Letter to the Editor

New Evidence that the Hydrophobic Effect and Dispersion Are Not Major Driving Forces for Nucleotide Base Stacking

The stacking of nucleotide bases is an enthalpically favorable process in aqueous solution, and it is widely accepted that this stacking attraction contributes to the stability of ordered DNA and RNA conformations. There has been considerable debate, however, regarding the origin of the stacking preference. Stacking has been attributed to the hydrophobic effect (Crothers and Ratner, 1968; Tazawa et al., 1980; Ts'o et al., 1969), to dispersion (i.e., the attractive component of van der Waals interactions) (Hanlon, 1966; Sowers et al., 1987), to polar interactions (Albergo and Turner, 1981; Sarai et al., 1988), and to dipole-induced dipole interactions (Bugg et al., 1971; however, see: Caillet and Claverie, 1975). These alternatives are not exclusive; all or any subset could operate simultaneously.

Friedman and Honig (1995) have recently reported a computational study that was intended to identify the sources of stacking affinity. They concluded that "nonpolar interactions, involving the hydrophobic effect and enhancement of van der Waals interactions caused by close packing, drive stacking." Polar interactions, represented in this model by Coulombic interactions among partial atomic charges, were concluded to oppose stacking. These deductions are not consistent with conclusions drawn from some experimental studies. For example, Albergo and Turner (1981) examined the effects of nonaqueous cosolvents on DNA conformational stability, and concluded that dipolar interactions between bases contributed to the stability of double helical forms.

The computationally derived conclusions of Friedman and Honig (1995) also appear to be inconsistent with experimental results that we have reported (Newcomb and Gellman, 1994a). Our experiments involved the spectroscopic examination of compounds 1–5 in aqueous solution (Fig. 1). Based on earlier work with related compounds (Leonard, 1979), we expected bis-adenine compound 1 to be partially stacked in water at room temperature. This stacking was detected via ^{1}H NMR (Newcomb and Gellman, 1994a): the two adenine ring protons of compound 1 are shifted upfield relative to the analogous resonances of compound 2 ($\Delta\delta = -0.27$ for H2 and -0.14 for H8). In contrast, we could not detect a significant difference between dinaphthyl compound 3 and mononaphthyl compound 4 for any of the seven naphthyl ring protons ($\Delta\delta \sim 0$ in each case). We therefore concluded that compound 3 does not experience significant naphthyl-naphthyl stacking in aqueous solution. If the dominant driving force for adenine-adenine stacking in compound 1 arose from a classical hydrophobic effect and/or dispersion, we would have expected dinaphthyl compound 3 to display at least as much stacking as compound 1. Based on these model studies, we concluded that adenine-adenine stacking in compound 1, and by extrapolation, in DNA and RNA, is predominantly driven by some type of polar interaction rather than by the hydrophobic effect or dispersion.

Friedman and Honig (1995) were cognizant of our findings, but, based on their own inspection of spectra that accompanied our report (Newcomb and Gellman, 1994b), they concluded that "there is evidence for naphthalene stacking in water." Specifically, Friedman and Honig (1995) suggested that "pronounced line broadening" in the spectrum of dinaphthyl compound 3 was indicative of stacking. Here we provide new ^{1}H-NMR data for compound 3 and reference compound 4. These data show that the line broadening noted by Friedman and Honig (1995) is an artifact of previous spectral acquisition conditions. The new data are consistent with our original interpretation, and therefore support our conclusion that neither the hydrophobic effect nor dispersion are dominant forces in base stacking.

Fig. 2 compares aromatic region ^{1}H-NMR data for compounds 3 and 4 in D_2O at room temperature. The lines are narrow in both spectra, and only very small variations ($\Delta\delta \leq 0.014$ ppm) among individual resonances are observed between the two spectra. (This conclusion is based on the assumption that the order of resonances along the ppm axis is identical for compounds 3 and 4. We further assume that the resonance at 7.911 ppm for compound 4 corresponds to the two resonances at 7.908 and 7.897 for 3, which is consistent with the results of integration.) These data were obtained on a 500-MHz spectrometer; our previous data (Newcomb and Gellman, 1994b) were obtained on a 270-MHz spectrometer. Because compound 3 must be examined at low concentration, analysis at 270 MHz required the collection of $>10{,}000$ scans, which in turn required $\sim$12 h. The line broadening observed in our original data for compound 3 (Newcomb and Gellman, 1994b) may have stemmed from imperfect temperature control during the acquisition period, leading to drift in the lock signal. The 500-MHz data for compound 3 (Fig. 2) required only 512 scans. For data collected with dilute samples at 270 MHz, we have previously reported that the uncertainty in $\Delta\delta$ is $\pm$ 0.02 ppm (Newcomb et al., 1995). The $\Delta\delta$ uncertainty in the 500-MHz data is obviously much smaller (Fig. 2), and these more precise data reveal that within the 270-MHz uncertainty limits, it was correct to conclude that there was no significant upfield shifting of any aromatic proton in compound 3 relative to the analogous proton in compound 4

