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

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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 peptides (alahel) 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 hetero-ion pairing that is observed in concentrated $Gdm^+{}_2SO_4{}^{2-}$ solutions. TPA^+ strongly stabilises helical conformations in alahel peptides indicating exclusion from the peptide bond. The observations are broadly consistent with predictions from 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 co-solutes 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 ($SO_4{}^{2-}$, $CO_3{}^{2-}$, Mg^{2+}) stabilise folded protein states and promote "salting out", whereas weakly-solvated ions with low charge density (SCN^-, Br^-, I^-, Rb^+) promote protein unfolding and "salting in".[1-3] Recent studies support the conclusion that protein-destabilising 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.

Recently we investigated the interactions of more complex ionic denaturants with protein moieties, exploring the contribution of counterion pairing using molecular dynamics simulations. These studies led to a series of predictions of the effects of tetrapropylammonium (TPA^+) chloride and $TPA^+{}_2SO_4{}^{2-}$ 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 TPA^+ Cl^- and $SO_4{}^{2-}$ salts on the conformational stabilities of trpzip peptides,[8] 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^+ is predicted to interact with the Trp indole group, since it shares essential properties of Gdm^+ (cation-π interaction propensity and weakly-hydrated quasi-planar faces that maximise dispersion forces), despite the otherwise disparate nature of the respective molecular structures.[7] TPACl and other tetraalkyl ammonium salts have been reported to be strong protein denaturants,[10] and these experiments also serve to address possible denaturant mechanisms.

Table 1. Amino Acid Sequences of Peptides[a]

Trpzip1	SWTWEGNKWTWK-NH_2
Trpzip2	SWTWENGKWTWK-NH_2
Alahel-21E2	Ac-AE$(QAAAA)_3$QAAY−NH_2

[a]Ac = acetyl; −NH_2 = C-terminal amide.

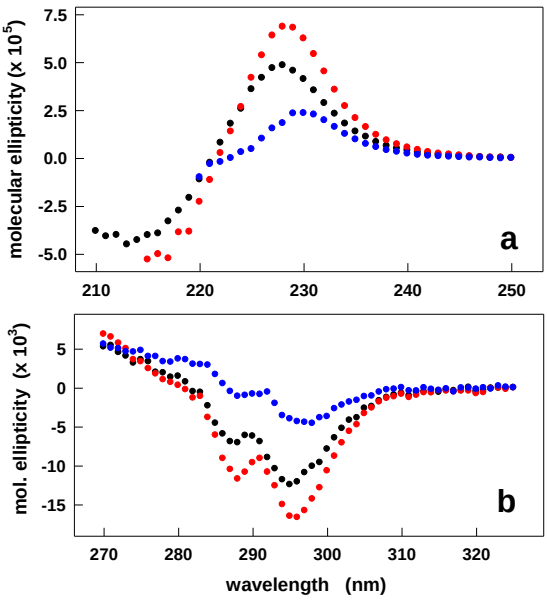

Figure 1. Far (a), and near (b) UV CD spectra of trpzip1 in 10 mM potassium phosphate buffer, pH 3.0, 42 °C in the absence (●) and presence of TPACl at 0.5 M (●) and 2.0 M (●) concentrations.

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, and which is manifest 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

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concentrations).[8,9] Surprisingly, TPACl stabilises trpzip1 at concentrations up to around 0.5-1.0 M concentrations. At higher concentrations TPACl strongly destabilises the folded state as indicated by loss of the exciton coupled CD signal; at a concentration of 2.5 M TPACl, trpzip1 is essentially fully unfolded (Figure 2a). We confirmed that the TPACl effects on the 227 nm CD signal report 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 states), 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 folded state trpzip population at TPACl concentrations of 0.5 M and the promotion of unfolded trpzip conformations at higher TPACl concentrations is observed in TPACl-mediated variations in the intensities of the conformation-dependent induced CD signals around 280-300 nm (Figure 1b) with folded state populations that match those inferred from the exciton-coupled CD bands. Very similar observations were made with trpzip2 (Table 1; not shown).

