard-sphere radii as inputs. However, these radii are not well defined. For both water and cosolvent, there are fairly wide ranges of reasonable values.

The hard-sphere radius of water as measured by experiment is typically around 1.35 Å, but different experiments give values ranging from 1.25 to 1.46 Å (Pierotti, 1965). In theoretical studies of water, the following radii have been used: 1.35 Å (a hard-sphere fluid with water's fractional free volume and number density has this radius; Pohorille and Pratt, 1990), 1.38 Å (Lee, 1985; obtained from solubility experiments of Pierotti (1965, 1976); see also next paragraph), 1.40 Å (the most probable water oxygen-oxygen distance; Prévost et al., 1996), 1.44–1.5 Å (obtained by fitting SPT with the water radius as an adjustable parameter, to free-energy data obtained via simulations of simple point charge (SPC) (Postma et al., 1982) and transferable intermolecular potential 4 point (TIP4P) (Floris et al., 1997) water models), and 1.58 Å (the Lennard-Jones σ parameter divided by 2; Prévost et al., 1996). Solvent probe radii of 1.4 Å (Lee and Richards, 1971; Shrake and Rupley, 1973) and 1.5 Å (Connolly, 1983) have been used to determine the solvent-accessible surface areas of macromolecules. There is not one unique hard-sphere radius for water.

Since many (co)solvents are less studied than water, their hard-sphere radii can be even more ambiguous. Some researchers (Wilhelm and Battino, 1972; Morel-Desrosiers and Morel, 1981) have argued that the most self-consistent hard-sphere radius for use in SPT is measured via a technique pioneered by Pierotti (1965). Solubilities of a series of nonpolar, spherical solutes (e.g., noble gases) are measured. When the data are extrapolated to zero polarizability, only the hard-sphere interaction remains. Matching to SPT yields the solvent's hard-sphere radius. These experiments are non-trivial, and hard-sphere radii have been obtained by other methods: fitting pressure-density data to a hard-sphere plus Lennard-Jones equation of state (Ben-Amotz and Herschbach, 1990; Ben-Amotz and Willis, 1993); fitting surface-tension (Mayer, 1963), isothermal compressibility (Mayer, 1963), and heats of vaporization data (Pierotti, 1976) to SPT; from cell theories of liquids (Salsburg and Kirkwood, 1953; Kobatake and Alder, 1962); and from gas-phase virial coefficients and viscosities (Hirschfelder et al., 1964 and references therein). The radii obtained by all of these experimental methods implicitly include solvent-solvent interactions and are therefore effective radii. Unfortunately, none of these methods can be used to obtain the hard-sphere radii of many biologically interesting cosolutes such as urea or sucrose since the methods assume that the molecule of interest is a liquid (or a gas) in its pure form. For cosolutes, the only available techniques measure the length dimensions of a molecule in isolation. These techniques include calculating molecular van der Waals volumes (Bondi, 1964; Edward, 1970; Gogonea et al., 1998), as well as actually measuring lengths on a space-filling model (Goldstein and Solomon, 1960; Schultz and Solomon, 1961). Radii from these methods do not include any solvent-solvent interactions. However, the relationship between molecular lengths and the hard-sphere radius of an equivalent sphere has not been fully determined (Gogonea et al., 1998).

Table 1 lists the hard-sphere radii of cosolvents (cosolutes) by various methods. For common organic solvents, for which experimental values are available, the radii vary by several tenths of an angstrom. This is not surprising since these molecules are not spherical and the solvent-solvent

TABLE 1 (Co)solvent and cosolute hard-sphere radii obtained from experiment and from calculations of molecular lengths vary depending on the method used to obtain them

	radius (Å)	
(co)solvent, cosolute	experimental	calculated
water	1.36[a], 1.375[bc], 1.385[d], 1.44–1.46[e], 1.46[f]	1.61–1.62[n], 1.70[o]
methanol	1.64–1.68[e], 1.73–1.75[a], 1.79[g], 1.84[h], 1.85[c], 1.86[d], 1.91[i], 1.92[f]	2.02[n], 2.02–2.05[p], 2.05[o]
ethanol	2.00[e], 2.06[a], 2.16[j], 2.17[ch], 2.18[d], 2.22[fi], 2.23[g]	2.30[n], 2.30–2.33[p], 2.33[o]
ethylene glycol	2.18[c], 2.22[ae], 2.31[f], 2.32[i]	2.24[q], 2.39–2.44[p], 2.44[o]
glycerol	2.60[f], 2.67[i]	2.67–2.73[p], 2.73[o], 2.74[q], 2.9[r]
acetone	2.24[e], 2.26[a], 2.42[f]	2.44[n], 2.48[o], 2.49[p]
CCl_4	2.57–2.58[a], 2.57–2.59[e], 2.68[hk], 2.69[d], 2.70[fi], 2.71[l], 2.94[g]	2.73[o], 2.74[p], 2.78[n]
benzene	2.50–2.51[a], 2.51–2.52[e], 2.61[k], 2.62[bj], 2.63[hl], 2.64[g], 2.65[f]	2.68[op]
dioxane	2.61[c], 2.62[m], 2.68[i]	2.64–2.70[p], 2.68[o]
glucose/glucopyranose	3.16[i]	3.21–3.28[p], 3.27–3.33[o], 3.9[r]
sucrose	3.98[i]	3.95–4.05[p], 4.02[o], 4.5[r]
urea		2.03[q], 2.3[r], 2.35[o]

Radii obtained from:

[a] isothermal compressibility, 283 *K*-303 *K* (Mayer, 1963).

[b] gas-solubility data (Pierotti, 1965).

[c] an empirical linear relationship between the Lennard-Jones σ parameter determined by gas solubilities and the molar volume (Brückl and Kim, 1981).

[d] solubility data (Pierotti, 1976, Liabastre, 1974).

[e] surface tensions, 283 *K*-303 *K* (Mayer, 1963).

[f] pressure-density data fit to hard-sphere + Lennard-Jones equation of state (Ben-Amotz and Herschbach, 1990).

[g] gas-phase viscosities (Hirschfelder et al., 1964).

[h] gas-solubility data (Wilhelm and Battino, 1971).

[i] volume-increment tables obtained by fitting pressure-density data to hard-sphere + Lennard-Jones equation of state (Ben-Amotz and Willis, 1993).

[j] heat of vaporization plus SPT (Pierotti, 1976).

[k] vapor pressure + a cell theory of liquids (Kobatake and Alder, 1962).

[l] heats of vaporization and molal volumes + a cell theory of liquids (Salsburg and Kirkwood, 1953).

[m] gas solubilities (Gallardo et al., 1983).

[n] van der Waals volume (Gogonea et al., 1998).

[o] van der Waals volume-increment tables (Edward, 1970).

[p] van der Waals volume-increment tables, with varying number of hydrogen bonds or type of ether group (Bondi, 1964).

[q] length measurements on a space-filling model (Goldstein and Solomon, 1960).

[r] length measurements on a space-filling model (Schultz and Solomon, 1961).

interactions implicitly included in the experimental values are experiment-dependent. Values obtained from molecular-length calculations tend to be larger than experimental values. Radius data for cosolutes are limited. We presume that if there were some way of obtaining them from experiment, there would be a similar variation in radii as for the (co)solvents.

SPT parameters used in this work

Because the cosolvents studied here are of comparable size to water, we treat water explicitly in our SPT calculations. We chose the following radii for water and cosolvents. The water radii (R_w), 1.35, 1.38, and 1.40 Å, represent those used in water studies (Pohorille and Pratt, 1990; Lee, 1985; Prévost et al., 1996) as well as the value measured from solubility experiments (Pierotti, 1965, 1976). The cosolvent radii, R_c, we chose are ethanol, 2.00, 2.15, and 2.30 Å; ethylene glycol, 2.20, 2.30, and 2.40 Å; sucrose, 3.85, 4.00, and 4.15 Å; and urea, 2.15, 2.25, and 2.35 Å. The radii values of ethanol and ethylene glycol span the observed range from experimental data and molecular-length calculations; for sucrose and urea, we have chosen a range of radii representing a possible spread of values around those obtained from molecular-length calculations.

