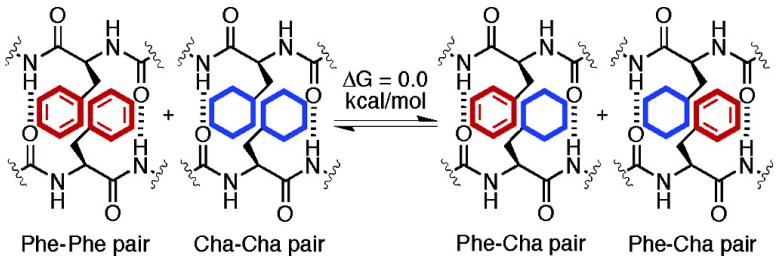
Communication

The Absence of Favorable Aromatic Interactions between β-Sheet Peptides

De Michael Chung, Yimeng Dou, Pierre Baldi, and James S. Nowick

J. Am. Chem. Soc., **2005**, 127 (28), 9998-9999• DOI: 10.1021/ja052351p • Publication Date (Web): 25 June 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 4 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 06/25/2005

The Absence of Favorable Aromatic Interactions between β-Sheet Peptides

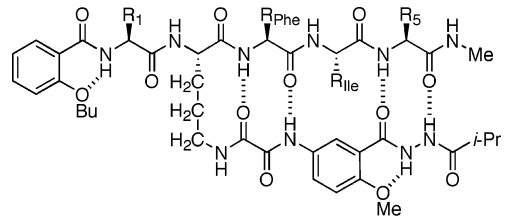
De Michael Chung, Yimeng Dou, Pierre Baldi, and James S. Nowick*

Departments of Chemistry and Computer Science and the Institute for Genomics and Bioinformatics, University of California, Irvine, Irvine, California 92697-2025

Received April 11, 2005; E-mail: jsnowick@uci.edu

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 of the non-hydrogen-bonded cross-strand pairs of antiparallel β-sheets are especially important and finds that they are not.

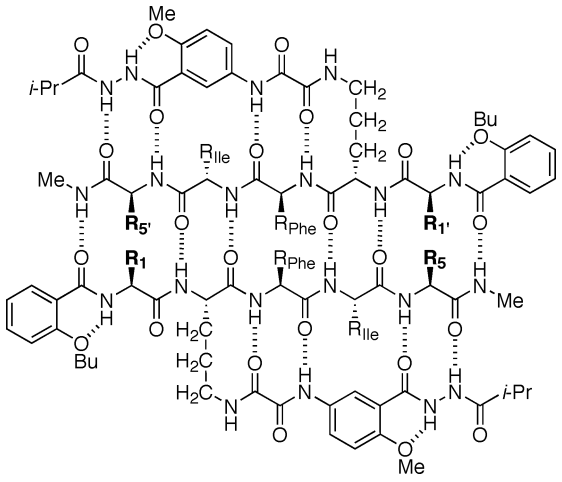
We recently reported a sensitive system for probing interactions between β-sheets.[7] The system consists of peptides of the general structure **1**, which contain the β-sheet template Orn(*i*-PrCO−Hao).[8]



1a $R_1 = R_5 = R_{Phe}$ **1c** $R_1 = R_{Phe}$ $R_5 = R_{Cha}$
1b $R_1 = R_5 = R_{Cha}$ **1d** $R_1 = R_{Cha}$ $R_5 = R_{Phe}$

These peptides form β-sheet homodimers in organic solvents. When the homodimers of different peptides are mixed, they equilibrate to form heterodimers, as well as homodimers (Chart 1). The position

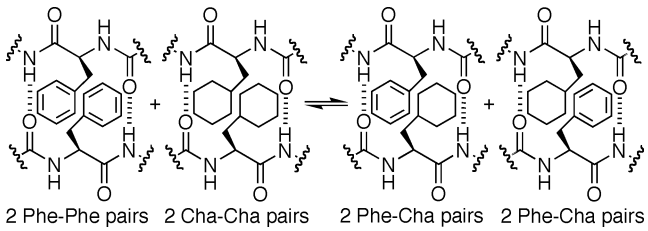
Chart 1. Homo- and Heterodimers of β-Sheet Peptides **1**



of the equilibrium, corrected for statistical effects, reflects the propensity of the first (R_1) and fifth (R_5) amino acids to interact within the non-hydrogen-bonded cross-strand pairs of β-sheets. To evaluate the roles of interactions between phenylalanine residues of the non-hydrogen-bonded cross-strand pairs of β-sheets, we have now studied the equilibria among peptides **1a**−**d**, which contain phenylalanine (Phe) and cyclohexylalanine (Cha) residues at the R_1 and R_5 positions.

