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# Complex Ion Effects on Polypeptide Conformational Stability: Chloride and Sulfate Salts of Guanidinium and Tetrapropylammonium

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Supporting Information

**ABSTRACT:** The effects of chloride and sulfate salts of tetrapropylammonium ($TPA^+$) and guanidinium ($Gdm^+$) on the conformational stabilities of tryptophan zipper (trpzip) and α-helical (alahel) peptides were measured by circular dichroism spectroscopy. Like $Gdm^+$, $TPA^+$ interacts with the planar tryptophan indole group, perturbing the conformational stability of trpzip peptides. $TPA^+$ effects are largely unaffected by sulfate, indicating an absence of the heteroion pairing that is observed in concentrated $Gdm_2SO_4$ solutions. $TPA^+$ stabilizes helical conformations in alahel peptides, indicating exclusion from the peptide bond. The observations are broadly consistent with predictions of molecular dynamics simulations [Mason, P. E.; et al. *J. Phys. Chem. B* **2009**, *113*, 3227−3234], indicating that the effects of complex ions on proteins are increasingly predictable in terms of ion hydration, complementary interactions with specific protein groups, and ion-pairing contributions.

The effects of cosolutes on the solubility and conformational stability of proteins have been of intense interest for decades.[1,2] For simple ions, the effects generally scale with solute charge density and hydration properties; thus, strongly solvated, high-charge-density ions ($SO_4^{2-}$, $CO_3^{2-}$) stabilize folded protein states and promote "salting out", whereas weakly solvated ions with low charge density ($SCN^-$, $Br^-$, $I^-$) promote protein unfolding and "salting in".[1−3] Recent studies support the conclusion that protein-destabilizing solutes, including the "classical" denaturants guanidinium ($Gdm^+$) and urea, interact directly with poorly solvated protein groups that are buried in the folded protein and are exposed upon unfolding.[4−6] Strongly solvated solutes interact poorly with weakly solvated protein groups and are excluded from the surface of unfolded proteins, promoting folded protein states.

We recently investigated the interactions of more complex ionic denaturants with protein moieties, exploring the contribution of counterion pairing using molecular dynamics (MD) simulations. These studies led to a series of predictions concerning the effects of tetrapropylammonium ($TPA^+$) chloride and $TPA_2SO_4$ on small peptides chosen to "dissect" specific interactions that contribute to the conformational stabilities of proteins.[7] An experimental test of some of these predictions is described here. In particular, we compare the effects of the salts TPACl and $TPA_2SO_4$ on the conformational stabilities of tryptophan zipper (trpzip) peptides,[8] which are small β-hairpin peptides with high folded-state stability resulting from cross-strand interactions between pairs of Trp indole groups (Table 1). $Gdm^+$ interacts with the Trp indole groups and is a denaturant of trpzip peptides.[9] $TPA^+$ was predicted to interact with the Trp indole group, since it shares some essential properties of $Gdm^+$ (propensity for cation−π interactions and weakly hydrated quasi-planar faces that maximize dispersion forces when interacting with planar nonpolar surfaces), despite the otherwise disparate nature of the respective molecular structures.[7] TPACl and other tetraalkylammonium salts have been reported to be strong protein denaturants,[10] and these experiments also served to address possible denaturant mechanisms.

**Table 1. Amino Acid Sequences of Peptides Used in This Work[a]**

| | |
|---|---|
| trpzip1 | SWTWEGNKWTWK-$NH_2$ |
| trpzip2 | SWTWENGKWTWK-$NH_2$ |
| alahel-E2 | Ac-AEQAAAAQAAAAQAAY-$NH_2$ |

[a] Abbreviations: Ac, acetyl; -$NH_2$, C-terminal amide.

The conformational stabilities of trpzip peptides are readily assessed using circular dichroism (CD) spectroscopy, making use of the strong exciton coupling between pairs of indole groups that interact in the folded conformation, which is manifested as a strong positive CD signal at 227 nm (Figure 1; the negative band near 215 nm is obscured by absorption from $TPA^+$ at high concentrations).[8,9] Surprisingly, TPACl stabilized trpzip1 at concentrations up to 0.5−1.0 M. At higher concentrations, TPACl strongly destabilized the folded state, as indicated by loss of the exciton-coupled CD signal; at a TPACl concentration of 2.5 M, trpzip1 was essentially fully unfolded (Figure 2a). We confirmed that the effects of TPACl on the 227 nm CD signal reported on TPA-induced changes in folded-state populations (rather than, for example, TPA-induced changes in the nature of indole−indole interactions in folded trpzip) by measuring the near-UV CD signal arising from structure-induced asymmetry in the Trp indole group in the same samples (Figure 1b). The enhancement of the folded-state trpzip population at TPACl concentrations of 0.5 M and the promotion of unfolded trpzip

