Aromatic Stacking Interactions in Aqueous Solution: Evidence That neither Classical Hydrophobic Effects nor Dispersion Forces Are Important

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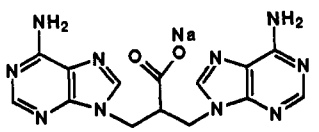
The association of aromatic surfaces with one another is believed to play a significant role in the folding and complexation behavior of biopolymers.[1] The forces that drive aromatic–aromatic interactions, however, are not yet clear. Hydrocarbon aromatic groups are generally considered to be "hydrophobic" moieties, because of the distinctive thermodynamic signature that benzene and derivatives show for transfer from the pure liquid to aqueous solution at room temperature (ΔH is small and positive, ΔS is large and negative, ΔC_p is large and positive).[2] This thermodynamic profile, also displayed by saturated hydrocarbons, has been commonly interpreted to imply that dissolution is opposed by water's preference for interacting with itself relative to interacting with the hydrocarbon, and that water molecules forced to reside near the nonpolar solute have excess energy that is manifested in a higher degree of order than that of bulk water.[3] In contrast, the thermodynamic signatures for self-association of purine and pyrimidine derivatives in aqueous solution (enthalpically favorable but entropically unfavorable)[4] have been interpreted to imply that these associations are driven by intrinsic attractions between the heterocyclic rings, rather than by their mutual exclusion from water.[4c] This conclusion has been reinforced by thermodynamic data for intramolecular stacking in dinucleotides[5] and in compounds in which pairs of heterocycles are connected via polymethylene chains.[6] The nature of the attraction between heterocycles is uncertain; both dispersion forces and interactions between partial charges within adjacent rings have been invoked.[4c,7] Furthermore, several workers have concluded that there is a "hidden" hydrophobic component to this type of interaction.[4c,8]

We present a direct comparison of the stacking tendencies of representative hydrocarbon (phenyl and naphthyl) and heterocyclic (adenine) aromatic moieties. (The term "stacking" is used here in a strictly geometric sense.) We have focused on intramolecular pairwise associations because in this situation the covalent linker determines which groups come together in solution. By choosing a linker that contains three sp^3 carbon atoms, we limit the aromatic–aromatic juxtapositions to those in which the aromatic rings are nearly parallel (either fully stacked or partially offset).[9–11] Our linkers contain carboxylate groups to provide sufficient aqueous solubility, in the monomeric state, for NMR study.

Adenine–adenine stacking was examined by comparing bis-adenine **1** to control compound **2**. Leonard et al. previously studied stacking with the neutral analogues (propylene linker), observing a hypochromicity at 258 nm of 15% for the bis-adenine relative to N^9-propyladenine in water at room temperature.[12] We observed 16% hypochromicity for **1** vs **2**, which indicates that the carboxylate substituent does not disrupt stacking. Stacking was



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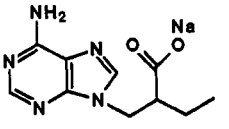

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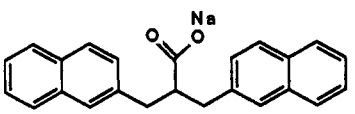
also detectable via upfield shifts of the adenine ring ^{1}H NMR resonances of **1** (0.5 mM) relative to **2** (2 mM) at 22 °C: H2 $\Delta\delta = -0.27$, and H8 $\Delta\delta = -0.14$. These shifts are similar in size to those resulting from stacking in the adenine dinucleotide ApA.[13] Optical measurements by Leonard et al. indicate a decrease in stacking at elevated temperatures,[12] and we observed smaller $\Delta\delta$ values for **1** vs **2** at 88 °C: −0.16 for H2 and +0.01 for H8. Consistent with prior reports in a number of systems,[6,14] we found no evidence of adenine–adenine stacking in DMSO solution.

No evidence of intramolecular naphthy–naphthyl association was detected for **3** in aqueous solution at 22 °C; the aromatic regions of the ^{1}H NMR spectra of **3** (0.28 mM) and **4** (4 mM) are identical.[15,16] Similar behavior was observed for the phenyl



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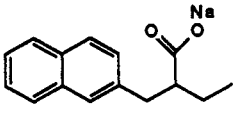

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analogues of **3** and **4**.[15] A crystal structure of the bis-phenyl compound in its free acid form (not shown) revealed the aromatic rings to be splayed apart, in contrast to the reported crystal structures of two propylene-linked heterocycles, 1,3-propanediyl bis(8-theophylline)[9a] and 1,3-propanediyl bis(1-thymine),[9b] both

