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Cation−π Interaction in Model α-Helical Peptides

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Abstract: Cation−π interactions are increasingly recognized as important in chemistry and biology. Here we investigate the cation−π interaction by determining its effect on the helicity of model peptides using a combination of CD and NMR spectroscopy. The data show that a single Trp/Arg interaction on the surface of a peptide can make a significant net favorable free energy contribution to helix stability if the two residues are positioned with appropriate spacing and orientation. The solvent-exposed Trp→Arg (i, $i+4$) interaction in helices can contribute −0.4 kcal/mol to the helix stability, while no free energy gain is detected if the two residues have the reversed orientation, Arg→Trp (i, $i+4$). The derived free energy is consistent with other experimental results studied in proteins or model peptides on cation−π interactions. However in the same system the postulated Phe/Arg (i, $i+4$) cation−π interaction provides no net free energy to helix stability. Thus the Trp→Arg interaction is stronger than Phe→Arg. The cation−π interactions are not sensitive to the screening effect by adding neutral salt as indicated by salt titration. Our results are in qualitative agreement with theoretical calculations emphasizing that cation−π interactions can contribute significantly to protein stability with the order Trp > Phe. However, our and other experimental values are significantly smaller than estimates from theoretical calculations.

Introduction

The native three-dimensional structure of a protein is determined by a delicate balance of weak noncovalent interactions. Hydrogen bonds, salt bridges, and the hydrophobic effect all play roles in folding a protein and establishing its final structure. While there have been many attempts to assess noncovalent interactions (for example, see refs 1−6), a complete understanding of protein stability requires consideration of the contribution of nonclassical noncovalent interactions as well, including aromatic−aromatic,[7,8] charge−dipole,[9,10] and cation−π[11−18] interactions. Cation−π interactions between a (partially) positively charged group and aromatic systems with delocalized π-electrons were first recognized and studied in the gas phase.[19−23] In recent years studies of model host−guest systems and analysis of biological macromolecular structures have revealed that cation−π interactions are one of the fundamental noncovalent interactions in solution.[24−26] Cation−π interactions in proteins have been identified in a number of important studies.[27−37] Mounting evidence implicates cation−π interactions[11,12,38,39] in the function of acetylcholine receptors,[40−43]

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enzyme−substrate binding[44,45] and catalysis,[46] toxin/K^+-channels,[47,48] protein−DNA binding,[49] as well as specific drug/ligand−receptor and antigen−antibody[50] interactions.

Computational studies of cation−π interactions in both the gas phase and aqueous media[51−57] have helped to clarify the forces involved. Analysis of various models implicates charge−quadrupole, charge−dipole, charge-induced dipole, charge transfer, dispersion forces, as well as a hydrophobic component. Although qualitative agreement can be found between the results of different calculations, there is still no quantitative consensus. Hence, it is important to obtain quantitative experimental data about the cation−π interaction energies in protein or polypeptide models in aqueous solution.

Efforts to screen the Protein Data Bank (PDB) for cation−π interactions often rely on geometric constraints, identifying situations where a cationic side chain displays a certain distance/angle relationship to an aromatic side chain. While useful qualitative information about these interactions can be derived from statistic analysis by applying such geometric criteria, there is no necessary correlation between energy and frequency.[58] Thus, it is not possible to obtain quantitative energetic information from this analysis. As pointed out by Gallivan and Dougherty, not all cation-aromatic contacts represent an energetically favorable cation−π interaction, since these can be repulsive as well as attractive.[59]

There is still a paucity of experimental studies on the energetics of cation−π interaction in protein or polypeptides.[13,15−18,60] We present here a study of two series of alanine-based peptides containing combinations of Arg with Trp or Phe located with different spacing and orientation. The first series of peptides is designed to allow quantitative evaluation of the role of an Arg/Trp cation−π interaction on helix stability, while the second was designed to study cation−π, anion−π as well as the potential polarizing effect of an aromatic ring on the stability of α-helix.[18] The present study focuses on cation−π interactions. Differential effects of spacing and orientation of Arg and Trp side chains on helix stability provide a way to measure small free energy differences. Our results rationalize the statistical observation that solvent-exposed (i, $i + 4$) cation−π interactions can stabilize proteins with the Trp/Arg interaction being stronger than the Phe/Arg interaction.

Materials and Methods

Peptide Synthesis and Purification. Peptides were synthesized by solid-phase peptide synthesis on a Rainin PS3 automated synthesizer using Rink resin (Advanced Chemtech) and Fmoc chemistry. Cleavage from the resin and removal of side-chain protecting groups was performed with 90% TFA in the presence of the scavengers anisole and H_2O. Crude peptides were precipitated in cold ether, dissolved in water, and lyophilized. Purification was performed by HPLC on a Delta Pak C18 reverse phase semipreparative column. Molecular weights were confirmed by MALDI mass spectrometry using a Kratos MALDI I linear time-of-flight spectrometer.

