The Interaction of Guanidinium Ions with a Model Peptide

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ABSTRACT In addition to promoting unfolded protein states, the denaturants urea and guanidinium (Gdm^+) accumulate at the surface of folded proteins at subdenaturing concentrations, a phenomenon that correlates with their denaturant activities. The enhanced accumulation of Gdm^+ relative to urea indicates different binding modes, or additional binding sites, for Gdm^+, and we recently proposed potential binding modes to protein functional groups for Gdm^+ based on the determination of the weak hydration properties of this complex cation. Here we describe molecular dynamics simulations of a model helical peptide, melittin, in a 3 M solution of GdmCl, to identify potential interactions with amino-acid side chains in a nondenatured polypeptide surface. The simulations indicate that Gdm^+ can interact with a number of planar amino-acid side chains (Arg, Trp, Gln) in a stacking manner, as well as more weakly with hydrophobic surfaces composed of aliphatic side chains, and that these interactions result in enhanced number densities of Gdm^+ at certain locations on the peptide surface. These observations provide molecular scale insight into the accumulation of Gdm^+ at protein surfaces that has previously been observed experimentally.

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Protein denaturants such as urea and guanidinium (Gdm^+) chloride preferentially accumulate at the surface of folded proteins, and the extent of this accumulation is related to their denaturant activities (1). Thus Gdm^+ has a partition coefficient, K^{nat}, for accumulation at the surface of bovine serum albumin, of ~1.6 relative to the bulk solution concentration, whereas K^{nat} for urea is ~1.1 (2). These observations reflect a general phenomenon, since protein-stabilizing solutes (osmolytes such as trimethylammonium oxide or strongly hydrated ions like sulfate) are excluded from the protein surface (2,3). Although the polypeptide backbone makes a strong contribution to solute effects on protein stability (3), preferential partitioning of solutes, including denaturants, can be measured with folded proteins. The nature of the solute-protein interactions that underlie this observation is not known at the molecular level.

Previous molecular dynamics (MD) simulations of proteins in urea identified hydrogen bonding with exposed polar groups as a mechanism for surface accumulation of urea (4–6). Recent studies from our groups indicate that the hydration properties of Gdm^+ might support alternative binding modes relevant to its surface accumulation and denaturant activity (7–9). Neutron diffraction with isotopic substitution demonstrates that Gdm^+ forms hydrogen bonds with water in the molecular plane, but is weakly hydrated above and below the molecular plane (7). The hydrophobic nature of the face of the Gdm^+ cation results in homo-ion pairing (i.e., stacking) in MD simulations of strongly denaturing salts [Gdm^+Cl^-; Gdm^+SCN^-] (8,9). This behavior indicates that Gdm^+ ions might stack against hydrophobic side chains, reducing the entropic cost of hydrophobic hydration by displacing waters (10,11), while also hydrogen-bonding to the backbone in unfolded proteins. Homo-ion stacking suggests that Gdm^+ should interact with the planar guanidine moiety of Arg, and possibly with aromatic side chains and planar side-chain amide groups. Such behavior might explain the enhanced preferential partitioning and denaturant activity of Gdm^+ over urea that is not fully represented in the relative activities of these denaturants to attenuate structure stabilized by hydrogen bonds (12).

To examine the interactions of the Gdm^+ ion with the surface of a folded polypeptide, we have run MD simulations of a helical peptide, melittin, in a solution of GdmCl. Melittin, the membrane-active toxin from bee venom (13), is a 26-amino-acid peptide with the sequence GIGAVLKVLTTG-LPALISWIKRKRQQ-NH_2. It is soluble in both the tetrameric α-helical form and the monomeric random coil (14). The melittin monomer does not normally exist as an α-helix, and the peptide only assumes this form in water as the structured tetramer. However, our goal was to use melittin as a model peptide with a representative mix of hydrophilic and hydrophobic groups exposed to water. For example, one face of the melittin helix has a hydrophobic surface made up largely of aliphatic side chains that are normally buried in the helical tetramer. Simulation of the monomer allows us to assess the interactions of Gdm^+ with hydrophobic regions not normally accessible in a folded polypeptide or proteins.