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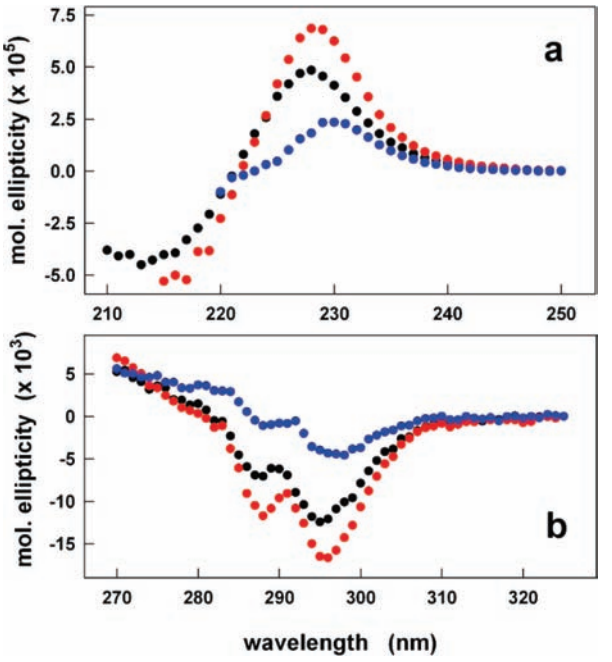


**Figure 1.** (a) Far- and (b) near-UV CD spectra of trpzip1 in 10 mM potassium phosphate buffer (pH 3.0) at 42 °C in the absence (black) and presence of TPACl at concentrations of 0.5 M (red) and 2.0 M (blue).

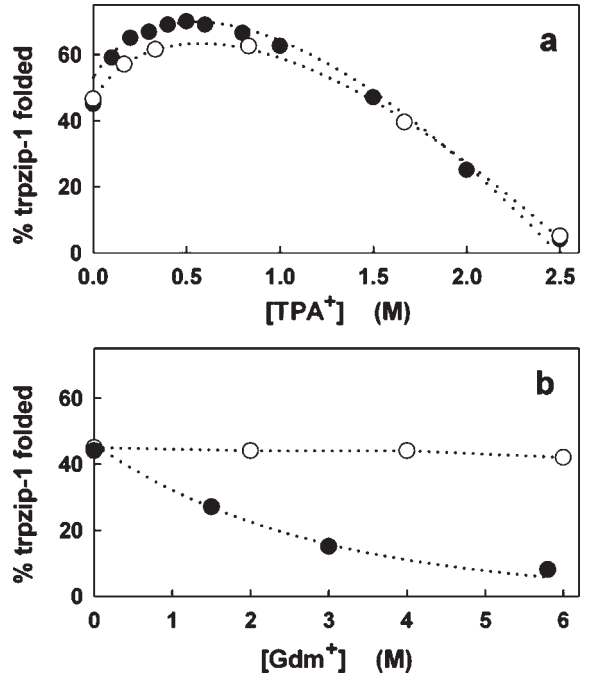


**Figure 2.** Effects of chloride (●) and sulfate (○) salts of (a) $TPA^+$ and (b) $Gdm^+$ on the folded-state population of trpzip1 in 10 mM potassium phosphate (pH 3.0) at 42 °C. Dotted lines have been drawn to guide the eye.

conformations at higher TPACl concentrations were observed in the TPACl-mediated variations in the intensities of the induced CD signals at 280−300 nm (Figure 1b), with folded-state populations matching those inferred from the exciton-coupled CD bands. Very similar observations were made with trpzip2 (Table 1; also see Figure S1 in the Supporting Information).

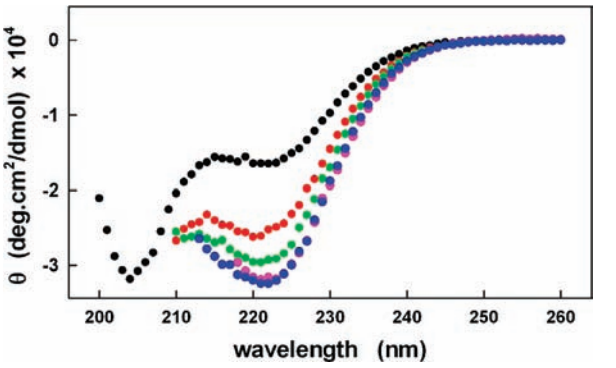


**Figure 3.** Far-UV CD spectrum (mean residue ellipticity) of alahel-E2 in 10 mM potassium phosphate buffer (pH 7.0) at 15 °C (black) and in the presence of TPACl at concentrations of 0.5 M (red), 1.0 M (green), 1.5 M (blue), and 2.0 M (pink).

