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A Free Energy Analysis of Nucleic Acid Base Stacking in Aqueous Solution

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ABSTRACT This paper reports a theoretical study of the free energy contributions to nucleic acid base stacking in aqueous solution. Electrostatic interactions are treated by using the finite difference Poisson-Boltzmann method and nonpolar effects are treated with explicit calculation of van der Waals interactions and/or free energy-surface area relationships. Although for some pairs of bases there is a favorable Coulombic interaction in the stacked conformation, generally the net effect of electrostatic interactions is to oppose stacking. This result is caused by the loss of favorable base-solvent electrostatic interactions, that accompany the partial removal of polar atoms from water in the stacked conformation. Nonpolar interactions, involving the hydrophobic effect and enhancement of van der Waals interactions caused by close-packing, drive stacking. The calculations qualitatively reproduce the experimental dependence of stacking free energy on purine-pyrimidine composition.

INTRODUCTION

It has been realized for some time that base stacking interactions provide a major driving force that stabilizes nucleic acid structure (Sturtevant et al., 1958), an idea reinforced by the fact that isolated bases stack in aqueous solution (Ts'o et al., 1962; Broom et al., 1967). The underlying physical forces that lead to base stacking are, however, not well understood and, indeed, the phenomenon has been attributed alternatively to electrostatic interactions (Newcomb and Gellman, 1994), the hydrophobic effect (Herskovits et al., 1961), and dispersion interactions (Hanlon, 1966). In this paper we address the relative roles of each of these contributions using theoretical methods that rely on a continuum treatment of solvent effects.

Experimental studies have not allowed an unambiguous characterization of the stacking phenomenon. The hydrophobic effect was originally implicated by the observation that organic compounds denature DNA (Herskovits et al., 1961) but, as has recently been emphasized by Newcomb and Gellman (1994), the fact that the enthalpy of stacking is negative appears to be at odds with this interpretation. These authors suggest that stacking is electrostatic in origin. However, negative enthalpy of stacking does not, in and of itself, imply that there is not a hydrophobic contribution to stacking. Hydrophobicity, qualitatively defined, is the reluctance of nonpolar molecules to dissolve in water and, by implication, the propensity of nonpolar molecules to associate in water. The term hydrophobic effect, in and of itself, says nothing about the sign of the enthalpy. For example, the enthalpy of transfer of pentane from the pure liquid to water is −2.0 kcal/mol at 25°C and becomes positive above 30°C (Gill et al., 1976; Privalov and Gill, 1988). For polar moieties such as nucleic acid bases, the presence of strong electrostatic interactions, which generally have a significant enthalpic component, further complicates the interpretation of thermodynamic measurements in terms of standard partitions of the free energy.

Theoretical studies have also failed to identify clearly the physical origins of stacking interactions. Calculations of the free energy of stacking of isolated bases and base pairs in vacuum, using classical potential functions, have found that favorable van der Waals interactions provide the largest contribution, accompanied by a smaller, yet significant, favorable or unfavorable electrostatic contribution (Devoe and Tinoco, 1962; Claverie et al., 1966; Cieplak and Kollman, 1988). Hunter (1993) recently calculated the electrostatic stacking free energy of base pairs in vacuum using a model that included out-of-plane partial charges representing the π electrons and found the van der Waals contribution to predominate and the electrostatic contribution to be positive. Quantum mechanical calculations of the stacking of bases and base pairs in vacuum have also found the van der Waals contribution to predominate but have usually found the electrostatic contribution to be favorable (Aida and Nagata, 1986).

A variety of approaches have been used to account for solvent effects on base stacking. Sinanoglu and Abdulnur (1964) treated solvation effects on the formation of the DNA duplex from single strands. They concluded that the hydrophobic effect, which they described in terms of a surface tension model, is the main driving force for the formation of the double helix. Pohorille and co-workers (1984) and also Danilov and Tolkh (1984) have studied internal energy contributions to the stacking of bases in water by Monte Carlo. They found that changes in water-water interactions, which they associate with the hydrophobic effect, is the main driving force for base stacking in solution. Kollman and co-workers carried out free energy molecular dynamics simulations that showed that the elec-

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trostatic contribution opposes stacking (Bash et al., 1987; Cieplak and Kollman, 1988; Dang and Kollman, 1990). These authors concluded that van der Waals interactions, including both base-base and base-water interactions, were primarily responsible for the formation of AA, AT, and GC stacked complexes (Cieplak and Kollman, 1988).

In the present paper we attempt to identify the physical origin of base stacking by using continuum solvation methods (Honig et al., 1993) to calculate the free energy of association of nucleic acid bases to form a variety of stacked complexes. The electrostatic free energy is partitioned into pairwise Coulombic interactions and desolvation effects. Nonpolar interactions are treated by using two different thermodynamic cycles to separate the contributions to the stacking free energy from van der Waals interactions and the hydrophobic effect.

This paper is a companion to our earlier work, which studied how the contributions to the base stacking energy of DNA vary with conformation (Friedman and Honig, 1992). Although total solvation free energies of nucleic acid bases have not been calculated previously with continuum methods, their success in treating small organic molecules suggests that they provide a satisfactory treatment of nucleic acid bases as well (Sitkoff et al., 1994). The speed of continuum methods, as distinct from explicit solvent methods, enables us to study a large number of stacked complexes. Our goal is not just to understand the stacking of isolated bases but also to use this relatively simple process to gain insight into the stability of the double helix and other nucleic acid structures.

METHODS

Theory

The free energy of base stacking is calculated with two different thermodynamic cycles. These are illustrated in Fig. 1 with AT stacking as an example. In cycle 1, the isolated bases are transferred from water into vacuum, the bases are stacked in vacuum, and the stacked complex is transferred to solution (Smith and Honig, 1994). In cycle 2, the bases are discharged in water, the remaining entitites, which have no partial charges, are stacked, and the stacked complex is recharged (Gilson and Honig, 1988).