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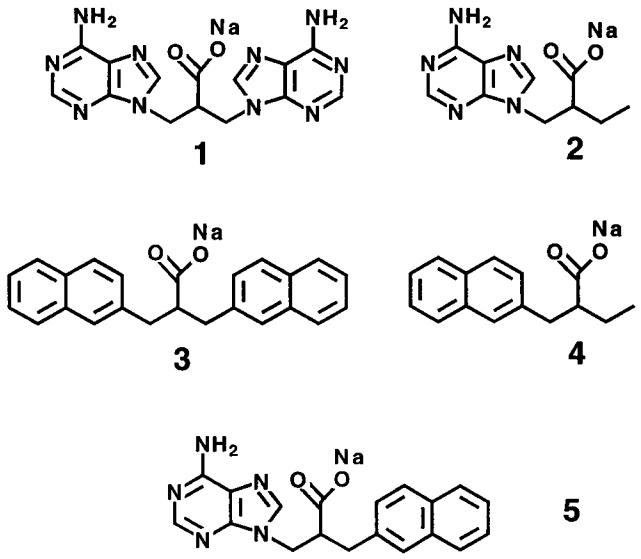

FIGURE 1 Compounds 1–5.

(Newcomb and Gellman, 1994a). Neither the spectral acquisition conditions nor the $\Delta\delta$ uncertainties were provided in our first report (Newcomb and Gellman, 1994a,b), which may have led to ambiguities in the interpretation of the original data.

Our original conclusion that there is no significant naphthyl-naphthyl stacking in compound 3 is supported by the new NMR data: the aromatic proton resonances of compounds 3 and 4 are very similar ($\Delta\delta < 0.02$). In contrast, all aromatic protons of diadenine compound 1 and of adenine-naphthyl compound 5 are shifted upfield by > 0.10 ppm relative to the analogous protons of control compounds 2 and 4. The small differences in resonance positions observed for compound 3 relative to compound 4 include both upfield and downfield shifts, whereas only upfield shifts are observed for compounds 1 and 5 relative to compounds 2 and 4.

If there were significant naphthyl-naphthyl stacking in 3, we would expect to detect this intramolecular proximity by ^{1}H NMR. The fact that the resonances of the adenine ring protons of diadenine compound 1 and of adenine-naphthyl compound 5 are shifted upfield to nearly the same extent, relative to monoadenine compound 2, suggests that stacked adenine and naphthyl groups exert comparable upfield shifts, unless the degree of stacking in compound 5 is much greater than in 1. Further evidence of the naphthyl group's ability to cause upfield ^{1}H-NMR shifts is found in our examination of dinaphthyl compounds with four-atom linkers (Newcomb et al., 1995) and in our study of cyclophanes in which pairs of aromatic rings are more rigidly juxtaposed than in compound 3 (Schladetzky et al., 1995). Therefore, it seems unlikely that significant NMR-invisible stacking occurs in 3.

Our results cast doubt on Friedman and Honig's conclusion that the drive for base stacking results largely from the hydrophobic effect and dispersion, even though Friedman and Honig (1995) did not perform calculations on our compounds or on any naphthalene-containing molecules. Identifying the source of the contradiction between our two sets of conclusions should provide insight on the origin of nucleotide base stacking proclivities and on the best methods for modelling such stacking experimentally and computationally.