The radii of the solutes—the amino acids, triglycine (3gly; all in their zwitterionic form), and diketopiperazine (DKP)—were obtained using van der Waals volume increments (Edward, 1970) and are listed in Table 2. We do not vary solute radii because it has been noted that $G_{\mathrm{solv}}^{\mathrm{ev}}$ values are not as sensitive to solute radii as to solvent radii (Morel-Desrosiers and Morel, 1981) and preliminary evidence (data not shown) indicate that this is also true of $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values.

Except in the section where n_w^{mix} was approximated by holding p_{hs} constant, n_w^{mix} values were obtained from the following experimentally measured cosolvent molarities and solution densities: 60 vol% (10.3 M) ethanol and 30 vol% (5.36 M) ethylene glycol: 0.9096 and 1.0405 g/ml, respectively, at 20°C (obtained by interpolating data from Wolf et al. (1985); 1 M sucrose: 1.127100 g/ml at 25°C (Liu and Bolen, 1995); 2 M urea: 1.028 g/ml at 25°C (D. W. Bolen and M. Auton, University of Texas Medical Branch, personal communication). The number densities of pure water (n_w^{wat}), 55.407 and 55.342 M at 20 and 25°C, respectively, were obtained from the corresponding mass densities (Weast, 1987), 0.9982063 and 0.9970480 g/ml. Note that, here, both water and cosolvent number densities are fixed by experiment; they are not adjustable parameters.

In studies of generic cosolvents, n_w^{mix} cannot be measured experimentally, and the approximation of holding p_{hs} constant has been used to obtain n_w^{mix} (Berg, 1990; Guttman et al., 1995; Saunders et al., 2000). Below, we test this approximation against calculations done with n_w^{mix} values obtained from experiment (per the previous paragraph). n_w^{mix} obtained from holding p_{hs} fixed was calculated by numerically solving the equation $p_{\mathrm{hs}}^{\mathrm{mix}}(R_w, R_c, n_w^{\mathrm{mix}}, n_c^{\mathrm{mix}}) = p_{\mathrm{hs}}^{\mathrm{wat}}(R_w, n_w^{\mathrm{wat}})$ for n_w^{mix} and taking the real root. $p_{\mathrm{hs}}^{\mathrm{wat}}$ and $p_{\mathrm{hs}}^{\mathrm{mix}}$ were calculated using Eqs. 6 and 7 with one species (i = water) and two species (i = {water, cosolvent}), respectively. n_w^{wat} is 55.407 M (20°C) and 55.342 M (25°C). Note that n_w^{mix} is a function of R_w, R_c, n_w^{wat}, and n_c^{mix}.

TABLE 2 Radii of amino-acid and protein-backbone-analog solutes

solute	radius (Å)
triglycine (3gly)	3.24
DKP	2.9
gly	2.4
ala	2.6
val	3.0
leu	3.1
ile	3.1
pro	2.9
ser	2.7
thr	2.9
phe	3.3
tyr	3.3
trp	3.5
lys	3.2
arg	3.3
his	3.1
asp	2.9
glu	3.0
asn	2.9
gln	3.1
met	3.1
cys	2.8

Radii were calculated using the van der Waals volume increment table of Edward (1970). The radii of aspartate and aspartic acid are the same, to two significant digits; the same is true of glutamate and glutamic acid, and charged and neutral histidine.

Hence, for transfer to a given solvent mixture (fixed n_c^{mix}), n_w^{mix} will vary as solvent radii are varied.

RESULTS AND DISCUSSION

To determine what role excluded volume plays in transfer processes, we have used SPT to model the excluded-volume portion, ΔG_{tr}^{ev}, of the total transfer free energy, ΔG_{tr} ($= \Delta G_{tr}^{ev} + \Delta G_{tr}^{i}$), of solutes from water to mixed solvents. We then compared the ΔG_{tr}^{ev} values and uncertainties with experimentally measured ΔG_{tr} values to see whether ΔG_{tr}^{i} can be usefully determined. In particular, we determined how uncertainties in solvent radii and in solvent density, necessary for the input parameters, translate into uncertainties in ΔG_{tr}^{ev}. Also, we checked how ΔG_{tr}^{ev} values calculated using an approximate method of obtaining n_w^{mix} compare to those calculated using experimentally determined n_w^{mix} values. The systems we studied are amino-acid solutes transferring from water to 60 vol% ethanol, 30 vol% ethylene glycol, 1 M sucrose, and 2 M urea.

ΔG_{tr}^{ev} is very sensitive to solvent radii

Cosolvent: ethylene glycol

We take the transfer of amino acids and 3gly from water to 30% ethylene glycol as a representative example. We have calculated ΔG_{tr}^{ev} using SPT with the pressure set to atmospheric pressure ($p = p_{atm}$) for three different water radii and three different cosolvent radii. The results are displayed in Fig. 1.

We make several observations. 1) For any given solute, the overall spread in ΔG_{tr}^{ev} values due to uncertainty in both cosolvent and water radii is a few kcal/mol, as large as or larger than ΔG_{tr}^{ev} itself. Compare this to the experimental ΔG_{tr} (not its uncertainty), which is an order of magnitude smaller, several tenths of a kcal/mol. For example, for the transfer of glycine, ΔG_{tr}^{ev} ranges from -0.3 kcal/mol ($R_c = 2.2$ Å, $R_w = 1.40$ Å) to $+1.2$ kcal/mol ($R_c = 2.4$ Å, $R_w =$