The free energy of stacking is treated as a sum of electrostatic and nonpolar terms:

$$\Delta G = \Delta G_{\mathrm{el}} + \Delta G_{\mathrm{np}} \tag{1}$$

The electrostatic terms are calculated as described previously (Gilson and Honig, 1988; Smith and Honig, 1994). These terms are identical for both cycles, because the electrostatic energy is a path-independent quantity. This is shown explicitly in the Appendix. The electrostatic free energy is obtained from the sum of the Coulombic and reaction field energies of the product minus that of the reactants:

$$\Delta G_{\mathrm{el}}(\epsilon_{\mathrm{i}}, \epsilon_{\mathrm{o}}) = \Delta G_{\mathrm{c}}(\epsilon_{\mathrm{i}}) + \Delta G_{\mathrm{rf}}(\epsilon_{\mathrm{i}}, \epsilon_{\mathrm{o}}) \tag{2}$$

where $G_{\mathrm{el}}(\epsilon_{\mathrm{i}}, \epsilon_{\mathrm{o}})$ is the total electrostatic free energy of a molecule with interior dielectric ϵ_{i} and outer dielectric ϵ_{o}. $G_{\mathrm{c}}(\epsilon_{\mathrm{i}})$, the Coulombic free energy, is defined here as the pairwise interaction of the partial charges of the molecule in a medium of dielectric constant ϵ_{i}. $G_{\mathrm{rf}}(\epsilon_{\mathrm{i}}, \epsilon_{\mathrm{o}})$, the reaction field free energy, is the free energy of transferring a molecule from a medium of dielectric ϵ_{i} to one of dielectric ϵ_{o}.

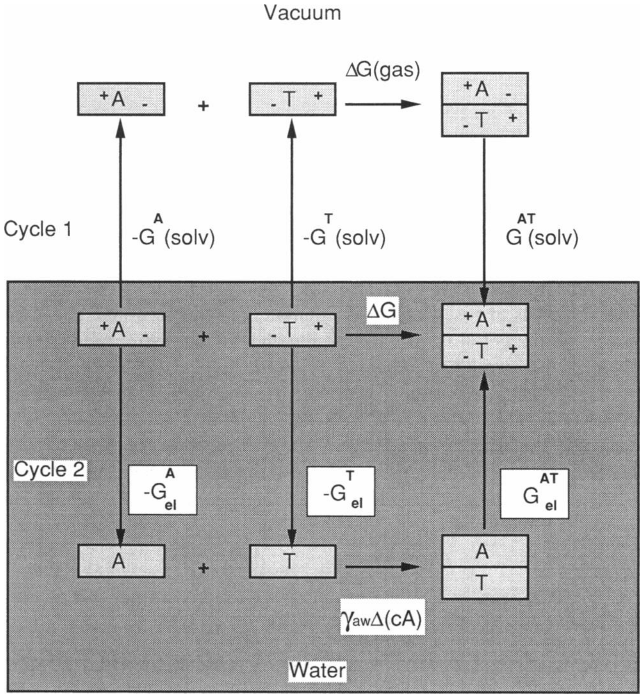

FIGURE 1 Two cycles for computing the base stacking free energy.

ΔG_{np} in the two cycles is given by

$$\Delta G_{\mathrm{np}}(v) = \Delta G_{\mathrm{LJ}} + \gamma_{\mathrm{vw}}\Delta(cA) \tag{3a}$$

$$\Delta G_{\mathrm{np}}(a) = \gamma_{\mathrm{aw}}\Delta(cA) \tag{3b}$$

ΔG_{LJ} is the Lennard-Jones (LJ) contribution. The quantities γ_{vw} and γ_{aw} are the microscopic surface tensions associated with the transfer of alkane from vacuum to water and from liquid alkane to water, respectively. The factor c describes the effect of the local curvature of the molecule on its surface free energy (Sharp et al., 1994; Nicholls et al., 1991). Because c will be different for the stacked complexes and isolated bases, it must be included in the difference between product and reactants in Eq. 3. Van der Waals interactions are not considered explicitly in cycle 2 and, rather, are assumed to be implicitly taken into account in the surface tension parameter, γ.

The stacking free energy in the first cycle may be written as the sum of gas phase and solvation terms:

$$\Delta G(v) = \Delta G(gas) + \Delta G(solv) \tag{4}$$

where

$$\Delta G(gas) = \Delta G_{\mathrm{el}}(gas) + \Delta G_{\mathrm{LJ}} \tag{5}$$

and ΔG_{el}(gas) is the electrostatic free energy of binding in the gas phase. ΔG(solv) is given by

$$\Delta G(solv) = \gamma_{\mathrm{vw}}\Delta(cA) + \Delta G_{\mathrm{el}}(solv) \tag{6}$$

where $\Delta G_{\mathrm{el}}(solv)$ is the electrostatic component of $\Delta G(solv)$.

Contributions to the free energy from translational, rotational, and vibrational motion are not calculated, in part because there is presently considerable uncertainty as to how the relevant terms should be treated (Peitzsch and McLaughlin, 1993; Janin and Chothia, 1978). The problem is circumvented here in the standard way, by calculating the relative binding free energies of two complexes for which the contributions from molecular motion should be similar.

Structures

TS'o and coworkers (Broom et al., 1967) have shown by analysis of nuclear magnetic resonance (NMR) ring current shifts that bases form stacked complexes in aqueous solution. Martel (1979) has observed by neutron diffraction that bases stack in solution in a parallel fashion with a separation of planes of ~3.4 Å. NMR data has been used to propose several models of bases stacked in solution in geometries other than ideal B-form (Broom et al., 1967; Helmkamp and Kondo, 1967; Aradi, 1990). However, because of uncertainties in the theoretical description of ring current shifts and the rapid conformational averaging of stacked bases in solution, the NMR results do not preclude significant populations of bases stacked in the ideal B-form. Hence we will choose the structures to be in the ideal B-form (rise = 3.4 Å, twist = 36, and all other helical parameters = 0), as have other theoretical studies of base stacking (Cieplak and Kollman, 1988; Dang and Kollman, 1990).