Mixing peptides **1a**−**d** in all six possible binary combinations provides a measure of the relative propensities of Phe and Cha to pair. Mixing peptides **1a** and **1b** in $CDCl_3$ solution, for example, generates heterodimer **1a·1b**, in addition to the original homodimers **1a·1a** and **1b·1b**. The **1a·1a** homodimer contains two Phe−Phe pairs, and the **1b·1b** homodimer contains two Cha−Cha pairs, while the two molecules of heterodimer that form upon their equilibration each contain two Phe−Cha pairs. (Chart 2 illustrates this exchange.)

Chart 2. Equilibration of Phe−Phe, Cha−Cha, and Phe−Cha Pairs



Conversely, mixing peptides **1c** and **1d** generates heterodimer **1c·1d**, in addition to the original homodimers **1c·1c** and **1d·1d**, and exchanges Phe−Cha pairs for Phe−Phe pairs and Cha−Cha pairs. The remaining four possible binary mixings of the peptides (**1a** with **1c**, **1a** with **1d**, **1b** with **1c**, and **1b** with **1d**) result in no net change in the number of Phe−Phe, Cha−Cha, and Phe−Cha pairs.

The position of the equilibrium in $CDCl_3$ 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 NH resonances are used to quantify the species present. Table 1 summarizes the results of the six mixing experiments.[9,10]

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 ($K \approx 4$), and no (<0.1 kcal/mol) preference is seen for any pairing combination.[11] 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

10.1021/ja052351p CCC: $30.25

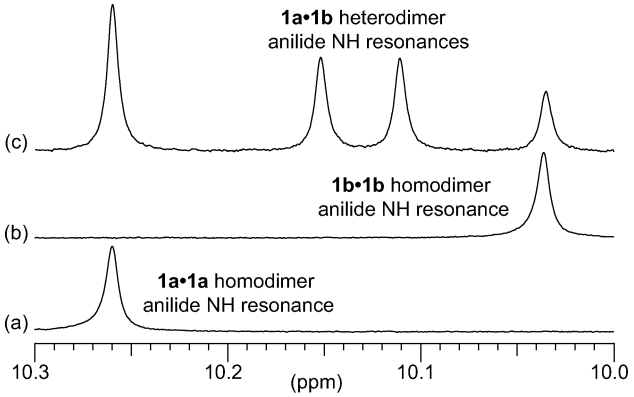


Figure 1. ^{1}H NMR spectra of the anilide NH groups of peptide **1a** (a), peptide **1b** (b), and a mixture of the two peptides (c). Spectra were recorded at 500 MHz and 253 K in $CDCl_3$ at (a) 2.0 mM **1a**, (b) 2.0 mM **1b**, (c) 2.1 mM **1a**, and 1.6 mM **1b**.

Table 1. Equilibria among Homo- and Heterodimers of Peptides **1**[a]

mixing experiment	K[b]	ΔG (kcal/mol)[c]
1a and **1b**	3.9	0.02
1c and **1d**	3.6	0.05
1a and **1c**	3.5	0.07
1a and **1d**	3.8	0.03
1b and **1c**	4.0	0.00
1b and **1d**	3.4	0.09

[a] $CDCl_3$, 253 K. [b] K = [heterodimer]^2/[1st homodimer][2nd homodimer]. [c] Statistically corrected free-energy difference ($\Delta G = -RT\ln(K/4)$).

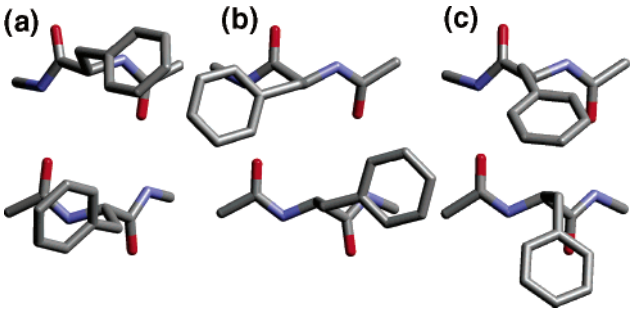


Figure 2. Representative non-hydrogen-bonded cross-strand Phe−Phe pairs at the interface between antiparallel β-sheets: (a) bovine neurophysin II (PDB ID 2bn2), (b) bifunctional enzyme and transcriptional coactivator DCoH (PDB ID 1dco), and (c) the insulin dimer (PDB ID 1mso).[12]

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.[13] 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.[14,15] 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.[3c,e] 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.[3b,d]

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.

