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Effect of Halogenation on Edge–Face Aromatic Interactions in a β-Hairpin Peptide: Enhanced Affinity with Iodo-Substituents

Chad D. Tatko and Marcey L. Waters*

Department of Chemistry, CB 3290, University of North Carolina, Chapel Hill, North Carolina 27599

mlwaters@email.unc.edu

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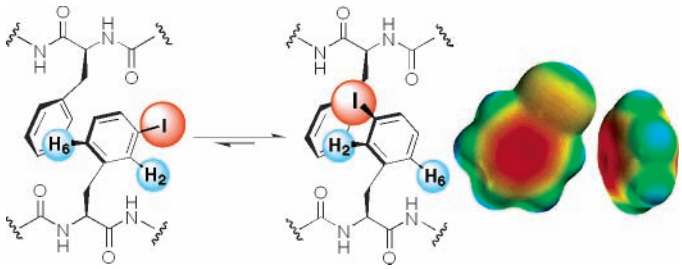
ABSTRACT



In a model β-hairpin peptide, we have found that the favorable interaction of cross-strand aromatic rings can be enhanced by up to 1 kcal mol^{-1} with halogen substituents. It appears that the polarizability of the halogen atoms accounts for the increase in stability and that there is a direct interaction between the N-terminal phenylalanine and the halogen atom. Thermal denaturation studies indicate that the interaction is enthalpically driven with an associated entropic cost. These findings have relevance to areas of molecular recognition and drug design.

C−X···π interactions, where X is a halogen, are prevalent in supramolecular structures such as host−guest systems and two-dimensional crystalline networks.[1] Recent studies of these interactions include work by Kobayashi et al., in which iodinated aromatic rings are bound more tightly than other aromatic guests within a cavitand in $CDCl_3$,[2] and a study by Dougherty, in which the recognition of neutral and cationic guests by a brominated cyclophane host in aqueous solution provides enhanced binding affinities.[3] Rotello and co-workers have demonstrated that a fluoride−π interaction is favorable for electron-deficient aromatic rings in $CDCl_3$.[4] In contrast, Hunter and co-workers have investigated the magnitude of the interaction of halomethyl groups with aromatic rings in $CDCl_3$ and found that the interactions are repulsive.[5] The nature and magnitude of halogen···aromatic interactions have biological significance, since halogenation is a common method for improving binding of pharmaceutical leads. A relevant biological example of a halogen−aromatic interaction is that of the thyroid hormone receptor bound to the thyroid hormone triiodothyroxine, T_3, in which Phe 269 and Phe 272 interact with the 5′-iodo group via their edge hydrogens (Figure 1).[6] However, it is evident that the magnitude and driving force for these interactions are not yet well defined, particularly in aqueous solution.

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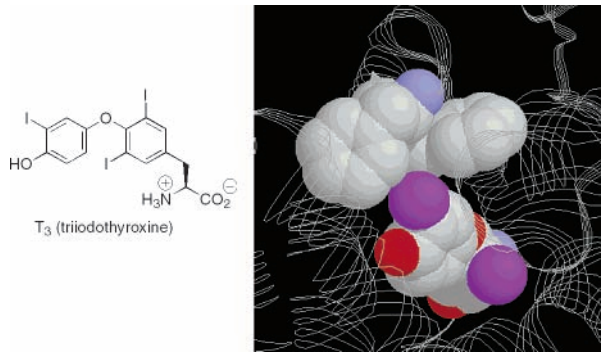

Figure 1. Structure of the thyroid hormone T_3 and crystal structure of T_3 bound to the thyroid hormone receptor indicating the interactions of the 5′-iodo group with Phe 269 and Phe 272. Phe 269, Phe 272, and T_3 are shown as space-filling models with oxygen in red, nitrogen in light blue, and iodine in purple (PDB code 1BSX).

We report here the investigation of halogen−aromatic interactions in the context of a β-hairpin peptide in aqueous solution (Figure 2). β-Hairpins have been shown to be useful

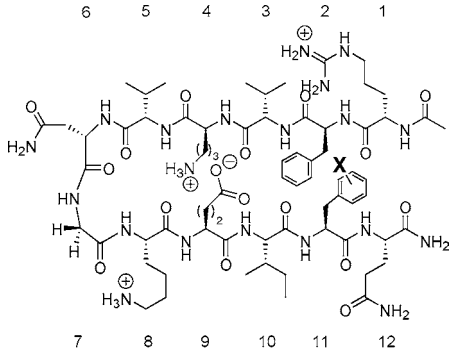

Figure 2. Peptide sequence used to probe the influence of substitution on edge−face interactions. In the case of iodo substituents, substituted Tyr was used in place of Phe 11.