Calculations and parameters

Structures of stacked dinucleotides were generated by using JUMNA (Lavery, 1990) and edited into stacked bases by insightII (Biosym, 1990). ΔG_{el} was calculated by using DelPhi (Nicholls et al., 1990) at 3.0 grid/Å resolution with the scaled solvation energy method (Nicholls and Honig, unpublished results). *A*, the surface area, and *c*, the curvature factor, were calculated by using Surfcv (Sridharan et al., 1992). A probe radius of 1.4 Å was used in all calculations. Calculations are performed both with $\epsilon_i = 1$ and $\epsilon_i = 2$. The former value is consistent with the force fields used in this work, which do not take electronic polarizability into account. Using a dielectric constant of 2 approximates the effects of electronic polarizability. For water we take $\epsilon_o = 78.9$ (abbreviated as 80), the experimental dielectric constant of water (Lide, 1991). For vacuum, $\epsilon_o = 1$.

The Amber, LJ, charge, and radii parameters were used to calculate the absolute stacking energies of 17 base complexes, consisting of the 16 different complexes that could be formed from A, T, G, and C, plus the UU complex. The 10–12 hydrogen bond term was omitted as in previous work (Cieplak and Kollman, 1988; Dang and Kollman, 1990; Friedman and Honig, 1992). Stacking energies are insensitive to inclusion of the 10–12 term. The methyl proton radius was taken to be 1 Å. In addition to Amber, the parameters of Poltev and co-workers (without the 10–12 term) (Zhurkin et al., 1981; Poltev and Shulyupina, 1986) and the optimized potentials for liquid simulations (OPI-S) parameter set (Jorgensen and Pranata, 1990) were used to calculate the relative self-stacking free energies of adenine and uracil (see below). The charge of the proton bonded to the glycosidic nitrogen is not given in the Amber or Poltev charge sets, because these sets describe nucleotides, where the glycosidic carbon is bonded to the glycosidic nitrogen. A charge was assigned to the proton bonded to the glycosyl nitrogen such that the base is electrically neutral.

The surface tension coefficients, γ, were calculated from the partition coefficients (K_p) of linear alkanes between water and either the gas phase (cycle 1) or the neat alkane phase (cycle 2). γ_{vw} is defined in the standard way from the relationship

$$\Delta G = -RT \ln K_p = \gamma_{vw} c A \tag{7a}$$

However, recently it has been shown that transfer free energies contain volume-dependent contributions to solubility and must thus be corrected to remove these terms if an appropriate surface area-dependent coefficient is to be extracted from partition experiments (DeYoung and Dill, 1990; Sharp et al., 1991). Although the need for such a correction has been a subject of some controversy (Ben-Naim and Mazo, 1993; Holtzer, 1992; Giesen et al., 1994), the issue has now been resolved in two recent papers (Chan and Dill, 1994; Kumar et al., 1995). It was found, based on a statistical mechanical analysis of chains on a lattice, that molar volume effects originate in solvent phases consisting of chain-like or asymmetric molecules and thus must be accounted for in partition experiments involving the alkane phase. Thus volume effects are likely to be small for vacuum-to-water transfer, so that it is possible to obtain γ_{vw} from Eq. 7a. In contrast, the calculation of γ_{aw} requires that volume effects be accounted for and these are derived form the Flory-Huggins expression, which can be written as (Flory, 1941; Huggins, 1941; Flory, 1953)

$$\Delta G = -RT \ln K_p - RT\left(1 - \frac{v^o(\mathrm{al}kane)}{v^o(water)}\right) = \gamma_{aw} c A \tag{7b}$$

where v^o(alkane) and v^o(water) are the molar volumes of an alkane and water, respectively. In practice γ_{vw} was derived from the 1 M standard state transfer free energy of alkanes from gas to water (Sharp et al., 1991) whereas γ_{aw} was obtained from Eq. 7b using the partition coefficients of alkanes between the neat alkane phase and water (Sharp et al., 1991). In addition, the effects of curvature on surface tension were taken into account as has been described previously (Sharp et al., 1991; Nicholls et al., 1991). The actual values for γ_{vw} and γ_{aw} depend on the atomic radii used to calculate the accessible surface area and are given for radii derived from different parameter sets in Table 1.

Comparison with experiment

A wide range of experimental measurements have been made of the stacking free energy of nucleic acid bases, nucleosides, and nucleotides in aqueous solution (Morcillo et al., 1987; Iza et al., 1988; Nakano and Igarashi, 1970; Sowers et al., 1987; Solie and Schellman, 1968; Rymden and Stilbs, 1985; Stokkeland and Stilbs, 1985; Broom et al., 1967; Ts'o et al., 1962; Mitchell and Sigel, 1978). Unfortunately, values measured by different groups for the same association reaction are often strongly at variance. For example, determinations of the 1 M standard state stacking free energy of AA (including base, nucleoside, and nucleotide forms) vary between −1.21 (Mitchell and Sigel, 1978) and −5.73 kcal/mol (Morcillo et al., 1987). Similarly, the variation in the same quantity for UU is between 0.29 (Ts'o et al., 1962) and −1.75 kcal/mol (Iza et al., 1988). Although determinations of the stacking free energy of a given complex by different workers vary, there is a consistency in the qualitative trends. Within the results of each group, stacking free energies in order of decreasing stability follow the order purine-purine > purine-pyrimidine,

TABLE 1 Nonpolar transfer parameters*

Potential function	Carbon radius (Å)	Hydrogen radius (Å)	γ_{vw} (kcal/(Å^{2}mol))	Correlation coefficient of vacuum-water fit (*r*)	γ_{aw} (kcal/(Å^{2}mol))	Correlation coefficient of alkane-water fit (*r*)
Amber	1.85	1.00	0.0064 ± 0.0017	0.965	0.058 ± 0.001	1.00
Poltev et al.	1.80	1.35	0.0056 ± 0.0016	0.963	0.051 ± 0.001	1.00
OPLS	1.96	1.40	0.0055 ± 0.0016	0.963	0.050 ± 0.001	1.00

All errors are within 95% confidence limits.

