Solvent Influence on Base Stacking

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ABSTRACT In this paper we present a detailed analysis of the base-stacking phenomenon in different solvents, using nanosecond molecular dynamics simulations. The investigation focuses on deoxyribo- and ribodinucleoside monophosphates in aqueous and organic solutions. Organic solvents with a low dielectric constant, such as chloroform, and solvents with intermediate dielectric constants, such as dimethyl sulfoxide and methanol, were analyzed. This was also done for water, which is highly polar and has a high dielectric constant. Structural parameters such as the sugar puckering and the base-versus-base orientations, as well as the energetics of the solute-solvent interactions, were examined in the different solvents. The obtained data demonstrate that base stacking is favored in the high dielectric aqueous solution, followed by methanol and dimethyl sulfoxide with intermediate dielectric constants, and chloroform, with a low dielectric constant.

INTRODUCTION

Base stacking is one of the driving forces responsible for the stabilization of the three-dimensional structure of DNA and RNA molecules. In contrast to the composition-dependent hydrogen bonding energy of Watson-Crick base pairing, base-stacking interactions are very sequence dependent, which was first confirmed from the analysis of a dodecamer crystal structure (Drew et al., 1981). A number of different experimental and theoretical methods have been used to investigate the base-stacking phenomenon, which has been attributed to electrostatic interactions, hydrophobic effects, and dispersion interactions (Hanlon, 1966; Herskovits et al., 1961; Newcomb and Gellman, 1994). The natural habitat for nucleic acids is an aqueous solution, containing a mix of salts as well as high-molecular-weight compounds, and structural properties of nucleic acids can be modulated by the dielectric nature of other molecular species with which they interact (Fogolari et al., 1997); proteins interacting with DNA and RNA may well exert a structural, or dynamic, influence and modify the properties of the nucleic acid by providing a hydrophobic environment, shielding it from the aqueous solution. It is therefore of interest to study one of the basic determinants of nucleic acid structure, base stacking, in surroundings with different dielectric properties.

One of the most popular model systems in this area is the ribodinucleoside monophosphate riboadenylyl-3′,5′-riboadenosine (rAprA), which has been extensively examined both dynamically, using nuclear magnetic resonance experiments (Kondo and Danyluk, 1976; Chachaty et al., 1980), and thermodynamically (Davis and Tinoco, 1968; Frechet et al., 1979; Johnson and Schleich, 1974; Kang et al., 1992; Lee and Tinoco, 1977; Lowe and Schellman, 1972; Ogasawara and Inoue, 1976; Poland et al., 1966; Powell et al., 1972; van Holde et al., 1965; Watts and Tinoco, 1978). The deoxyribodinucleoside monophosphate deoxyribothymidylyl-3′,5′-deoxyribothymidine (dTpdT) has also been investigated experimentally and theoretically (Broyde et al., 1978; Cantor and Schimmel, 1980; Frechet et al., 1979; Thiyagarajan and Ponnuswamy, 1978).

A theoretical tool that can give insight into the dynamic behavior and the free energy of stacking is molecular dynamics (MD) simulations (McCammon and Harvey, 1987). Simulations have been performed of both a stacked and an unstacked conformation of a ribodinucleoside monophosphate in aqueous solution (Norberg and Nilsson, 1994a,b). A transformation to a more stacked form during the simulation was observed for the unstacked structure. In the simulation of the stacked structure, it was found that the stacked conformation was maintained during the whole simulation. From these simulations the distance between the glycosidic base nitrogen atoms was found to correlate well to the degree of stacking. Other theoretical investigations of base stacking have focused on the aromatic base-base interactions and have not taken into account the dynamical backbone and/or the influence of solvent interactions (Aida, 1988; Pohorille et el., 1984). Solvent interactions are known to affect the conformational state of nucleic acids, and base stacking has been found to be favored in aqueous solution (Cantor and Schimmel, 1980; Lowe and Schellman, 1972).

