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Cooperative hydrophobe aggregation mediated by interfacially active alcohols

Ashish V. Sangwai, Luigi P. Verdoni, Henry S. Ashbaugh*

Department of Chemical and Biomolecular Engineering, Tulane University, New Orleans, LA 70118, USA

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ABSTRACT

The aggregation of isobutane and toluene in aqueous solutions of 5 wt% alcohols of varying polarity is studied using molecular dynamic simulations. In the absence of alcohol these hydrophobes are predominantly monomeric in water with minimal clustering. Simple alcohols like methanol and ethanol barely perturb the hydrophobe cluster size distribution. More complex alcohols with added carbon and hydroxyl units, on the other hand, can induce cooperative aggregation of the hydrophobic solutes and alcohols, with mean cluster sizes greater than that observed for the hydrophobes or alcohols alone. The tendency for an alcohol to induce cooperative aggregation is strongly correlated with its affinity for sitting at an oil/water interface, as quantified by the free energy of transferring a single alcohol molecule from bulk water to a water/octane interface. The onset of aggregation occurs over a narrow range of adsorption free energies centered about −3.2 kcal/mol. Alcohols with adsorption free energies below this value tend to promote hydrophobic aggregation, and alcohols with adsorption free energies above this value are indifferent to aggregation. The tendency for alcohols to induce hydrophobic aggregation is also correlated with their tendency to lower the size selective barrier to transport of cargo greater than ~30 kDa in mass across the nuclear pore complex in eukaryotic cells, which regulates traffic between the nucleus and cytoplasm. The transport barrier within the central channel of the nuclear pore complex is composed of tangled natively unfolded proteins with repeat sequences rich in leucine and phenyalanine, which isobutane and toluene are side chain analogs of. Our results suggest interfacial active alcohols promote leucine and phenyalanine aggregation, subsequently opening holes within the nuclear pore complex's transport barrier to provide alternate routes for cargo translocation.

1. Introduction

The aggregation of nonpolar solutes in water is presumptively driven by the hydrophobic effect, succinctly summarized by the adage "oil and water don't mix" [1,2]. From a phenomenological point of view, the unfavorable hydration free energies of small isolated hydrophobes in water drives their association into larger aggregates that minimize their collective surface area exposed to water and drives the free energy down [3,4]. On a macroscopic scale this aggregation is readily observed as bulk phase separation, but on a microscopic scale heterogeneous solutes with both hydrophobic and hydrophilic characteristics can help drive self-assembly into complex molecular self-assemblies such as micelles, membranes, and globular proteins. In the case of soluble proteins, for example, the constituent nonpolar side chains along the polypeptide backbone reside predominantly in the protein core while polar and charged side chains prefer the exposed interface with water. Molecular simulations of hydrophobes in water, however, find that at low concentrations above infinite dilution, nonpolar solutes tend to be well dispersed with limited aggregation [3,5–7]. A recent spectroscopic analysis of soluble and insoluble alcohols in water concluded that below the solubility limit these mixtures appear random rather than aggregated [8].

The addition of alcohol cosolvents to water can alter the solubility of nonpolar groups and tune hydrophobic interactions. In addition to varying the cosolvent concentration, the hydrophilic–lipophilic balance number (HLB) [9] of the alcohol can be varied by the choice of alcohol used altering the relative affinity of the alcohol to hydrophobic solutes. Like surfactants, alcohols can adsorb at air/water interfaces to lower the surface tension [10,11], accentuating their properties as cosolvents for hydrophobic solutes. Native proteins denature when alcohols of increasing hydrophobicity are added to their solutions [12], while the hydrophobic interactions between nonpolar solutes such as methane and ethane decrease as ethanol is added to solution

* Corresponding author. Tel.: +1 5048628258.
E-mail address: hanka@tulane.edu (H.S. Ashbaugh).

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[13]. At the length scale of molecular aggregates, the exposed hydrophobic surface area is thought to be a controlling factor in determining their hydration free energy. Adsorption of alcohols could thereby stabilize aggregates by lowering their interfacial free energy [4]. Whereas, simpler alcohols like 1-propanol decrease the interfacial tension of water near hydrophobic surfaces [14], diols and alcohols with longer hydrocarbon groups, e.g., 1,2-hexanediol, can form aggregate assemblies [15]. The interplay between an alcohol's HLB number, its propensity to sit at an interface, and the solubilization of hydrophobic groups, however, is poorly understood because of complexity of water–alcohol interactions. Even though ethanol causes denaturation of proteins, glycerol, for example, can stabilize tertiary structures [16,17]. An understanding of the interactions between mixtures of nonpolar solutes and alcohols would therefore shed light on how alcohols tune hydrophobic interactions.