CD Measurements. Stock solution concentrations were determined by tryptophan/tyrosine absorbance in 6 M Guanidine HCl ($\epsilon_{275} = 5400/1450\ M^{-1}\ cm^{-1}$).[61] Stock solutions were prepared in 10 mM phosphate buffer (pH = 7.0) at a concentration of 500−1500 μM. CD measurements were performed at a peptide concentration of 50 μM in 10 mM phosphate buffer pH 7 at 4 °C unless otherwise specified. Concentration-dependent CD measurements were conducted over a range of peptide concentrations from 10 to 400 μM. Salt concentration variations were performed by preparing a 3 M NaCl/KF solution in 10 mM phosphate buffer and diluting to 0.5, 1.0, and 2.5 M NaCl/KF. CD measurements were recorded on an Aviv DS 60 CD spectrometer equipped with a temperature controller. The helix content of each peptide was determined from the mean residue CD at 222 nm, $[\theta]_{222}$ (deg cm^2 dmol^{-1}) corrected for the length of the chains according to Manning and Woody.[62] The wavelength of the instrument was calibrated using (+)-10-camphorsulfonic acid.[63]

CD Data Analysis. Analysis of the free energy contribution of side chain−side chain interactions from CD data was carried out using a modified Zimm−Bragg multistate helix−coil transition model described previously.[64,65] The relation between fraction helicity (f) and CD is taken as $f = [\theta]_{222}/[\theta]^\circ_{222}$, where $[\theta]^\circ_{222}$ is the estimated molar residue CD signal at 222 nm, −34 000 for an α helix of 24 residues and −32 000 for an α-helix of 18 residues.[65]

In addition to the nucleation constant σ and a set of helix propagation constants s_i corresponding to each species of amino acid (i) in the sequence, the model explicitly introduces an additional equilibrium constant $\gamma = \exp(-\Delta G/RT)$, where ΔG refers to the interaction between side chains. The weighting for a chain of N residues is generated recursively from the weights of shorter chains using difference equations described by Gans et al.[64] and Yang et al.[65] Side chain−side chain interactions spaced at (i, $i + 4$) are weighted by the additional stability constant γ. The nucleation constant is assumed to be independent of sequence, with a value of 0.004.[65] Intrinsic helix propensities were taken to be sAla = 1.5, sArg = 1.1, sGlu = 0.43, sOrn = 0.53, sTrp = 0.33, sPhe = 0.33 and sTyr = 0.45.[66,67] We fit the CD data to the helix−coil transition model for individual peptides,[4,64] and also tested global fits to CD data on groups of peptides to establish the significance of differences in γ values.

NMR Spectroscopy. ^{1}H NMR spectra were collected on a Varian UNITY 500 spectrometer. We used the States method[68] to obtain phase-sensitive clean-TOCSY[69,70] spectra using a mixing time of 80 ms.

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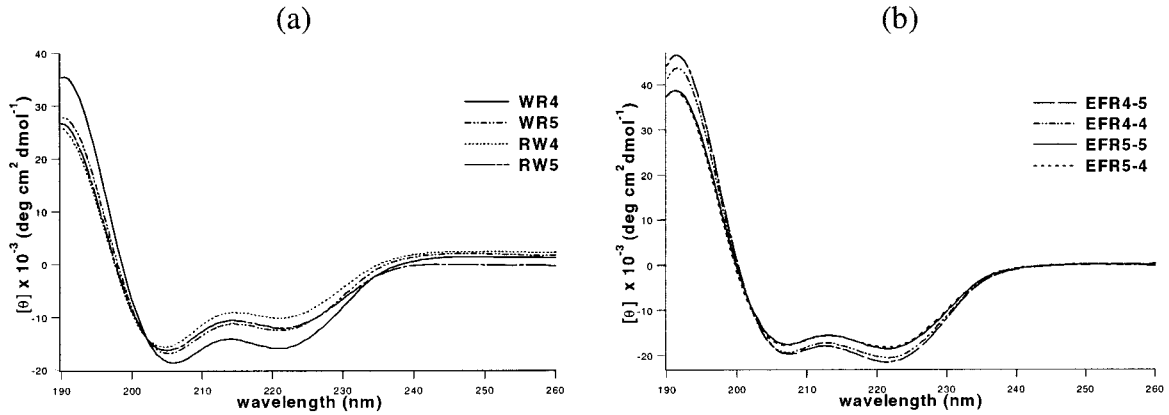

Figure 1. Circular dichroism spectra of W/R and EFR peptides in 10 mM phosphate buffer pH 7 at 4 °C. The peptide concentrations are 50 μM in each case as determined by tyrosine or tryptophan absorbance at 275 nm.

NOESY experiments[71,72] were run with a mixing time of 400 ms. Water suppression was achieved using a Watergate sequence.[73] Each 2D data set contained 512 FIDs with 2K complex data points each, obtained by collecting 64 added free induction decays after 4 dummy scans. Spectra were Fourier transformed in both t_2 and t_1 dimensions after apodization with a shifted square sine bell function, typically with an 80° phase shift. Zero filling was done in the t_1 dimension to obtain a final matrix of 2048 × 1024 real points. NMR data were processed using VNMR (version 6.1A). Samples were prepared by dissolving peptides in 10 mM phosphate buffer (pH = 7, 10% D_2O) to a concentration of ca. 5 mM. The sodium salt of 3-(trimethysilyl)-[3,3,2,2-^{2}H] propionic acid was used as an internal chemical shift reference.