Another method that has been applied to the study of the free energy of stacking is potential of mean force (PMF) calculations (Beveridge and DiCapua, 1989; Straatsma and McCammon, 1992). In a PMF investigation of the stacking between the 9-methyladenine and 1-methylthymine bases, the backbone was not included (Dang and Kollman, 1990). All combinations of bases in the A-RNA ribodinucleoside monophosphates and the B-DNA deoxyribodinucleoside monophosphates have been examined in aqueous solution by using potential of mean force calculations (Norberg and Nilsson, 1995b,c). In these studies the stacking free energies

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of going from the stacked structure to an unstacked conformation were determined and found to be very sequence dependent. Stacking preferences followed the general sequence purine-purine > purine-pyrimidine ≥ pyrimidine-purine > pyrimidine-pyrimidine. This order has also been obtained experimentally (Ts'o et al., 1963). In another PMF investigation the entropy and enthalpy of the base stacking process for the ribodinucleoside monophosphate rAprA were determined (Norberg and Nilsson, 1995d). In this study the base stacking process of rAprA was found to be very temperature dependent and shown to be enthalpy driven.

In the present study the stacking abilities of ribo- and deoxyribodinucleoside monophosphates in aqueous and organic solutions are examined in the model systems rAprA and dTpdT, using extensive nanosecond unrestrained molecular dynamics simulations and potential of mean force calculations. The stacking propensity was investigated in aqueous solution and in organic solvents such as chloroform ($CHCl_3$), dimethyl sulfoxide (DMSO), and methanol (MeOH). In these solvents the dielectric constants ranged from 78.3 for water to 4.8 for chloroform; dimethyl sulfoxide and methanol had intermediate dielectric constants (Lide, 1993).

MATERIALS AND METHODS

Nucleic acid structures

The starting structures of the ribodinucleoside monophosphates riboadenylyl-3′,5′-riboadenosine (rAprA) and the deoxyribodinucleoside monophosphate deoxyribothymidylyl-3′,5′-deoxyribothymidine (dTpdT) were built from x-ray fiber diffraction data (Arnott et al., 1976). The structure of rAprA was generated in single-stranded stacked standard A-RNA form, and that of dTpdT was generated in single-stranded stacked standard B-DNA form. The distance between the glycosidic base nitrogen atoms was 4.736 Å and 4.417 Å for rAprA and dTpdT, respectively. To obtain charge neutralization, a sodium counterion was placed on the bisector of the phosphate oxygens of each structure. Thereafter the two structures were energy minimized by 100 cycles of steepest descent.

Aqueous and organic solvents

The boxes of solvents were generated using the charges and the nonbonded Lennard-Jones parameters as displayed in Table 1. Below we describe the setup procedure of the water, methanol, dimethyl sulfoxide, and chloroform boxes in detail. A cubic box of water with a side length of 25.0 Å was built from the TIP3P water model (Jorgensen et al., 1983). The rAprA and dTpdT structures were solvated in water boxes, and all of the water molecules that had the oxygen atom closer than 2.8 Å to any heavy atom of each structure were removed. Thereafter the systems were energy minimized by 100 cycles of steepest descent and 3000 cycles of adopted-basis set Newton-Raphson (Brooks et al., 1983), using harmonic constraints with a force constant of 1.0 kcal · mol^{-1} · $Å^{-2}$ on the atoms of the solutes.

TABLE 1 The charges and nonbonded parameters of the solvents used

	q (e)	σ (Å)	ϵ (kcal/mol)
$CH(CHCl_3)$	0.420	3.800	0.080
$Cl(CHCl_3)$	−0.140	3.470	0.300
CH_3(DMSO)	0.160	2.019	0.160
O(DMSO)	−0.459	1.650	0.066
S(DMSO)	0.139	2.000	0.202
CH_3(MeOH)	0.285	2.119	0.207
H(MeOH)	0.400	1.000	0.000
O(MeOH)	−0.685	1.723	0.170
$H(H_2O)$	0.417	0.4000	0.046
$O(H_2O)$	−0.834	3.1506	0.1521

In the case of MeOH, a 25.0-Å-side cubic box was also used to solvate the rAprA and dTpdT molecules. This box was generated using united-atom MeOH parameters (Jorgensen, 1986), for which the influence of hydrogen bonding on microscopic properties of MeOH has been analyzed (Guàrdia et al., 1994). In each system MeOH molecules with the oxygen atom closer than 3.0 Å to any heavy atom of the solute were deleted. The solutes in each system were harmonically constrained by using a force constant of 20.0 kcal · mol^{-1} · $Å^{-2}$ during the energy minimization: first 100 steps of steepest descent and then 3000 steps of adopted-basis set Newton-Raphson.