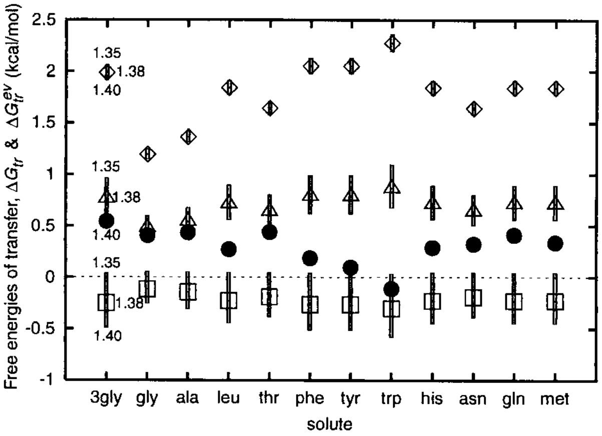

FIGURE 1 ΔG_{tr} and ΔG_{tr}^{ev} values for the transfer of amino-acid solutes from water to 30% ethylene glycol. For any solute, the overall spread/uncertainty in ΔG_{tr}^{ev} (several kcal/mol) due to variation in solvent radii is as large or larger than the ΔG_{tr}^{ev} value itself, and much larger than ΔG_{tr}. For each solute (along the *x*-axis) there are plotted one experimental apparent ΔG_{tr} (●) (Nozaki and Tanford, 1965) (converted to the molarity scale), and nine (for 3 × 3 water/cosolvent radius pairs) SPT-calculated ΔG_{tr}^{ev} values (three open symbols, □, △, and ◇, for $R_c = 2.2$, 2.3, and 2.4 Å, respectively, with rectangular bars through them). The rectangular bars indicate how ΔG_{tr}^{ev} varies as water's radius is changed but the cosolvent radius is kept fixed. The open symbol is located at the ΔG_{tr}^{ev} value with $R_w = 1.38$ Å; the ends of the rectangular bars are at the ΔG_{tr}^{ev} values with $R_w = 1.35$ and 1.40 Å. Whether ΔG_{tr}^{ev} with $R_w = 1.35$ Å is at the upper or lower end of the rectangular bar (the value with $R_w = 1.40$ Å being at the opposite end) is independent of the solute and is only a function of cosolvent size. The water radii are marked on the figure only for the first solute (in this case 3gly) for clarity. Therefore, for any particular solute and choice of R_c, if ΔG_{tr}^{ev} with $R_w = 1.35$ Å and the same R_c is at the top (bottom) of the bar for 3gly, then it is also at the top (bottom) for that solute. For example, for the transfer of tyrosine, if $R_c = 2.2$ Å (□), ΔG_{tr}^{ev} with $R_w = 1.35$ Å is at the top of the rectangular bar, as it is for 3gly with $R_c = 2.2$ Å, and is equal to 0.039 kcal/mol; with $R_w = 1.38$ Å and 1.40 Å, the values are, from the locations of □ and the bottom of the corresponding bar, -0.26 and -0.51 kcal/mol, respectively. It turns out that for the three cosolvent radii considered here, increasing water's radius always increases favorability of transfer (ΔG_{tr}^{ev} with $R_w = 1.40$ Å is always at the bottom of the bar). This is not the case for all cosolvent radii. See for example, the data for transfer to aqueous sucrose and urea (Figs. 3 and 4). (The experimental ΔG_{tr} were measured at 25°C, whereas our calculations were at 20°C, the temperature at which solution density data were available (Wolf et al., 1985). However, the qualitative conclusions we make should not be affected by a 5° temperature change.)

1.35 Å), a spread of 1.5 kcal/mol, whereas $\Delta G_{tr} = 0.4$ kcal/mol; for tryptophan, ΔG_{tr}^{ev} ranges from -0.6 to 2.4 kcal/mol, a spread of 3.0 kcal/mol, whereas $\Delta G_{tr} = -0.1$ kcal/mol. 2) Not only is there a large uncertainty in ΔG_{tr}^{ev}, but even the sign of ΔG_{tr}^{ev} is not known. From SPT calculations, one cannot determine whether excluded-volume interactions favor or disfavor transfer. 3) The uncertainty in water radius alone (with cosolvent radius fixed), can lead to an uncertainty in ΔG_{tr}^{ev} larger than the experimental ΔG_{tr} itself. Looking again at transfer of tryptophan, if $R_c = 2.3$ Å (△), ΔG_{tr}^{ev} ranges from 0.68 to 1.09 kcal/mol, a spread of 0.41 kcal/mol, due only to a 0.5 Å change in water radius. Compare this to $\Delta G_{tr} = -0.1$ kcal/mol. 4) Typically, a 0.1 Å change in cosolvent radius translates to ~1 kcal/mol change in ΔG_{tr}^{ev}.

Cosolvents ethanol, sucrose, and urea: results are qualitatively the same as for ethylene glycol

In Figs. 2–4, we show ΔG_{tr}^{ev} values (calculated with $p = p_{atm}$) and experimental ΔG_{tr} values for the transfer of amino acid and backbone-analog solutes from water to 60% ethanol, 1 M sucrose, and 2 M urea, respectively.

We make the same principal observations as with the transfer to ethylene glycol. 1) Uncertainties in ΔG_{tr}^{ev} are as large as or larger than the values themselves, and an order of magnitude larger than ΔG_{tr} values. 2) Sometimes the sign of ΔG_{tr}^{ev} cannot be determined. 3) If one were to calculate ΔG_{tr}^{i} ($= \Delta G_{tr} - \Delta G_{tr}^{ev}$), the uncertainty in ΔG_{tr}^{i} would also be several kcal/mol.

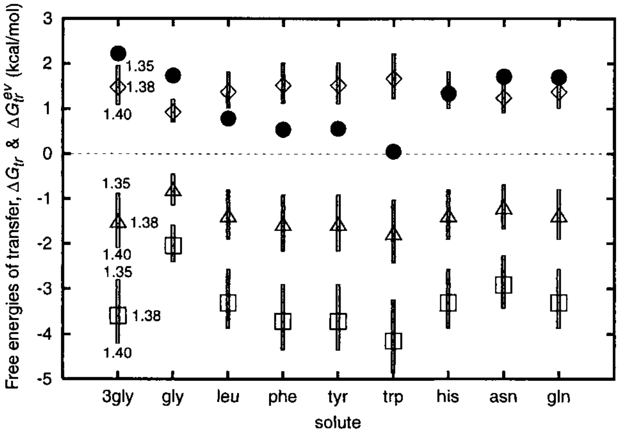

FIGURE 2 ΔG_{tr} and ΔG_{tr}^{ev} values for the transfer of amino acid solutes from water to 60% ethanol. Experimental apparent ΔG_{tr} values (●) (Nozaki and Tanford, 1971) were again converted to the molarity scale. SPT-calculated ΔG_{tr}^{ev} ($p = p_{atm}$) were obtained with $R_c = 2.00$ (□), 2.15 (△), and 2.30 Å (◇). ΔG_{tr} values were measured at 25°C, whereas ΔG_{tr}^{ev} were calculated at 20°C, the temperature at which density data were available (Wolf et al., 1985). See caption of Fig. 1 for more details on interpreting the figure.

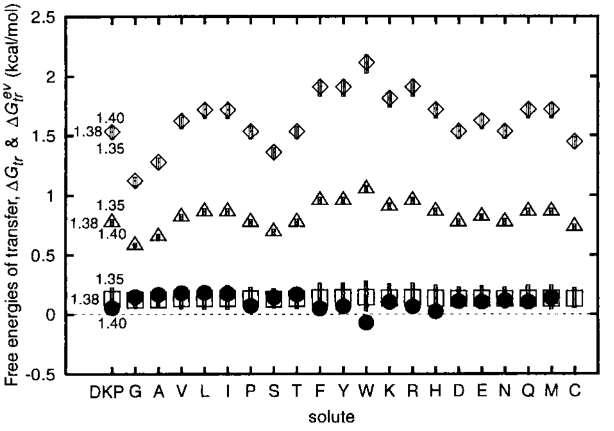

FIGURE 3 ΔG_{tr} and ΔG_{tr}^{ev} values for the transfer of amino-acid solutes from water to 1 M sucrose. Experimental apparent ΔG_{tr} values (●) are from Liu and Bolen (1995). SPT-calculated ΔG_{tr}^{ev} ($p = p_{atm}$) with $R_c = 3.85$ (□), 4.00 (△), and 4.15 Å (◇) are indicated by open symbols. Both ΔG_{tr} and ΔG_{tr}^{ev} were obtained at 25°C. See caption of Fig. 1 for more details on interpreting the figure.