Table 1. Sequences of Peptides Used in This Study

peptide	sequence[a]
WR5	Ac−OOAAAA**W**AAAA**R**AAAAOO−NH_2
WR4	Ac−OOAAAAA**W**AAA**R**AAAAOO−NH_2
RW5	Ac−OOAAAA**R**AAAA**W**AAAAOO−NH_2
RW4	Ac−OOAAAA**R**AAA**W**AAAAAOO−NH_2
EFR5−5	Ac−OOAAAA**E**AAAA**F**AAAA**R**AAAAOOY−NH_2
EFR5−4	Ac−OOAAAA**E**AAAA**F**AAA**R**AAAAAOOY−NH_2
EFR4−5	Ac−OOAAAAA**E**AAA**F**AAAA**R**AAAAOOY−NH_2
EFR4−4	Ac−OOAAAAA**E**AAA**F**AAA**R**AAAAAOOY−NH_2

[a] Ac = acetyl; O = ornithine; A = alanine; W = tryptophan; R = arginine; E = glutamic acid; F = phenylanaline; Y = tyrosine.

Results

Peptide Design. As demonstrated in several previous studies[18,74,75] on model alanine helical peptides, the high helix-forming propensity of alanine allows short peptides to form helical structures in water without side-chain interference. Our strategy relies on positioning ornithine residues outside the range of the target interactions to provide solubility and prevent aggregation. Strict conservation of composition within a series of these peptides avoids differences in intrinsic helical propensity in each chain. Differences in helicity within a series thus reflect positional effects or side chain−side chain interactions. This strategy avoids differences in reference states that can complicate evaluating the interactions specified.

The first series of peptides were 18-mers with a Trp residue in each peptide serving for concentration determination (Table 1). The peptide WR4 allows Trp and Arg side chains to interact on the surface of the helix with residues at (i, $i + 4$) positions. As control we use peptide WR5 in which the Trp and Arg side chains are spaced at positions that prevent their interaction in a helix (i, $i + 5$). This arrangement places the two side chains at sites on noncontiguous faces of a helix and far apart. In these two peptides, we shift the position of Trp while keeping the Arg residue in the same position. We designed RW4/RW5 to investigate the effect of orientation on the interaction. Table 1 lists four other peptides: EFR4-4 and EFR5-4 were designed to allow Phe and Arg side chains to interact, whereas EFR4-5 and EFR5-5 serve as controls. Orientation effects are not considered in this series of model peptides. Tyr residues serve for concentration determination in the latter series.

CD Analysis. The CD spectra (Figure 1) show that all peptides are helical, with characteristic minima at 222 and 208 nm and an isodichroic point near 202 nm, consistent with a monomeric two-state helix−coil transition in all peptides. In each case, the helix content is independent of concentration from 10 to 400 μM (data not shown). A similar alanine peptide with 13 contiguous alanines flanked by pairs of ornithine residues at each end was shown to be monomeric in an analytical ultracentrifugation study.[74] Thus, the helical structure in each peptide is stabilized by intramolecular interactions under the conditions studied.

It is known that aromatic side chains can influence the CD spectra of helical peptides.[76] The CD data suggest that the WR4 helix is significantly more stable than the control WR5, while RW4 is slightly less helical than the control RW5 (Table 2). Since in each case, there is a single Trp side chain present, its intrinsic differential effect on the CD spectra should be small. The CD data of the first series peptides are consistent with the hypothesis that (i, $i + 4$) Trp/Arg interactions stabilize α-helix. However, Trp and Arg residues interact to stabilize helix only in the N→C orientation in our model system. In the second series of peptides, both EFR4-4 and EFR5-4 are comparable in helicity to the corresponding controls EFR4-5 and EFR5-5. Thus, the

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Table 2. Observed Helicity and Energetics for the Peptides

peptide	$-[\theta]_{222} \times 10^{-3}$ (deg cm^2 dmol^{-1})[a]	f_H[b]	$\Delta\Delta G$ (kcal mol^{-1})[c]
WR5	11.1	0.347	0
WR4	13.5	0.422	−0.4
RW5	9.66	0.302	−
RW4	8.68	0.271	/
EFR4-5	20.8	0.612	−
EFR4-4	20.4	0.600	0
EFR5-5	18.3	0.538	−
EFR5-4	18.1	0.532	0

[a] In 10 mM phosphate buffer pH 7 at 4 °C. The peptide concentrations are 50 μM as determined by tyrosine or tryptophan absorbance at 275 nm. [b] The relationship between fraction helicity and molar ellipticity is $f_H = [\theta]_{222}/[\theta]_{222}^{\circ}$, where $[\theta]_{222}^{\circ} = -34\,000$ or $-32\,000$ is the estimated molar residue CD signal at 222 nm for an α-helix of 24 residues or 18 residues. [c] The free energies of side-chain interactions were computed using an algorithm based on the Zimm−Bragg helix−coil transition model, with the nucleation parameter $\sigma = 0.004$ and values of the helix propensities from amino acids Ala, Trp, Arg, Glu, Phe, Tyr, and Orn.

corresponding Phe-Arg interaction is not detected by CD spectra. Table 2 summarizes the CD data and free energies of the peptides relative to those of the controls. At this point the results show that properly positioned cation and aromatic residues on solvent-exposed surface can provide free energy for peptide/protein stabilization. The results imply that Trp is a better aromatic residue than Phe in the ability to form cation−π interactions, consistent with other experimental studies.[13,15−18,60]

NMR Analysis. NMR can provide a wealth of important structural information for the peptides studied. For the purpose of this study, we are interested in the interactions between the side chains of Arg and aromatic residue Trp or Phe. An intrinsic feature in the design of the peptides is that we can assign proton signals for the side chains of these residues straightforwardly through TOCSY spectra. Figure 2a shows a strip of the TOCSY spectrum for the R12 side chain in WR4. Only characteristic signals for the indole side chain of W8 appear in the aromatic region of the ^{1}H spectrum. Figure 2, b and c, shows that the aromatic ring of W8 makes extensive contacts with the R12 side chain as evinced by the strong NOE cross-peaks between W8 aromatic and R12 side-chain protons. For a comparison, Figure 2d shows the assignment of the R12 side chain in WR5, where we see no NOE interactions at all between W7 and R12 as shown in Figure 2, e and f. The only NOEs shown in these regions are those between the aromatic protons of W7 and its own side-chain protons. The NMR data for WR4/WR5 are consistent with the CD data and provide independent evidence that Trp and Arg can interact and stabilize the α-helix in the $(i, i + 4)$ positions.

