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Insight into Indole Interactions from Alkali Metal Chloride Effects on a Tryptophan Zipper β-Hairpin Peptide

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The introduction of tryptophan residues in cross-strand register in small β-hairpin "tryptophan zipper" (trpzip) peptides can result in extraordinary stabilization of the folded hairpin conformation.[1] The contributions to structural stabilization in trpzips are not fully defined but may include intrinsic contributions from the indole−indole interaction itself and from factors such as the efficient screening of the peptide backbone by the indole side chain. The NMR structures of trpzips[1] demonstrate that the indole−indole interaction has a significant electrostatic component since it has a T-offset geometry which maximizes the interaction of partial charges in the indole side chains.[1,2]

In a recent analysis of denaturant effects on trpzip peptides, we observed destabilization of trpzips by certain alkali metal chlorides,[3] which we explore in more detail here. These observations allow us to further characterize the nature of the tryptophan-induced stabilization in trpzips and the nature of the indole−indole interaction. We have chosen to study trpzip1 (see Table 1 for peptide sequences) since it has a convenient degree of folding (60% at 42 °C)[1,3] that allows the effects of both structural stabilization and destabilization to be determined by circular dichroism (CD) spectroscopy. However, the indole side chains do not constitute the only contribution to structural stability in trpzip peptides; trpzip1 has an E5-K8 salt bridge, and potentially four cross-strand hydrogen bonds.[1] To make a qualitative separation of the effects of electrolytes on the different contributions to stability in trpzip1, we have also measured the effects of alkali metal chlorides on two other peptides (Table 1) MrH4a (at 31 °C)[4] and alahel (also called E7; at 15 °C).[3,5] Alahel is stabilized largely by (helical) hydrogen bonds and thus allows characterization of salt effects on hydrogen bonds. MrH4a has a salt bridge involving the C-terminal carboxyl group and the N-terminal K1 and K2 residues, and (potentially) cross-strand hydrogen bonds. The main contribution to stability is the cross-strand cluster of aliphatic side chains involving L3, V5, I12, and V14,[4] and as a result, this peptide shows a significant cold denaturation. Measurements on the β-hairpin peptides, trpzip1 and MrH4a, were made at pH 2.5 to protonate the carboxyl component of salt bridges and thus eliminate this contribution to stability.[3] The proportion of folded state of trpzip1 and MrH4a at pH 2.5 was 54% (42 °C) and 25% (31 °C), respectively; the value for MrH4a was based on the reported value of 31% for the folded state at basic pH's at 31 °C,[4] and using the ratio of molecular ellipticities (222 nm) obtained at pH 2.5 and 7.5, after estimating a correction for the ellipticity of the unfolded peptide (Supporting Information).

The accessible CD spectral region of trpzip1 in the absence and presence of 2 molar concentrations of alkali metal chlorides is shown in Figure 1. The full CD spectra of trpzips contain negative and positive bands centered near 215 and 227 nm, respectively, resulting from exciton coupling between pairs of Trp indole groups from opposing strands of the β-hairpin.[1] Only the positive band can be recorded in high salt concentrations under the accessible experimental conditions (trpzip1 at concentrations in the 50−100 μM range is precipitated by some alkali salts, so all measurements were made at 10 μM in 1 cm cuvettes). The intensity of the positive band is proportional to the concentration of the folded state.[1,3] The absence of a significant contribution to the CD signal from alkali metal chloride effects on the folded state structure was established using the highly stabilized trpzip2 peptide at room temperature (Figure S3), and the salts have minimal effects on the CD spectrum of a peptide corresponding to the C-terminal pentapeptide of trpzip1, a model for the unfolded state (Figure S3).

Of the alkali metal chlorides, Li has a small stabilizing effect on trpzip1, Na is effectively neutral in its effects, whereas K and, more strongly, Rb destabilize trpzip1 (Figure 1). We determined *m* values that correspond to the dependence on the alkali metal chloride concentration of the free energy of the folded state (relative to the unfolded state), for each of the peptides in Table 1, using the methods described in ref 3. These analyses are based on the linear extrapolation method (LEM)[6] in which the equilibrium constant, K_{FU}, that defines the ratio of folded and unfolded peptide

Table 1. Amino Acid Sequences of Peptides

Trpzip1	SWTWENGKWTWK−NH_2
MrH4a	KKLTVSINGKKITVSA
Alahel(E7)	Ac-AAQAAAEQAAAAQAAY−NH_2[a]

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

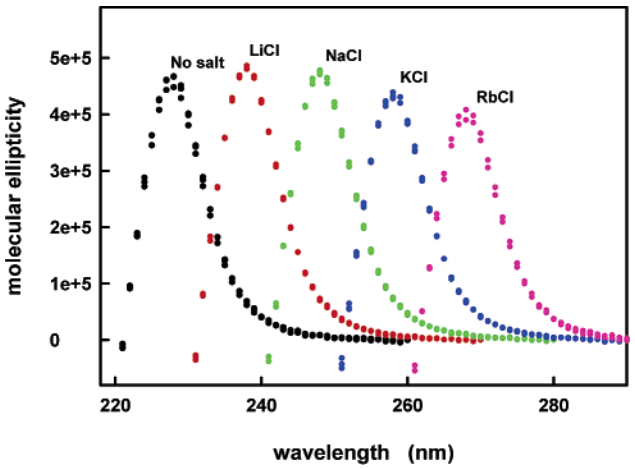

Figure 1. Far UV CD of trpzip1 in salt-free 10 mM phosphate buffer, pH 2.5, and in buffer containing 2 M concentrations of the alkali metal salts (spectra from three separate samples of each salt). The wavelength scale corresponds to the salt-free spectra; all other sets of spectra are offset for clarity.

