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Environmental Modulation of Protein Cation-π Interactions

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The cation-π interaction has recently emerged as a potentially important member of the noncovalent forces involved in the structural and functional properties of proteins.[1] To investigate the free energy contributions made by cation-π interactions to the global stability of proteins, this type of interaction has been studied in a number of model systems including monomeric α-helices,[2−5] a β-hairpin,[6] and a coiled-coil peptide.[7] These studies have shown that stabilizing cation-π interactions may or may not be formed depending on the specifics of the engineered cation-π pair and the peptide scaffold used. For those combinations in which stabilizing interactions are formed, the bond energies tend to be weak. The strength of various cation-π interactions placed on the surface of monomeric α-helices are all ≥ -0.4 kcal mol^{-1},[4,5] except for a W/H pair in which the interaction energy was reported to be ca. -1 kcal mol^{-1}.[3] Similarly, the bond energies of four different cation-π interactions (F/K, F/R, W/K, and W/R) studied in a β-hairpin are in the -0.20 to -0.48 kcal mol^{-1} range.[6] For the three combinations (R/F, R/Y, and R/W) engineered in a coiled-coil system, only the R/F pair provided a stabilizing force.[7]

A feature common to the cation-π peptide model systems is that the engineered aromatic and basic residues are fully or highly solvent exposed. This is however not the case for a typical protein cation-π interaction.[8,9] Although these interactions are often found near the protein surface, the exposure of the interacting residues varies. The solvation of cation-π interactions is intermediate between the solvation of aromatic residues, which tend to be buried, and the solvation of cationic residues, which in contrast tend to be highly solvent exposed.[9] In an effort to characterize a system that more closely mimics the properties of naturally occurring cation-π interactions, we determined the interaction energies between a buried Trp and partly solvated Lys, Arg, and His residues. Changes in the interaction energy upon increasing the solvation of the W/K cation-π pair were also investigated. Our results suggest that changes in solvation can tune the interaction energy between a Trp and a Lys by at least 0.9 kcal mol^{-1}.

The structurally characterized α_3W model protein was used for these studies.[10] The peptide chain of α_3W contains repeating *a*-*g* heptad segments and it folds into three interacting α-helices in water.[11] The protein sequences of α_3W and variants used in this work are listed in Table S1 in the Supporting Information. The central helix of α_3W contains the W32-E33-E34-L35-K36-K37-K38 heptad in which a cation-π interaction is present between heptad *a* residue W32 and heptad *e* residue K36.[10] A solvent accessible surface area (ASA) analysis of the α_3W NMR structure[10] shows that the protein strictly follows the expected heptad repeat pattern with buried residues in heptad *a* and *d* positions and more solvent exposed residues in the heptad *b*, *c*, *e*, *f*, and *g* positions (see Supporting Information, Table S2). The average ASA of W32 and K36 across the family of NMR structures are 2.6 $\pm$ 1.4% and 24.2 $\pm$ 1.9%, respectively.

To determine the strength of the W32/K36 cation-π interaction, a double-mutant cycle (DMC)[12,13] was constructed by measuring the global stability (ΔG) of four proteins: α_3W, two single-site variants in which either K36 was changed to an Ala (W32/A36) or W32 changed to a Val (V32/K36), and a double-site variant in which W32 and K36 were changed to a Val and an Ala, respectively (V32/A36). The W32/K36 interaction energy ($\Delta\Delta G$) was determined to -0.73 ± 0.08 kcal mol^{-1} by subtracting the stability of the single-site variants W32/A36 and V32/K36 from the stability of α_3W and the double-site variant V32/A36:[12]

$$\Delta\Delta G_{\mathrm{W32/K36}} = \Delta G_{\mathrm{W32/K36}} + \Delta G_{\mathrm{V32/A36}} - \Delta G_{\mathrm{W32/A36}} - \Delta G_{\mathrm{V32/K36}}$$

The W32/K36 interaction is salt insensitive, which is consistent with earlier studies.[2,4,13] Only a -0.14 kcal mol^{-1} increase in the interaction energy was observed as the KCl concentration was raised from 15 mM to 1.0 M. Table 1 lists ΔG values of α_3W and the W32/A36, V32/K36, and V32/A36 variants obtained at pH 5.5, in 15 mM or 1.0 M KCl, and the derived $\Delta\Delta G_{\mathrm{W32/K36}}$ energies. ΔG values were acquired by chemical denaturation. Figure S1 (Supporting Information) displays typical data and provides experimental details.