We note two further minor points. 1) Increasing the cosolvent radius always disfavors transfer to the mixed solvent (ΔG_{tr}^{ev} increases as R_c increases). When the cosolvent radius grows, the free volume of the mixed solvent always shrinks, whereas the free volume in pure water is unaltered. Hence, the work of transfer increases. However, if water's radius grows, it is not clear whether transfer is more or less favored. E.g., for transfer to 1 M sucrose, Fig. 3, if $R_c = 4.15$ Å (◇), increasing R_w causes an increase in ΔG_{tr}^{ev}, whereas the opposite is true at $R_c = 3.85$ Å (□); a similar pattern is seen for transfer to 2 M urea (Fig. 4). Increasing water's radius shrinks the free volume in both the

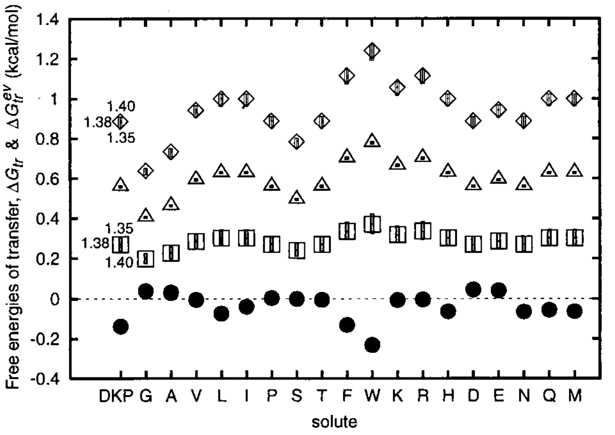

FIGURE 4 ΔG_{tr} and ΔG_{tr}^{ev} values for the transfer of amino acid solutes from water to 2 M urea. Experimental apparent ΔG_{tr} values (●) are from Wang and Bolen (1997). SPT-calculated ΔG_{tr}^{ev} ($p = p_{atm}$) were obtained with $R_c = 2.15$ (□), 2.25 (△), and 2.35 Å (◇). Both ΔG_{tr} and ΔG_{tr}^{ev} were obtained at 25°C. See caption of Fig. 1 for more details on interpreting the figure.

pure water and the mixed solvent states, and it is not clear a priori which will dominate. 2) If we were to assume that ΔG_{tr} is dominated by the excluded-volume interaction and that soft interactions are negligible (i.e., $\Delta G_{\mathrm{tr}}^{\mathrm{i}} \sim 0$), then $\Delta G_{\mathrm{tr}}^{\mathrm{ev}} \sim \Delta G_{\mathrm{tr}}$. If we further assume that the water and amino acid solute radii used here are accurate, then by letting the cosolvent radius be an adjustable parameter and fitting $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ to ΔG_{tr}, we can predict the cosolvent hard-sphere radius from SPT and ΔG_{tr} values. Preliminary estimates (not shown) indicate that the radii determined this way appear to be consistent, independent of whether p is set to p_{hs} or p_{atm} and independent of moderate changes in cosolvent molarity. The predicted radii are approximately: ethanol: 2.2–2.3 Å; ethylene glycol: 2.2–2.3 Å; sucrose: 3.8–3.9 Å; urea: 2.0–2.1 Å. Of course, assuming that soft interactions are negligible is a conjecture; we also do not know how robust the predicted radii are to a wider variety of types and sizes of solutes.

Why is ΔG_{tr}^{ev} so sensitive to solvent radii?

The answer lies with the solvation free energy, $G_{\mathrm{solv}}^{\mathrm{ev}}$, from which the sensitivity arises. (It has already been shown that $G_{\mathrm{solv}}^{\mathrm{ev}}$ is strongly dependent on solvent radii (Wilhelm and Battino, 1972; Lucas, 1976; Morel-Desrosiers and Morel, 1981; Pohorille and Pratt, 1990; Madan and Lee, 1994), and there is no a priori reason to believe that this sensitivity cancels and a new one arises for transfer processes, since the initial and final solvent environments can be entirely unrelated.) First, let us make a simple physical argument for where the various terms in SPT come from, and then we will discuss the size-sensitivity issue. Imagine the solvent being composed of tiny wax beads filling a closed jar. The free volume is comprised of the empty spaces between the beads. Now, imagine heating the jar so that the beads melt and become one solid clump at the bottom. The free volume stays the same, but now the free and occupied volumes are completely separated. The probability of inserting a cavity center in this melted-bead system (ignoring interface effects) is the probability of picking a location in the free space (V_{free}) relative to the total space (V_{tot}). Using the definition of ξ_3:

$$P(R) = \frac{V_{\mathrm{free}}}{V_{\mathrm{tot}}} = 1 - \xi_3 \text{ (melted-bead system).} \tag{8}$$

Then, using Eq. 1,

$$\frac{G_{\mathrm{solv}}^{\mathrm{ev}}(R)}{kT} = -\ln(1 - \xi_3) \text{ (melted-bead system).} \tag{9}$$

We identify this with the first term (Eq. 2) in SPT's $G_{\mathrm{solv}}^{\mathrm{ev}}(R)$. The remaining three cavity-size dependent terms in $G_{\mathrm{solv}}^{\mathrm{ev}}(R)$ (Eqs. 3–5) must come from the solvent's parsing up the free volume into molecule-sized pieces. Around each solvent molecule is a shell of thickness R which, although unoccupied, is unavailable for placing a cavity center due to steric overlap. Hence, the volume, V_{avail}, actually available for insertion of a cavity center is less than the unoccupied volume, V_{free}. In general,

$$P(R) = \frac{V_{\mathrm{avail}}}{V_{\mathrm{tot}}} \tag{10}$$

Multiplying top and bottom by V_{free}, taking the negative logarithm and using Eq. 1 and the definition of ξ_3, we obtain

$$\frac{G_{\mathrm{solv}}^{\mathrm{ev}}(R)}{kT} = -\ln(1 - \xi_3) - \ln\frac{V_{\mathrm{avail}}}{V_{\mathrm{free}}}. \tag{11}$$

Comparing to SPT's $G_{\mathrm{solv}}^{\mathrm{ev}}(R)$ (Eqs. 2–5), we now identify the three cavity-size dependent terms (Eqs. 3–5) with $-\ln(V_{\mathrm{avail}}/V_{\mathrm{free}})$.

Now, let us return to the size-sensitivity issue. The first term in $G_{\mathrm{solv}}^{\mathrm{ev}}(R)$, $-\ln(1 - \xi_3) = -\ln(V_{\mathrm{free}}/V_{\mathrm{tot}})$, does not depend on the solute radius, and only weakly on the solvent radii. The remaining three terms (Eqs. 3–5), equal to $-\ln(V_{\mathrm{avail}}/V_{\mathrm{free}})$, give rise to the strong size dependence. Let's look more closely at $-\ln(V_{\mathrm{avail}}/V_{\mathrm{free}})$ to see why this is. Fig. 5 shows a two-dimensional depiction of a binary solvent of hard circular disks with a fractional free volume of 0.6. The black circles are the solvent disks; the gray areas

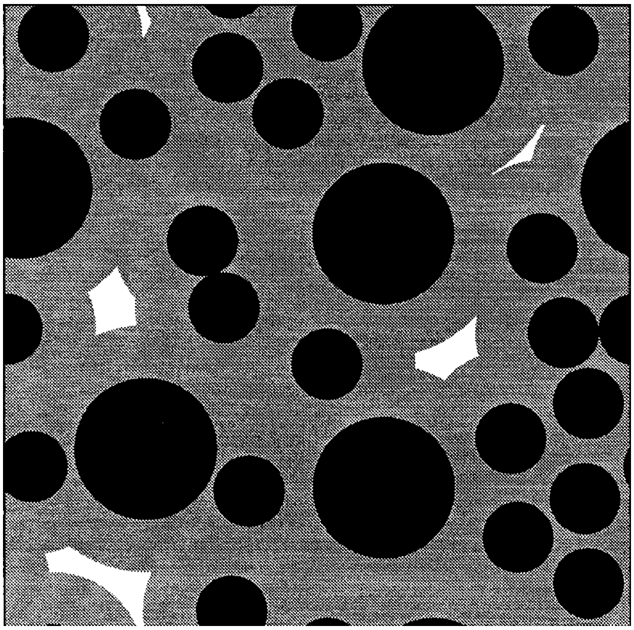

FIGURE 5 The volume available for cavity insertion is significantly smaller than the free volume. This is a random snapshot of a two-dimensional binary fluid of hard disks, indicated by black circles, with a total fractional occupancy (packing fraction) of 0.40. The smaller disks have a radius of 1, the larger a radius of 2. The gray regions are areas that are unoccupied, yet unavailable for the insertion of a cavity with a radius of 1.5. The volume available for insertion (*white regions*) is significantly smaller than the "free" but unavailable (*gray*) regions. If one attempts to insert a cavity the same size as the larger disks (radius 2), there is no region in this configuration available for insertion.