*Sitkoff, 1994.

TABLE 2 Electrostatic contribution to the base stacking free energy (kcal/mol)*

Complex	$\Delta G_c(1)$	$\Delta G_{rf}(1,80)$	$\Delta G_{el}(1,80)$	$\Delta G_c(2)$	$\Delta G_{rf}(2,80)$	$\Delta G_{el}(2,80)$
AA	−0.44	2.43	1.99	−0.22	1.23	1.01
AT	0.43	1.42	1.85	0.21	0.56	0.78
AG	−1.53	3.15	1.61	−0.77	1.70	0.94
AC	0.59	0.97	1.56	0.30	0.49	0.79
TA	−1.18	2.35	1.18	−0.59	1.17	0.58
TT	0.26	1.05	1.31	0.13	0.57	0.71
TG	−1.42	2.47	1.05	−0.71	1.29	0.58
TC	0.56	0.48	1.04	0.28	0.08	0.36
GA	−2.39	4.30	1.90	−1.20	2.13	0.94
GT	0.77	1.04	1.81	0.38	0.54	0.92
GG	2.30	−0.36	1.94	1.15	0.05	1.19
GC	−2.86	4.38	1.52	−1.43	2.25	0.82
CA	−0.65	2.09	1.44	−0.32	0.95	0.63
CT	−0.43	1.89	1.46	−0.21	0.95	0.74
CG	−2.96	4.11	1.15	−1.48	2.11	0.63
CC	2.86	−1.51	1.36	1.44	−0.66	0.58
UU	0.22	0.82	1.03	0.11	0.41	0.52

*Amber charge parameters and radii (Weiner et al., 1986).

pyrimidine-purine > pyrimidine-pyrimidine (Ts'o, 1974; Saenger, 1984; Morcillo et al., 1987; Iza et al., 1988; Nakano and Igarashi, 1970; Solie and Schellman, 1968; Broom et al., 1967; Ts'o et al., 1962; Mitchell and Sigel, 1978).

For quantitative comparison, we limit ourselves to relative stacking free energies determined by the same group. The quantity $\Delta\Delta G^0_{AA,UU} = \Delta G^0_{AA} - \Delta G^0_{UU}$ varies between −1.35 (Broom et al., 1967; Ts'o et al., 1962) and −3.98 kcal/mol (Morcillo et al., 1987; Iza et al., 1988). The former value is derived from experiments performed on nucleosides rather than isolated bases and was obtained under concentration conditions where the bases are believed to polymerize rather than dimerize (Ts'o et al., 1962). The latter value is derived from experiments performed at low concentration and was inferred by the authors as characterizing a dimerization, rather than a polymerization, process (Morcillo et al., 1987; Iza et al., 1988).

RESULTS

Electrostatic contributions to stacking free energies calculated with the Amber parameter set for 17 stacked complexes are given in Table 2. In all cases, the electrostatic contribution opposes binding. The Coulombic contribution is either favorable or unfavorable depending on the individual case. The reaction field contribution is usually unfavorable, because stacking involves the partial removal of water from the surface of a polar molecule and its replacement with a less polarizable medium (the other base). However, in four cases, CC and GG, with the Amber set, and UU with the Amber and Poltev sets, the latter of which is not shown, this contribution is favorable. In such cases, like partial charges are brought close together upon stacking and favorable reaction-field contributions result from the interaction of these fixed charges with the induced charges of their neighbors. When such contributions dominate, ΔG_{rf} will be negative. When $\epsilon_i = 2$, ΔG_c, ΔG_{rf}, and ΔG_{el} are approximately one-half as large, as are the corresponding quantities when $\epsilon_i = 1$.

The nonpolar contributions to the stacking free energy is given in Table 3. LJ and nonpolar solvation contributions favor stacking for all complexes. With Amber parameters, $\Delta G_{np}(v)$ is ~50% larger than $\Delta G_{np}(a)$. $\Delta G_{np}(v)$ is also significantly larger than $\Delta G_{np}(a)$ when the OPLS and Poltev parameter sets are used (not shown), although by differing amounts.

The LJ term in order of decreasing contribution to complex stability follows the order purine-purine ≫ purine-pyrimidine > pyrimidine-purine > pyrimidine-pyrimidine. Purines have more heavy atoms than pyrimidines, so they have larger LJ interactions. Similarly, with a few exceptions, the quantities $\gamma_{vw}\Delta(cA)$ and $\gamma_{aw}\Delta(cA)$ follow the same order as they depend on changes in surface area upon complex formation. $\Delta G_{np}(v)$ follows the same order as ΔG_{LJ}, because ΔG_{LJ} is the dominant contribution to $\Delta G_{np}(v)$. In fact, ΔG_{LJ} is a good approximation to $\Delta G_{np}(v)$. The electrostatic contribution to base stacking does not correlate with the purine or pyrimidine composition or ordering in the stacked complex. This is because the electrostatic free energy depends on the details of charge distribution and not just on atomic size or surface area.