The effects of changing the interacting cationic residue were investigated. A DMC based on the W32/R36, W32/A36, V32/R36, and V32/A36 proteins, provided a $\Delta\Delta G_{\mathrm{W32/R36}}$ of -0.71 ± 0.06 kcal mol^{-1} (Table 1). There is no significant change in the strength of the cation-π interaction as K36 is changed to an Arg. ΔG values for the W32/H36, W32/A36, V32/H36, and V32/A36 proteins were obtained at pH 5.5 and 9.0 (Table 1; Supporting Information, Figure S1). A pH titration of the W32/H36 protein provided a H36 pK_A of 7.2 $\pm$ 0.1 (not shown). The interaction energy derived from the pH 5.5 and 9.0 DMC data sets consequently represents W32 paired with protonated H36$^+$ and neutral H36^0, respectively.

The W32/H36 interaction is stronger at lower pH and overall weaker than the W32/K36 and W32/R36 interactions. $\Delta\Delta G$ is determined to -0.48 ± 0.08 kcal mol^{-1} for the W32/H36$^+$ pair and -0.32 ± 0.02 kcal mol^{-1} for the neutral W32/H36^0 pair (Table 1).[14]

Modulation of the W/K interaction as a function of solvent exposure was investigated. To make more solvated W/K pairs in α_3W, the Trp was moved to heptad *b* position 33 to pair up with heptad *f* residue K37 (Figure 1B), and to heptad *c* position 34 to pair up with heptad *g* residue K38 (Figure 1C). The aim was to rotate the Trp around the axis of the central helix from the protein interior to the exterior (Figure 1). Increases in the overall solvation of the W33/K37 and W34/K38 pairs, relative to the W32/K36 pair, are predicted to arise mainly from a change in the Trp environment. As noted above, the α_3W structure displays a tightly preserved helical heptad pattern (Supporting Information, Table S2). The average ASA of heptad *a* residues in α_3W are 3 $\pm$ 2% and heptad

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Table 1. Thermodynamic Properties of α_3W and Variants[a]

*i,i+*4 pair[b]	ΔG (pH 5.5)[c]	ΔG (1 M KCl)[d]	$\Delta\Delta G$
W32/K36	-3.39 ± 0.05	-4.71 ± 0.02	W32/K36
W32/A36	-2.90 ± 0.01	-4.28 ± 0.01	
V32/K36	-4.68 ± 0.02	-5.67 ± 0.01	-0.73 ± 0.08[c]
V32/A36	-4.93 ± 0.06	-6.11 ± 0.01	-0.87 ± 0.03[d]
W32/R36	-3.42 ± 0.02		W32/R36
V32/R36	-4.73 ± 0.01		0.71 ± 0.06[c]
ΔG (pH 5.5)[c]		ΔG (pH 9.0)[e]	
W32/H36	-2.66 ± 0.04	-2.44 ± 0.01	W32/H36
W32/A36	-2.90 ± 0.01	-3.01 ± 0.02	
V32/H36	-4.20 ± 0.03	-3.61 ± 0.02	-0.48 ± 0.08[c]
V32/A36	-4.93 ± 0.06	-4.51 ± 0.01	-0.32 ± 0.02[e]
W33/K37	-4.67 ± 0.01		W33/K37
W33/A37	-4.40 ± 0.01		
E33/K37	-4.93 ± 0.02		
E33/A37	-4.72 ± 0.01		-0.06 ± 0.02[c]
W34/K38	-4.42 ± 0.01		W34/K38
W34/A38	-4.56 ± 0.01		
E34/K38	-4.93 ± 0.02		
E34/A38	-4.93 ± 0.06		0.15 ± 0.07[c]

[a] ΔG (kcal mol^{-1}), global protein stability; $\Delta\Delta G$ (kcal mol^{-1}), interaction energy derived from double mutant cycle analysis.[14] ΔG values were derived from 2 to 3 separate experiments. [b] See Supporting Information, Table S1, for complete protein sequences. [c] Sample conditions: 10 mM sodium acetate, 15 mM KCl, pH 5.5. [d] Sample conditions: 10 mM sodium acetate, 1.0 M KCl, pH 5.5. [e] Sample conditions: 10 mM TRIS/HCl, 15 mM KCl, pH 9.0.