model systems for the investigation of noncovalent interactions because the influence of a side chain−side chain interaction is reflected in the stability of the hairpin.[7,8] In this system, we find that aryl halides, and iodo substituents in particular, provide significant stabilization. The interaction energies correlate with both polarizability and hydrophobicity. However, thermal denaturation studies indicate that the interaction is enthalpically driven with a concomitant entropic cost. In addition, stronger interactions result in a decreased change in heat capacity, which is inconsistent with increased burial of hydrophobic surface area. Hence, these findings suggest that increased dispersion forces may be the source of the enhanced stability, which are related to increased polarizability. These studies provide insight into the nature of halogen−aromatic interactions in aqueous solution and have relevance to medicinal chemistry and biomolecular recognition.

The peptide system used to investigate the effect of substitution on the edge−face interaction is the based on a previously reported β-hairpin with a Phe in positions 2 and 11 that demonstrated a lateral edge−face interaction,[8a] with the exception that the substituents of Phe 11 were varied in the current study (Figure 2). The peptides under investigation have an overall charge of +2 to promote solubility and prevent aggregation. The peptides also include an Asn−Gly turn sequence that has been shown to promote hairpin formation via a type I′ turn.[9] All peptides were characterized by MALDI mass spectrometry and NMR, including COSY, TOCSY, and ROESY. Variation of the aromatic edge−face interaction between positions 2 and 11 was monitored by the effect on β-hairpin stability, which is determined by comparison of H_α chemical shifts,[10] glycine chemical shifts,[11] and by NOE analysis (see Supporting Information). In our previous study we showed that Phe 11 interacted via its 2,6-hydrogens with the face of Phe 2.[8a] Hence, substitution at the 3-, 4-, and 5-positions was investigated to determine the effect on the edge−face aromatic interaction. A methyl substituent was also investigated as a hydrophobic control. All substituents occur on phenylalanine except for the iodo substituents that occur on tyrosine. To demonstrate that the presence of a 4-hydroxyl group does not affect the stability of these peptides, an F2Y11 cross-strand mutant was examined and shown to have a global peptide stability within the error of the F2F11 peptide, 40 and 39%, respectively (Table 1). Consequently, any difference between the peptide containing 3-I tyrosine and other substituted Phe residues can be attributed to the iodo substituent and not the hydroxyl group.

The stabilization gained from the halogen substitutions is shown in Table 1. The maximum stability achieved from a monosubstituted aromatic ring is 0.54 kcal mol^{-1} and up to 1.05 kcal mol^{-1} for the system with two iodo substituents. Interestingly, energetic gains over the unsubstituted Phe are only achieved when substitution is in the 3- and 3,5-positions and not the 4-position. Moreover, there is no linear correlation between σ_{meta} or σ_{para} and the observed stabilities of the 3-substituted peptides (see Supporting Information). In

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Table 1. Stability of β-Hairpin Peptides with Substituents on Phe 11 at 298 K as Determined from the Gly Chemical Shift Method[11]

substituent	Gly $\Delta\delta$	fraction folded	$\Delta G°$	$\Delta\Delta G°$
3-Me	0.286	0.51	−0.03	−0.30
H	0.216	0.39	0.27	–
3-F	0.243	0.44	0.15	−0.12
3-Cl	0.295	0.53	−0.07	−0.34
3-Br	0.325	0.58	−0.2	−0.47
3-I-Tyr	0.340	0.61	−0.27	−0.54
3,5-F_2	0.259	0.49	0.03	−0.24
3,5-I_2-Tyr	0.433	0.78	−0.74	−1.01
4-Me	0.214	0.384	0.28	0.01
4-OH	0.213	0.382	0.28	0.01
4-F	0.224	0.402	0.24	−0.03
4-Cl	0.217	0.390	0.27	0.00

[a] Units of ΔG are kcal mol^{-1}. Error is $\pm$0.05 kcal mol^{-1}

contrast, the stabilities correlate quite well with both polarizability[12] and hydrophobicity,[13] as determined from substituted benzene (Figure 3).[14] These findings argue against a substantial inductive effect on the interaction energy and suggest that the increased stability observed with the 3-substituted Phe may be the result of a direct halogen−Phe interaction.[15]

