Aromatic interactions in model systems

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A thorough knowledge of noncovalent interactions is crucial to the understanding of biological complexity. One of the less well understood but significant weak interactions in nature is the aromatic interaction. Recent studies have provided new insight into the driving force, stability and selectivity of these interactions. The contribution of solvophobic and electrostatic interactions have been shown to be inextricably linked. Moreover, the influence of electrostatic and solvophobic components on the selectivity of aromatic interactions has been demonstrated.

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Abbreviation
Cha cyclohexylalanine

Introduction

Aromatic interactions are ubiquitous in nature. They are believed to provide stability to duplex DNA [1]; they have been proposed to contribute to the unique properties of thermophilic proteins [2•]; they may play a role in aggregation of amyloid β in Alzheimer's disease [3]; and they are common motifs in biomolecular recognition. Extensive work has focused on these interactions to determine their importance as a recognition element, including statistical analyses of the Protein Data Bank [4,5•,6–8], mutation studies in proteins [9] and protein–nucleic-acid complexes [10,11], and theoretical studies [12••]. Model systems have also proven to be an informative method of investigating the nature and significance of aromatic interactions as molecular recognition elements in biological and non-biological systems. This review presents a general background on aromatic interactions and recent studies of aromatic interactions in solution-phase synthetic and biological model systems that have advanced the understanding of this biologically important noncovalent interaction.

The nature and geometry of aromatic interactions

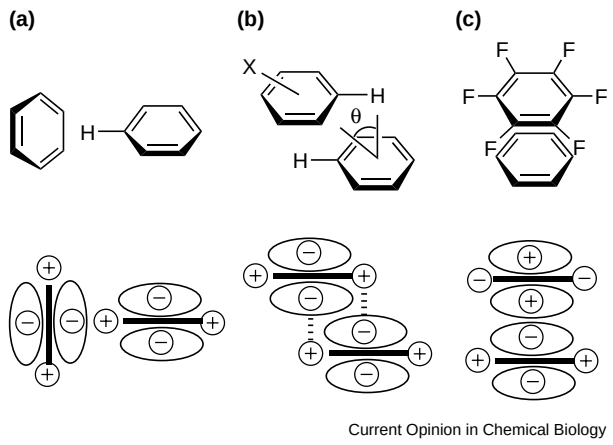
Aromatic interactions have been proposed to consist of van der Waals, hydrophobic and electrostatic forces [12••]. The relative contribution and magnitude of each of these components is still under investigation. This is complicated by the fact that aromatic groups interact in one of several geometries, depending on the nature of the rings involved. Nonetheless, aromatic interactions are intriguing molecular recognition elements because they are expected to be strong in water because of the hydrophobic component of the interaction yet, at the same time, the interaction should be selective if the electrostatic component is significant, thus providing the best features of both hydrophobic interactions and hydrogen bonding.

Several geometries are attractive, and have been proposed on the basis of the electrostatic component of the interaction (Figure 1) [13]. The electrostatic component has been proposed to arise from interactions of the quadrupole moments of the aromatic rings. Although benzene has no net dipole, it has an uneven distribution of charge, with greater electron-density on the face of the ring and reduced electron-density on the edge, which gives rise to the quadrupole moment. The edge-face geometry (Figure 1a), which can be considered a CH–π interaction, is found in benzene in the solid state, and is commonly observed between aromatic residues in proteins. The offset stacked orientation (Figure 1b) is also commonly found in proteins and is the geometry of base stacking in DNA. In this geometry, more surface area is buried, and the van der Waals and hydrophobic interactions are increased. This orientation appears to be more common when the electron density on the face of one or both rings is reduced. A third possible geometry is the face-to-face stacked orientation (Figure 1c). This is commonly observed with donor–acceptor pairs and compounds that have opposite quadrupole moments, such that the interaction between the faces of the rings is attractive. The benzene–perfluorobenzene interaction is an excellent example of this type of aromatic interaction, and has been calculated to provide –15.5 kJ mol^{-1} in stability [14]. In an elegant study, Cozzi and Siegel [15] have demonstrated the electrostatic contribution to aromatic interactions in the face-to-face stacked conformation. However, questions still exist regarding the importance of electrostatics relative to dispersion and hydrophobic forces in the edge–face and offset–stacked geometries, which are the geometries commonly found in nature.