The total free energy is given in Table 4. Because electrostatic interactions oppose complexation, our calculations predict that nonpolar interactions drive base stacking. For a given complex, with Amber parameters, the free energy calculated with the first cycle is, on average, ~70% larger than calculated with the second cycle, independent of the internal dielectric constant. $\Delta G(v)$ in order of decreasing stability follows the order purine-purine ≫ purine-pyrimidine > pyrimidine-purine >pyrimidine-pyrimidine with a 2-kcal/mol spread between the least stable purine-purine and the most stable pyrimidine-pyrimidine. Some members of each class have similar stacking free energies, but the trend holds between the average stacking free energy of each class. For $\Delta G(a)$, purine-purine complexes are again

TABLE 3 Nonpolar contributions to the base stacking free energy (kcal/mol)*

Complex	ΔG_{LJ}	$\gamma_{vw}\Delta(cA)$	$\Delta G_{np}(v)$	$\Delta G_{np}(a)$
AA	−7.95	−0.58	−8.52	−5.24
AT	−6.64	−0.53	−7.17	−4.81
AG	−8.08	−0.57	−8.65	−5.17
AC	−6.06	−0.50	−6.56	−4.50
TA	−5.93	−0.49	−6.42	−4.46
TT	−5.75	−0.48	−6.23	−4.39
TG	−5.83	−0.46	−6.30	−4.21
TC	−5.36	−0.46	−5.82	−4.20
GA	−8.69	−0.61	−9.31	−5.57
GT	−7.17	−0.57	−7.74	−5.12
GG	−9.11	−0.62	−9.74	−5.63
GC	−6.73	−0.54	−7.27	−4.89
CA	−5.87	−0.49	−6.36	−4.46
CT	−5.62	−0.48	−6.10	−4.38
CG	−5.75	−0.47	−6.22	−4.24
CC	−5.26	−0.46	−5.72	−4.16
UU	−5.11	−0.43	−5.54	−3.92

*Amber LJ parameters and radii (Weiner et al., 1986).

TABLE 4 Total base stacking free energies (nonpolar plus electrostatic contributions) (kcal/mol)*

	$\epsilon_i = 1$		$\epsilon_i = 2$	
Complex	$\Delta G(v)$	$\Delta G(a)$	$\Delta G(v)$	$\Delta G(a)$
AA	−6.53	−3.24	−7.51	−4.23
AT	−5.32	−2.96	−6.39	−4.04
AG	−7.03	−3.55	−7.71	−4.23
AC	−5.00	−2.95	−5.78	−3.72
TA	−5.25	−3.28	−5.84	−3.88
TT	−4.92	−3.09	−5.52	−3.68
TG	−5.25	−3.16	−5.72	−3.63
TC	−4.78	−3.16	−5.46	−3.85
GA	−7.41	−3.66	−8.37	−4.63
GT	−5.93	−3.32	−6.82	−4.20
GG	−7.79	−3.69	−8.54	−4.44
GC	−5.76	−3.37	−6.45	−4.06
CA	−4.92	−3.02	−5.73	−3.83
CT	−4.64	−2.92	−5.36	−3.65
CG	−5.07	−3.09	−5.59	−3.61
CC	−4.36	−2.80	−5.13	−3.58
UU	−4.51	−2.89	−5.02	−3.40

*Amber charges, LJ parameters, and radii (Weiner et al., 1986).

the most stable, with members of the classes of pyrimidine-containing complexes interspersed in order of stability. These trends hold whether the internal dielectric constant is 1 or 2. Hence the total free energies reproduce the experimental trends.

The absolute and relative total stacking free energies of the AA and UU complexes calculated according to the Amber and OPLS potential set and the potential set of Poltev et al. are given in Table 5. The value of $\Delta\Delta G^0_{AA,UU}$ calculated with most of the parameters and cycles used in this work falls within or close to the experimental range of -1.35--3.98 kcal/mol. Only the OPLS parameter set with the second cycle yields results contrary to experiment.

TABLE 5 Relative and absolute total base stacking free energies (nonpolar plus electrostatic contributions) of AA and UU (kcal/mol)

	$\epsilon_i = 1$		$\epsilon_i = 2$	
Complex	$\Delta G(a)$	$\Delta G(a)$	$\Delta G(v)$	$\Delta G(a)$
Amber*				
AA	−6.53	−3.24	−7.51	−4.23
UU	−4.51	−2.89	−5.02	−3.40
$\Delta\Delta G_{AA,UU}$	−2.02	−0.35	−2.49	−0.82
Poltev et al.‡				
AA	−4.53	−3.90	−5.21	−4.58
UU	−3.51	−3.32	−3.77	−3.57
$\Delta\Delta G_{AA,UU}$	−1.02	−0.58	−1.45	−1.01
OPLS§				
AA	−4.79	−1.76	−6.30	−3.27
UU	−3.94	−2.70	−4.43	−3.20
$\Delta\Delta G_{AA,UU}$	−0.85	0.95	−1.87	−0.07

*Amber charges, LJ parameters, and radii (Weiner et al., 1986).

‡Poltev et al. charges, LJ parameters, and radii (Zhurkin et al., 1981).

§OPLS charges, LJ parameters, and radii (Jorgensen and Pranata, 1990).

DISCUSSION

The major finding of this study is that electrostatic interactions oppose base stacking whereas nonpolar interactions favor stacking. This is consistent with the observation that observed stacking free energies are roughly proportional to molecular size, which is expected for nonpolar, but not for electrostatic, interactions. The results obtained here are consistent with the explicit water molecular dynamics calculations of Kollman and co-workers (Bash et al., 1987; Cieplak and Kollman, 1988; Dang and Kollman, 1990).

In the following we partition the nonpolar contribution to stacking into enhanced LJ interactions as a result of close-packing and hydrophobic interactions. It should be emphasized that in the partition of the free energy used here, there is a hydrophobic contribution to the association of polar as well as nonpolar molecules, which results from the exclusion of water from the interface. In polar molecules, the electrostatic contribution is then added to the hydrophobic contribution, as shown in Fig. 1.

Whether the nonpolar contribution to base stacking can be associated entirely with hydrophobicity depends on whether base stacking can be treated in terms of the association of two liquid phases (Nicholls et al., 1991). This will clearly not be correct if, for example, the stacked complex is solid-like, in that interbase LJ interactions are stronger in the complex than base-water interactions. In this case, there will be uncompensated LJ interactions that are caused by closer packing in the complex than in the uncomplexed state. As LJ interactions are calculated explicitly in cycle 1, the difference between $\Delta G_{np}(v)$ and $\Delta G_{np}(a)$ may be attributable to enhanced dispersion interactions in the complex as a result of close packing. That is, if the nonpolar component could simply be modeled by the burial of nonpolar surface area, as in cycle 2, the nonpolar contribution to the binding free energy computed by both cycles should, in principle, be the same. However, where they are not the same, which, for the potential models studied, includes every case studied except the UU complex with the potential set of Poltev and co-workers (not shown), this suggests that enhanced dispersion interactions favor complex formation.