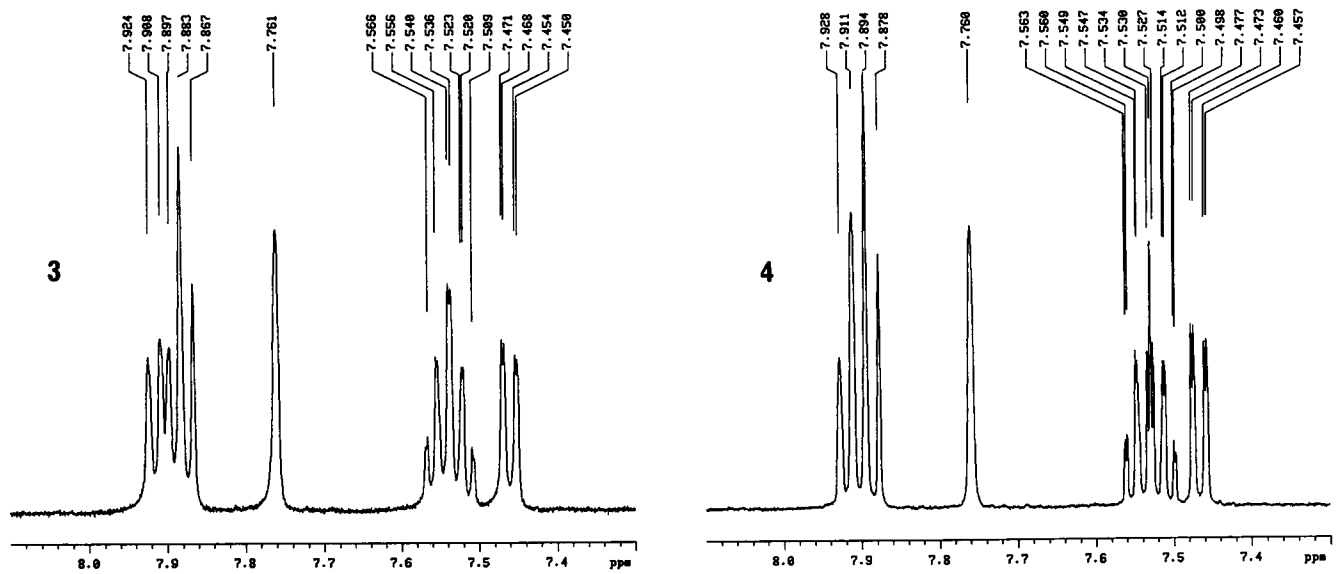

FIGURE 2 Aromatic region ^{1}H-NMR spectra of compounds 3 (0.5 mM) and 4 (1.8 mM) in D_2O at 24°C. Both spectra were acquired on a Varian Unity 500-MHz spectrometer equipped with a 5-mm ^{1}H/^{19}F probe. The HOD peak was presaturated before data acquisition, and 512 scans were acquired in each case. No line broadening was applied in the processing of the data. Spectra were referenced externally to a 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid, sodium salt standard.

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S. H. Gellman, T. S. Haque, and L. F. Newcomb

Department of Chemistry, University of Wisconsin Madison, Wisconsin

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Response to S. H. Gellman, T. S. Haque, and L. F. Newcomb

The experiments of Gellman and co-workers described in the preceding paper (Gellman et al., 1996) and in earlier work (Newcomb and Gellman, 1994a,b) provide an interesting approach to the problem of parsing the various free energy contributions to base stacking. Implicit in the logic of the experiments is that the chemical shifts due to naphthyl-naphthyl (compound 3), naphthyl-adenine (compound 5), and adenine-adenine (compound 1) stacking are approximately equal. Given this assumption, the observation that naphthyl-naphthyl stacking clearly produces smaller shifts than in the other two molecules, each of which contains at least one polar ring system, leads Gellman et al. (1996) to conclude that hydrophobic interactions do not drive base stacking and, rather, that polar interactions are implicated. This clearly contradicts our study, which argues that because of desolvation effects polar interactions oppose base stacking, whereas nonpolar interactions drive ring systems to aggregate (Friedman and Honig, 1995). However, the assumption that the chemical shifts produced by stacking in each of the three compounds are equal is unlikely to be correct. Indeed once the different sources of the chemical shift expected from stacking in compounds 1, 3, and 5 are considered, the measurements of Gellman and co-workers may actually support our theoretical calculations.

As naphthyl groups are nonpolar, ring-current effects presumably are responsible for the chemical shifts in compound 3 relative to compound 4. The magnitude of the shift depends on the unknown relative orientation and distance of the two rings in the stacked conformation. When one of the stacked moieties is polar (adenine in this case) another factor needs to be taken into account. Specifically, it is well known that polar molecules undergo large changes in dipole moment on transfer from polar to nonpolar solvents (because of changes in the reaction field of the solvent). Moreover, the change in dipole moment is proportional to the original dipole moment. Stacking of an adenine against either another adenine or a naphthyl group effectively removes solvent from one face of the ring and will inevitably lead to a reduction in the adenine dipole moment. This should result in significant chemical shifts because the charge distribution near each nucleus would be affected. Determining the relative magnitudes of dipole-moment changes and ring-current effects as well as their dependence on stacking geometry is essential if the data of Gellman and co-workers are to be interpreted properly. In the absence of

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Address reprint requests to Dr. Barry Honig, Department of Biochemistry and Molecular Biophysics, Columbia University, 630 West 168th Street, New York, NY 10032. Tel.: 212-305-7970; Fax: 212-305-6926; E-mail: honig@bass.bioc.Columbia.edu.

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