For the other two peptides RW4/RW5 in the series, the CD data show no free energy gain from possible $(i, i + 4)$ cation−π interactions. It is important to know whether the side chains of Arg and Trp in RW4 are still close in space. Figure 3a shows the strip of the TOCSY spectrum for the side chain R8 of RW4. As shown in the NOESY spectrum of RW4 (Figure 3, b and c), there is only one very weak NOE interaction between the side-chain protons of the two residues. This suggests that our failure to detect a significant free energy contribution to the stability of the peptide reflects the lack of any substantial interaction. Figure 3, d and e, shows regions of the TOCSY and NOESY spectra for peptide RW5. The strip of the TOCSY spectrum shows the assignment of the R8 side chain; as expected there are no NOE cross-peaks between the side-chain protons of R8 and those of W13. Thus, for the four peptides in this

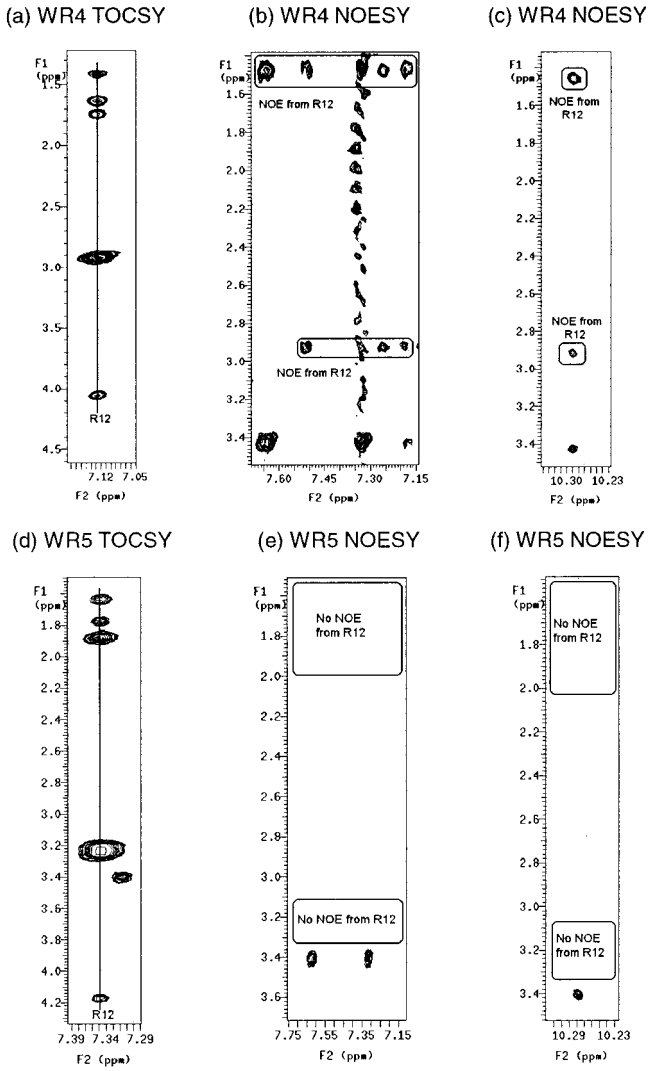

Figure 2. Regions of TOCSY and NOESY spectra for WR4 and WR5 peptides. The strips of TOCSY spectra show the assignment of the Arg side chains of the peptides. The shown NOESY spectra are the expanded regions where the possible NOE cross-peaks between Arg side chain and the Trp indole ring protons could be identified.

series, NMR and CD data present a coherent picture: the Trp and Arg side chains interact in a strongly orientation-dependent manner.

For the EFR peptides, the NMR spectra show diagnostic features of typical helical peptides. As an example, the fingerprint region of the TOCSY spectrum and the amide region of the NOESY spectrum for EFR4-5 is shown in Figure 4, a and b. Figure 4a shows the assignment of all non-alanine residues and a number of alanine residues. In Figure 4b strong i to $i + 1$ amide−amide NOEs are detected across the whole backbone chain of the peptide, with some weak to medium NOEs between i and $i + 3/i + 4$. Consistent with the CD spectra, this points to a large population of each peptide being in α-helical conformation.

