Polar Interactions with Aromatic Side Chains in α-Helical Peptides: Ch···O H-Bonding and Cation−π Interactions

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The native structure of proteins is stabilized by a large number of individually weak forces; a complete understanding of folding implies the need to evaluate the contribution of each of these, including ion pairs, solvent interactions, and H-bonds.[1] While the traditional picture of globular proteins emphasizes burial of nonpolar side chains to form a hydrophobic core, close proximity of aromatic side chains and positive or negative charges is a feature of many protein structures; the crystal structure of the HIV gp120 envelope glycoprotein and CD4 glycoprotein complex reveals multiple interactions between a Phe and Arg of the receptor CD4 with conserved Asp, Glu, and Trp residues from gp120.[2] Two types of selective interactions between aromatic and polar residues have been identified: (i) nonclassical H-bonding between weakly acidic aromatic C−H groups and electronegative acceptors[3] and (ii) so-called cation−π association between the face of an aromatic ring and positive charges.[4] C−H···O bonding was inferred initially by Pauling on the basis of a thermodynamic argument,[5] and confirmed by IR spectroscopy, X-ray diffraction and neutron diffraction of crystals of small organic molecules.[6] C−H···O hydrogen bonding has been implicated in stabilizing many protein structures, by C^{α}−H groups and other C−H groups adjacent to electron-withdrawing amino and carbonyl groups.[7] Theoretical and experimental studies have also demonstrated the ability of aromatic C−H groups to act as hydrogen-bond donors in gp120.[2,3] A theoretical study of benzene and formamide suggests that the aromatic C−H can interact with a CO group with a free energy in the range of 0.6−1.5 kcal/mol.[3c] The second type of interaction is referred to as a cation−π association, whereby a positively charged ion associates with the face of the π ring system via a quadrupolar interaction.[4b] Recent calculations imply that cation−π interactions with Lys or Arg and aromatic residues stabilize proteins by 2−3 kcal/mol more than salt bridges because of the lower extent of desolvation required for access to the π ring.[4f]

EFR5-5 Ac-OOAAAA**E**AAAA**F**AAAA**R**AAAAOOY-NH_2
EFR5-4 Ac-OOAAAA**E**AAAA**F**AAA**R**AAAAOOY-NH_2
EFR4-5 Ac-OOAAAAA**E**AAA**F**AAAA**R**AAAAOOY-NH_2
EFR4-4 Ac-OOAAAAA**E**AAA**F**AAA**R**AAAAOOY-NH_2

Figure 1. Peptide sequences: A = alanine, O = orninthine, E = glutamic acid, F = phenylalanine, R = arginine, Y = tyrosine, Ac = acetyl.

Given the potential impact of these interactions on the stability of native proteins, we have determined the free energy of interactions between Phe and Glu or Arg side chains by positioning them on the surface of model peptides and measuring their effect on helix content. The free energies of a variety of side chain−side chain interactions have been calibrated by spacing the groups appropriately in short-helix-forming peptides.[8] The dependence of helix content on spacing allows this method to probe interactions between pairs or combinations of side chains with high sensitivity: interactions as weak as 400 J/mol have been evaluated using this system,[8c,f] including ion pairs,[8b−g] H-bonds,[8g,h] hydrophobic interactions,[8i−l] and helix-capping effects.[9] Figure 1 lists the four peptides used in this study: in EFR4-5 the Phe and Glu side chains can interact on the surface of a helical model peptide in water; in EFR5-4 the Arg and Phe side chains can interact; EFR4-4 considers the possibility that the three residues can interact cooperatively, via polarization of the aromatic ring; in EFR5-5, the control peptide for this study, neither the Arg nor the Glu side chain can interact if the peptide is α-helical. Effects of N−C directionality are not considered in this series of models.[8h] However, other peptide models have demonstrated the ability of Arg side chains to form stabilizing interactions in the C→N orientation.[4g,8b] The far UV CD spectra show that all four model peptides are highly helical, with characteristic minima at 222 and 208 nm, and an isodichroic point at 202 nm (available upon request).[8a] The CD signals are concentration-independent over a range from 10 to 400 μM; a related peptide containing 13 contiguous alanine side chains was shown to be monomeric by equilibrium centrifugation.[10] Thus, the helical structure in these peptides is intramolecular under the conditions studied. EFR4-4 and EFR4-5 are significantly more

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Table 1. Helicity and Energetics of Peptides

peptide	$-[\theta]_{222} \times 10^{-3}$ (deg cm^2 dmol^{-1})[a]	f_H[b]	$\Delta\Delta G$ (kcal mol^{-1})[c]
EFR4-5	20.8	.61	−0.53
EFR4-4	20.4	.61	−0.53
EFR5-5	18.3	.54	−
EFR5-4	18.1	.53	0.00

[a] In 10 mM phosphate buffer pH 7 at 4 °C. The peptide concentrations are 50 μM as determined by tyrosine absorbance at 275 nm. [b] The relationship between fraction helicity and molar ellipticity is $f_H = -[\theta]_{222}/34\,000$, where −34 000 is the estimated molar residue CD signal at 222 nm for an α-helix of 24 residues.[8b] [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 for amino acids Ala, Glu, Arg, Phe ,and Orn. Details are presented in ref 8b.

helical than the control peptide, EFR5-5, while EFR5-4 is comparable in helicity to the control. Table 1 summarizes the CD data and free energies of the peptides relative to the control.[8b] Composition is conserved in the series, avoiding standard state differences that make it difficult to evaluate individual interactions from peptide models.