are spaces which are unoccupied, yet unavailable for insertion of a cavity. Only the white regions are available for cavity-center insertion. Despite the fact that a full 60% of the volume is unoccupied and free, only a tiny fraction of that volume is actually available for cavity insertion. Using SPT, we calculated $V_{\mathrm{avail}}/V_{\mathrm{free}}$ for several of the solvents studied here and showed that it is typically fractions of a percent (data not shown). If the solvent molecules grow a little, the white regions shrink. Hence, $V_{\mathrm{avail}}/V_{\mathrm{free}}$ is tiny and shrinks as the solvent size grows. Taking the negative logarithm of this approaching-zero value gives a result which rapidly blows up. To summarize, as the solvent size grows, the probability of inserting a molecular-sized (or larger) cavity, $P(R)$, goes asymptotically to zero. The associated work of cavity insertion, proportional to $-\ln P(R)$, is extremely sensitive to this near-zero $P(R)$ and blows up rapidly as solvent size increases and $P(R)$ decreases.

This explanation of why the work of creating a hard cavity is strongly solvent-size-dependent is general and should apply to all liquid systems, not just the hard-sphere systems described by SPT. Hence, we believe that estimating the excluded-volume portion of cavity insertion or transfer will always be extremely sensitive to solvent size, regardless of the theory or modeling one uses to determine it. Indeed, the sensitivity of $G^{\mathrm{ev}}_{\mathrm{solv}}$ on solvent size has been observed in more detailed models (Pohorille and Pratt, 1990; Madan and Lee, 1994). The best estimate of $G^{\mathrm{ev}}_{\mathrm{solv}}$ and $\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$ will probably lie with atomic-resolution models and simulations. Even then, there will be some uncertainty in $G^{\mathrm{ev}}_{\mathrm{solv}}$ and $\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$ due to solvent-size issues, since even atomic van der Waals radii are not precisely known (Bondi, 1964). Cruder models of solvent molecules, such as the spheres used in SPT, will likely always yield large uncertainties in $G^{\mathrm{ev}}_{\mathrm{solv}}$ and $\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$, because of the ambiguity in replacing a complex-shaped solvent molecule by one or a few parameter(s).

Why is the sign of ΔG^{ev}_{tr} not predictable?

It is perhaps commonly assumed that the addition of a "neutral" cosolvent that excludes more volume than water will always increase the work of opening a cavity. However, as we have seen, this is not necessarily true. The short answer to why the sign of $\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$ is not predictable is that the work of transferring a cavity depends sensitively on the relative densities of the two solvents. Why, then, are the densities such that $\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$ is near zero? That is, why is the volume available for cavity insertion approximately the same for the mixed solvents and water? We cannot give a definitive reason, but offer a few suggestions:

First, for the four systems we studied, amino acids transferring to aqueous ethylene glycol, ethanol, sucrose, and urea, the experimental ΔG_{tr} values are near zero, so perhaps one should expect $\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$ to be near zero as well.

Second, for any solvent, the free volume is determined by an interplay between the soft and the hard interactions. If the molecules are too close together, hard interactions become strongly unfavorable; if there's too much empty space, there's an energetic price of fewer soft interactions. Therefore, crudely, the free volumes of most fluids should be comparable when the soft interactions are comparable. Then, the amount of space available for cavity insertion should be similar.

Third, from an excluded-volume point of view, one can think of the mixed solvent as starting with a system of only water and then growing $n^{\mathrm{mix}}_{\mathrm{w}}$ of the water molecules to cosolvent size. $\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$ should then be closely related to how the free energy of cavity formation changes as the cosolvents are grown, i.e., to $\partial G^{\mathrm{ev}}_{\mathrm{solv}}/\partial R_{\mathrm{c}}$. How does $\partial G^{\mathrm{ev}}_{\mathrm{solv}}/\partial R_{\mathrm{c}}$ behave? 1) To open a cavity takes work because solvent molecules are constrained to not occupy the cavity. For constant total fractional free volume and cosolvent molarity, as the size of the cosolvents increases, the number of waters decreases. Hence, $G^{\mathrm{ev}}_{\mathrm{solv}}$ decreases because fewer particles are constrained. Alternatively, the free volume is less subdivided, so the available volume is greater. 2) On the other hand, the fractional free volume of solvents tends to increase with size; liquid alkanes are one example (see, e.g., Hesse et al. (1996). An increase in packing fraction causes $G^{\mathrm{ev}}_{\mathrm{solv}}$ to increase. 3) A push-pull relationship between items 1 and 2 makes it hard to determine whether $\partial G^{\mathrm{ev}}_{\mathrm{solv}}/\partial R_{\mathrm{c}}$, and hence $\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$, should be positive or negative.

$\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$ is also very sensitive to solvent density

The experimental solvent density, ρ, is used in conjunction with the experimental cosolvent molarity to determine the molarity of water in the mixed solvent, $n^{\mathrm{mix}}_{\mathrm{w}}$. Here, we show that $\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$ is also extremely sensitive to ρ, as previously noted by (Berg, 1990). Fig. 6 shows $\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$ values for the transfer of amino acids to 30 vol% ethylene glycol, calculated with ρ set to the experimental density and to the experimental density plus and minus 1%. A change of density of 1% yields a change in $\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$ of 0.2 to 0.4 kcal/mol, depending on the solute. For transfer to 60 vol% ethanol, 1 M sucrose, and 2 M urea, the change in $\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$ due to a 1% change in ρ is 0.2 to 0.3, 0.2 to 0.5, and 0.2 to 0.4 kcal/mol, respectively. These variations are comparable to the experimental ΔG_{tr} magnitudes themselves.

$\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$ is so sensitive to ρ for essentially the same reason it is also sensitive to the solvent radii: changing ρ alters the amount of space occupied by solvent, which in turn strongly alters the work of inserting a cavity. To avoid substantial errors in $\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$ due to uncertainties in ρ, it is necessary to measure ρ to high precision, e.g., with a precision densitometer.

p_{hs} versus p_{atm}: the behavior of $\Delta G^{\mathrm{ev}}_{\mathrm{tr}}$ is qualitatively the same

It is still not clear which pressure, p_{hs} or p_{atm}, to use in the pV term in the cavity formation work (Eq. 5) (Shimizu et al.,

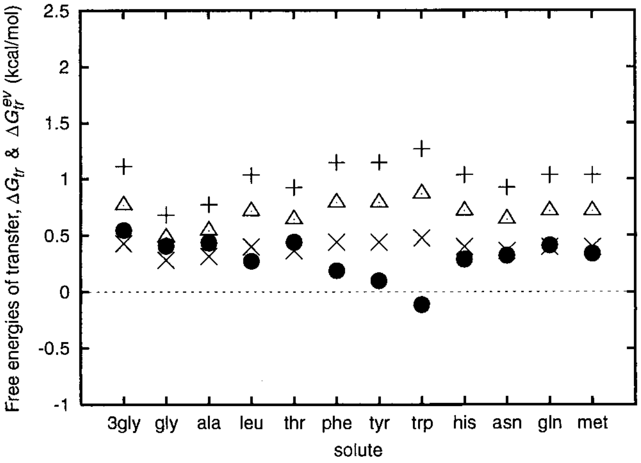

FIGURE 6 $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ is also sensitive to ρ. Plotted are data for the transfer of amino acids to 30 vol% ethylene glycol, with $R_{\mathrm{w}} = 1.38$ Å and $R_{\mathrm{c}} = 2.3$Å. $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ was calculated with ρ set to the experimental density (△, same as in Fig. 1) and to the experimental density plus 1% (+) and minus 1% (×). The solid circles (●) are the experimental apparent ΔG_{tr} values (Nozaki and Tanford, 1965) converted to the molarity scale. A change of density of ±1% yields a change in $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ of 0.2 (gly) to 0.4 (trp) kcal/mol.