Figure 5 shows regions of the TOCSY and NOESY spectra for peptides EFR4-4 and EFR5-4. In detail, Figure 5, a and b, shows strips from the TOCSY spectrum of EFR4-4 with the assignments for the side chains of R16 and E8. In the NOESY spectrum of Figure 5c, extensive and strong NOE interactions are seen between the side-chain protons of E8/R16 and those of F12. Figure 5, d−f, shows the corresponding TOCSY and

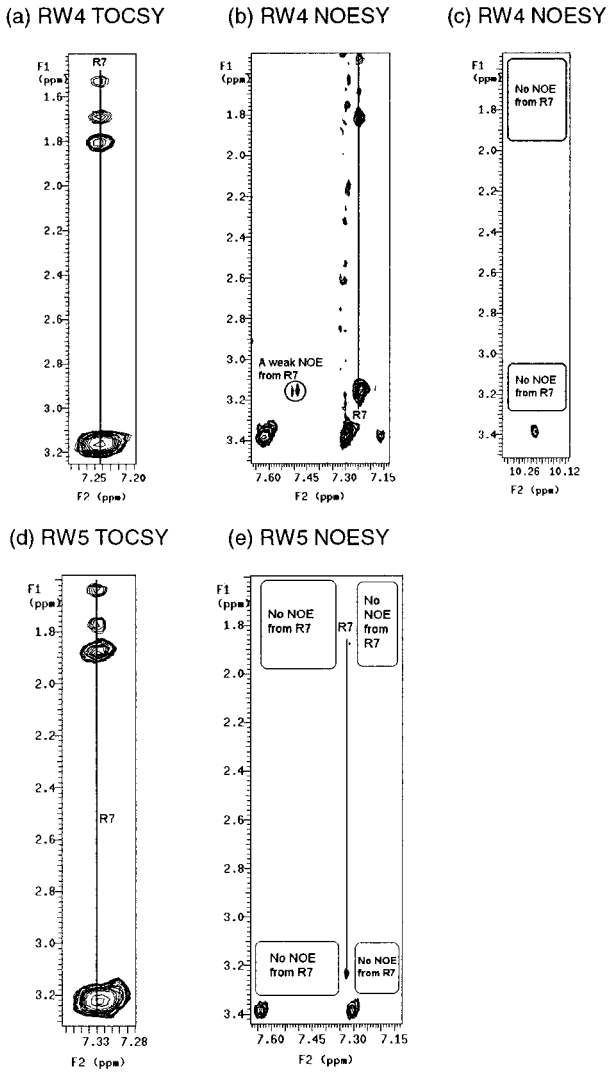

Figure 3. Regions of TOCSY and NOESY spectra for RW4 and RW5 peptides. The strips of TOCSY spectra show the assignment of the Arg side chains of the peptides. The shown NOESY spectra are the expanded regions where the possible NOE cross-peaks between Arg side chain and the Trp indole ring protons could be identified.

NOESY spectra for EFR5-4. Figure 5, d and e, shows the assignments of the E7 and R16 side chains. In Figure 5f there is at least one strong NOE cross-peak between aromatic protons of F12 and the side chain of R16 but no NOE from E7. In the sequences of both peptides, Phe and Arg are spaced at (i, i + 4) positions, and the NOESY spectra of both peptides show strong NOE interactions between the side-chain protons of Phe and Arg residues. However, the CD data in Table 2 suggest that these interactions fail to provide energetic stabilization to the peptides compared to that for the control peptides EFR4-5 and EFR5-5. We note that there are no NOEs detected between the Phe and Arg side-chain protons in EFR4-5 and EFR5-5 (data not shown) as expected.

Salt Titration. Neutral salts were added to the peptide solutions to assess the dependence of cation−π interactions on ionic strength. In the reference peptides WR5 and RW5, clear effects of added salts can be seen in the CD spectra:[75,77] helicity increases with added neutral salts as has been found previously (Figure 6). The peptides with (WR4) or potentially with (RW4) cation−π interactions show a similar trend in the salt titration CD measurement. We see no screening effect of salt on the cation−π interaction. The effects differ from those on salt bridges, for which neutral salts screen the interaction and peptides with salt bridges lose helicity relative to (or gain less helicity than) the control peptides as salt is added.[75,77] In the EFR peptides, both pairs behave similarly in terms of the dependence of helicity on salt concentration: chloride salts initially stabilize helix in EFR5-4 and its control peptide EFR5-5 up to about 1 M and then become destabilizing due to their apparent chaotropic effect. Similar but smaller effects were detected for both EFR4-4 and its control peptide EFR4-5 (see Figure 3b of ref 18).

Discussion

In a survey of crystal structures Gallivan and Dougherty[59] found roughly one favorable cation−π interaction for every 77 residues. This study showed that there is a preference for Arg over Lys for cationic residues and a preference for Trp over Tyr/Phe for the aromatic partner. About 20% of all Arg residues and about 26% of all Trp residues form a stabilizing interaction on the basis of the energetic criteria. The percentages would be much higher if the analysis were based only on geometric definitions: for example, over 70% of all Arg residues are near an aromatic residue. They also noted a large preference for cation−π interaction between i and i + 4 residues in helical structures. Another survey also by Gallivan and Dougherty[78] revealed that many of the energetically significant cation−π interactions are exposed to solvent, consistent with an earlier conclusion by Flocco and Mowbray.[33]

Peptide models have played a major role in efforts to define the contribution of specific amino acid side-chain interactions to stabilizing helical structures. In peptides the background can be specified precisely, the effects of weak interactions between side chains can be readily detected by CD or NMR, and quantitative free energy values can be derived by fitting CD spectral data to helix−coil transition models. Examples include hydrogen bonds,[1,2,79] salt bridges,[4,75,80] aromatic and hydrophobic interactions,[5,6,81] helix-capping,[82−84] and so forth. We demonstrate here that model peptides are also an ideal system to study the solvent-exposed cation−π interaction on the surface of the helix.