Of specific interest in regard to the question of ion pairing in salts of complex ions is a comparison of the effects of the sulfate salts of $TPA^+$ and $Gdm^+$ on the folded-state populations of trpzip. These data are shown in Figure 2. GdmCl is a denaturant of trpzip peptides, whereas the $Gdm_2SO_4$ salt has no effect on trpzip peptide conformational stability.[11] Since sulfate (as the $Na^+$ or $K^+$ salt) has little effect on the conformational stability of trpzip peptides,[11] we attribute the sulfate-induced reversal of $Gdm^+$ effects on trpzip conformational stability to $Gdm^+$−sulfate ion pairing. The effect of sulfate on the $TPA^+$-induced perturbation of trpzip conformational stability is quite different; $TPA_2SO_4$ has very similar effects on trpzip peptides as TPACl. These observations are consistent with the expectation that $SO_4^{2-}$ can form ion pairs with $Gdm^+$,[11,12] particularly at the high ion concentrations at which Hofmeister effects are manifested (and thus reverse the effects on polypeptide conformational stability arising from $Gdm^+$−polypeptide interactions), whereas sulfate cannot form ion pairs with $TPA^+$ and thus has little influence on $TPA^+$ interactions with polypeptide moieties (Trp indole groups in the case of trpzip peptides) that affect folded-state stabilities.

A further prediction based on consideration of the properties of the complex $TPA^+$ and $Gdm^+$ cations relates to interactions with the peptide bond.[7] $Gdm^+$ is a denaturant of H-bonded secondary structure as a result of competition for H-bonding to the peptide carbonyl and/or "stacking" interactions with the planar $\pi$-bonded peptide group, as observed for $Gdm^+$ side-chain amides in MD simulations[6] and predicted in a study of denaturant effects on peptide amide hydrogen−deuterium exchange.[13] $TPA^+$ cannot compete with waters for H-bonding, and while "stacking" interactions with isolated amides should be feasible, conformational restrictions against access to the peptide bond in polypeptide structure is expected to limit such interactions of the large $TPA^+$ cation with the peptide bond.

Accordingly, we found that TPACl stabilizes polypeptide conformations for which intramolecular H-bonding dominates the stability of the folded state. Figure 3 shows CD spectra of alahel-E2 (Table 1) in the absence and presence of increasing TPACl concentrations. For simple helical peptides in water, the ellipticity at 222 nm provides a robust measure of the population of helical states.[9] At 15 °C, ∼33% of peptide bonds are in α-helical conformations in alahel-E2, allowing both stabilizing and destabilizing effects to be measured. TPACl increased the magnitude of the ellipticity at 222 nm, indicating stabilization of H-bonded helical conformations. This contrasts with the


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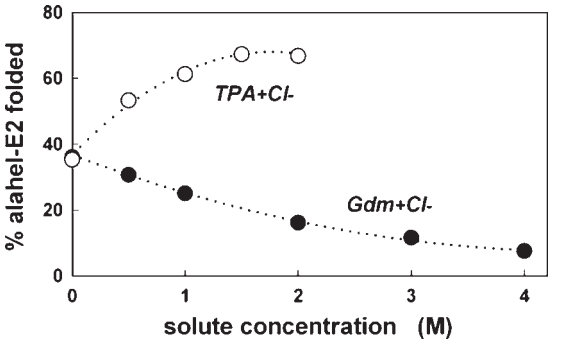


**Figure 4.** Cosolute dependence of the helical content of alahel-E2 in solutions of TPACl (○) and GdmCl (●). Buffer conditions are given in the Figure 3 caption. Dotted lines have been drawn to guide the eye.

well-characterized destabilizing effect of GdmCl on helical conformations in alahel peptides (see Figure 4).[9]

These observations support the conclusion that the effects of complex ions on noncovalent interactions that contribute to the stability of the protein folded state can be understood in terms of molecular complementarity between the ion and protein moieties and between the ion and its counterion.[6,7,9,11] Notably, the effects of TPACl and $TPA_2SO_4$ on trpzip and alahel peptides largely conform to predictions made on the basis of analysis of MD simulations of peptide interactions with TPA salts.[7] The absence of significant counterion pairing between $TPA^+$ and sulfate conforms to Collins' "law of matching water affinities",[14] as the large, low-charge-density $TPA^+$ cation is expected to be weakly hydrated, in contrast to the more strongly hydrated sulfate anion. Thus, sulfate has little effect on the interaction of $TPA^+$ with trpzip peptides; this contrasts with the reversal by sulfate of the denaturating effects of $Gdm^+$ on trpzip peptides (Figure 2b).[11] Although $Gdm^+$ is a weakly hydrated cation, the hydration properties of the planar $Gdm^+$ ion are asymmetric, with the low-hydration surfaces existing above and below the molecular plane.[15] In the molecular plane, $Gdm^+$ forms H-bonds to water, and it is these H-bonded waters that are displaced when sulfate forms ion pairs with $Gdm^+$, an interaction that is promoted by the complementary geometry of pairs of in-plane NH groups on $Gdm^+$ and pairs of oxygen H-bond acceptors on the tetrahedral sulfate dianion.[12,16]