A cubic box of DMSO molecules was generated to have a side length of 31.6 Å. The united-atom DMSO parameters (Jorgensen, personal communication) had been applied earlier in a MD study of the enzyme subtilisin (Zheng and Ornstein, 1996). The box was first energy minimized by 200 cycles of steepest descent. The rAprA and dTpdT molecules were solvated in the DMSO boxes, and the DMSO molecules for which the sulfur atoms were closer than 3.0 Å from any heavy atom of the solutes were removed. Thereafter the systems were energy minimized by 200 cycles of steepest descent and 1000 cycles of adopted-basis set Newton-Raphson, and the rAprA and dTpdT were constrained using a harmonic potential with a force constant of 20.0 kcal · mol^{-1} · $Å^{-2}$.

The box of $CHCl_3$ molecules was built with a side length of 32.9 Å and relaxed using 200 steps of steepest descent minimization. The united-atom $CHCl_3$ parameters (Jorgensen et al., 1990) have previously been adopted to analyze conformations of peptides (Mierke and Kessler, 1993). Each of the rAprA and dTpdT structures was solvated in $CHCl_3$ boxes, and all of the $CHCl_3$ molecules with carbon atoms closer than 3.0 Å to any heavy atom of the structure were removed. In each system energy minimizations of 200 cycles of steepest descent and 1000 cycles of adopted-basis set Newton-Raphson were performed, and the solutes were harmonically constrained using a force constant of 20.0 kcal · mol^{-1} · $Å^{-2}$.

Potential of mean force

As a reaction pathway of the stacking-unstacking process, the R_{NxNy} (Nx, Ny = N1-pyrimidine, N9-purine) distance between the glycosidic base nitrogen atoms was used (Norberg, 1995). This simple reaction coordinate correlates well with stacking (Norberg and Nilsson, 1994b), while still allowing the bases and backbone to be flexible. The umbrella sampling method (Beveridge and DiCapua, 1989; Straatsma and McCammon, 1992) was used to obtain the potential of mean force along this reaction coordinate:

$$w(R_{NxNy}) = -k_B T \ln \rho^*(R_{NxNy}) - E_{restr}(R_{NxNy}) + C$$

where k_B is the Boltzmann constant, T is the temperature, $\rho^*(R_{NxNy})$ is the probability distribution along the reaction pathway, and C is a normalization constant. In the umbrella sampling we used a harmonic restraining potential,

$$E_{restr} = k(R_{NxNy} - R_{ref})^2$$

where k is the force constant, R_{NxNy} is the distance between the glycosidic base nitrogen atoms, which in a particular simulation window was restrained to the reference value R_{ref}. The force constant was set at 16.0 kcal · mol^{-1} · $Å^{-2}$, except for the two outermost simulations, in which the force constant was 32.0 kcal · mol^{-1} · $Å^{-2}$. In an earlier study of a ribotrinucleoside diphosphate, a two-dimensional PMF calculation was

performed using a harmonic restraining potential for two reaction pathways (Norberg and Nilsson, 1996a).

Here we describe the parameters used in the MD simulations for the PMF calculations. In all of the simulations, periodic boundary conditions were utilized. A relative dielectric constant of 1.0 was applied, and the nonbonded list was updated every 20 steps. The trajectories were calculated using the CHARMM program (Brooks et al., 1983) with the all-atom nucleic acid parameters (MacKerell et al., 1995). To allow for a time step of 0.002 ps in the integration of the equations of motion, all hydrogen atom-heavy atom bond lengths were constrained with the SHAKE algorithm (Ryckaert et al., 1977). The integration of the equations of motion was carried out with the Verlet algorithm (Verlet, 1967) in all of the PMF calculations. The nonbonded interactions were smoothly shifted to zero at a cutoff of 11.5 Å (Brooks et al., 1983), and the coordinates were saved every 20 steps. In the simulations a temperature of 300 K was maintained by periodically checking a window of ±5 K. All of the PMF calculations were performed on DEC AXP 3000/400 workstations.