Reports on cation−π interaction energies in the gas phase from both experimental results[19−22] and calculations[12,54] suggest that the magnitude ranges from −10 to over −30 kcal/mol in different systems. However, the solvation energy of a cation is of the same order or even larger in magnitude[38,47] than the interaction energy in the gas phase. Hence, the final free energy contribution from the cation−π interaction is determined by the balance of two opposing influences: a favorable attraction between the cation and aromatic groups and an unfavorable energy penalty from desolvation. A computational study of

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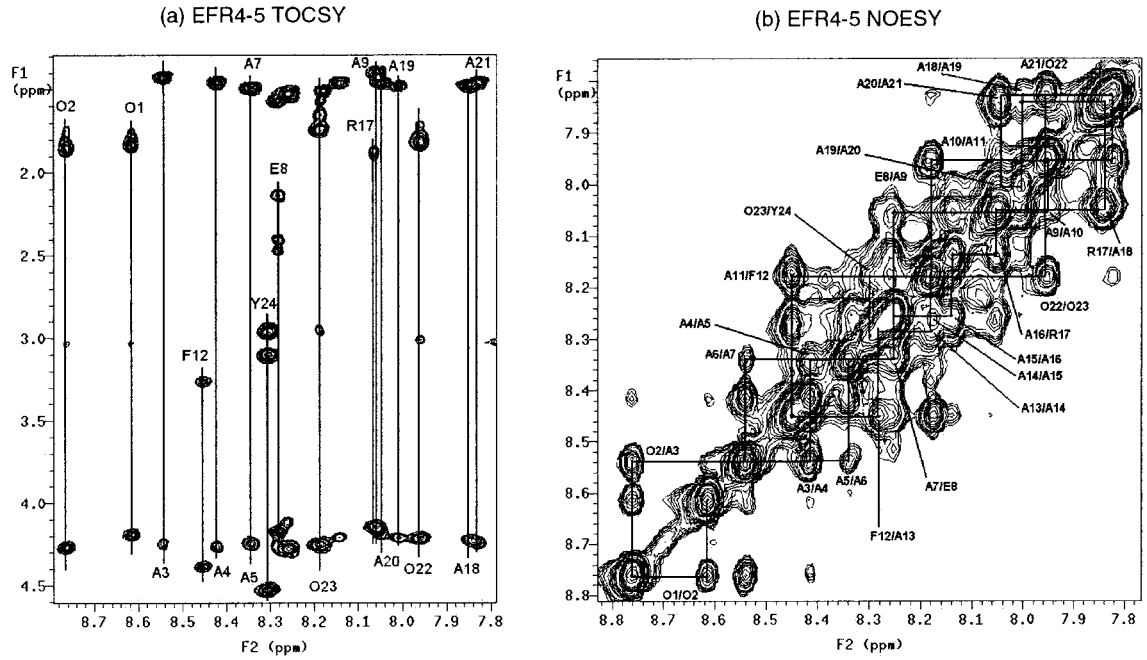

Figure 4. (a) Fingerprint region of the TOCSY spectrum for EFR4-5 peptide. The assignments of each residue of the peptide are shown. (b) Amide region of the NOESY spectrum for EFR4-5. The connectivity between *i* and *i* + *1* amides in the peptide backbone is shown.

cation−π interactions in aqueous media revealed that a cation−π interaction can contribute as much as −5.5 kcal/mol in water, which is much larger than the −2.2 kcal/mol found for a salt bridge.[78] Experimental studies in protein systems using double mutant cycle analysis[13,17] or in model peptide systems by CD measurements and pH titration[14,15] revealed a cation−π contribution of −0.5 to −1 kcal/mol, depending on the systems studied. These numbers are quantitatively well below those from theoretical calculations.

In an early study on barnase[13] an aromatic-histidine cation−π interaction between Trp94 and His18 was found to contribute −0.8 to −1 kcal/mol to the stability of the protein by comparing the interactions from both neutral and charged histidine. While histidine serves as a system that is relatively easy to manipulate, a statistical survey of the PDB pointed out that the most frequent cation−π interactions involve Arg and Trp residues. Furthermore, any change in the protonation state of a His residue can potentially cause local conformational arrangements, a problem faced by altering any specific residue inside a protein in mutational studies. This effect is hard to evaluate even if detailed structural data are available.

Fernandez-Recio et al.[17] studied the energetics of a His-Phe cation−π interaction in apoflavodoxin using double cycle mutations and monitoring the pK_a shifts of His by NMR. They concluded that the His-Phe cation−π interaction contributes about −0.5 kcal/mol to the stability of the protein, well below theoretical estimates. Nevertheless, local conformational rearrangements could potentially affect other noncovalent interactions, so that once again the effect is very hard to dissect and quantify.

An earlier peptide study analyzed the ability of His and Trp to interact on the surface of an α-helix.[15] The authors found that W→H stabilizes in an (*i*, *i* + 4) orientation only, with a Δ*G* of −0.8 kcal/mol, slightly higher than the value their group reported for the His-Phe cation−π interaction, which is expected due to the properties of the indole ring compared to benzene.[54] Neither the (*i*, *i* + 3) nor the H→W orientation stabilizes helix. However, intrinsic in the design of these peptides, there are potential artifactual interactions involving R and H, E, or W that may influence the results.