Solvent effects on aromatic interactions

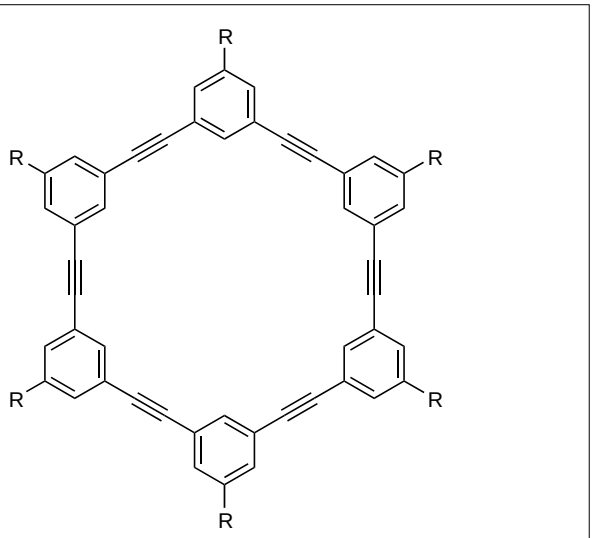
Several groups have recently published solvent studies on aromatic interactions to address the importance of electrostatic and solvophobic components in aromatic interactions. A solvent study by Cammers-Goodwin [16] and co-workers suggests that water interacts preferentially with the hydrogens of an aromatic ring, influencing the propensity for stacking. Moore and co-workers [17,18••] have designed both cyclic and linear systems made up of *m*-phenylene ethynylene units that spontaneously associate in polar solvents (Figure 2). The driving force for association is believed to be stacking of the aromatic rings. The fact that association occurs preferentially in polar solvents suggests that solvophobic forces influence this system significantly. However, folding is highly dependent on the nature of the substituents on the rings, suggesting an electronic tuning of the interactions

Figure 1



Geometries of aromatic interactions. (a) edge-face; (b) offset stacked; (c) face-to-face stacked.

Figure 2



Cyclic *m*-phenylene ethynylenes.

through minimization of repulsive quadrupole–quadrupole interactions [19]. Intriguingly, these molecules were also found to fold in aromatic solvents, which was unexpected. The same phenomenon has also been observed in *m*-diethynylbenzene macrocycles [20].

Gellman and co-workers [21] have probed the solvent effect on a simple aromatic interaction in the context of a secondary amide model system (Figure 2, R = H; Ar_1 = phenyl). In this system, the *E* to *Z* ratio of amide rotamers is influenced by the Ar_2-group and the solvent. When Ar_2 = H, the *Z*-isomer is favored as a result of reduced steric interactions between side chains relative to the *E*-isomer and the *E*:*Z* ratio is independent of solvent (water or chloroform). By contrast, when Ar_2 = naphthyl or biphenyl, the amount of *E*-isomer is fourfold higher in water than in chloroform. In addition, the amount of *E*-isomer increases with increasing temperature. Both the solvent dependence of the rotamer populations and the temperature dependence of the equilibrium constant are consistent with a hydrophobically driven association between the aromatics groups.

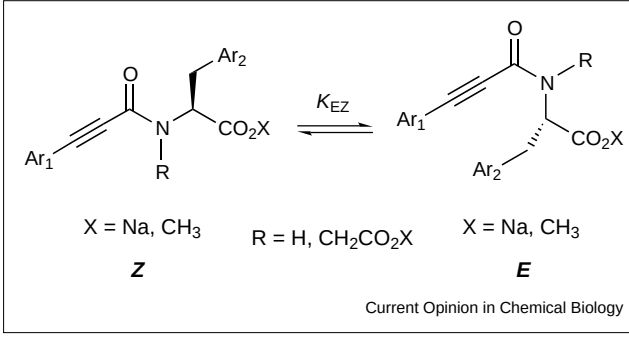
Iverson and co-workers [22••] have performed an elegant solvent study to address the nature of aromatic interactions. Three different aromatic pairs were shown to have varying degrees of sensitivity to solvent polarity, depending on the nature of the electrostatic interaction between the two rings (Figure 4). For the donor–acceptor pair, which interact in the face-to-face stacked orientation, a strong solvent effect was observed, in which the interaction was stronger in more polar solvents. This suggests that dispersion and/or hydrophobic interactions dominate. In the case of the *self*-association of the electron-poor aromatic (**1**, Figure 4), although there is the possibility of greater burial of surface area, these molecules are found to stack in the offset orientation in the solid state, as predicted by the electrostatic model. Moreover, the solvent effect was an order of magnitude smaller for this system, presumably because less surface area is buried. The *self*-association of the electron-rich aromatic ring (**2**), which was the weakest of the three interactions and is believed to interact in the edge–face orientation, showed almost no solvent dependence. Thus, it appears that the electrostatic component influences the geometry of the interaction, which in turn influences the magnitude of the solvent effect on the interaction. The range of strengths of these interactions in water were about –6.7 to –18.8 kJ mol^{-1}. It is also worth noting that this study demonstrates the potential for selective aromatic interactions.

