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Excluded volume contribution to cosolvent-mediated modulation of macromolecular folding and binding reactions

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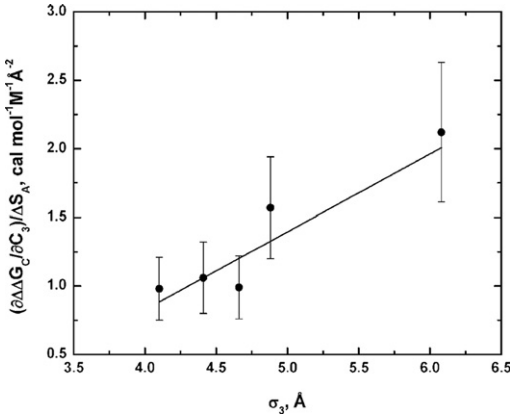
HIGHLIGHTS

- We study the role of cosolvents in folding and binding reactions of proteins and DNA.
- We calculate the m-values and changes in preferential hydration for these reactions.
- The excluded volume contribution is significant and needs to be taken into account.
- Our results provide new insights into interpretation of cosolvent-dependent data.

GRAPHICAL ABSTRACT



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ABSTRACT

Water-miscible cosolvents may stabilize or destabilize proteins, nucleic acids, and their complexes or may exert no influence. The mode of action of a specific cosolvent is determined by the interplay between the excluded volume effect and direct solute–cosolvent interactions. Excluded volume refers to the steric exclusion of water and cosolvent molecules from the space occupied by solute, an event accompanied by a decrease in translational entropy. In thermodynamic terms, the excluded volume effect is modeled by creating a cavity which is sufficiently large to accommodate the solute and which is inaccessible to surrounding molecules of water and cosolvent(s). An understanding of the relationship between the energetic contributions of cavity formation and direct solute–cosolvent interactions is required for elucidating the molecular origins of the stabilizing or destabilizing influence of specific cosolvents. In this work, we employed the concepts of scaled particle theory to compute changes in free energy of cavity formation, $\Delta\Delta G_C$, accompanying the ligand–protein binding, protein dimerization, protein folding, and DNA duplex formation events. The computations were performed as a function of the concentration of methanol, urea, ethanol, ethylene glycol, and glycine betaine. Resulting data were used in conjunction with a previously developed statistical thermodynamic algorithm to estimate the excluded volume contribution to changes in preferential hydration, $\Delta\Gamma_{21}$, and interaction, $\Delta\Gamma_{23}$, parameters and m-values associated with the reactions under study. The excluded volume contributions to $\Delta\Gamma_{21}$, $\Delta\Gamma_{23}$, and m-values are very significant ranging from 30 to 70% correlating with the size of the cosolvent molecule. Our results suggest that a pair of "fully excluded cosolvents" with negligible solute–solvent interactions may differ significantly with respect to their excluded volume contributions to $\Delta\Gamma_{21}$, $\Delta\Gamma_{23}$, and m-values thereby differently influencing the equilibrium of the reaction being sampled. This notion has implications for understanding the long-standing observation that, in osmotic stress studies, various osmolytes may produce significantly distinct estimates of hydration/dehydration for the same reaction.