Simulation details are available in the Supplementary Material. An 8 ns NVE-ensemble simulation (the first 0.5 ns used as equilibration) was calculated using CHARMM (15). The system consisted of a 44.7 Å cube containing 125 GdmCl units, one melittin, six Cl^- counterions, and 2319 TIP3P waters (16). Density maps were calculated for Gdm^+

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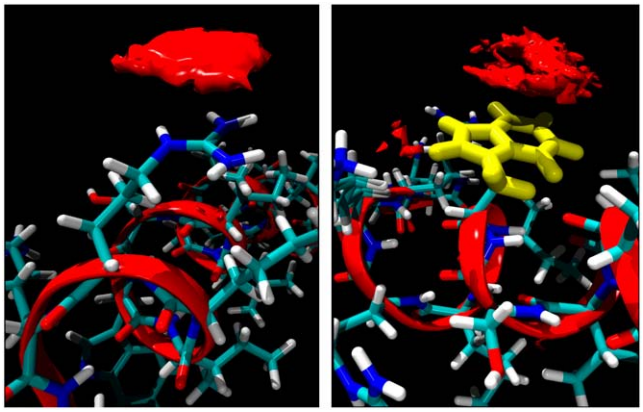

FIGURE 1 Atom density of Gdm^+ around melittin side chains Arg^{22} (*left*) and Trp^{19} (*right*), displayed using VMD (20). The Gdm^+ contours are displayed at a number density of 4.4-times the bulk number density of Gdm^+ for both figures.

nitrogen atoms relative to melittin, as has previously been done for water around small rigid solutes (17,18). The size and flexibility of the melittin helix make it more difficult to analyze Gdm^+ density around the peptide than in previous applications (19), and only local densities could be compared due to motional smearing on a larger scale.

Apart from some fraying at the N- and C-termini, and bending near Pro^{14}, the helix remains largely intact throughout the simulation. The ion densities are statistically converged and temporally stable on this timescale; the average densities for the two halves of the simulation are statistically equivalent. As predicted, the Gdm^+ ions were found to bind weakly to melittin by stacking against the hydrophobic groups of the peptide. In addition, Gdm^+ ions also complex with the like-charged guanidine groups of Arg^{22} and Arg^{24} in a stacked manner (Fig. 1) similar to that found for Gdm^+ ions in GdmCl and GdmSCN solutions (8,9). Preferential partitioning of Gdm^+ by weak stacking interactions was also observed for the indole group of Trp^{19} (Fig. 1) and the planar side-chain amides of the $Gln^{25}/^{26}$ residues (see Supplementary Material).

Interaction of Gdm^+ with melittin side chains results in displacement of waters from the hydration surface. This is illustrated by the hydration of the indole group of Trp^{19}. At least one Gdm^+ ion occupies a position within the hydration sphere (Gdm^+ carbon atoms within 4.5 Å of an indole atom) of the Trp indole group for virtually the entire simulation (Fig. 2), although not all of these interactions involve stacking modes. For short periods of the trajectory with no indole-Gdm^+ interactions, the indole group has 13–16 waters in its hydration volume (within 4.5 Å). The average number of waters hydrating the Trp indole in the full simulation is 10.9, indicating a significant displacement of hydrating waters by Gdm^+-indole interactions.

Water displacement from weakly hydrated surfaces of other side chains occurs in a similar manner. Fig. 3 illustrates that the guanidine group of Arg interacts with hydrating waters via in-plane hydrogen bonding and with Gdm^+ by a stacking interactions (Fig. 1). The latter interaction results in the displacement of waters from the surface above the plane of the guanidine group. Gdm^+ aligns adjacent to the nonpolar surface composed of aliphatic amino-acid side chains, although the atom density for the denaturant is diffusely distributed compared to that for the interaction with the planar π-systems of Arg, Trp, and Gln (Fig. 3 and Supplementary Material).