To investigate this issue further, nonpolar contributions, as calculated with Amber parameters, are plotted in Fig. 2 versus the change in accessible area upon complex formation. The slope of the plot of ΔG_{LJ} is 0.113 kcal/(Å^2 mol), whereas that of $\Delta G_{np}(v)$ is 0.118 kcal/(Å^2 mol) and that of $\Delta G_{np}(a)$ is 0.047 kcal/(Å^2 mol). Hence in this example, enhancement of LJ interactions as a result of close packing contributes 0.071 kcal/(Å^2 mol), which is significantly larger than the hydrophobic contribution defined by $\Delta G_{np}(a)$. For the OPLS parameter set, the enhancement of LJ interactions as a result of close packing contributes 0.058 kcal/(Å^2 mol), whereas for the set of Poltev and co-workers, the effect amounts to only 0.016 kcal/(Å^2 mol), which is significantly smaller than the hydrophobic contribution de-

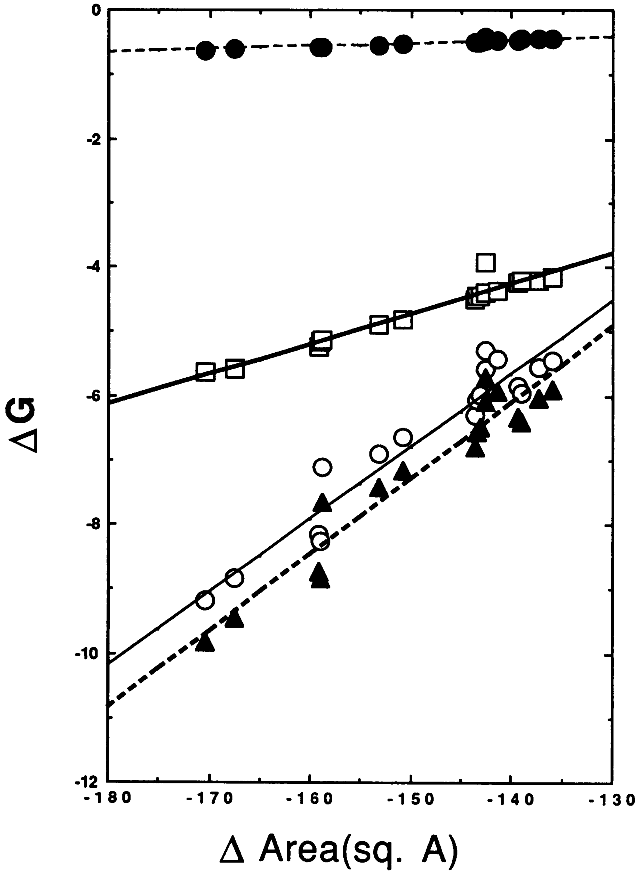

FIGURE 2 Dependence of nonpolar contributions to the stacking energy on change in surface area. ○, ΔG_{LJ} of individual stacked complexes. ———, Best straight line for ΔG_{LJ} versus area. ●, $\gamma_{vw}\Delta(cA)$ of individual stacked complexes. - - - -, Best straight line for $\gamma_{vw}\Delta(cA)$ versus area. □, $\gamma_{aw}\Delta(cA)$ of individual stacked complexes. ———, Best straight line for $\gamma_{aw}\Delta(cA)$ versus area. ▲, $\Delta G_{np}(\nu)$ of individual stacked complexes. - - - -, Best straight line for $\Delta G_{np}(\nu)$ versus area.

fined by $\Delta G_{np}(a)$. It follows from this analysis that both hydrophobicity and enhancement of LJ interactions as a result of close packing are responsible for base stacking, although their relative contributions are parameter dependent and are thus difficult to isolate.

Newcomb and Gellman (1994a) have recently used NMR to study a series of molecules consisting of two aromatic groups linked by a flexible chain. They found that, in aqueous solution, two linked adenines exhibited changes in chemical shifts relative to a single adenine attached to the same linker chain. An adenine linked to a naphthalene exhibited similar effects. In contrast, two linked naphthalenes did not exhibit corresponding large shifts, which Newcomb and Gellman interpreted as implying the absence of stacking. However, interpretation of their spectra is complicated by pronounced line broadening and small shifts that may reflect dynamic averaging between stacked and unstacked conformations (Newcomb and Gellman, 1994b). In dimethylsulfoxide (DMSO), no changes in chemical shifts were observed for any of the molecules. Because Newcomb and Gellman concluded that the naphthalenes do not stack in water, they suggested that nucleic acid base stacking is caused by electrostatic rather than nonpolar interactions. However, as we have stated, there is evidence for naphthalene stacking in water. In addition, the electrostatic mechanism proposed does not explain the changes in chemical shifts in the adenine-naphthalene molecule. Furthermore, as DMSO has a much lower dielectric constant than water (4.7 vs. 78.9 at 25°C (Lide, 1991)), the electrostatic contribution to stacking in DMSO is expected to be more favorable than in water. As the bases seem to stack in water and not DMSO, stacking in water must be a result of nonelectrostatic contributions, which is consistent with the conclusions of this study.

Appendix

Equivalence of the electrostatic free energy in the two cycles

Our basic approach to the calculation of the electrostatic free energy has already been given (Gilson and Honig, 1988). However, the thermodynamic cycles used in the previous publication are somewhat different than those used in the present work. Hence it is necessary to derive the expressions for the electrostatic free energy in cycles 1 and 2 and show that they are equal.