1999 and references therein). On the one hand, Neff and McQuarrie (1973) advocate the use of p_{hs} as a consistent separation of the interaction into hard and soft parts. In contrast, Pierotti (1976) has argued, SPT "is used primarily as a means of determining the reversible work required to introduce a hard-sphere molecule into a real fluid whose molecules behave as hard cores but whose volume and pressure . . . are determined by the real intermolecular potentials. . . ." Pierotti (1976) thus uses p_{atm} and then the pV term becomes negligible. The choice presumably depends on how G_{solv} is dissected and on which interactions are being apportioned to the excluded-volume portion of the free energy ($G_{\mathrm{solv}}^{\mathrm{ev}}$). We point out that if SPT with $p = p_{\mathrm{hs}}$ is used, soft interactions are still included in $G_{\mathrm{solv}}^{\mathrm{ev}}$. The soft interactions determine the experimental fluid density which is then used as an input parameter.

To gauge how choice of pressure affects $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$, we recalculated the $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ data in Figs. 1–4, this time using $p = p_{\mathrm{hs}}$ in place of p_{atm}. Fig. 7 shows the results for the transfer of amino acid solutes from water to 30% ethylene glycol. Comparing to the calculations with $p = p_{\mathrm{atm}}$ (Fig. 1), we make the same observations as before (section 3.1): $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ is very sensitive to solvent radii; the sign of $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ can be either positive or negative; uncertainties in $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ due to uncertainties in solvent radii are larger than experimental ΔG_{tr} values. The main difference between $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values calculated with $p = p_{\mathrm{hs}}$ versus p_{atm} is that both the uncertainties and the magnitudes of $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ are a factor of two or three larger with $p = p_{\mathrm{hs}}$. Similar conclusions can be drawn for the transfer of amino acids to 60% ethanol, 1 M sucrose, and 2 M urea (data not shown).

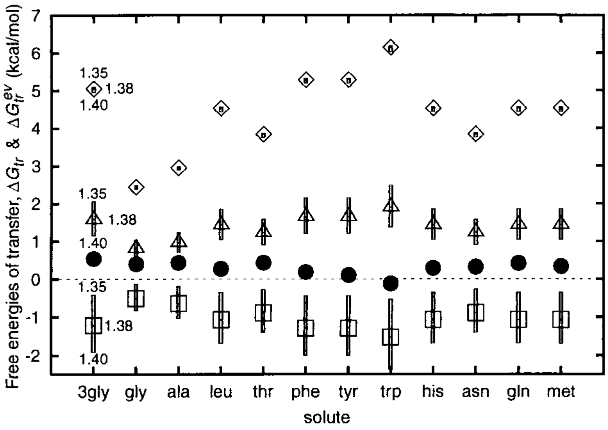

FIGURE 7 ΔG_{tr} and $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values for the transfer of amino-acid solutes from water to 30% ethylene glycol. Same as Fig. 1 except that p was set to p_{hs}.

Approximating $n_{\mathrm{w}}^{\mathrm{mix}}$ by holding p_{hs} constant: $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values and uncertainties are an order of magnitude smaller

For calculations of $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ from water to a generic aqueous solvent, $n_{\mathrm{w}}^{\mathrm{mix}}$ cannot be obtained from experiment but must be calculated via some other method. Applying the Gibbs-Duhem relation to the SPT portion of the equation of state, equivalent to holding p_{hs} fixed at pure water's value ($p_{\mathrm{hs}}^{\mathrm{wat}}$) (Guttman et al., 1995), is one method of obtaining $n_{\mathrm{w}}^{\mathrm{mix}}$ for any aqueous mixed solvent (Berg, 1990; Guttman et al., 1995).

Formally, this is an approximation. The Gibbs-Duhem relation certainly applies to the entire equation of state, but not necessarily to a part of it. Keeping p_{hs} constant leads to the unrealistic conclusion that the (very positive) hard-sphere pressure and the (very negative) pressure due to the soft interactions must combine to make up the (nearly zero) atmospheric pressure. If p_{hs} is fixed, then, since atmospheric pressure is constant, the pressure due to soft interactions must also be constant, irrespective of the cosolvent. However, different cosolvents have different soft interactions, so this cannot be true.

In practice, does this approximation predict reasonable $n_{\mathrm{w}}^{\mathrm{mix}}$ values? Our first test was to calculate solution densities using the approximate $n_{\mathrm{w}}^{\mathrm{mix}}$ values and compare them to experimental densities. Table 3 lists the calculated ρ values of 30% ethylene glycol (20°C), and the percentage differences from the experimental value. Tables 4, 5, and 6 show the same for 60% ethanol (20°C), 1 M sucrose (25°C), and 2 M urea (25°C), respectively. (Note that $n_{\mathrm{w}}^{\mathrm{mix}}$ and hence the calculated ρ values depend on the radii of both water and cosolvent.) The constant-p_{hs} approximation predicts the solution density fairly well, to within a few percentage points of the real value, for aqueous ethylene glycol, sucrose, and urea. The approximation is less good for aqueous ethanol, where the deviations from the correct value can be more

TABLE 3 The calculated densities, ρ, of 30% ethylene glycol (20°C) using $n_{\mathrm{w}}^{\mathrm{mix}}$ values obtained by holding p_{hs} fixed at $p_{\mathrm{hs}}^{\mathrm{wat}}$, and the percent differences from the experimental ρ

R_{w} (Å)	R_{c} (Å)	predicted ρ (g/ml)	difference from experimental ρ (percent)
1.35	2.20	1.050	0.9
1.35	2.30	1.019	−2.1
1.35	2.40	0.985	−5.3
1.38	2.20	1.058	1.7
1.38	2.30	1.027	−1.3
1.38	2.40	0.994	−4.5
1.40	2.20	1.063	2.1
1.40	2.30	1.032	−0.8
1.40	2.40	1.000	−3.9

The experimental ρ is 1.0405 g/ml, obtained by interpolating data from (Wolf et al., 1985).

than 10%. The poorer quality of the approximation for 60% ethanol is probably due to its higher weight concentration of cosolvent (480 mg/ml, as opposed to 330, 340, and 120 mg/ml for 30% ethylene glycol, 1 M sucrose, and 2 M urea, respectively).

Our second test was to compare our previous $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values calculated with the experimentally obtained $n_{\mathrm{w}}^{\mathrm{mix}}$ values, which we denote in this section as $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(ex)$, with those obtained using the approximate $n_{\mathrm{w}}^{\mathrm{mix}}$ values, $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(cp)$. Figs. 8–11 show the $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(cp)$ values for transfer of amino acid solutes from water to aqueous ethylene glycol, ethanol, sucrose, and urea, respectively. Comparing them to the corresponding $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(ex)$ values (Figs. 1–4), we observe that the $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(ex)$ and $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(cp)$ are qualitatively different. 1) $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(ex)$ values and uncertainties are an order of magnitude larger than $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(cp)$ values and uncertainties. The latter are typically one-tenth of a kcal/mol—comparable to the experimental ΔG_{tr} values. With the constant-p_{hs} approximation, $\Delta G_{\mathrm{tr}}^{\mathrm{i}}$ can be usefully determined. 2) $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(cp)$ values are still sensitive to solvent radii. The uncertainties in $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(cp)$ are still significant relative to the $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(cp)$ magnitudes. 3) For the four mixed solvents studied, $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(cp) > 0$. In other words, transfer to the mixed solvent is always unfavorable. This was also the case in previous studies with different solutes and solvents (Berg, 1990; Guttman et al., 1995). In addition, the fractional volume occupancies of the four mixed solvents are greater than that of water (data not shown).