Acknowledgment. The authors thank the NSF and NIH for grant support (CHE-0213533, EIA-0321390, and Biomedical Informatics Training Grant LM-07443-01).

References

(1) Burley, S. K.; Petsko, G. A. *Science* **1985**, *229*, 23−28.
(2) (a) Meyer, E. A.; Castellano, R. K.; Diederich, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210−1250. (b) Waters, M. L. *Biopolymers* **2004**, *76*, 435−445.
(3) (a) Russell, S. J.; Cochran, A. G. *J. Am. Chem. Soc.* **2000**, *122*, 12600−12601. (b) Cochran, A. G.; Skelton, N. J.; Starovasnik, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5578−5583. (c) Tatko, C. D.; Waters, M. L. *J. Am. Chem. Soc.* **2002**, *124*, 9372−9373. (d) Russell, S. J.; Blandl, T.; Skelton, N. J.; Cochran, A. G. *J. Am. Chem. Soc.* **2003**, *125*, 388−395. (e) Kiehna, S. E.; Waters, M. L. *Protein Sci.* **2003**, *12*, 2657−2667.
(4) (a) Serrano, L.; Bycroft, M.; Fersht, A. R. *J. Mol. Biol.* **1991**, *218*, 837−846. (b) Smith, C. K.; Regan, L. *Science* **1995**, *270*, 980−982. (c) Distefano, M. D.; Zhong, A.; Cochran, A. G. *J. Mol. Biol.* **2002**, *322*, 179−188.
(5) (a) Paliwal, S.; Geib, S.; Wilcox, C. S. *J. Am. Chem. Soc.* **1994**, *116*, 4497−4498. (b) Newcomb, L. F.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 4993−4994. (c) Newcomb, L. F.; Haque, T. S.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 6509−6519. (d) Adams, H.; Carver, F. J.; Hunter, C. A.; Morales, J. C.; Seward, E. M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1542−1544. (e) Kim, E.; Paliwal, S.; Wilcox, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 11192−11193. (f) Carver, F. J.; Hunter, C. A.; Jones, P. S.; Livingstone, D. J.; McCabe, J. F.; Seward, E. M.; Tiger, P.; Spey, S. E. *Chem.−Eur. J.* **2001**, *7*, 4854−4862.
(6) (a) Jorgensen, W. L.; Severance, D. L. *J. Am. Chem. Soc.* **1990**, *112*, 4768−4774. (b) Chipot, C.; Jaffe, R.; Maigret, B.; Pearlman, D. A.; Kollman, P. A. *J. Am. Chem. Soc.* **1996**, *118*, 11217−11224.
(7) (a) Nowick, J. S.; Chung, D. M. *Angew. Chem., Int. Ed.* **2003**, *42*, 1765−1768. (b) Chung, D. M.; Nowick, J. S. *J. Am. Chem. Soc.* **2004**, *126*, 3062−3063.
(8) Nowick, J. S.; Lam, K. S.; Khasanova, T. V.; Kemnitzer, W. E.; Maitra, S.; Mee, H. T.; Liu, R. *J. Am. Chem. Soc.* **2002**, *124*, 4972−4973.
(9) Equilibration is rapid at 253 K. When solutions of **1c** and **1d** 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% CD_3SOCD_3 to a solution of **1c** and **1d** in $CDCl_3$ has no significant effect on K.
(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.
(12) (a) Chen, L.; Rose, J. P.; Breslow, E.; Yang, D.; Chang, W.-R.; Furey, W. F., Jr.; Sax, M.; Wang, B.-C. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4240−4244. (b) Cronk, J. D.; Endrizzi, J. A.; Alber, T. *Protein Sci.* **1996**, *5*, 1963−1972. (c) Smith, G. D.; Pangborn, W. A.; Blessing, R. H. *Acta Crystallogr., Sect. D* **2003**, *59*, 474−482.
(13) (a) Wouters, M. A.; Curmi, P. M. G. *Proteins: Struct., Funct., Genet.* **1995**, *22*, 119−131. (b) Hutchinson, E. G.; Sessions, R. B.; Thornton, J. M.; Woolfson, D. N. *Protein Sci.* **1998**, *7*, 2287−2300.
(14) http://www.igb.uci.edu/servers/icbs/
(15) Dou, Y.; Baisnée, P.-F.; Pollastri, G.; Pécout, Y.; Nowick, J.; Baldi, P. *Bioinformatics* **2004**, *20*, 2767−2777.

JA052351P