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1. Introduction

Elucidation of the balance of forces governing protein and nucleic acid recognition events, including self-assembly (*e.g.*, folding) and binding, is required to gain molecular insights into the functional activity of biomacromolecules. In general, reactions of assembly, self-assembly, and binding can be viewed as substitution of solute–solvent interactions by intrasolute (folding) or intersolute (binding) interactions [1]. Modulation of solute–solvent interactions (solvation) by introducing water-soluble organic compounds (cosolvents) may shift the equilibrium between the reactants and the products of a biochemical reaction [2,3]. Depending on the chemical nature of the cosolvent, proteins, nucleic acids, and their complexes may be stabilized or, in contrast, destabilized to the point of denaturation and/or dissociation. The stabilizing or destabilizing action of a cosolvent can be expressed in terms of the m-value or the differential preferential hydration, $\Delta\Gamma_{21}$, and interaction, $\Delta\Gamma_{23}$, parameters [2,4–6]. The m-value for a reaction with an equilibrium constant, K, is defined as $m = -(\partial\Delta G^\circ / \partial C_3)_{T,P}$, where $\Delta G^\circ = -RT\ln K$ is the standard free energy of the reaction, and C_3 is the molar concentration of cosolvent [5,6]. The differential preferential interaction, $\Delta\Gamma_{23}$, and hydration, $\Delta\Gamma_{21}$, parameters are defined as $\Delta\Gamma_{21} = (\partial \ln K / \partial \ln a_1)_{T,P}$ and $\Delta\Gamma_{23} = -(N_3 / N_1)\Delta\Gamma_{21} = (\partial \ln K / \partial \ln a_3)_{T,P}$, respectively, where a_1 and a_3 are the activities of water and cosolvent, respectively; and N_1 and N_3 are the numbers of moles of water and cosolvent, respectively [2,4]. By assuming the activity coefficient of the cosolvent of unity ($a_3 \approx C_3$), the m-value, $\Delta\Gamma_{21}$, and $\Delta\Gamma_{23}$ are linked *via* $m \approx (RT / C_3)\Delta\Gamma_{23} = -(RT / C_1)\Delta\Gamma_{21}$, where C_1 is the molar concentration of water.

Solute–cosolvent interactions that take place on the background of solute-water and cosolvent-water interactions are weak [2–4,7]. Therefore, cosolvent concentrations within the molar range are, generally, needed for a detectable modulation of the extent of folding and binding reactions involving proteins or nucleic acids. A great deal of theoretical and experimental effort has gone into characterizing and modeling the interactions of cosolvents with proteins and nucleic acids [2–4,8–26]. In a series of recent studies, we have developed a statistical thermodynamic model in which solute–cosolvent interactions are governed by a balance between the free energy of cavity formation, ΔG_C (the excluded volume effect), and the free energy of direct solute–cosolvent interactions, ΔG_I [21,25,27]. Direct solute–cosolvent interactions are viewed within the context of a modified solvent exchange model in which binding of cosolvent to solute is accompanied by a release of waters of hydration to the bulk [13,28]. Based on this approach, we have derived analytical equations for the m-value, $\Delta\Gamma_{21}$, and $\Delta\Gamma_{23}$ for protein folding/unfolding transitions [25]. We have characterized the effect of urea, a quintessential protein denaturant, and glycine betaine, a protein stabilizer, on the native-to-unfolded transitions of proteins of varying size [25]. The computed dependences of the m-values, $\Delta\Gamma_{21}$, and $\Delta\Gamma_{23}$ on the concentrations of urea and glycine betaine have been found to qualitatively reproduce those for real proteins thereby lending credence to the model [25].

The present work aims at gaining a further understanding of the balance of forces governing cosolvent-induced shifts in reaction equilibria. Specifically, we estimate the nonstoichiometric contribution of cavity formation (excluded volume) to the m-values and changes in preferential hydration, $\Delta\Gamma_{21}$, and interaction, $\Delta\Gamma_{23}$, parameters for folding/unfolding and association/dissociation reactions. These events are mimicked by sphere-to-spherocylinder transitions and merger of spherical and/or spherocylindrical geometric figures.