We first performed an equilibration of each system during a period of 40 ps, with the restrained distance set to 4.5 Å, using a force constant of 16.0 kcal · mol^{-1} · $Å^{-2}$. Conformational space was thereafter sampled by performing MD simulations in 18 windows, with R_{ref} varying from 3.5 Å to 12.0 Å in 0.5-Å intervals. The sampling was started from the MD simulation with R_{ref} = 4.5 Å; in each window the simulation was started from a snapshot of the preceding window, and after 5 ps of equilibration conformations were sampled for 40–60 ps. In the R_{ref} = 5–10 Å range, the structures were in transition between stacked and unstacked states, and longer simulation times were used. From a MD simulation in aqueous solution of an unstacked conformation of the ribodinucleoside monophosphate guanylyl-3′,5′-uridine, the majority of the states were obtained in the R_{ref} = 6–9 Å range (Norberg and Nilsson, 1994b). The total simulation time for the PMF calculation for one system was 965 ps, and for all eight systems it was 7.72 ns. The PMF surfaces were processed by the weighted histogram analysis method (Kumar et al., 1992; Boczko and Brooks, 1993). An error analysis of the PMF calculations has been performed for similar systems (Norberg and Nilsson, 1995c, 1996c), indicating that the protocol used for the PMF calculations is adequate.

Unrestrained simulations

To understand the effects on the nucleic acid structures from the organic solvents, free unrestrained nanosecond MD simulations were carried out. The rAprA and dTpdT structures were simulated in all four solvents. The leapfrog algorithm (van Gunsteren and Berendsen, 1990) was used for the integration of the equations of motion, and the coordinates were saved every 200 steps. To allow for a time step of 0.002 ps, all hydrogen atom-heavy atom bond lengths were constrained with the SHAKE algorithm (Ryckaert et al., 1977). Periodic boundary conditions were applied in the simulations, and a relative dielectric constant of 1.0 was used. After every 20 steps the nonbonded list was updated and the nonbonded interactions were smoothly shifted to zero at a cutoff of 11.5 Å by the atom based force-shift method (Brooks et al., 1985). First the systems were heated from 0 K to 300 K over a 10-ps period. Thereafter the eight simulations were continued for 1000 ps. A total simulation time of 8.08 ns was performed for the eight unrestrained MD simulations on an IBM SP2 system. The last 400 ps of every simulation was used for the analysis.

RESULTS AND DISCUSSION

Potential of mean force

From potential of mean force calculations along a reaction pathway, we obtained free energy surfaces for rAprA and dTpdT in aqueous and organic solutions. In aqueous solution a well-defined minimum, which corresponds to a stacked A-RNA-like conformation, was obtained at ~4.7 Å. A wider minimum located at about the same position was found for rAprA in MeOH, but the unstacked states were lower in free energy in MeOH compared to aqueous solution (Fig. 1). For DMSO the minimum was shifted toward larger R_{N9N9} distances, and the unstacked states had quite high free energies. In $CHCl_3$ the unstacked states were of very low energy, and no significant barrier between stacked and unstacked states was observed. Stacking of dTpdT has been found experimentally to be favored in aqueous solution compared to methanol (Cantor and Schimmel, 1980). In Fig. 2 the stacking free energy of dTpdT is displayed for aqueous and organic solutions. Only in aqueous solution was a well-defined minimum observed for dTpdT, and the free energy of the unstacked states was higher than in the less polar solvents.

Unrestrained simulations

The structural stability of MD simulations is often characterized by the root mean square (rms) displacement of atomic positions from a suitable reference structure, usually the starting structure. The time evolutions of the rms deviations (rmsds) for rAprA in aqueous, chloroform, dimethyl sulfoxide, and methanol solutions are displayed in Fig. 3. In all four cases the rms deviation was found to be stable throughout the simulation. The smallest rms deviation from the initial structure for the nonhydrogen atoms of rAprA was obtained in methanol (0.82 Å during the last 400 ps), and in aqueous solution the rms deviation was slightly larger (0.91 Å). In DMSO the rms deviation for rAprA was 1.07 Å, and a clearly larger value was determined in $CHCl_3$ (1.53 Å). When all atoms were included in the calculation of the rmsd, the rms deviation for rAprA in the four solvents increased by ~0.2 Å. In the different solvents the standard deviations of the rmsd of rAprA were found to be 0.19–0.28 Å. rAprA stacks very well in aqueous solution (Norberg and Nilsson, 1995c,d), and in this study rAprA showed good stacking ability in both aqueous and organic solutions, but with a rms deviation 0.62 Å higher in the low dielectric solvent, $CHCl_3$, compared to the high dielectric aqueous solution, consistent with the PMF result (Fig. 1), that the free energy minimum occurs at larger base separations and

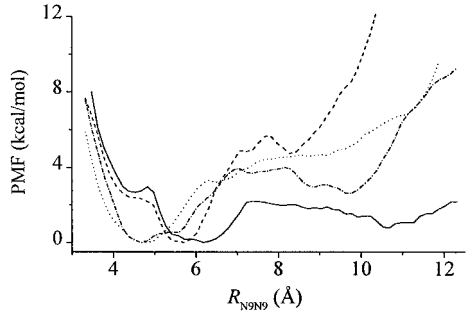

FIGURE 1 Free energy surfaces of stacking for the ribodinucleoside monophosphate rAprA in $CHCl_3$ (——), DMSO (– – –), MeOH (– · – · –), and aqueous (·····) solution.