TABLE 4 Same as table 3 except for 60% ethanol (20°C)

R_{w} (Å)	R_{c} (Å)	predicted ρ (g/ml)	difference from experimental ρ (percent)
1.35	2.00	1.040	14.4
1.35	2.15	0.961	5.7
1.35	2.30	0.873	−4.1
1.38	2.00	1.053	15.8
1.38	2.15	0.976	7.2
1.38	2.30	0.889	−2.3
1.40	2.00	1.062	16.7
1.40	2.15	0.985	8.3
1.40	2.30	0.899	−1.2

The experimental ρ is 0.9096 g/ml, obtained by interpolating data from Wolf et al. (1985).

TABLE 5 Same as table 3 except for 1 M sucrose (25°C)

R_{w} (Å)	R_{c} (Å)	predicted ρ (g/ml)	difference from experimental ρ (percent)
1.35	3.85	1.127	0.0
1.35	4.00	1.105	−2.0
1.35	4.15	1.081	−4.1
1.38	3.85	1.130	0.3
1.38	4.00	1.108	−1.7
1.38	4.15	1.085	−3.8
1.40	3.85	1.133	0.5
1.40	4.00	1.111	−1.5
1.40	4.15	1.087	−3.5

The experimental ρ is 1.127100 g/ml (Liu and Bolen, 1995).

Why are $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(cp)$ and $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(ex)$ qualitatively different? Although the constant-p_{hs} approximation can predict ρ to within a few percent, we showed above that $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ is very sensitive to ρ. In that light, it is not surprising that the values obtained for $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(cp)$ and $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(ex)$ are not the same. Why is the magnitude of $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(cp)$ so much smaller than that of $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(ex)$? p_{hs} is a measure of the frequency with which the hard-sphere solvent molecules bump the surface of a cavity (Reiss, 1966). The work to grow a cavity involves pushing aside solvent molecules at the cavity surface (Reiss, 1966) and is therefore closely related to p_{hs}. We expect the work to transfer a cavity between two fluids with the same p_{hs} ($\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(cp)$) should be much smaller than between two fluids with different p_{hs} values ($\Delta G_{\mathrm{tr}}^{\mathrm{ev}}(ex)$).

The unfavorability of the water-to-mixed-solvent transfer is probably due to the increased fractional volume occupancy of the latter. Why, then, is the fractional volume occupancy of the mixed solvent greater when p_{hs} is the same for both solvents? Let us assume that the fractional volume

TABLE 6 Same as table 3 except for 2 M urea (25°C)

R_{w} (Å)	R_{c} (Å)	predicted ρ (g/ml)	difference from experimental ρ (percent)
1.35	2.15	1.018	−1.0
1.35	2.25	1.007	−2.1
1.35	2.35	0.995	−3.3
1.38	2.15	1.021	−0.8
1.38	2.25	1.010	−1.8
1.38	2.35	0.998	−3.0
1.40	2.15	1.023	−0.6
1.40	2.25	1.012	−1.6
1.40	2.35	1.000	−2.8

The experimental ρ is 1.028456 g/ml (D. W. Bolen and M. Auton, University of Texas Medical Branch, personal communication).

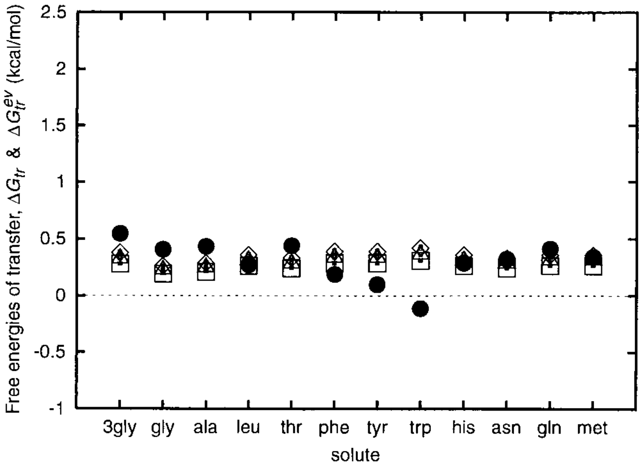

FIGURE 8 ΔG_{tr} and $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values for the transfer of amino acid solutes from water to 30% ethylene glycol. Same as Fig. 1 except that $n_{\mathrm{w}}^{\mathrm{mix}}$ was not obtained from the experimental ρ but rather by fixing the hard-sphere pressure of the aqueous ethylene glycol solution to that of water, $p_{\mathrm{hs}}^{\mathrm{mix}} = p_{\mathrm{hs}}^{\mathrm{wat}}$.

occupancy is constant and we will show that, to first order, p_{hs} will decrease. We again think of the mixed solvent as starting with all waters, and then growing $n_{\mathrm{w}}^{\mathrm{mix}}$ of them to cosolvent size. For constant fractional volume occupancy, the number of waters must decrease as the cosolvents grow. Since pressure is roughly proportional to the total number of molecules, the pressure should decrease. Hence, at a fixed p_{hs}, the solvent with larger molecules will generally have a higher fractional volume occupancy.

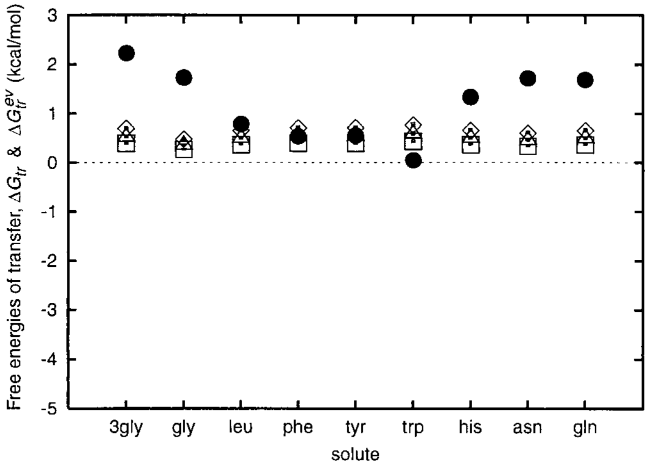

FIGURE 9 ΔG_{tr} and $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values for the transfer of amino acid solutes from water to 60% ethanol. Same as Fig. 2 except that $n_{\mathrm{w}}^{\mathrm{mix}}$ was not obtained from the experimental ρ but rather by fixing the hard-sphere pressure of the aqueous ethylene glycol solution to that of water, $p_{\mathrm{hs}}^{\mathrm{mix}} = p_{\mathrm{hs}}^{\mathrm{wat}}$.

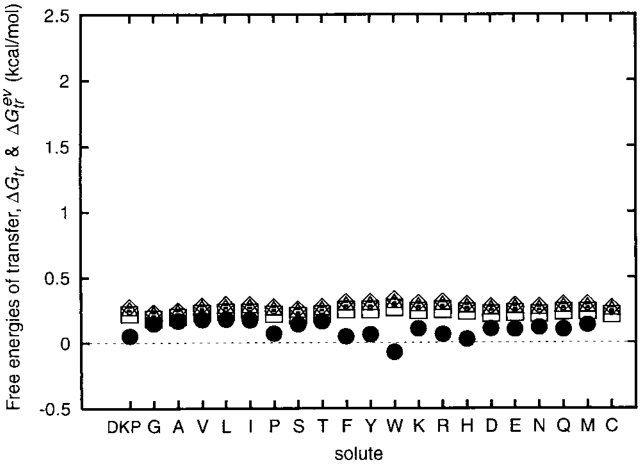

FIGURE 10 ΔG_{tr} and $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values for the transfer of amino acid solutes from water to 1 M sucrose. Same as Fig. 3 except that $n_{\mathrm{w}}^{\mathrm{mix}}$ was not obtained from the experimental ρ but rather by fixing the hard-sphere pressure of the aqueous ethylene glycol solution to that of water, $p_{\mathrm{hs}}^{\mathrm{mix}} = p_{\mathrm{hs}}^{\mathrm{wat}}$.