Excluded volume is an entropic effect originating from the expulsion of water (the principal solvent) and cosolvent molecules from the space occupied by solute molecules. In other words, there is a solution domain that cannot be occupied by water and cosolvent molecules, thereby causing the latter to exhibit a reduced translational entropy. Thermodynamically, the effect of excluded volume can be modeled by creating a cavity in solution, which is sufficiently large to accommodate the solute and which is inaccessible to the surrounding molecules of water and cosolvent(s). Cavity creation in an aqueous solution is a costly thermodynamic undertaking. In the absence of other compensating interactions, the thermodynamic cost of cavity creation (ΔG_C) would result in a shift in the reaction equilibrium towards the more compact state(s); folding will be favored over unfolding, while association will be favored over dissociation. When cosolvents are added to water, the cost of cavity formation, generally, increases (although, theoretically, ΔG_C may decrease with an increase in cosolvent concentration [29]). An increase in ΔG_C will, in turn, facilitate a further shift in the reaction equilibrium towards the more compact state(s). The situation, however, may be reversed with cosolvent favoring the expanded state(s) if the free energy of direct solute–cosolvent interactions, ΔG_I, prevails over the free energy of cavity formation, ΔG_C. An understanding of the relationship between ΔG_C and ΔG_I is fundamentally important for elucidating the molecular origins of the stabilizing or destabilizing nature of individual cosolvents.

These considerations have implications for osmotic stress-based measurements of changes in hydration accompanying protein and nucleic acid reactions [30,31]. As mentioned above, the free energy of cavity formation, ΔG_C, generally, increases as the concentration of a cosolvent increases. In the absence of the offsetting influence of direct solute–cosolvent interactions, cosolvent molecules would move away from the solute in an attempt to decrease the cost of cavity formation, ΔG_C. Consequently, a region of reduced cosolvent concentration will be created around the solute. This additional exclusion of cosolvent will enhance preferential hydration of the solute. This nonspecific increase in preferential hydration has impact on the values of $\Delta\Gamma_{21}$ and $\Delta\Gamma_{23}$ and, thus, needs to be evaluated and taken into account for rigorous interpretation of experimental data in terms of the differential number of water (or cosolvent) molecules solvating the solute in its folded/unfolded or associated/dissociated states.

2. Method

Despite its being a hard-sphere theory, scaled particle theory (SPT) has been applied with success to free energy calculations in aqueous solutions and has produced results in good agreement with experimental and simulation data [32–38]. We use SPT to compute changes in free energy of cavity formation for four reactions of biological significance, namely, ligand–protein binding, protein dimerization, protein folding, and DNA duplex formation. These reactions were modeled by transitions between spherical and/or spherocylindrical geometric states with the volume of the reactants being equal to that of the products, an approach introduced by Graziano when analyzing cold-induced protein denaturation [36]. The volumes of a sphere with a radius r and a spherocylinder with a spherocylindrical curvature a and a cylindrical length l are given by $V_M = (4 / 3)\pi r_S^3$ and $V_M = \pi a^2[(4 / 3)a + l]$, respectively. The solvent accessible surface areas of a sphere and a spherocylinder are expressed *via* $S_A = 4\pi(r_S + 1.4)^2$ and $S_A = 2\pi(a + 1.4)[2(a + 1.4) + l]$, respectively (1.4 Å is the radius of a water molecule).

The protein–ligand binding event studied in this work was geometrically approximated by merging two spherical particles with radii of 10 (protein) and 3 (ligand) Å into a larger sphere with a radius of 10.0892 Å (complex). The molecular volume of the complex is chosen to be equal to the sum of the volumes of the associating spheres. A net change in solvent accessible surface area, ΔS_A, associated with the reaction is equal to $4\pi[(10.0892 + 1.4)^2 - (10 + 1.4)^2 - (3 + 1.4)^2] = -218$ Å^2. The free energy of cavity formation, ΔG_{CS}, for each sphere was calculated as a function of cosolvent concentrations based on the concepts of SPT [32,37,38]:

$$\Delta G_{CS} = RT\{-\ln(1-\xi_3) + [6\xi_2/(1-\xi_3)r_S + 12\xi_1/(1-\xi_3)]r_S^2 + \left[18{\xi_2}^2/(1-\xi_3)^2\right]r_S^2 \quad (1)$$

where r_S is the radius of a spherical solute; $\xi_i = (\pi/6)\Sigma_j N_A C_j {\sigma_j}^i$; N_A is