An interesting application of alcohols to changing biological function through moderating hydrophobic interactions is in transport across the nuclear pore complex (NPC). The NPC is a 50+ MDa proteinaceous complex that sits in the nuclear membrane and regulates transport between the nucleus and cytoplasm of eukaryotic species [18]. The NPC contains a central channel ~38 nm in diameter that spans the nuclear membrane and is lined with natively unfolded proteins [18,19]. The NPC passively allows species smaller than ~30 kDa to pass while blocking transport of larger species unless accompanied by a karyopherin transport protein [20]. Analysis of the natively unfolded proteins lining the pore reveals they are enriched in the tetrameric repeat sequences F*x*FG and GLFG (where *x* is a wildcard amino acid side chain, G is glycine (G), and F and L are the hydrophobic side chains phenylalanine and leucine) which are essential to the NPC's function [21]. Two competing theories of the function of FG-nups in regulating transport are the selective phase and virtual gating models [22,23]. In the selective phase model the hydrophobic side chains form aggregates that turn the NPC interior into an associatively cross-linked gel [24]. Karyopherins have highly conserved surface hydrophobic sites that bind to F*x*FG and GLFG sequences and permit these transport receptors to jump between hydrophobic domains to traverse the pore [25]. In the virtual gating model the FG-nups are proposed to act as entropic bristles that block passage to all but the karyopherins, which selectively bind to the hydrophobic side chains to negotiate the pore [26]. Alcohols have been shown to reversibly disrupt the permeability of the NPC, with the efficiency of an alcohol at lowering the transport barrier correlated with its hydrophobicity. For example, 1,2-hexanediol and 1,6-hexanediol have been shown to open up the NPC, while 1,2,3-hexanetriol has little effect on transport [27,28]. Simpler alcohols like methanol and propanol can lower the transport barrier, however, only at increased concentrations [20]. In the context of either barrier model, the alcohols are thought to interfere with FG-nup side chain interactions to moderate transport.

In this study, we use molecular simulations to examine the impact of adding alcohol cosolvents to water on moderating hydrophobic interactions. The hydrophobic species we examine are toluene and isobutane, which are side chain analogs of phenylalanine and leucine, respectively. We examine a range of 12 different alcohols from simple mon-ols, like methanol and ethanol, to di-ols, tri-ols, and a tetra-trol with up to seven carbon units. Simulations are conducted of aqueous solutions of hydrophobes with no added alcohol, aqueous solutions of alcohols with no added hydrophobes, and mixtures of water, alcohol, and hydrophobes to examine the impact of added alcohol on the formation of hydrophobic clusters in solution. In addition we perform simulations of alcohol adsorption at a water/octane interface to quantify the interfacial affinities of the alcohols. In the concluding section we comment on the potential role alcohols may play in moderating NPC transport based on our results.

2. Methods

Isothermal–isobaric molecular dynamics simulations were performed using GROMACS 4.0 [29]. The alcohols considered in this study were methanol, ethanol, 1-propanol, *tert*-butanol, 1,2-butanediol, 1,2-pentanediol, 1,2-hexanediol, 1,6-hexanediol, 1,2,3-hexanetriol, 1,2,3,4-hexanetetrol, 1,3-heptanediol, and 1,2-heptanediol. The alcohols vary from mon-ols to tetra-ols to cover a broad range of functionality. Toluene, isobutane, octane (a representative organic liquid), and alcohols were modeled with Generalized Amber Force Field for organic molecules [30], while water was modeled using the TIP3P potential [31]. Gaussian ab initio calculations were performed for the alcohols and nonpolar solutes using the B3LYP hybrid functional and 6-31G* basis set to optimize geometries and calculate atom centered partial charges using the RESP method [32,33]. Initial randomized system configurations were generated using PACKMOL [34]. Energy minimization was performed by steepest descent over 20,000 steps. A time step of 2 fs was used to integrate the equations of motion. The temperature and the pressure were maintained at 300 K and 1 atm using a modified Berendsen thermostat with stochastic sampling [35] and the Berendsen barostat [36]. Non-bonded interactions were truncated beyond 8 Å with a continuum correction for longer range contributions to the Lennard–Jones interaction. Long-range electrostatic interactions were evaluated using Particle Mesh Ewald [37]. Bonds involving hydrogen were constrained using LINCS algorithm [38].

In a first series of simulations, we examined mixtures of water with hydrophobes (isobutane or toluene), with alcohols, and with hydrophobes and alcohols. In the simulations of hydrophobes and water 10 isobutanes or toluenes were placed in a box of 2000 water molecules, corresponding to 1.6 wt% isobutane and 2.5 wt% toluene in water. In the simulations of alcohols and waters, 5 wt% alcohol was placed in a box of approximately 2000 water molecules, where the number of waters was adjusted slightly up or down to meet the target alcohol concentration. In the simulations of hydrophobe, alcohol, and water mixtures, 10 isobutanes or toluenes were added to the 5 wt% aqueous alcohol solutions. The simulation box was cubic with an edge length of approximately 40 Å. The systems were equilibrated for 5 ns, followed by a production run of 50 ns. 500,000 configurations were saved during the production run to analyze thermodynamic averages. Hydrophobic groups from either isobutane, toluene, or the alcohols were judged to be in the same cluster if any carbon atom on different molecules lay within 6 Å of one another [5–7]. This separation was chosen since it corresponds to the first minimum in the methane–methane RDF in water, defining the primary contact shell between carbon units. The criterion here is relevant for analysis of hydrophobe aggregation discussed below.

In the second series of simulations, we evaluated the potential-of-mean force (PMF) for pulling an alcohol through a liquid water/octane interface to quantify each alcohol's adsorption affinity. The water and octane phases contained 453 and 50 molecules, respectively, in a rectangular box with approximate dimensions of $20 \times 20 \times 68$ Å. The volumes of the aqueous and organic phases were approximately equal. The potential-of-mean (PMF) for pulling each alcohol through the liquid/liquid interface along the elongated *z*-axis was determined using umbrella sampling [39]. The alcohol centers-of-mass were restrained by a harmonic potential over 31 windows with minima in the restraint potential spaced 1 Å apart. A spring constant of 3 kcal/(mol Å^2) was used, permitting sufficient overlap between adjacent windows. The alcohol was initially placed in the bulk aqueous phase. Subsequent