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(2) (a) Privalov, P. L.; Gill, S. J. *Pure Appl. Chem.* **1989**, *61*, 1097 and references therein. (b) Phenylalanine is generally considered to be one of the most hydrophobic of the common amino acids; see: Makhatadze, G. I.; Privalov, P. L. *J. Mol. Biol.* **1990**, *213*, 375 and references therein. (c) In many efforts to assess the contribution of nonpolar surface burial to protein conformational stability, aromatic and aliphatic hydrocarbon moieties have been considered to be functionally equivalent; see: Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8069. Livingstone, J. R.; Spolar, R. S.; Record, M. T. *Biochemistry* **1991**, *30*, 4237. (d) A recent effort to evaluate hydration energetics for hydrocarbon moieties has led to the conclusion that aliphatic groups are hydrophobic but aromatic groups are not: Makhatadze, G. I.; Privalov, P. L. *J. Mol. Biol.* **1993**, *232*, 639, 660.

(3) For leading references, see: Muller, N. *Acc. Chem. Res.* **1990**, *23*, 23.

(4) (a) Gill, S. J.; Downing, M.; Sheats, G. F. *Biochemistry* **1967**, *6*, 272. (b) Farquhar, E. L.; Downing, M.; Gill, S. J. *Biochemistry* **1968**, *7*, 1224. (c) Ts'o, P. O. P. In *Basic Principles in Nucleic Acid Chemistry*; Ts'o, P. O. P., Ed.; Academic Press: New York, 1974; Vol. I, Chapter 6 and references therein. (d) See also: Dewey, T. G.; Raymond, D. A.; Turner, D. H. *J. Am. Chem. Soc.* **1979**, *101*, 5822.

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(6) For leading references, see: (a) Leonard, N. J. *Acc. Chem. Res.* **1979**, *12*, 423. (b) Constant, J. F.; Laugaa, P.; Roques, B. P.; Lhomme, J. *Biochemistry* **1988**, *27*, 3997.

(7) (a) Topal, M. D.; Warshaw, M. M. *Biopolymers* **1976**, *15*, 1775. (b) Sowers, L. C.; Shaw, B. R.; Sedwick, W. D. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 790. (c) Sarai, A.; Mazur, J.; Nussinov, R.; Jernigan, R. L. *Biochemistry* **1988**, *27*, 8498. (d) Albergo, D. D.; Turner, D. H. *Biochemistry* **1981**, *20*, 1413.

(8) (a) Crothers, D. M.; Ratner, D. I. *Biochemistry* **1968**, *7*, 1823. (b) Ts'o, P. O. P.; Kondo, N. S.; Robins, R. K.; Broom, A. D. *J. Am. Chem. Soc.* **1969**, *91*, 5625. (c) Tazawa, I.; Koike, T.; Inoue, Y. *Eur. J. Biochem.* **1980**, *109*, 33.

(9) Crystal structures of a bis-thymine and a bis-theophylline, both containing propylene linkers, show intramolecularly stacked conformations: (a) Rosen, L. S., Hybl, A. *Acta Crystallogr.* **1971**, *B27*, 952. (b) Frank, J. K.; Paul, I. C. *J. Am. Chem. Soc.* **1973**, *95*, 2324.

(10) In order to assess the aromatic–aromatic geometries allowed by the propylene linker, we carried out conformational searches for 1,3-diphenylpropane using the "Multiconformer" mode of MacroModel v3.0, with both the MM2 and OPLS/A force fields (Mohamdi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, C.; Change, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440 and references therein). All of the resulting local minima with the phenyl groups near one another had the rings roughly parallel (fully stacked or offset). Perpendicular phenyl juxtapositions are apparently not allowed by the three-atom linker.

(11) For analysis of interactions between phenyl rings at the 1- and 8-positions of a naphthalene spacer, see: Cozzi, F.; Cinquini, M.; Annuziata, R.; Siegel, J. S. *J. Am. Chem. Soc.* **1993**, *115*, 5330 and references therein.

(12) Leonard, N. J.; Ito, K. *J. Am. Chem. Soc.* **1973**, *95*, 4010.

(13) Chan, S. I.; Nelson, J. H. *J. Am. Chem. Soc.* **1969**, *91*, 168.

(14) Bolte, J.; Demuynck, C.; Lhomme, J. *J. Med. Chem.* **1977**, *20*, 1607.

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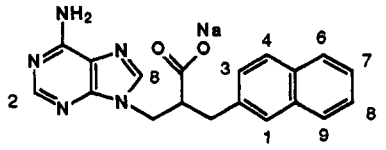
of which show intramolecular stacking. (The angles between the mean planes of the linked heterocycles in these structures are 9.3° and 6.6°, respectively.) Although one must be cautious in comparing the behavior of flexible molecules in solution and the solid state, it seems noteworthy that the trend we detect under dynamic conditions is mirrored in the available crystallographic data.

