Measurements of Molecular Electrostatic Field Effects in Edge-to-Face Aromatic Interactions and CH-π Interactions with Implications for Protein Folding and Molecular Recognition

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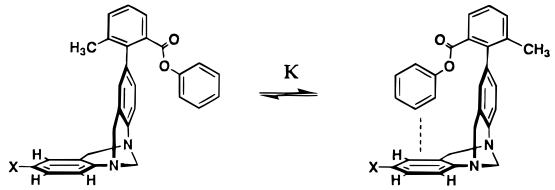
Edge-to-face aromatic interactions have been invoked to explain the stability of certain protein folding motifs.[1] While observations and speculations involving these interactions have been reported in many papers in chemistry[2] and structural biology,[3] the origin of these interactions is imperfectly understood. This project was undertaken to determine how important the electrostatic component of this interaction might be when compared to other effects, particularly the London dispersion force.[4] The work is based on the following premise: if aryl−aryl interactions have a larger electrostatic component than simple alkyl−aryl interactions,[5,6] aryl−aryl interactions should be more sensitive than alkyl−aryl interactions to changes in molecular electrostatic potential (MEP).

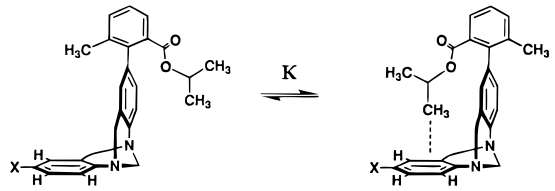
We described the design of a "molecular torsion balance" for the quantitative evaluation of side-chain interactions in 1994.[7] Such molecules are models for protein folding and allow precise measurements of folding energies. Molecules **1(a−g)** and **2(a−g)** (Scheme 1) have two gently restricted conformational states (folded and unfolded, Figure 1). The geometry of the folded forms has been determined in the crystalline state (X-ray diffraction) and in solution (NMR) for several analogues. Folded and unfolded states are separated by a barrier >18 kcal/mol, and direct observation of the populations of the two states is easily accomplished by NMR spectroscopy at 25 °C.

Electron-donating groups and electron-withdrawing groups increase and decrease, respectively, the negative electrostatic potential on the π-face of an aromatic ring. To investigate the effect such changes in aryl ring MEP might have on folding events driven by aryl contacts, we synthesized phenyl, isopropyl, and methyl esters with a series of substituents (X, Scheme 1).[8]

Scheme 1. Models for Protein Folding



1(a-g) X = (a) -NO_2, (b) -CN, (c) -Br, (d) -I, (e) -CH_3, (f) -OH, (g) -NH_2



2(a-g) X = (a) -NO_2, (b) -CN, (c) -Br, (d) -I, (e) -CH_3, (f) -OH, (g) -NH_2

Substituent effects on alkyl−aryl and aryl−aryl could then be directly compared.

We favored isopropyl as an alkyl group because diffraction data on our isopropyl esters proved that alkyl−aryl contacts occurred in the folded state and because the side chains of amino acids valine, leucine, threonine, and isoleucine have all been proposed to interact with aromatic rings in proteins and peptides.[9]

The changes in free energy of folding in these molecular torsion balances (and a third series composed of **3a−g**, the methyl ester analogues of **1a−g**) were determined by ^{1}H NMR spectroscopy in chloroform at 298 K (Table 1). (Folding energies were previously found to be insignificantly affected by solvent polarity and solvent bulk.[7,10])

The methyl esters were used as controls because the C−H proton of the methyl ester is too far from the target aryl ring for contact. Any small conformational selection seen for the methyl ester could only be the result of long-range polar interactions or solvent effects. The methyl ester shows very little preference for either state.

The folding energies of the isopropyl esters and phenyl esters are 0.5 ± 0.1 kcal/mol and 0.3 ± 0.1 kcal/mol, respectively, for all substituents (Table 1). This insignificant difference in substituent effect argues against a dominating electrostatic component in edge-to-face interactions. Molecules containing electron-withdrawing groups (NO_2 and CN) show folding similar to or greater than those containing electron-donating groups (NH_2 and OH). Furthermore, the isopropyl ester folding energy is greater than the phenyl folding energy, even though the C−H contact point on the phenyl ester is more positively charged than the corresponding isopropyl C−H.[11] (In prior work on congeners of **2e**, we noted that a cyclohexyl ester also folds more favorably than a phenyl ester.[7]) The data contradict the notion that electrostatic forces are important for on-face aromatic interactions but are consistent with Jorgensen's semiempirically based prediction that London dispersion forces should be the predominant driving force for this type of interaction.[4c]

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(10) The standard free energy of folding was calculated from the equilibrium constants (folding ratio) by the usual methods. Sample concentration did not significantly affect the folding ratio. Mole fractions of solutes were calculated on the basis of multiple NMR integration of the isopropyl methyl groups and the biaryl methyl group proton signals. The observed interaction energy for phenyl and isopropyl esters was modified by subtracting the methyl ester folding energy to compensate for this very small remote effect, but the conclusions of this paper are not changed by omitting this correction.