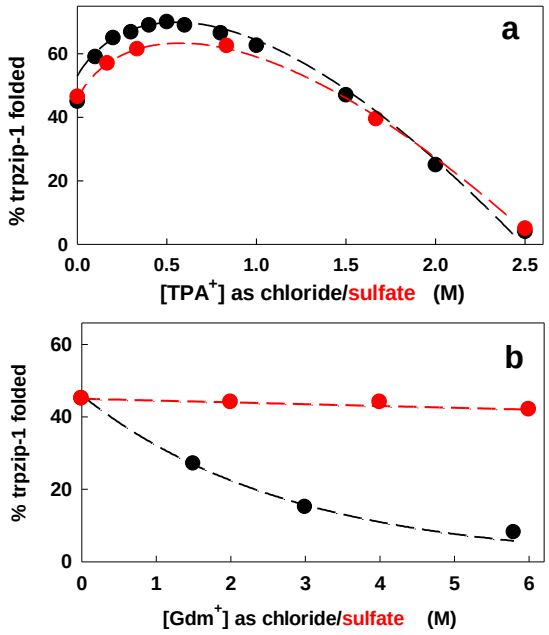

Figure 2. Effects of TPA^+ (a) and Gdm^+ (b) chloride (black) and sulfate (red) salts on the folded state population of trpzip1 in 10 mM potassium phosphate, pH 3.0, 42 ºC. Dotted lines are drawn to guide the eye.

Of specific interest 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 trpzip folded state populations. These data are shown in Figure 2. As previously shown, GdmCl is a denaturant of trpzip peptides whereas the $(Gdm^+)_2SO_4^{2-}$ salt has no effect on trpzip peptide conformational stability.[11] Since sulfate (as Na^+ or K^+ salts) has no 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 TPA^+-induced perturbation of trpzip conformational stability is quite different; $TPA^+{}_2SO_4^{2-}$ has very similar effects on trpzip peptides as TPA^+Cl^-. These observations are consistent with the expectation that SO_4^{2-} can ion pair with Gdm^+,[11,12] particularly at the high ion concentrations at which Hofmeister effects are manifest (and thus reverse effects on polypeptide conformational stability arising from Gdm^+ - polypeptide interactions), whereas sulfate cannot ion pair 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 from consideration of the properties of the complex TPA^+ and Gdm^+ cations relates to interactions with the peptide bond.[7] Gdm^+ is a denaturant of hydrogen (H)-bonded secondary structure as a result of competition for H-bonding to the peptide carbonyl and/or "stacking" interactions with the planar π-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 restriction against access to the peptide bond in polypeptide structure is expected to limit such interactions of the large TPA^+ cation with the peptide bond.

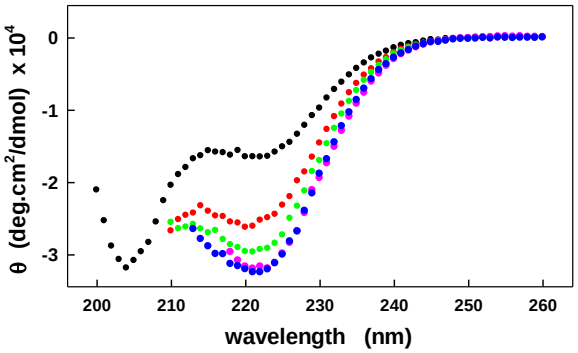

Figure 3. Far UV CD spectrum (mean residue ellipticity) of alahel-21E2 in 10 mM potassium phosphate buffer, pH 7.0 at 15 oC (black), and in the presence of TPACl at concentrations of 0.5M (red), 1.0 M (green), 1.5 M (blue) and 2.0 M (pink).