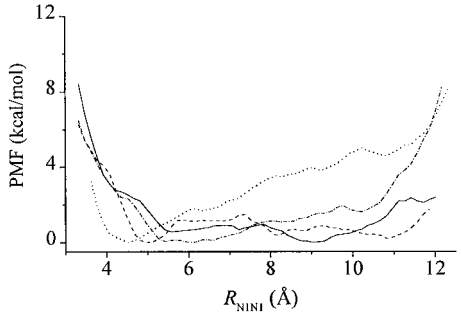

FIGURE 2 Free energy surfaces of stacking for the deoxyribodinucleoside monophosphate dTpdT in $CHCl_3$ (——), DMSO (– – –), MeOH (– · – · –), and aqueous (·····) solution.

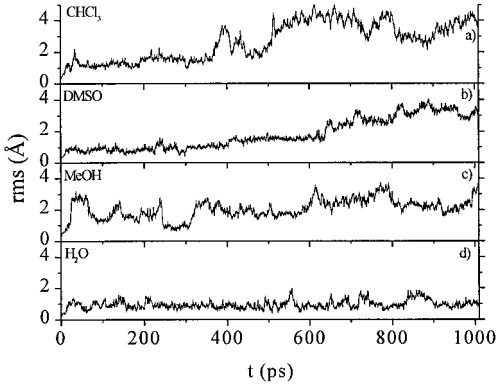

FIGURE 4 Root mean square atomic deviations versus time for all heavy atoms of the deoxyribodinucleoside monophosphate dTpdT in (*a*) $CHCl_3$, (*b*) DMSO, (*c*) MeOH, and (*d*) aqueous solution.

that the extended conformations have lower free energies in $CHCl_3$.

For dTpdT the rms deviation from the initial structure in aqueous solution was kept stable during the whole simulation. A rms deviation of 1.03 Å was obtained for all heavy atoms of dTpdT in water, and a deviation of 1.34 Å was obtained for all atoms (Fig. 4). This is consistent with earlier investigations in which the free energy of the unstacked states was found to be lower, with a less favorable base-base interaction energy, for dTpdT than for rAprA (Norberg and Nilsson, 1995b,c). In the organic solvents the rmsd for dTpdT still fluctuated on the nanosecond time scale, indicating a lower stability for the stacked state. Among the organic solvents the most stable rms deviation was found for dTpdT in MeOH (2.50 Å) for all of the heavy atoms, and larger values of rmsd for dTpdT were observed in DMSO and $CHCl_3$.

From the rms deviation for both rAprA and dTpdT we get a clear indication that base stacking is favored in aqueous solution with a high dielectric constant compared to organic solutions with low dielectric constants. For rAprA, which has displayed a high stacking propensity in aqueous solution (Fig. 1), a more or less stacked conformation was maintained, even in low dielectric solvents, but for dTpdT with intermediate stacking ability (Fig. 2) the bases became much more flexible in the low dielectric solvents compared to the situation in aqueous solution, further destabilizing the stacked conformation.

To visualize the base-base distance and orientation during the MD simulation, the distance (Fig. 5) between the glycosidic base nitrogen atoms, R_{NxNy} (Nx, Ny = N1-pyrimidine, N9-purine), and the angle (Fig. 6) between the normal vectors of the bases were calculated. The initial distance between the glycosidic base nitrogen atoms for rAprA and dTpdT was 4.736 Å and 4.417 Å, respectively. During the last 400 ps the R_{N9N9} distance was 4.7 Å for rAprA in aqueous solution (Fig. 6 *b*). In MeOH the R_{N9N9} distance increased by 0.35 Å, and in DMSO the R_{N9N9} distance was even higher (Figs. 6 *a* and 5 *b*). In the low dielectric $CHCl_3$, the R_{N9N9} distance was stable at 5.76 Å with a fluctuation of 0.2 Å (Fig. 5 *a*).