These results are consistent with the hypothesis that *i*, *i* + 4 Phe-Glu interactions stabilize α-helix. However, the corresponding Phe-Arg interaction does not. In addition a longer-range Glu-Phe-Arg interaction can be ruled out, since EFR4-4 and EFR4-5 have comparable helicity. The NOESY data show that both the Glu and Arg are close enough to make contacts with the Phe in the *i*, *i* ± 4 position, despite that fact that the Arg does not contribute to any additional helicity (Figure 2).[11] A statistical study of cation−π interactions in proteins as well as theoretical calculations implies that these are stabilizing,[4a] particularly at solvent-exposed positions.[4f] Contrary to expectation this interaction in our peptide model in water fails to increase helicity. Chemical shifts of the two Phe C_β hydrogens are degenerate, demonstrating that the ring is freely spinning.[11b] Flipping of the ring might impede the ability of the guanido group of arginine to associate with either face of the ring; in any case the interaction is not sufficient to compensate for the entropic cost of the ring motion. On the other hand the Cξ of the Phe that interacts with glutamate may be less affected by rotation around the Cβ−Cξ axis of the side chain. A Phe-His interaction studied in a model peptide system appears to be stronger than the Phe-Arg one we study here,[8m] due perhaps to the ease of orienting the rigid His ring relative to the flexible Arg side chain.

pH and salt titrations provide additional information concerning the interactions involved (Figure 3). Neutralization of Glu increases helicity in the control peptide; this is consistent with the higher helix propensity of Glu^0 relative to that of Glu$^-$.[8g]

EFR4-5 also increases in helix content at pH 3, but by less than the control. Thus, part of the helix stabilization at neutral pH is electrostatic in origin, as was found in the case of Phe-His.[8m] Screening the electrostatic interactions by increasing salt concentration shows a similar effect. Chloride salts initially stabilize helix up to about 1 M and then become destabilizing due to their chaotropic effect.[8c] This is seen in EFR5-5; both EFR4-4 and EFR4-5 show a smaller effect, indicating screening by the salt of the stabilizing interaction(s) present. Both experiments are consistent with the presence of an electrostatic contribution in the Glu-Phe interaction. Change in salt concentration affects helicity of EFR5−4 as it does the control EFR5−5. Thus, we do not detect an electrostatic interaction between the Phe and Arg side chains.

(11) (a) Experimental procedures are the same as in ref 8b. (b) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.

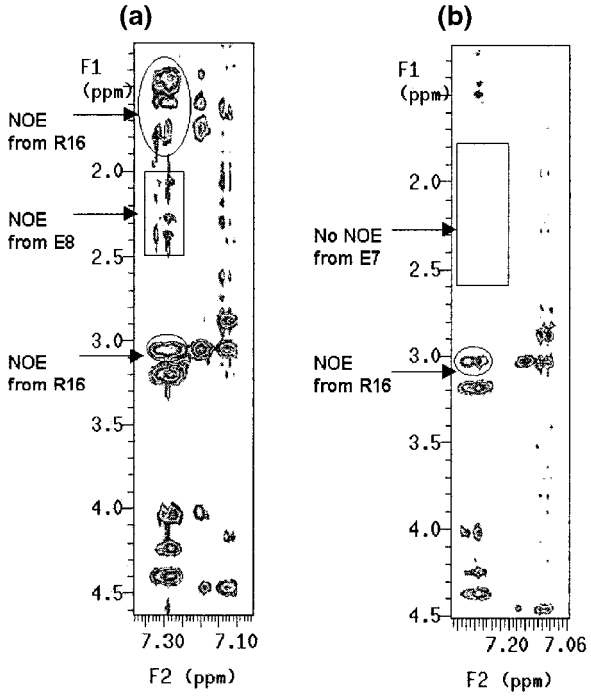

Figure 2. Region of the NOESY spectrum of (a) EFR4-4 and (b) EFR5-4, indicating the interaction between aromatic side-chain protons of F and the side-chain protons of E or R. For EFR5-5 (data not shown), there is no NOE interaction from either E7 or R17.

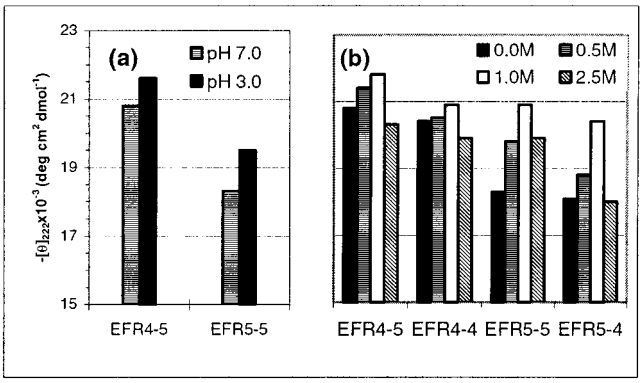

Figure 3. (a) The effect on the helicity of EFR4-5 upon titration of the Glu compared to the control, EFR5-5, in 10 mM phosphate buffer at pH 7 and pH 3 at 4 °C. (b) The effect on helicity of the model peptides by increasing NaCl concentrations in 10 mM phosphate buffer pH 7 at 4 °C.

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