In contrast to the differences between $Gdm^+$ and $TPA^+$, each molecule has poorly hydrated, quasi-planar faces that are complementary to the poorly hydrated planar indole rings. $Gdm^+$ destabilizes H-bonded secondary structure in helical peptides by competing with water for H-bonding interactions with the backbone peptide carbonyl and/or by stacking with the weakly hydrated surfaces of the planar $\pi$-bonded peptide amide. These interactions also conform to a "law" of matching water affinities, with the weakly hydrated surfaces of the complex $Gdm^+$ cation interacting with the weakly hydrated surfaces of groups buried in the interior of folded proteins. The $TPA^+$ cation is too large to stack its weakly hydrated surfaces with the peptide bond, even in the case of a peptide having small side chains. In this particular respect, $TPA^+$ is more similar to a "crowding" solute than the stabilizing osmolytes that are preferentially excluded from interacting with the peptide bond;[17,18] $TPA^+$-induced folding of alahel peptides may have a contribution from effects on solvent free energy as waters hydrating the peptide groups are released into the bulk phase upon formation of the helical structure.[19]

The $TPA^+$-induced stabilization of folded-state conformations in trpzip peptides at concentrations up to 0.5−1.0 M

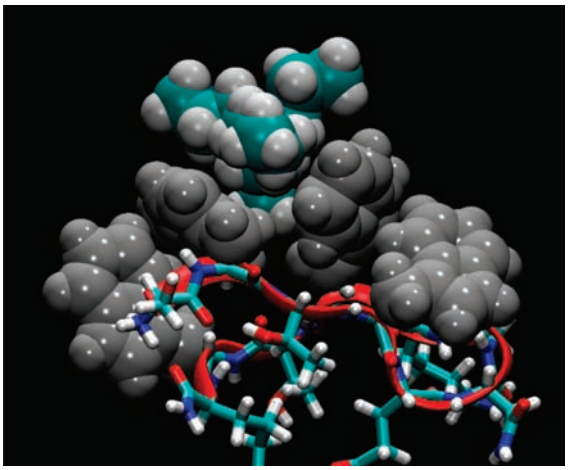

**Figure 5.** Model for a potential stabilizing interaction of $TPA^+$ with two indole side chains of trpzip1. Trpzip 1 is modeled on the NMR structure of the peptide, and the tryptophan indole side chains and $TPA^+$ cation are modeled using the space-filling-atom representation.

remains to be explained but may result from the complementary nature of the structure of $TPA^+$, which has the size and shape to interact simultaneously with each member of the central pair of indole groups in trpzip peptides, as modeled in Figure 5. We prefer this explanation to one that involves opposing destabilizing ($TPA^+$−indole) and stabilizing ($TPA^+$−peptide bond) interactions, since a combination of these effects would not be expected to produce the observed concentration-dependent, biphasic effect on trpzip peptide folded-state stability.

A remaining major uncertainty in the nature of the interactions of these complex cations with proteins relates to interactions with aliphatic side chains that are largely buried in folded protein states. While the planar $Gdm^+$ cation is structurally complementary to the planar $\pi$-bonded aromatic and peptide amide groups in proteins, it is not clear whether the cation can interact productively with the nonplanar "lumpy" aliphatic groups of proteins. Geometrical considerations indicate that displacement of weakly hydrated waters from lumpy aliphatic surfaces should not lead to strong favorable contributions from dispersion forces, a consideration that is supported by recent evidence for poor interactions between $Gdm^+$ and isopropyl groups in water.[20] Since the nonpolar surfaces of the $TPA^+$ cation are aliphatic, this cation may be more effective in disrupting favorable hydrophobic contributions to protein stability arising from burial of aliphatic side chains. Indeed since $TPA^+$ stabilizes H-bonded secondary structure (Figures 3 and 4), attenuation of nonpolar contributions to protein stability seems to be the only possible explanation for the reported denaturant activity of TPA salts.[10] A more detailed investigation of the denaturant activity of TPACl, involving measuring the concentration dependence of folded-state free energies ($m$ values) of proteins, is therefore likely to be insightful.

## ■ ASSOCIATED CONTENT

**Supporting Information.** Experimental details and spectroscopic data on TPACl effects on trpzip2. This material is available free of charge via the Internet at http://pubs.acs.org.

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