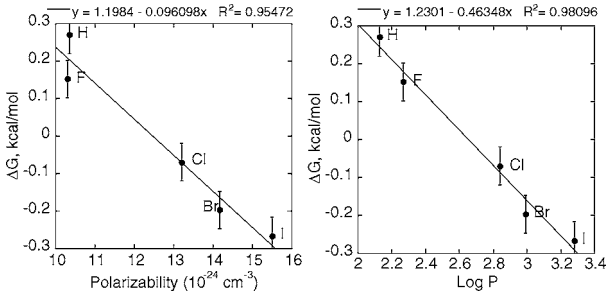


Figure 3. Correlation between the stability of the 3-substituted peptides and (a) polarizability and (b) Log *P*. Log *P* is the partition coefficient for substituted benzenes between water and *n*-octanol.[13]

To investigate the effect of the halogen on the geometry of interaction between Phe 11 and Phe 2, we measured the upfield shift of the aromatic protons of Phe 11 (Table 2). In the parent peptide, the 2,6-protons on Phe 11 are shifted upfield by approximately 0.4 ppm relative to the random coil chemical shift.[4] This is due to close proximity of the degenerate 2,6-protons on Phe 11 to the face of the phenyl ring in position 2. Substitution in the 3-position results in inequivalency of the 2- and 6-protons in Phe 11, such that the favored geometry of interaction with Phe 2 can be determined by comparison of the upfield shift of H_2 and H_6 of Phe 11 (Scheme 1). In all 3-substituted systems, the 2-proton is shifted upfield to a greater extent than the 6-proton (Table 2). This indicates that there is a preference for the side of the aromatic ring that has the halogen substituent to interact with the face of Phe 2, supporting a direct interaction between Phe 2 and the halogen substituent on Phe 11. Interestingly, H_2 is not shifted upfield to as great an extent for the 3-iodo substituent as for others, despite the fact that it is the most stabilizing substituent. The large size of the iodo group may prevent H_2 from making as close a contact with the face of Phe 2 in favor of the halogen$\cdots$ aromatic interaction.

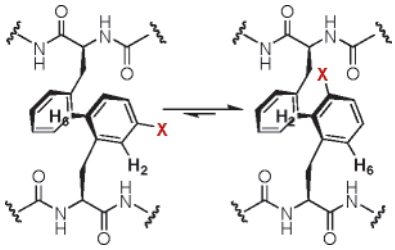
Given the apparent directionality of this interaction, which orients the halide substituent toward the Phe 2 ring (Scheme 1), we surmised that disubstitution of Phe 11 in the 3- and 5-positions should result in an interaction that is twice the magnitude of a single substitution because the two rotameric edge−face interactions are now equivalent. This is indeed the case. The 3-F substituent provides −0.12 kcal mol^{-1} over

Table 2. Upfield Shift of the Phe 11 Aromatic Protons for Peptides Containing 3-Substituents Relative to Random Coil Chemical Shifts at 298 K[a]

	$\Delta\delta$, ppm					% shift of H_2
substituent	H_2	H_3	H_4	H_5	H_6	(H_2/(H_2 + H_6))
3-Me	0.52	–	0.10	0.15	0.41	56
H	0.32	0.11	0.05	0.11	0.32	–
3-F	0.44	–	0.07	0.11	0.30	59
3-Cl	0.65	–	0.00	0.13	0.37	64
3-Br	0.74	–	0.10	0.15	0.40	65
3-I-Tyr	0.59	–	–	−0.10	0.20	74
3,5-F_2	0.44	–	0.07	–	0.44	–
3,5-I_2-Tyr	0.35	–	–	–	0.35	–

[a] Change in chemical shift of each aryl resonance was determined relative to 7-residue control peptides with the sequence Ac-NGKEI**X**Q-NH_2 (See Supporting Information).

Scheme 1. Equilibrium between the Two Inequivalent Edge−Face Interactions between Phe 2 and Substituted Phe 11



(12) Polarizability values were taken from: Lide, D. R. *CRC Handbook of Chemistry and Physics*, 84th ed.; CRC Press: New York, 2004; pp **10**-169 to **10**-173.

(13) Log *P* values were taken from: Lide, D. R. *CRC Handbook of Chemistry and Physics*, 84th ed.; CRC Press: New York, 2004; page **16**-44.

(14) It is interesting to note that the hydroxyl substituent in the 3-I-Tyr peptide does not influence its correlation with either polarizability or hydrophobicity.