Baldwin's group[14] used a model helical peptide to study the interaction between Phe and His (oriented from N→C). Titration of His in this peptide series demonstrates a decrease in helicity with the decrease of pH as measured by CD whether the interacting residues are positioned at the end or middle of a helix. They did not quantify the interaction energy, and their model (AAKAA)*n* background allowed for additional interactions between the K and F residues in some peptides and between the K and H residues in others, which may or may not contribute to the interactions being investigated.

Ting et al.[16] engineered a cation−π interaction in the interior of staphylococcal nuclease (SNase) using unnatural amino acid mutagenesis. They assigned a value of −2.6 kcal/mol to this cation−π interaction. However, this number depends on the accuracy of estimation of the differential solvation energies between different mutants. Furthermore, it is unclear whether the engineered cation−π interaction in SNase is mono-, di-, or tridentate, and therefore whether the value of −2.6 kcal/mol should be divided by 2 or 3 to give −1.3 or −0.87 kcal/mol, respectively, for the energetic value of a unitary cation−π interaction.

Gallivan and Dougherty[78] concluded that a single interaction between an Arg and an aromatic residue could stabilize protein structure by more than −5 kcal/mol, and demonstrated statistically that cation aromatic contacts occur frequently at positions of (*i*, *i* + 4), implying that cation−π interactions are common in α-helices.[59] This and other experimental studies suggest that these interactions do not provide as large an energetic gain as they calculated. The results in our model helical peptides demonstrate that the interaction of Trp→Arg (N→C) at positions of (*i*, *i* + 4) contribute about −0.4 kcal/mol to the peptide

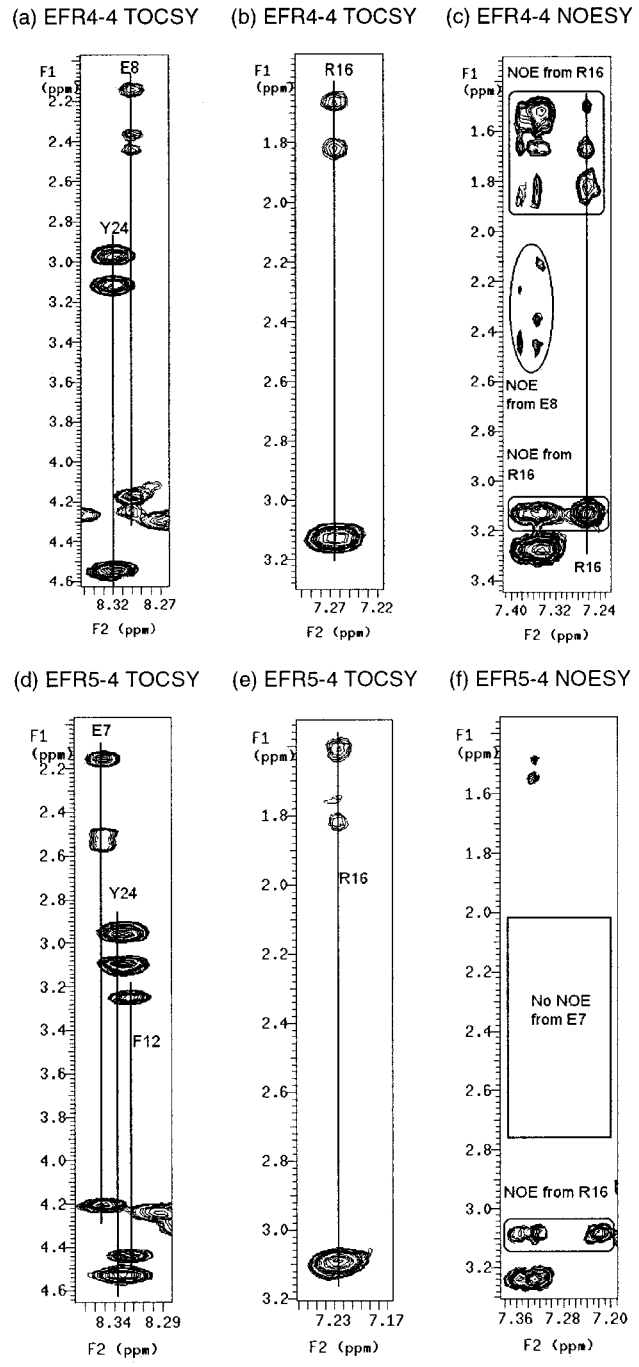

Figure 5. Regions of TOCSY and NOESY spectra for EFR4-4 and EFR5-4 peptides. The strips of TOCSY spectra show the assignment of Glu and Arg side chains of the peptides. The shown NOESY spectra are the expanded regions from which the possible NOE cross-peaks between Glu/Arg side-chain and the Phe aromatic ring protons will be identified.

stability, while we fail to detect any significant free energy gain from putative Arg→Trp (N→C) and Phe→Arg (N→C) cation−π interactions.