The residence times for Gdm^+ ions around both the Trp^{19} and Arg^{22} side chains were ~30 ps, which was almost the same as the lifetime for Gdm^+-Gdm^+ interactions. The residence times for Gdm^+ ions adjacent to neutral hydrophilic residues (Ser, Gln, Thr) were also similar to those adjacent to the hydrophobic residues (Ala, Val, Ile), which is somewhat different from previous findings with urea (4–6). Averaged over the simulation, ~7.6 Gdm^+ ions bound to the peptide. This coordination number does not equate to an accumulation relative to the bulk Gdm^+ concentration, a consequence of the very high positive charge (+6) of the peptide. We ran two further 4-ns simulations in which the net positive charge of the peptide was reduced to +2 and +1, respectively, first by deprotonating the amino groups of the N-terminus and Lys-7,21,23, and secondly by additionally deamidating the C-terminus. The coordination number for Gdm^+ peptide-interactions was 11.0 in the +1 simulation, corresponding to a local concentration of Gdm^+ of 1.13 relative to the bulk concentration (Supplementary Material Table), and further enhancement of the negative surface charge density is expected to yield local concentrations approaching that measured experimentally for bovine serum albumin. As expected, Gdm^+ interacts strongly with the C-terminal carboxylate group in the +1 simulation (not shown).

These observations indicate that the experimentally observed accumulation of Gdm^+ at the protein surface (1,2) can be understood in terms of the properties of this complex cation. While a dominant interaction of urea with surface groups in protein simulations involves hydrogen bonding with polar side-chain functions (4–6), the unique hydration properties of the Gdm^+ ion (7) support alternative interaction modes involving stacking with side-chain planar and hydrophobic

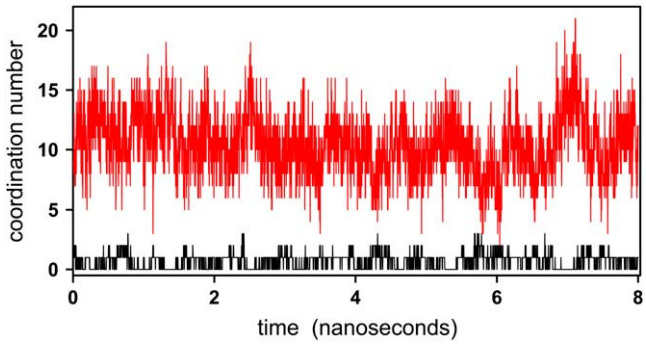

FIGURE 2 Number of hydration waters (*red*) and Gdm^+ ions (*black*) within 4.5 Å of a Trp^{19} indole heavy atom.

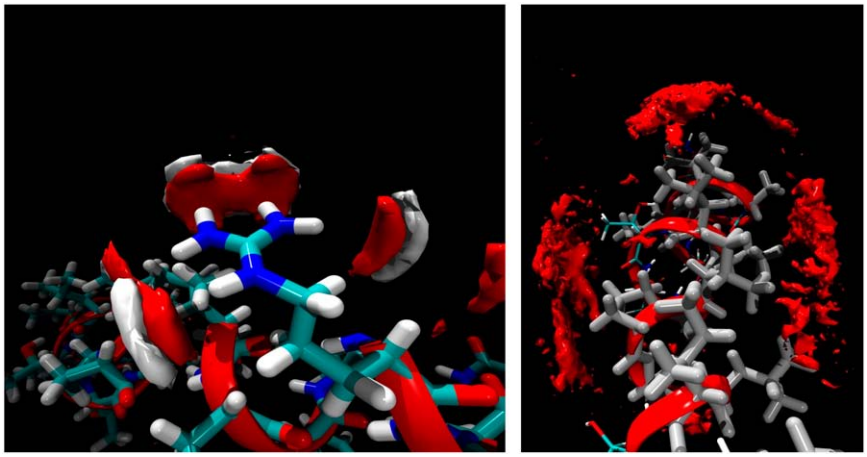

FIGURE 3 Density of solvent components around selected side chains of melittin. The left-hand panel shows water atom density (*red*, O atoms; *white*, H) around Arg^{22} guanidinium. The right-hand panel is Gdm^+ N atom density around the hydrophobic side chains in the N-terminal region. The contour level is 2.6-times the bulk number density of these nuclei.

groups. The existence of these binding modes is supported by experimental observations of extremely high sensitivity to Gdm^+ denaturation of tryptophan-zipper peptides in which side-chain indole-indole interactions provide the dominant contribution to the stability of the folded state (12). This strong stacking with side-chain aromatic groups may also explain the particularly effective promotion of water solubility of the aromatic amino acids by GdmCl (21). Overall, these observations reinforce the utility of MD simulations in providing interpretations of the interactions of ions at the protein surface at the molecular scale (e.g., (22)).

SUPPLEMENTARY MATERIAL

An online supplement to this letter can be found by visiting BJ Online at http://www.biophys.org.

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