(15) Direct interaction between the substituent and aromatic ring in an aromatic−aromatic interaction has been observed for two other model systems. See ref 4 and: Rashkin, M. J.; Waters, M. L. *J. Am. Chem. Soc.* **2002**, *124*, 1860−1861.

the unsubstituted Phe 11, whereas the 3,5-difluoro-substituted Phe 11 is −0.24 kcal mol^{-1} more stable than the parent peptide. Moreover, the 3-I substituent provides −0.54 kcal mol^{-1} relative to the unsubstituted case, whereas the 3,5-diiodo Phe 11 provides −1.01 kcal mol^{-1}. Indeed, the magnitude of stabilization of the β-hairpin gained by the incorporation of two iodo substituents is remarkable, given the modest change in structure. Interestingly, whereas the upfield shift of H_2 in 3,5-F_2-Phe 11 is the same as that of 3-F-Phe 11 (Table 2), the extent of upfield shifting of H_2 in 3,5-I_2-Phe 11 is actually less than that of 3-I-Phe 11. This may reflect a change in the geometry of interaction favoring the halogen···aromatic interaction over the CH···π interaction.

To further investigate the proximity of the 3-substituent on Phe 11 to the face of the aromatic ring in Phe 2, we examined the upfield shift of the 3-CH_3 substituent relative to its random coil chemical shift since it provides similar stability as a 3-chloro group and is NMR active. We found that the CH_3 resonance in the 3-methyl-Phe 11 is shifted upfield by 0.16 ppm relative to its random coil value, suggesting that it is in close proximity to the face of Phe 2. Moreover, the upfield shift of the 3-methyl substituent is greater than that observed for the 3-hydrogen of the unsubstituted peptide. Although this is not conclusive evidence, it is consistent with a direct interaction between the 3-substituent and Phe 2.

We performed thermal denaturation studies to determine the impact of the 3-substituents on the enthalpy and entropy of folding (Figure 4). Inspection of Table 3 indicates that as

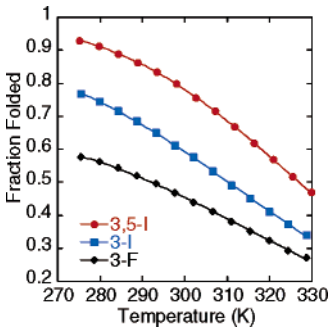


Figure 4. Thermal denaturation of the (a) 3-F-, 3-I-, and 3,5-I_2-substituted peptides at pH 4.2. The curve represents the best fit to the van't Hoff equation.[10] The temperature was calibrated with MeOH and ethylene glycol standards.

the β-hairpin stabilities increase from 3-F to 3-I and 3,5-I_2 substituents, the enthalpic term becomes more favorable with a concomitant entropic cost. An enthalpic driving force for folding is not expected if the hydrophobic effect is the primary driving force for folding of the β-hairpin. An enthalpic driving force for folding has been observed for a number of well-folded β-hairpins containing an aromatic cluster[8,16] and has been attributed to what Diederich has called the nonclassical hydrophobic effect.[17] Moreover, the

Table 3. Thermodynamic Parameters of Folding at 298 K as Determined from van't Hoff Analysis[a]

X	$\Delta H°$	$\Delta S°$	$\Delta Cp°$
3-F	−4280 (40)	−14.6 (0.1)	−90 (10)
3-I	−6250 (40)	−20.1 (0.1)	−60 (10)
3,5-I_2-Tyr	−8910 (40)	−27.2 (0.1)	−20 (10)

[a] Units: $\Delta H°$, cal mol^{-1}; $\Delta S°$, cal mol^{-1} K^{-1}; and $\Delta Cp°$, cal mol^{-1} K^{-1}. All parameters are determined from the fitting to the van't Hoff equation.[10] The error is determined from the fitting.

change in heat capacity decreases with increasing stability of the peptide. This is also unexpected if the classic hydrophobic effect is the primary driving force for folding, for which a *greater* change in heat capacity is expected with burial of more hydrophobic surface area. Thus, taken together, the increasing enthalpic drive and decreasing change in heat capacity with increasing peptide stability are suggestive of increased dispersion forces as the source of increased stability.

In conclusion, we have found that the direct interaction of a halogen atom substantially enhances an edge−face interaction in aqueous solution. The variation of 1 kcal/mol in going from the unsubstituted Phe 11 to the 3,5-diiodo substituent is remarkable for such a modest perturbation in structure. Moreover, it is similar in magnitude to the stability gained from a much more significant substitution of Ala for Trp found in another β-hairpin system.[18] The NMR data supports a direct interaction between the halogen and Phe 2, although we cannot differentiate between a halogen···π interaction and an ArH···halogen interaction. The interaction is enthalpic in nature and results in a significant decrease in the change in heat capacity upon folding, which has not been observed in other enthalpically driven β-hairpins. We expect that the thermodynamic gains from such modest variation in structure will have wide applicability in the fields of molecular recognition, supramolecular chemistry, and medicinal chemistry.

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Supporting Information Available: NMR assignments and characterization of all peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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