Can we rationalize our present results and reconcile the significant difference between theoretical calculations and experimental measurements? The CD and NMR data demonstrate that there is substantial cation−π interaction between Trp and Arg when the residues are in Trp→Arg (N→C) orientation, whereas neither CD nor NMR data detect any significant interaction from Trp/Arg when the residues are in the opposite orientation, Arg→Trp (N→C). This strong effect of orientation is not new or limited only to the cation−π interactions studied. We and other groups have reported a similar effect in the case of salt bridges.[75,80] We reason that in a standard α-helix, the side chains of larger residues orient toward the N terminus of the helix probably to satisfy the stereochemical requirement for each chiral residue. Arg has a longer side chain than Trp and hence can interact with Trp such that Arg's side chain lies in an energetically favorable conformation when two residues are positioned in the orientation Trp→Arg (i, i + 4). When two residues are oriented as Arg→Trp (i, i + 4), possibly some energetic strain within the peptide (either between side chains or between side chains and backbones) develops if they are forced to interact with each other. Our data, for RW4 with the orientation Arg→Trp (N→C), show that the combined free energetic cost from both entropy and steric strain is too large to overcome. A second factor in the orientation effect concerns the Arg side chain, which has been found to interact strongly with neighboring methyl groups of Ala that are N-terminal (but not C-terminal) to it (Garcia and Sanbonmatsu, private communication). This preference would naturally favor pairwise interactions with Arg as the partner in the C-terminal direction, and not the opposite.

In the series of EFR peptides, we detect NOEs by NMR yet fail to observe any stabilizing effect from CD data. All four of these peptides have the orientation Phe→Arg (N→C). Thus, the orientation effect seen in RW4 is not the reason. A more likely explanation is that the penalty paid for desolvation and entropy is comparable for the cation−π interactions in Trp/Arg and Phe/Arg, while the energetic gain from Trp/Arg is absolutely greater than that from Phe/Arg according to both gas-phase experimental results and theoretical calculations.[19−22,54] Hence, if we detect a stabilizing value of −0.4 kcal/mol for Trp/Arg, it is not surprising to find that the effect for Phe/Arg is smaller, perhaps beyond the detection limit in CD measurements, which we estimate to be ±0.1 kcal/mol. The only puzzle is why the theoretical predictions deviate so significantly from the experimental results: computed estimates of the interaction energy exceed all the experimental data. One possible source is from the uncertainty associated with estimating the energetics of solvation/desolvation processes in water. A less obvious issue is that in the theoretical calculations almost all studies assume the interacting partners are in an optimized geometry. On one hand this is advantageous because it makes results from different calculations comparable to each other. On the other hand, in a system as complicated as a protein, many interactions contribute cooperatively to the overall stability of the system with each individual interaction being weak and compromised to some extent so as to allow a more complete set of interactions to achieve the most favorable global stabilization for the whole system. This analysis suggests that for each individual interaction within a protein, the geometry may not be optimized individually. Hence, the results on cation−π interactions based on an idealized and optimized geometry may be significantly larger than the experimental results and may be considered as an upper limit.

Conclusions

We have used a combination of circular dichroism and nuclear magnetic resonance to evaluate the helix content of two series of peptides in which a Trp/Arg or Phe/Arg was placed at i, i +

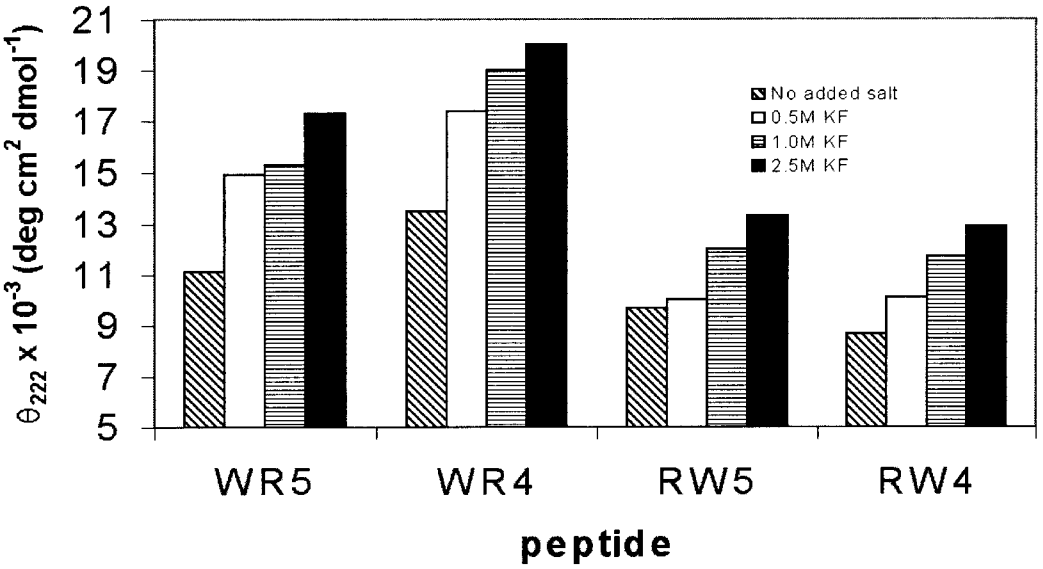

Figure 6. Salt concentration dependence of the observed helicity for the Trp/Arg peptides. Comparison of the effect of increasing KF concentration on the cation−π interaction in model peptides.

4 in N→C/C→N orientation. Analysis of the free energy contribution of the cation−π interactions using a modified Zimm−Bragg multistate helix−coil transition model shows that the solvent-exposed Trp→Arg (i, $i + 4$) interaction in helices can contribute about −0.4 kcal/mol to helix stability, while no free energy gain is detected if the two residues are positioned otherwise, Arg→Trp (i, $i + 4$). We have also investigated Phe/Arg (i, $i + 4$) cation−π interactions and found that it provides negligible free energy to the stability of the peptides studied. The results suggest that Trp→Arg interactions are indeed stronger than Phe→Arg, and are not sensitive to the screening effect of adding neutral salt as has been observed for salt bridges.

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