In aqueous solution the R_{N9N9} distance of rAprA was found to be equal to that of the crystal structure, indicating that the stacked state was well preserved throughout the MD

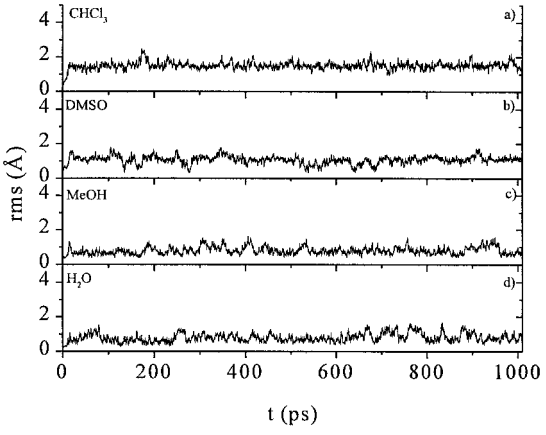

FIGURE 3 Root mean square atomic deviations as a function of time for all heavy atoms of the ribodinucleoside monophosphate rAprA in (*a*) $CHCl_3$, (*b*) DMSO, (*c*) MeOH, and (*d*) aqueous solution.

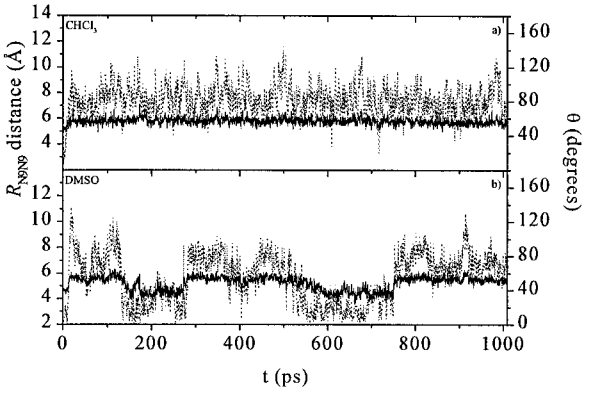

FIGURE 5 The time evolution of the distance between the glycosidic nitrogen base atoms (——), and the angle θ (·····) between the normal vectors of the bases for the ribodinucleoside monophosphate rAprA in (*a*) $CHCl_3$ and (*b*) DMSO.

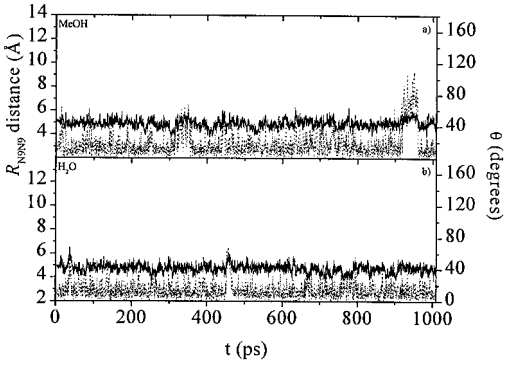

FIGURE 6 The time evolution of the distance between the glycosidic nitrogen base atoms (——) and of the angle θ (·····) between the normal vectors of the bases for the ribodinucleoside monophosphate rAprA in (*a*) MeOH and (*b*) aqueous solution.

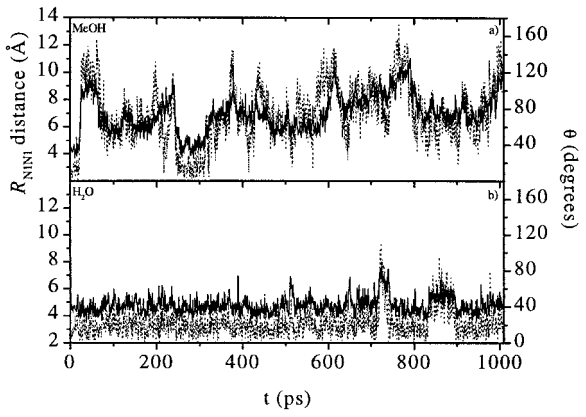

FIGURE 8 The time evolution of the distance between the glycosidic nitrogen base atoms (——) and of the angle θ (·····) between the normal vectors of the bases for the deoxyribodinucleoside monophosphate dTpdT in (*a*) MeOH and (*b*) aqueous solution.

simulation. A quite interesting observation was that the R_{N9N9} distance increased for rAprA in organic solvents and that the largest value of the R_{N9N9} distance was found for the low dielectric $CHCl_3$ solvent.