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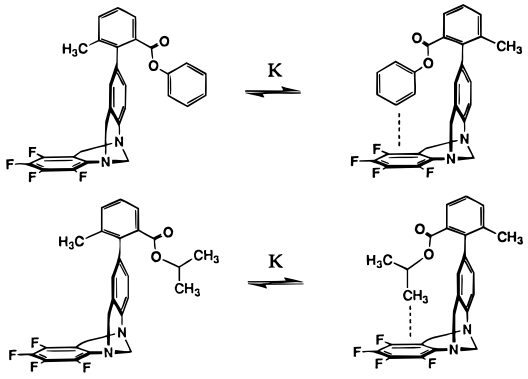
Table 1. Folding Energies of Methyl, Isopropyl, and Phenyl Esters **1**−**3**

entry	group X	$\Delta G°_{fold}$ (±10%)[a] methyl ester (**3**)	$\Delta G°_{fold}$ (±10%)[b] isopropylester[b] (**2**)	$\Delta G°_{fold}$ (±10%)[c] phenyl ester[c] (**1**)
1	NO_2	0.11	−0.51	−0.21
2	CN	0.06	−0.64	−0.30
3	I	−0.06	−0.46	−0.23
4	Br	0.02	−0.54	−0.26
5	CH_3	−0.04	−0.44	−0.27
6	OH	−0.03	−0.47	−0.23
7	NH_2	−0.06	−0.34	−0.18

[a] Free energies in kcal/mole. [b] $\Delta G°_{fold} = \Delta G°_{fold}$, isopropyl ester (observed) − $\Delta G°_{fold}$, methyl ester. [c] $\Delta G°_{fold} = \Delta G°_{fold}$, phenyl ester (observed) − $\Delta G°_{fold}$, methyl ester.

Scheme 2. Molecular Torsion Balances Designed to Study the Effects of a Reversal of Electrostatic Potential in the Aromatic Ring



Dispersion forces scale with polarizability. Nitro and cyano groups will change the polarizability of the aromatic ring and might invalidate a comparison that focuses solely on electrostatic effects. Edge fluorination offers a means of perturbing the MEP while having little effect on polarizability. Multiple fluorination of an aryl ring creates a positively charged centroid and a negative rim but has little effect on polarizability. Hexafluorobenzene and benzene have quadrupole moments of opposite sign but similar magnitude.[12] A perfluorinated torsion balance was prepared to further illuminate the nature of CH-π interactions (Scheme 2).

The experimental folding energies ($\Delta G°_{fold}$) of the phenyl and isopropyl esters of the tetrafluorinated system (in $CDCl_3$ at 25 °C) were determined to be −0.43 and −0.56 kcal/mol, respectively. Again, the isopropyl ester folds more exothermically than the phenyl ester. The energies are similar to those observed for other substituted benzene molecules in Table 1. Radical modification of the MEP by tetrafluorination of the face-donating component does not affect its interaction with phenyl or isopropyl groups. Taken together, the above experiments cast substantial doubt on the "importance" of electrostatic interactions in edge-to-face binding events.

The higher folding observed for isopropyl esters compared to that for phenyl esters is consistent with the dominance of dispersion forces in both types of CH-π interaction because there is more surface contact in an isopropyl−phenyl pairing than in a phenyl−phenyl edge-to-face pairing. The importance of multiple C−H contacts in CH-π interactions has been documented by Hirota.[13] Alkyl-π interactions have received less attention than aryl-π interactions, despite the frequency of alkyl−aromatic side chain juxtapositions in proteins. In water, hydrophobic effects will further increase the advantage of alkyl−aryl contacts because alkane fragments are less soluble than aromatic fragments.

These results support the idea that the dominant cause of these two types of attractive interactions is similar. The above data should be considered carefully by those who may propose that aryl−aryl contacts (and not aryl−alkyl contacts) are important in the kinetic and thermodynamic aspects of protein folding. In relation to projects involving receptor design or bioactivity optimization, these data illustrate that for simple aromatic rings, changes in aryl substituents that lead to substantial changes in MEP will have little effect on those receptor−substrate interactions attributable to edge-to-face binding.

In this work, we used substituent effects, a venerable tool of the physical organic chemist, to examine the nature of the CH-π interaction and to test the widely held beliefs that aryl−aryl interactions have an important electrostatic component and that such interactions are not like other side-chain interactions. The experiments support a conclusion that the electrostatic potential of the aromatic ring is *not* a dominant aspect of the aryl−aryl interaction. The results should encourage increased emphasis on the importance of London dispersion forces in on-face aryl interactions involving neutral components.[14] Work in progress is examining charged side-chain interactions with aryl rings. We hope this paper stimulates new quantitative experimental and theoretical studies of weak noncovalent interactions relevant to biological structure and function.

Acknowledgment. We thank the National Institutes of Health and the University of Pittsburgh for support of these studies.

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