Accordingly, we find that TPACl strongly stabilizes polypeptide conformations for which intramolecular H-bonding dominates folded state stability. Figure 3 shows CD spectra of alahel-21E2 (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 ºC, around 50% of peptide bonds are in α-helical conformations in alahel-21E2, allowing both stabilizing and destabilizing effects to be measured. TPACl increases the magnitude of the ellipticity at 222 nm, indicating stabilization of H- bonded helical conformations; at a concentration near 1.5 M TPACl, the peptide is essentially fully helical (Figure 4). This contrasts with the well-characterized destabilizing effect of GdmCl on helical conformations in alahel peptides (see Figure 4).[9]

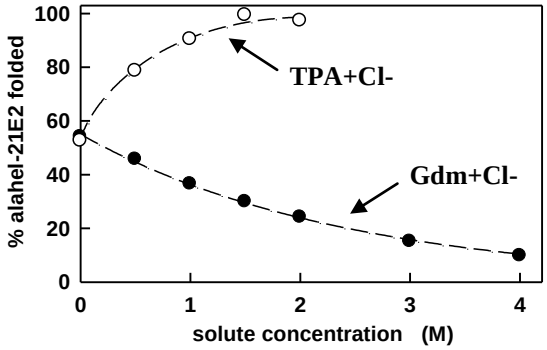

Figure 4. Co-solute dependence of the helical content of alahel-21E2 in solutions of TPACl (o) and GdmCl (●). Buffer conditions are in the legend to Figure 3. Dotted lines are drawn to guide the eye.

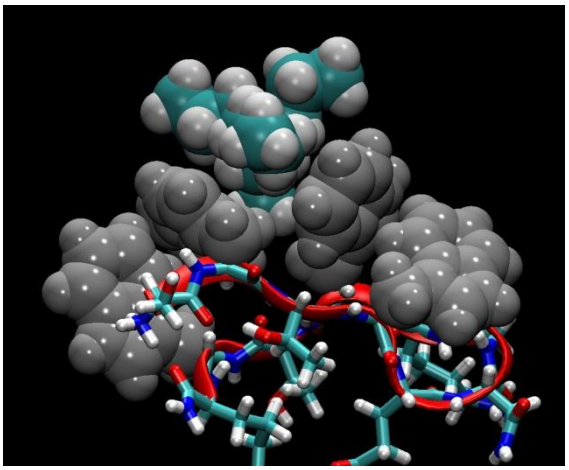

Figure 5. Model for a potential conformational stabilizing interaction of TPA^+ with two indole side chains of trpzip1. Trpzip 1 is modeled on the NMR structure of the peptide, and the tryptophan indole side chains and TPA+ cation are modeled with space-filling atom representation.

These observations support the conclusion that the effects of complex ions on non-covalent interactions that contribute to protein folded state conformational stability 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 sulfate on trpzip and alahel peptides largely conform to predictions made from 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] since 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 contrast 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 hydrogen 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] In contrast to these differences between Gdm^+ and TPA^+, each of the molecules has poorly hydrated, quasi-planar faces that are complementary to the poorly hydrated planar indole rings; in each case the interaction is promoted by cation-π contributions. Gdm^+ destabilises hydrogen-bonded secondary structure in helical peptides by interacting with the peptide bond by competing with water for H-bond interactions with the peptide carbonyl and/or by making stacking interactions with the weakly hydrated surfaces of the planar π-bonded peptide amide. We note that the nature of these interactions also conform to a "law" of matching water affinities, with weakly hydrated surfaces of the complex Gdm^+ cation interacting with the weakly hydrated surfaces of groups buried in the interior of folded proteins. However the TPA^+ cation is simply too large to stack its weakly hydrated quasi-planar surfaces with the peptide bond even in the case of a peptide having small side chains. In this respect TPA^+ has properties similar to protein-stabilising osmolytes which are excluded from interaction with the peptide bond.[16]

TPA^+-induced stabilization of folded state conformations in trpzip peptides at concentrations up to 0.5-1.0 M remains to be explained but may result from the complementary nature of the structure of TPA^+ that has the size and shape to interact simultaneously with each of the central pair of indole groups in trpzip peptides as modeled in Figure 5. We prefer this explanation to one that involves opposing destabilising (TPA^+-indole interactions) and stabilising (TPA^+-peptide bond interactions), since a combination of these effects is not expected to produce the observed concentration-dependent, biphasic effect on trpzip peptide folded state stability.