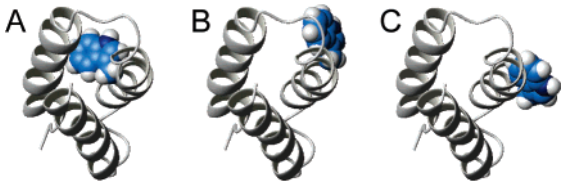

Figure 1. Investigating the strength of a W/K cation-π interaction as a function of solvent exposure. (A) The central helix of α_3W contains the *a-g* heptad segment, W32-E33-E34-L35-K36-K37-K38, in which a cation-π interaction is present between heptad *a* residue W32 and heptad *e* residue K36. W32 is essentially completely buried in the hydrophobic core of α_3W. To make more solvent exposed W/K pairs, the tryptophan was moved to (B) heptad *b* position 33 to pair up with heptad *f* residue K37 and to (C) heptad *c* position 34 to pair up with heptad *g* residue K38.

b and *c* residues 39 $\pm$ 11%. K36, K37, and K38 are all partly exposed (ASA being 24, 45, and 29%, respectively). Major differences in the solvent exposure of W33 and W34 relative to W32 are consistent with their fluorescence spectra (Supporting Information, Figure S2). The emission maxima of the W33/K37, W33/A37, W34/K38, and W34/A38 spectra are 28.5 ± 0.5 nm red-shifted relative to the Em_{max} of the α_3W spectrum. Large changes in quantum yields are also observed. We note that these spectral changes are not due to an overall destabilization of the helical scaffold. The helical contents of the four W33 and W34 proteins are close to that of α_3W (73 $\pm$ 5% vs 76% based on θ_{222} values), and they are all more stable than α_3W (Table 1).

Interestingly, DMC analyses provide an interaction energy of -0.06 ± 0.02 kcal mol^{-1} for the W33/K37 pair and predict a nonstabilizing interaction between W34 and K38 (Table 1; Supporting Information, Figure S3). These results are in agreement with the data obtained from the peptide model systems in which the engineered cation-π interactions are either nonstabilizing or weak.[2-7] Since cation-π interactions have no or very minor salt dependencies (Table 1),[2,4,13] the one order of magnitude difference in the interaction energy between W32/K36 and the W33/K37 and W34/K38 pairs appears not to arise from a change in the electrostatic milieu. Thus, the main parameter modulating the W/K interaction energy is most likely differences in solvation.

W32/K36 represents the first W/K cation-π interaction for which both the structure[10] and the interaction energy are known. This allows us to test theoretical methods developed to identify cation-π interactions. The CaPTURE[9] program correctly identifies W32/K36 as an energetically significant cation-π interaction although it overestimates the bond energy by a factor of $\sim$3.

In summary, the strength of cation-π interactions formed by a buried Trp and a partly solvated Lys, Arg, or His range from -0.8 to -0.5 kcal mol^{-1} and rank as W/K $\approx$ W/R > W/H. Upon increasing the solvent exposure of the W/K pair, the interaction energy drops from -0.73 to -0.06 and $+0.15$ kcal mol^{-1}. These results suggest that changes in solvation can tune the W/K interaction energy by at least 0.9 kcal mol^{-1}. The observed solvation dependence in the interaction energy further suggests that values derived from highly solvent exposed peptide model systems represent lower limit estimates for the free energy contribution of cation-π interactions to the stability of proteins.

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Supporting Information Available: Protein sequences of α_3W and variants (Table S1), accessible surface area analysis of the α_3W NMR structure (Table S2), typical protein denaturation plots and experimental details (Figures S1 & S3), and fluorescence spectra of the W32, W33, and W34 proteins (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Table 1 shows that the V32 proteins are more stable than those containing a Trp in position 32. The observed increase in stability most likely reflects a local structural relaxation occurring in the protein core upon changing W32 to a Val. The accuracy of the DMC analyses relies on the assumption that the energy associated with the W32 to V32 structural reorganization is uncoupled to the identity of the amino acid at position 36. That is, the 32 and 36 mutations are independent and protein reorganization terms will cancel in the DMC.[12] This assumption can be tested by comparing the difference in the W32/A36 and V32/A36 ΔG values (2.03 kcal/mol) to the difference in the W32/A36 and V32/K36 ΔG values after correcting for a Lys to Ala change in helical propensity (2.04 kcal/mol).[14] Similar calculations for the R36 & H36 DMC analyses predict that the average discrepancy is in the order of 0.03 kcal mol^{-1}. We conclude that the assumption made is valid.

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