Substituent effects on aromatic interactions

Hunter and co-workers [23] have designed a system for measurement of both edge–face and stacking interactions through the use of a chemical double mutant cycle (Figure 5). With this system, they have found that there is an electronic effect on edge–face interactions in chloroform. Changes in the substituents, X and Y, gave a linear free-energy relationship with interaction energies from +1.2 kJ mol^{-1} to –4.6 kJ mol^{-1}, with the unsubstituted edge–face interaction worth –1.4 kJ mol^{-1}. This is in contrast to previous work by Wilcox and co-workers [24,25], in which little electronic effect was observed and dispersion forces were suggested to be the most important component in edge–face interactions.

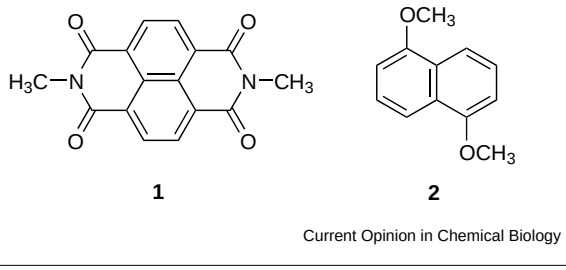
There have been some questions about the design of Hunter's system for double mutant cycles, including the rigidity of the 1,1-diphenylcyclohexyl subunit [26].

Figure 3



Gellman's system for measuring aromatic interactions in aqueous and organic solvents.

Figure 4



Aromatic compounds used in Iverson's solvent study.

Replacing this group with the more rigid 7,7-diphenylnorborane group results in an observed edge–face interaction energy near zero [26]. However, Hunter has recently established that his double-mutant cycle accounts for the lack of rigidity in the backbone and has shown that three different double mutant cycles all result in a value of approximately –1.4 kJ mol^{-1} for the interaction of two unsubstituted phenyl rings [27]. Resolution of this discrepancy will have to await further study.

Hunter has extended his double mutant cycles to the study of face-to-face stacking interactions [28]. In agreement with the electrostatic model proposed by Hunter and Sanders [13], Hunter has found that the interaction of anthracene with a 2,6-dimethylanilide derivative in a similar system to that in Figure 5 is slightly repulsive (+0.5 kJ mol^{-1}), whereas the interaction of the pentafluorophenyl ring with the electron-rich anilide system is modestly attractive (–0.4 kJ mol^{-1}) [28]. Further studies of the interactions of the pentafluorophenylamide with substituted anilides indicates that the interaction is only favorable for electron-rich rings, as expected [29].

Two groups have recently reported the direct interaction between substituents on one ring with the aromatic framework of the other. Rotello and co-workers [30] found that the position of a fluorine on a phenyl ring has a significant influence on its interaction with a flavin ring in chloroform. Because of the non-uniform electrostatic surface of a flavin ring, the fluorophenyl ring interacted in a favorable manner when the fluorine was in the *meta*- or *para*-position, but less favorably relative to the unsubstituted phenyl when the fluorine was in the *ortho*-position. This system provides a good example of polar interactions influencing the magnitude of aromatic interactions. Rashkin and Waters [31] have studied substituent effects in a model system for offset stacking between two phenyl rings in water. Although *para*-substituents on one ring showed little effect on interaction energies, electronegative substituents in the *meta*-positions gave larger interaction energies. Modeling suggested that the *meta*-substituents may interact directly with the electron-poor hydrogens of the other ring. These results were interpreted as an electrostatic interaction between the substituent of one ring and the hydrogens on the other.