A major uncertainty remains in the nature of interaction of these complex cations with proteins, and this relates to interactions with the aliphatic side chains that are largely buried in folded protein states. While the planar Gdm^+ cation is structurally complementary to the planar π-bonded aromatic and peptide amide groups in proteins, it is not clear whether the cation can interact productively with the non-planar "lumpy" aliphatic groups of proteins. Geometrical considerations indicate that displacement of weakly hydrated waters from lumpy aliphatic surfaces will not lead to strong favourable dispersion force contributions, a consideration that is supported by recent evidence for poor interactions between Gdm^+ and isopropyl groups in water.[17] Since the non-polar surfaces of the TPA^+ cation are aliphatic, this cation may be more effective in disrupting favourable hydrophobic contributions to protein stability arising from burial of aliphatic side chains. Indeed since TPA^+ strongly stabilises H-bonded secondary structure (Figures 3 and 4), attenuation of non-polar contributions to protein stability seems 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 protein folded state free energies (*m* values), is therefore likely to be insightful.

ACKNOWLEDGMENTS

REFERENCES

(1) Hofmeister, F. *Arch. Exp. Pathol. Pharmakol.* **1888**, *24*, 247-260.
(2) Collins, K. D.; Washabaugh, M. W. *Q. Rev. Biophys.* **1985**, *18*, 323-422.
(3) Baldwin, R. L. *Biophys. J.* **1996**, *71*, 2056-2063.
(4) Moglich, A.; Krieger, F.; Kiefhaber, T. *J. Mol. Biol.* **2005**, *345*, 153-162.
(5) Ma, L.; Pegram, L.; Record, M. T.; Cui Q. *Biochemistry* **2010**, *49*, 1954–1962.
(6) Mason, P. E.; Brady, J. W.; Neilson, G. W.; Dempsey, C. E. *Biophys. J.* **2007**, *93*, L4−L6.
(7) Mason, P. E.; Dempsey, C. E.; Vrbka, L.; Heyda, J.; Brady, J. W.; Jungwirth, P. *J. Phys. Chem. B* **2010**, *113*, 3227–3234.
(8) Cochran, A. G.; Skelton, N. J.; Starovasnik, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5578-5583.
(9) Dempsey, C. E.; Piggot, T. J.; Mason, P. E. *Biochemistry* **2005**, *44*, 775-781.
(10) von Hippel, P. H.; Wong, K.-Y. *J. Biol. Chem.* **1965**, *240*, 3909-3923.
(11) Dempsey, C. E.; Mason, P. E.; Brady, J. W.; Neilson, G. W. *J. Am. Chem. Soc.* **2007**, *129*, 15895-15902.
(12) Mason, P. E.; Dempsey, C. E.; Neilson, G. W.; Brady, J. W. *J. Phys. Chem. B* **2005**, *109*, 24185-24196.
(13) Lim W. K.; Rösgen J.; Englander, S. W. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 2595-2600.
(14) Collins, K. D. *Biophys. Chem.* **2006**, *119*, 271-281.
(15) Mason, P. E.; Neilson, G. W.; Dempsey, C. E.; Barnes, A. C.; Cruickshank, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4557−4561.
(16) Street, T. O.; Bolen, D. W.; Rose, G. D. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 13997-14002.
(17) Mason, P. E.; Dempsey, C. E.; Neilson, G. W.; Kline, S. R.; Brady, J. W. *J. Am. Chem. Soc.* **2009**, *131*, 16680-16696.