CONCLUSION

Scaled-particle theory, a theory describing the excluded-volume and packing interactions of hard-sphere fluids, is very useful for the qualitative understanding of the basic properties and behaviors of liquids. However, its use in making (semi)quantitative estimates of the excluded-volume contributions to solvation and the transfer free energies of solutes may be problematic. In this work, we investigated how typical uncertainties in solvent radii, R_{i}, and number densities, n_{i}, translate into uncertainty in $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ as calculated via SPT. Our test systems were the transfer of amino-acid

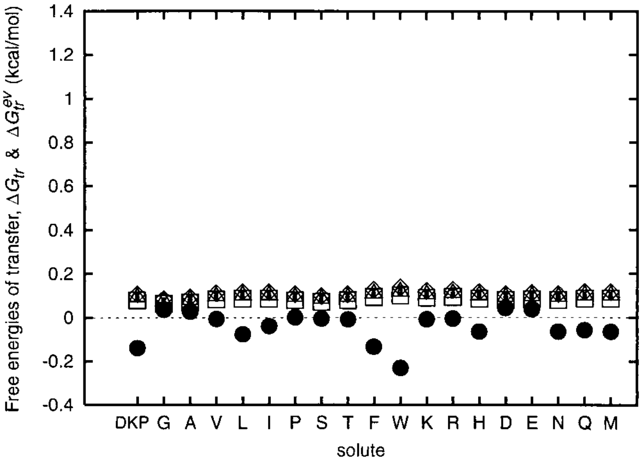

FIGURE 11 ΔG_{tr} and $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values for the transfer of amino acid solutes from water to 2 M urea. Same as Fig. 4 except that $n_{\mathrm{w}}^{\mathrm{mix}}$ was not obtained from the experimental ρ but rather by fixing the hard-sphere pressure of the aqueous ethylene glycol solution to that of water, $p_{\mathrm{hs}}^{\mathrm{mix}} = p_{\mathrm{hs}}^{\mathrm{wat}}$.

solutes from water to aqueous ethylene glycol, ethanol, sucrose, and urea.

We find that the uncertainty in $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ due to uncertainty in solvent radii is large. $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ is extremely sensitive to input solvent radii. Unfortunately, reducing a complex molecular shape into a single radius parameter is not a well defined process; different measures yield different radii (see Table 1). If input water and cosolvent radii are allowed to range among typical values found in the literature, $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values vary by several kcal/mol, an uncertainty as large as the $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values themselves and an order of magnitude larger than the experimental total transfer free energies. The spread in $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ due to variation in water's radius alone can be larger than ΔG_{tr}. Typically, a 0.1 Å uncertainty in solvent radius translates into ~1 kcal/mol uncertainty in $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$. Hence, making semiquantitative calculations of the excluded-volume and soft-interaction portions of the transfer free energy using SPT may not lead to very useful estimates. The resulting values may err in their sign and/or be incorrect by several kcal/mol.

$\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ can be either positive or negative. From an excluded-volume point of view, adding a solvent molecule that is larger than a water molecule does not mean that the work of cavity formation should increase; i.e., adding neutral crowding/background molecules does not necessarily reduce the solubility of a "neutral" solute. Which solvent environment is favored depends on their relative densities. This is in contrast to other studies of crowding, which predict that addition of neutral crowding molecules to the solvent always decreases solute solubility (i.e., $\Delta G_{\mathrm{tr}}^{\mathrm{ev}} > 0$; Minton, 1983; Berg, 1990; Guttman et al., 1995).

$\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ is also very sensitive to the solvent density, ρ, which is used to determine the water molarity in the mixed solvent, $n_{\mathrm{w}}^{\mathrm{mix}}$. A 1% error in ρ results in an error of 0.2 to 0.5 kcal/mol in $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$. Again, this error is comparable to ΔG_{tr} itself.

Because $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ is so sensitive to ρ, in studies of excluded-volume interactions of generic cosolvents, whose solution densities cannot be measured experimentally, a very accurate theory is needed to predict ρ (or $n_{\mathrm{w}}^{\mathrm{mix}}$). Otherwise, errors in predicted ρ values can lead to incorrect semiquantitative and sometimes even incorrect qualitative conclusions. For example, when $n_{\mathrm{w}}^{\mathrm{mix}}$ is approximated by holding the hard-sphere (not atmospheric) pressure constant and equal to that of water (only hard interactions are used to determine $n_{\mathrm{w}}^{\mathrm{mix}}$ relative to $n_{\mathrm{w}}^{\mathrm{wat}}$), the resulting $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values are typically several tenths of a kcal/mol and, for the systems studied, they are always positive (see results here and in Berg, 1990 and Guttman et al., 1995). These $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values are qualitatively different from those calculated here using the $n_{\mathrm{w}}^{\mathrm{mix}}$ values obtained from the experimental densities; the latter $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ values are an order of magnitude larger (several kcal/mol) and can be either sign.

Why is $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ (and the solvation free energy, $G_{\mathrm{solv}}^{\mathrm{ev}}$, in general) so sensitive to solvent size and number density? Even though the free (unoccupied) volume of a typical fluid is fairly large (50–60% of the total volume; see, e.g., Pierotti, 1976), the amount of volume actually available for insertion of a cavity is many orders of magnitude smaller (typically fractions of a percent for molecular-sized solutes; see, e.g., Fig. 5). As the solvent size grows or the number density increases, the probability of inserting a cavity, $P(R)$, goes asymptotically to zero. The associated work of cavity insertion, proportional to $-\ln P(R)$, is extremely sensitive to this near-zero $P(R)$ and blows up rapidly as $P(R)$ decreases. This argument is general and not limited to SPT. Hence, we believe that estimating the excluded-volume portion of cavity insertion or transfer in liquid solvents will always be sensitive to solvent size and density, regardless of the theory or modelling used to determine it.

If one wishes to use SPT to make semiquantitative estimates of excluded-volume interactions, one needs to determine the solvent radii and number densities to high precision. Errors of a few percent can make a significant difference in $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$. If these values cannot be precisely obtained, it is necessary to show that one's results are robust to typical variations/uncertainties in solvent radii and densities. One must also choose which pressure (p_{hs} versus p_{atm}) to use and justify the choice. Ideally, to minimize uncertainties due to the ambiguities in the input parameters and in the choice of pressure, it is best to use atomic-resolution models.

We thank Kevin Silverstein for pointing us to the SPT and water literature, Matt Auton and Prof. Wayne Bolen for sending us data on aqueous urea solutions, and Prof. Wayne Bolen for encouragement. We are grateful to Prof. Tom Record and the reviewers for suggesting the study of $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ with $n_{\mathrm{w}}^{\mathrm{mix}}$ determined by fixing p_{hs} of the mixed solvent at water's value, for one of the suggestions of why the sign of $\Delta G_{\mathrm{tr}}^{\mathrm{ev}}$ is not easily predictable, and for clarifying the separation of G_{solv} into excluded-volume and soft-interaction terms. This work was supported by grant GM28093 from the National Institutes of Health.

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