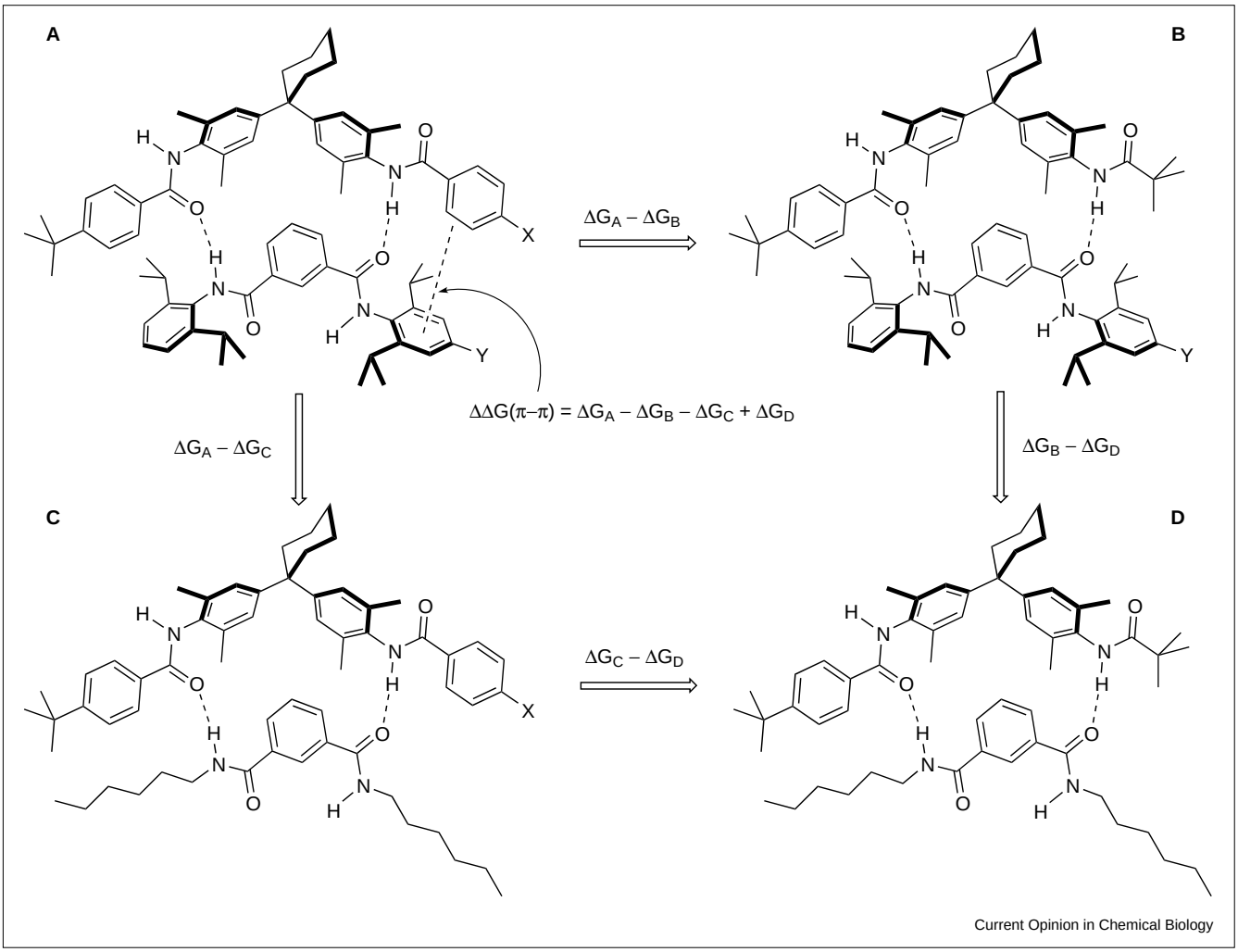
Aromatic interactions in peptides

Aromatic interactions have recently been studied in the context of peptide secondary structure. Butterfield and Waters [32] have found that aromatic interactions between two phenylalanines in the *i* and *i+4* residues in an α-helix can provide up to –3.3 kJ mol^{-1} to the stability of an α-helix in water. The geometry is believed to be an edge–face interaction. In a comparison of cross-strand interactions between phenylalanines and cyclohexylalanines (Cha) in a β-hairpin peptide in water, Tatko and Waters [33•] found that Phe residues show a preference for self-association. The Phe residues were found to interact in an edge–face geometry despite being solvent exposed. Moreover, the Phe–Phe cross-strand pair was found to be enthalpically more favorable and entropically less favorable than the Cha–Cha interaction, suggesting that a classical hydrophobic interaction is not the driving force for the Phe–Phe association.