The total electrostatic free energy of a molecule or complex is the sum of Coulombic and reaction field terms (Gilson and Honig, 1988):

$$G_{el}(\epsilon_i, \epsilon_o) = G_c(\epsilon_i) + G_{rf}(\epsilon_i, \epsilon_o) \tag{A1}$$

For cycle 1,

$$\Delta G_{el}(gas) = \Delta G_c(\epsilon_i) + \Delta G_{rf}(\epsilon_i, 1) \tag{A2}$$

For each of the reactants and the product in cycle 1,

$$G_{el}(solv) = G_{rf}(\epsilon_i, \epsilon_o) - G_{rf}(\epsilon_i, 1) \tag{A3}$$

Taking the difference in G_{el}(solv) between reactant and product and combining Eqs. A3 and A4 gives the free energy of cycle 1:

$$\Delta G_{el}(\nu) = \Delta G_c(\epsilon_i) + \Delta G_{rf}(\epsilon_i, \epsilon_o) \tag{A4}$$

That this is also the electrostatic free energy of cycle 2 follows from inspection of Fig. 1.

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REFERENCES

Aida, M. and C. Nagata. 1986. An ab initio molecular orbital study on the stacking interaction between nucleic acid bases. *Int. J. Quant. Chem.* 39:1253–1261.

Aradi, F. 1990. ^{1}H NMR chemical shift study on the stacking interaction between purine and 6-methylpurine. *Magn. Reson. Chem.* 28: 1040–1044.

Bash, P., U. C. Singh, R. Langridge, and P. Kollman. 1987. Free energy calculations by computer simulation. *Science.* 236:564–568.

Ben-Naim, A., and R. M. Mazo. 1993. Size dependence of the solvation free energies of large solutes. *J. Phys. Chem.* 97:10829–10834.

Biosym, 1991. Insight II. Biosym, San Diego, CA.

Broom, A. D., M. P. Schweizer, and P. O. P. Ts'o. 1967. Interaction and association of bases and nucleosides in solutions. V. Studies of the association of purine nucleosides by vapor pressure osmometry and by proton magnetic resonance. *J. Am. Chem. Soc.* 89:3612–3622.

Chan, H. S., and K. A. Dill. 1994. Solvation: effects of molecular size and shape. *J. Chem. Phys.* 101:7007–7026.

Cieplak, P., and P. A. Kollman. 1988. Calculation of the free energy of association of nucleic acid bases in vacuo and water solution. *J. Am. Chem. Soc.* 110:3734–3739.

Claverie, P., B. Pullman, and J. Caillet. 1966. Van der Waals-London interactions between stacked purines and pyrimidines. *J. Theor. Biol.* 12:419–434.

Dang, L. X ., and P. A. Kollman. 1990. Molecular dynamics simulations study of the free energy of association of 9-methyladenine and 1-methylthymine bases in water. *J. Am. Chem. Soc.* 112:503–507.

Danilov, V. I., and I. S. Tolokh. 1984. Nature of the stacking of nucleic acid bases in water: a Monte Carlo simulation. *J. Biomol. Struct. Dyn.* 2:119–130.

Devoe, H., and I. Tinoco. 1962. The stability of helical polynucleotides: base contributions. *J. Mol. Biol.* 4:500–517.

DeYoung, L. R., and K. A. Dill. 1990. Partitioning of nonpolar solutes into bilayers and amorphous *n*-alkanes. *J. Phys. Chem.* 94:801–809.

Flory, P. J. 1941. Thermodynamics of high polymer solutions. *J. Chem. Phys.* 9:660–661.

Flory, P. J. 1953. Principles of Polymer Chemistry. Cornell University Press, Ithaca, NY.

Friedman, R. A., and B. Honig. 1992. The electrostatic contribution to DNA base stacking interactions. *Biopolymers.* 32:145–159.

Giesen, D. J., C. J. Cramer, and D. G. Truhlar. 1994. Entropic contributions to the free energy of solvation. *J. Phys. Chem.* 4141–4147.

Gill, S. J., N. F. Nicholls, and I. Wadso. 1976. Calorimetric determination of enthalpies of solution of slightly soluble liquids. II. Enthalpy of solution of some hydrocarbons in water and their use in establishing the temperature dependence of their solubilities. *J. Chem. Thermodyn.* 8:445–452.

Gilson, M. K., and B. Honig. 1988. Calculation of the total electrostatic energy of a macromolecular system: solvation energies, binding energies, and conformational analysis. *Proteins.* 4:7–18.

Hanlon, S. 1966. The importance of London dispersion forces in the maintenance of the deoxyribonucleic acid helix. *Biochem. Biophys. Res. Commun.* 23:861–867.

Helmkamp, G. K., and N. S. Kondo. 1967. Preferred orientations in purine stacking. *Biochim. Biophys. Acta.* 145:27–30.

Herskovits, T. T., S. J. Singer, and E. P. Geiduschek. 1961. Nonaqueous solutions of DNA: denaturation in methanol and ethanol. *Arch. Biochem. Biophys.* 94:99–114.

Holtzer, A. 1992. The use of Flory-Huggins theory in interpreting partitioning of solutes between organic liquids and water. *Biopolymers.* 32:711–715.

Honig, B., K. A. Sharp, and A.-S. Yang. 1993. Macroscopic models of aqueous solutions: biological and chemical applications. *J. Phys. Chem.* 97:1101–1109.

Huggins, M. L. 1941. Solutions of long chain polymers. *J. Chem. Phys.* 9:440–441.

Hunter, C. A. 1993. Sequence-dependent DNA structure: the role of base stacking interactions. *J. Mol. Biol.* 230:1025–1054.

Iza, N., M. Gil, J. L. Montero, and J. Morcillo. 1988. Self-association of uracil in aqueous solution: study of dilute solutions by normal and second derivative UV absorption spectroscopy. *J. Mol. Struct.* 175: 19–24.

Janin, J., and C. Chothia. 1978. Role of hydrophobicity in the binding of coenzymes. *Biochemistry.* 17:2943–2948.