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Table 2. *m* Values (cal mol^{-1} M^{-1}) for Solute Effects on Peptide Stability

	Trpzip[a] (42 °C)	MrH4a[a] (31 °C)	alahel[b] (15 °C)
LiCl	*-26*	*−34*	12
NaCl	*0*	*−34*	7
KCl	65	*−34*	6
RbCl	111	*−34*	6
Urea	126[c]	n.d.	20[c]
GdmCl	358[c]	n.d.	28[c]

[a] The *m* values are per mole of peptide. [b] The *m* values are per amino acid residue; *m* values for alahel determined between 0.5 and 2 M electrolyte concentrations since alahel is stabilized by salt at concentrations up to 0.5 M (see ref 3). [c] The *m* values from ref 3. Negative *m* values indicate stabilizing effects. Values in italic text were determined from triplicates of samples at a single (generally 2 M) solute concentration; n.d. = not determined.

is measured as a function of molar solute concentration, and *m* is determined from eq 1, where K_0 is the value of K_{FU} in the absence of solute, *R* is the gas constant (1.987 cal mol^{-1} K^{-1}) and *T* is in Kelvins. The denaturant dependence of helix content in alahel was analyzed using the Zimm−Bragg theory[7] in which the helix propagation parameter, *s*, and s_0 for the solute-free peptide replace K_{FU} and K_0 in eq 1. The *m* values for solute effects on the helical stability are per residue values, whereas for the β-hairpin peptides, the *m* values are per mole of peptide. These data are listed in Table 2.

$$\ln(K_{FU}) = \ln K_0 - \frac{m[\text{solute}]}{RT} \qquad (1)$$

The data demonstrate that differences in alkali metal chloride effects on trpzip1 are dominated by interactions involving the indole groups. In particular, MrH4a is slightly stabilized by each of the chlorides at pH 2.5, as expected for a conformation stabilized largely by the hydrophobic effect. There is little difference, however, between the effects of the different chloride salts. The contribution from hydrogen bonding to stabilization of the β-hairpin peptides, trpzip1 and MrH4a, is not straightforward; NMR studies have indicated that for a peptide very similar to MrH4a cross-strand hydrogen bonds do not form with favorable geometries and make negligible contribution to the folded state stability.[8] While part of the effects of the electrolytes on trpzip1 stability may arise from effects on hydrogen bonds, the data on alahel indicate that these are likely to be small. In addition, the order of effectiveness in attenuating structure in alahel is reversed compared with the effects of alkali metal ions in trpzip1; for example, LiCl, which is most effective in destabilizing alahel, slightly stabilizes trpzip1 (Figure 1 and Table 2). Although the possibility arises that the very small electrolyte effects on MrH4a result from coincidental balances of destabilizing effects on hydrogen bonds and stabilizing effects on the hydrophobic contribution, the systematic effects of the alkali metal chlorides on trpzip1 that relates to ion charge density cannot easily be explained by consideration of effects on hydrogen bonds and/or the contribution from the hydrophobic effect.

We conclude that the effects of the alkali metal chlorides highlight aspects of the indole−indole and indole−solute interactions. Each of the salts is likely to have a small stabilizing contribution in trpzip1 due to enhancement of the hydrophobic effect (as seen with MrH4a), and this is most apparent with Li^+, a small, strongly solvated, high charge density cation. These salts cannot attenuate the electrostatic contribution to the indole−indole interaction by the classical effect arising from charge screening by solvated ions since the electrostatic contribution to the indole−indole interaction is short range.[1,2] The short range nature of the indole−indole electrostatic interaction means that only direct ion contacts are effective in attenuating this interaction. While Li^+ has the highest charge density of the cations studied here, it also has an unusually strong hydration shell for an alkali earth cation,[9] so that it is largely excluded from direct interaction with the indole groups. The K^+ and especially Rb^+ ions are more weakly solvated, however, and can interact directly with the weakly solvated faces of the indole groups, as observed in crystal structures of alkali metal salt chelates containing pendant indole groups[10] in a cation−π interaction.[11] Thus we conclude that the destabilization of trpzip1 by low charge density monovalent cations results from weak interactions with the indole side chain of Trp.

These data demonstrate the inherent stability of the indole−indole interaction in high concentrations of *high* charge density cations, despite a significant electrostatic contribution to the interaction. The data also highlight the essential simplicity of molecular interactions in these systems. The present and previous[3] studies demonstrate that stabilizing contributions are attenuated by solutes of complementary nature. Thus while Rb^+ is almost as effective as urea in denaturing trpzip1, it is around 3-fold less effective than the guanidinium cation (Gdm^+). The latter is also a weakly solvated, low charge density cation,[12,13] but interacts more effectively with the indole group due to its complementary planar nature.[3] Urea and Gdm^+ are effective denaturants of structures stabilized by hydrogen bonds, whereas hydrogen bonds are only weakly destabilized by simple monovalent electrolytes[3] (Table 2).

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Supporting Information Available: Spectroscopic data and graphical analyses for determination of *m* values. This material is available free of charge via the Internet at http://pubs.acs.org.

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