Carboxylate **5** (0.5 mM) was compared to control compounds **2** (1 mM) and **4** (1 mM) in order to examine the stacking propensity between a hydrocarbon/heterocyclic aromatic pair.[15,17]



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The $\Delta\delta$ values determined in aqueous solution at 22 °C indicated considerable stacking: adenine H2 = –0.28, H8 = –0.12; naphthyl H1 = –0.42, H3 = –0.32, H4 = –0.31, H6 = –0.19, H7 = –0.11, H8 = –0.11, H9 = –0.31. Little evidence of stacking could be detected for **5** in DMSO solution, which is similar to the behavior of bis-adenine **2**. The naphthyl–adenine stacking in **5** did not appear to be as effectively disrupted by elevated temperature as was adenine–adenine stacking in **2** (for **5** in aqueous solution at 88 °C, the $\Delta\delta$ values were adenine H2 = –0.23, H8 = –0.13; and naphthyl H1 = –0.38, H3 = –0.26, H4 = –0.29, H6 = –0.15, H7 = –0.07, H8 = –0.07, H9 = –0.25).

General conclusions about the nature of aromatic stacking interactions may be drawn from our results if we assume (i) that adenine is representative of all nucleotide bases and related heterocycles and (ii) that the juxtaposition(s) of aromatic groups allowed by the propylene linker are representative of "stacked" geometries commonly achieved in nucleic acids. Our data suggest that the favorable stacking between two heterocyclic moieties, or between a hydrocarbon and a heterocycle, is not a result of the "hydrophobic effect" that opposes the aqueous solubility of aromatic and nonaromatic hydrocarbons. If this classical hydrophobic effect were an important driving force for aromatic stacking, we should have observed stacking in bis-naphthyl **3**. We also conclude that dispersion attraction is not a dominant promoter of stacking in **1** or **5**, because this source of attraction should have been available to bis-naphthyl **3**. Our observations seem most consistent with stacking arising in **1** and **5** from attractive interactions between partial positive and negative charges on atoms in the neighboring aromatic groups. This mechanism of attraction has been proposed for related systems by others,[4c,17] often based upon intermolecular potential energy calculations,[1b,7c,d,18] although previous workers have usually concluded that dispersion and/or hydrophobic effects were also important. If the partial charge interaction hypothesis is correct, then it seems curious that water does not disrupt the stacking interaction as effectively as does DMSO. It is possible that water is not well suited to solvation of partially charged atoms when those atoms occur in an extended planar array. If this last supposition is correct, then aromatic stacking in aqueous solution may be viewed as resulting, at least in part, from a nonclassical hydrophobic effect.

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Supplementary Material Available: ^{1}H NMR comparisons of aromatic resonances among **1–5** and data on the aggregation of **1**, **2**, **4**, and **5** in aqueous solution (12 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(15) Spectroscopic data may be found in the supplementary material.

(16) Aggregation of various substrates was examined by monitoring the concentration dependence of ^{1}H NMR chemical shifts in D_2O at room temperature (data may be found in the supplementary material). Data for **1**, **2**, **4**, and **5** indicated that aggregation does not occur at the concentrations used for the measurements described in the text. The low solubility of bis-naphthyl **3** precluded a concentration dependence study; however, the similarity of the aromatic proton chemical shifts of **3** and **4** indicates that **3** does not aggregate at the concentration used for our studies (0.28 mM). The possibility that **3** forms conventional micelles at this concentration was further ruled out by the observation that the hydrophobic dye orange OT was not solubilized by a 0.3 mM aqueous solution of **3**.

(17) Enthalpically driven intramolecular stacking has been detected between an aromatic group and a diketo piperazine ring in a number of solvents: Kopple, K. D.; Marr, D. H. *J. Am. Chem. Soc.* **1967**, *89*, 6193.

(18) Aromatic–aromatic attraction arising from partial charges within the aromatic systems may be attributed computationally to Coulombic interactions or multipole–multipole interactions (e.g., dipole–dipole, dipole–quadrupole, quadrupole–quadrupole), depending upon how the intermolecular potential function has been constructed and parameterized. For leading references, see: (a) Karlström, G.; Linse, P.; Wallqvist, A.; Jönsson, B. *J. Am. Chem. Soc.* **1983**, *105*, 3777. (b) Pawliszyn, M.; Szcesniak, M. M.; Scheiner, S. *J. Phys. Chem.* **1984**, *88*, 1726. (c) Hunter, C. A.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5525. (d) Jorgensen, W. L.; Severance, D. L. *J. Am. Chem. Soc.* **1990**, *112*, 4768. (e) Hunter, C. A. *J. Mol. Biol.* **1993**, *230*, 1025.