Jorgensen, W. L., and J. Pranata. 1990. Importance of secondary interactions in triply hydrogen bonded complexes: guanine-cytosine vs. uracil-2,6-diaminopyridine. *J. Am. Chem. Soc.* 112:2008–2010.

Kumar, S. K., I. Szleifer, K. Sharp, P. Rossky, R. A. Friedman, and B. Honig. 1995. Size dependence of free energies. I. A Flory-Huggins approach. *J. Phys. Chem.* 99:8382–8391.

Lavery, R. 1990. JUMNA. Laboratoire de Biochimie Theorique CNRS, Institut de Biologie Physico-Chimique, Paris.

Lide, D. R. 1991. CRC Handbook of Chemistry and Physics. CRC Press, Boca Raton, FL.

Martel, P. 1979. Base crystallization and base stacking in water. *Eur. J. Biochem.* 96:213–219.

Mitchell, P. R., and H. Sigel. 1978. A proton nuclear-magnetic-resonance study of self-stacking in purine and pyrimidine nucleosides and nucleotides. *Eur. J. Biochem.* 88:149–154.

Morcillo, J., E. Gallego, and F. Peral. 1987. A critical study of the application of ultraviolet spectroscopy to the self-association of adenine, adenosine, and 5′-AMP in aqueous solution. *J. Mol. Struct.* 157: 353–369.

Nakano, N. I., and S. J. Igarashi. 1970. Molecular interactions of pyrimidines, purines, and some other heteroaromatic compounds in aqueous media. *Biochemistry.* 9:577–583.

Newcomb, L. F., and S. H. Gellman. 1994a. Aromatic stacking interactions in aqueous solution: evidence that neither classical hydrophobic effects nor dispersion forces are important. *J. Am. Chem. Soc.* 116:4993–4994.

Newcomb, L. F., and S. H. Gellman. 1994b. Aromatic stacking interactions in aqueous solution: evidence that neither classical hydrophobic effects nor dispersion focus are important. *J. Am. Chem. Soc.* 116:4993–4994, Supplementary material.

Nicholls, A., K. A. Sharp, and B. Honig. 1990. DelPhi. Dept. of Biochemistry and Molecular Biophysics, Columbia University, New York.

Nicholls, A., K. A. Sharp, and B. Honig. 1991. Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins.* 11:281–296.

Peitzsch, R. M., and S. McLaughlin. 1993. Binding of acylated peptides and fatty acids to phospholipid vesicles: pertinence to myristoylated proteins. *Biochemistry.* 32:10436–10433.

Pohorille, A., L. R. Pratt, S. K. Burt, and R. D. MacElroy. 1984. Solution influence on biomolecular equilibria: nucleic acid base associations. *J. Biomol. Struct. Dyn.* 1:1257–1280.

Poltev, V. I., and N. V. Shulyupina. 1986. Simulation of interactions between nucleic acid bases by refined atom-atom potential functions. *J. Biomol. Struct. Dyn.* 3:739–763.

Privalov, P. L., and S. J. Gill. 1988. Stability of protein structure and hydrophobic interaction. *Adv. Protein Chem.* 39:191–234.

Rymden, R., and P. Stilbs. 1985. Nucleotide aggregation in aqueous solution. *Biophys. Chem.* 21:145–156.

Saenger, W. 1984. Principles of Nucleic Acid Structure. Springer-Verlag, New York.

Sharp, K., D. Sitkoff, and B. Honig. 1994. Correlating solvation free energies and surface tensions of hydrocarbon solutes. *Biophys. Chem.* 51:397–409.

Sharp, K. A., A. Nicholls, R. M. Fine, and B. Honig. 1991. Reconciling the magnitude of the microscopic and macroscopic hydrophobic effects. *Science.* 252:106–109.

Sharp, K. A., A. Nicholls, R. Friedman, and B. Honig. 1991. Extracting hydrophobic free energies from experimental data: relationship to protein folding and theoretical models. *Biochemistry.* 30:9686–9697.

Sinanoglu, O., and S. Abdulnur. 1964. Hydrophobic stacking of bases and the solvent denaturation of DNA. *Photochem. Photobiol.* 3:333–342.

Sitkoff, D., K. A. Sharp, and B. Honig. 1994. Accurate calculation of hydration free energies using macroscopic solvent models. *J. Phys. Chem.* 98:1978–1988.

Smith, K. C., and B. Honig. 1994. Evaluation of the conformational free energies of loops in proteins. *Proteins Struct. Funct. Genet.* 18:119–132.

Solie, T. N., and J. A. Schellman. 1968. The interaction of nucleosides in aqueous solution. *J. Mol. Biol.* 33:61–77.

Sowers, L. R., B. R. Shaw, and W. D. Sedwick. 1987. Base stacking and molecular polarizability: effect of a methyl group in the 5-position of pyrimidines. *Biochem. Biophys. Res. Commun.* 148:790–794.

Sridharan, S., A. Nicholls, and B. Honig. 1992. A new vertex algorithm to calculate solvent accessible surface areas. *Biophys. J.* 61:A174.

Stokkeland, I., and P. Stilbs. 1985. A multicomponent self-diffusion NMR study of aggregation of nucleotides, nucleic acid bases and some derivatives in aqueous solution with divalent metal ions added. *Biophys. Chem.* 22:65–75.

Sturtevant, J. M., S. A. Rice, and E. P. Geiduschek. 1958. The stability of the DNA helical molecule in solution. *Disc. Faraday Soc.* 25:138–149.

Ts'o, P. O. P., editor. 1974. Bases, nucleosides, and nucleotides. *In* Basic Principles of Nucleic Acid Chemistry. Academic Press, New York. 454–584.

Ts'o, P. O. P., I. S. Melvin, and A. C. Olson. 1962. Interaction and association of bases in aqueous solutions. *J. Am. Chem. Soc.* 85:1289–1296.

Zhurkin, V. B., V. I. Poltev, and V. L. Florent'ev. 1981. Atom-atom potential functions for conformational calculations of nucleic acids. *Mol. Biol. (Russ.)* 14:1116–1130.