Aromatic interactions of heteroaromatic rings

Another area of recent investigation is the role of heteroatoms in aromatic interactions. Several studies of nucleic acids have attempted to address this, as well as studies of synthetic model systems. In a study of aromatic stacking in duplex DNA in which a wide range of aromatics were investigated, Kool and co-workers [34••] have found that polarizability and buried surface area correlate best with stacking of a 5′-dangling base, whereas the dipole moment and the hydrophobicity, as measured by log P (the partition coefficient between octanol and water), do not correlate well. These results were interpreted as indicating that hydrophobic interactions provide the greatest contribution to stacking interactions. The discrepancy between surface area and log P is explained by the fact that many of these compounds, particularly the natural bases, can simultaneously bury surface area and interact with water through their polar groups on the edges of the rings, resulting in the better correlation with buried surface area than log P.

Figure 5



Hunter's double mutant cycle for measuring edge-face interactions. Deletion of one or the other aromatic ring (A to B or A to C) results in loss of the aromatic interaction, but may also have other secondary effects on the strengths of the hydrogen bonds and other contributing interactions. The double mutant, D, accounts for these secondary changes, such that the sum $\Delta\Delta G(\pi–\pi)$ represents only the energy of the edge–face interaction.

Quadrapole–quadrapole interactions were also proposed to be important for electron-poor rings such as 4-nitroindole. Rosemeyer and Seela [35] have also shown that the polarizability of the dangling base correlates with stability of the duplex better than hydrophobicity as measured from RP-HPLC retention times, and have interpreted this as indicating that polarizability, rather than hydrophobicity, is the most important factor in stabilizing duplex DNA. A general difficulty with interpreting the results from dangling-base studies is that the geometry of interaction in these systems typically is not known. For example, Richert and co-workers [36•] found that duplex DNA with a 5′-quinolone cap was significantly stabilized relative to the uncapped sequence. However, detailed structural analysis of this system indicates that the quinolone does not cap the terminal T:A base pair, but in fact disrupts the terminal pair in favor of quinolone–adenine stacking against the penultimate G:C base pair.

Gellman and co-workers [37•] have studied the role of heteroatoms in aromatic interactions in their amide model system (Figure 3, R = CH_2CO_2X). In this system, the number and location of nitrogen atoms in two aromatic rings, Ar_1 and Ar_2, were varied, and the effect on amide rotomer population was determined in water. The *E*-rotamer places Ar_1 and Ar_2 in close proximity, and a crystal structure indicates that Ar_1 and Ar_2 interact in an offset-stacked geometry in this rotamer. In this system, Gellman has found that, in general, an increase in the number of heteroatoms in either or both Ar_1 and Ar_2 resulted in an increase in the *E*-rotomer, presumably due to a favorable aromatic interaction. This argues for a significant polar component to the interaction. Nonetheless, when the experiments were performed in chloroform, very little effect was observed, indicating that aqueous solvent is required for significant interaction.

Conclusions

Recent studies of aromatic interactions have provided new insight into the nature of these biologically important non-covalent interactions in terms of their driving force, stability and selectivity. It is clear that the hydrophobic effect plays a role in these interactions, but it is not the sole factor involved in these attractive interactions. Moreover, its contribution depends on the geometry of the interaction, which is influenced by electrostatics. Recent work has demonstrated that aromatic interactions can provide *selectivity* as well as stability. Selective association between different aromatics demonstrates the importance of electrostatics to the magnitude of the interaction. Moreover, the observed selectivity between aromatic and aliphatic groups in water demonstrates the fundamental differences between aromatic and purely hydrophobic interactions. The advances in understanding of aromatic interactions should prove useful in their application in molecular and biomolecular recognition.

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