The Absence of Favorable Aromatic Interactions between β-Sheet Peptides

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Favorable contacts between aromatic rings are generally thought to be important in protein folding and interactions.1,2 Examples of the importance of aromatic interactions have been reported and studied in peptide, protein, and small-molecule model systems, as well as through computational studies.3-6 These studies have provided varying answers on the exact magnitude of interactions between simple aromatic rings, with values ranging from 0 to 3 kcal/mol, depending on the system studied. Experimental solution-phase studies that compare related interactions have afforded values in the lower half of this range. This paper asks whether interactions between phenylalanine residues provide varying answers on the exact magnitude of interactions.

The position of the equilibrium in CDCl3 solution is readily determined by 1H NMR spectroscopy. The anilide or hydrazide NH resonances of the homo- and heterodimers are resolved, and these species are easily and accurately quantified by fitting these resonances with Lorentzian functions. Figure 1 illustrates the 1H NMR spectra from a typical mixing experiment, in which the anilide or hydrazide anilide or hydrazide NH resonances are used to quantify the species present. Table 1 summarizes the results of the six mixing experiments.

Analysis of the equilibrium constants in Table 1 reveals no significant preference for the formation of Phe–Phe pairs, or more specifically that two Phe–Phe pairs and two Cha–Cha pairs are of comparable stability to four Phe–Cha pairs. The equilibria in all six experiments are essentially statistical (K ≈ 4), and no (<0.1 kcal/mol) preference is seen for any pairing combination.7 Had there been a preference, K would have been significantly greater than 4 for 1a and 1b, significantly less than 4 for 1c and 1d, and essentially 4 for all other pairings.

The absence of favorable aromatic interactions between β-sheet peptides 1 is surprising, in light of the widely held belief that aromatic interactions are important in proteins. Statistical studies of the frequencies of Phe–Phe pairing within protein β-sheets provides insight into this interesting finding. The pairing of
phenylalanine with phenylalanine occurs with exceptionally high frequency in the hydrogen-bonded cross-strand pairs of antiparallel β-sheets, but does not occur with unusually high frequency in non-hydrogen-bonded cross-strand pairs. This difference suggests that favorable Phe–Phe contacts may not readily occur in the non-hydrogen-bonded cross-strand pairs of antiparallel β-sheets.

A survey of Phe–Phe pairs in the Interchain β-Sheet (ICBS) Database corroborates that little significant contact occurs between the aromatic rings in the non-hydrogen-bonded cross-strand pairs of antiparallel β-sheets at the interface between polypeptide chains. Figure 2 illustrates the structures of three representative Phe–Phe pairs from proteins in the ICBS Database. None of these structures, and only few other structures within the Database, show significant contact between the aromatic rings.

These findings would appear to contradict Tatko and Waters’ report of 0.55 kcal/mol stabilization of an intramolecular cross-strand Phe–Phe interaction within the non-hydrogen-bonded cross-strand pairs of a β-hairpin peptide. One possible explanation for this discrepancy is the type of model system used. The present model system involves preorganized β-sheets, rather than peptide β-hairpin structures. The β-hairpin structures are likely more flexible than typical protein β-sheets and may more easily form a highly twisted β-sheet that permits favorable cross-strand interactions within the non-hydrogen-bonded pairs.

The absence of favorable aromatic interactions in the present system is noteworthy, given the attention that aromatic interactions in peptides and proteins have received. Even though contacts between aromatic rings are favorable when they are of suitable geometry, the energetic price of achieving suitable geometries appears to offset the energetic benefits of such contacts in the current model system, as well as in proteins. This model system, although limited to organic solvents, offers a number of advantages over β-hairpin peptide model systems because it achieves the results of a double-mutant cycle experiment in a single step, does not involve an unfolded state, provides a highly sensitive direct thermodynamic readout, and allows six complementary experiments to be performed with just four peptides.

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References

9. Equilibration is rapid at 253 K. When solutions of α, β, and c are mixed at 213 K and the mixture is allowed to warm to 253 K in the NMR spectrometer, an equilibrium ratio of homo- and heterodimers is formed within minutes.
10. Addition of dimethyl sulfoxide does not affect the ratio of homo- and heterodimers. Addition of up to 16.7% CD3SOCD3 to a solution of α, β, and c has no significant effect on the preference (−0.1 kcal/mol) in these experiments corresponds to 0.05 kcal/mol from a traditional double-mutant cycle experiment.
11. Each of the first two mixing experiments is equivalent to a double-mutant cycle experiment in which two pairs of residues are mutated. The absence of preference (<0.1 kcal/mol) in these experiments corresponds to <0.05 kcal/mol from a traditional double-mutant cycle experiment.
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