The rAprA bases were found to be nearly parallel to each other through the whole aqueous MD simulation (Fig. 6 *b*). In MeOH the angle between the rAprA bases was somewhat larger than in aqueous solution (Fig. 6 *a*). The angle between the rAprA bases increased even more in DMSO, and in $CHCl_3$ the angle was on average 81° during the last 400 ps, indicating that the bases were almost perpendicular to each other (Fig. 5, *a* and *b*).

In aqueous solution the R_{N1N1} distance for dTpdT was 4.9 Å, which is slightly larger than the value of the crystal structure (Fig. 8 *b*). The stacked conformation of dTpdT was found to be preserved during the whole aqueous MD simulation. In the three organic solvents the R_{N1N1} distance showed major fluctuations (Figs. 7, *a* and *b*, and 8 *a*).

The angle between the bases of dTpdT was 27° on average, showing a slight disturbance from the stacked state in aqueous solution (Fig. 8 *b*). In the organic solvents the bases of dTpdT were observed to be very flexible (Figs. 7, *a* and *b*, and 8 *a*).

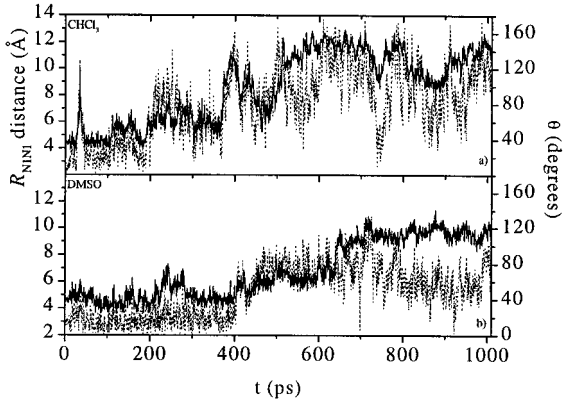

FIGURE 7 The time evolution of the distance between the glycosidic nitrogen base atoms (——) and of the angle θ (·····) between the normal vectors of the bases for the deoxyribodinucleoside monophosphate dTpdT in (*a*) $CHCl_3$ and (*b*) DMSO.

To determine the proportions of exposed and buried surface area, we calculated the solvent-accessible surface area (ASA) of AprA and dTpdT (Lee and Richards, 1971). The ASA in aqueous solution was determined by using a spherical probe of 1.4 Å corresponding to the average radius of a water molecule (Alden and Kim, 1979). The ASA of rAprA in aqueous solution was observed to be very stable throughout the MD simulation, with an average of 691 $Å^2$ over the last 400 ps (Fig. 9 *d*). In aqueous solution the ASA of dTpdT was 692 $Å^2$ (Fig. 10 *d*), which is the same as has been calculated for a single dTpdT structure (see table 2 in Norberg and Nilsson, 1995b). The standard deviation of the ASA in aqueous solution was 20 $Å^2$ for dTpdT, compared to 10 $Å^2$ for rAprA.

The organic solvents $CHCl_3$, DMSO, and MeOH and water have significantly different molecular sizes. Therefore we have to apply different probe radii for the different solvents. For $CHCl_3$ we used a probe radius of 2.3 Å, and for MeOH a probe radius of 1.7 Å. These values of the probe radius have been applied in a recent report of the pulmonary surfactant lipoprotein SP-C (Kovacs et al., 1995). The probe radius for DMSO was estimated to 2.1 Å from its size, compared to the size of a molecule of chloroform, methanol, or water. In MeOH the ASA of rAprA was determined to be 749 $Å^2$, and for DMSO and $CHCl_3$ we obtained 837 $Å^2$ and 882 $Å^2$, respectively (Fig. 9, *a–c*). In the three organic solvents the ASA of rAprA was stable during the whole MD simulation, but for dTpdT there were major variations (Fig. 10, *a–c*), coincident with variations in the base-base orientations (Figs. 7, *a* and *b*, and 8 *a*).

The conformation of the furanose ring can be described by the pseudorotation phase angle (Altona and Sundaralingam, 1972), which can be calculated from the endocyclic sugar torsion angles. Changes of the furanose ring can also be seen from the maximum out-of-plane pucker (Saenger, 1988). The pseudorotation phase angle was determined for

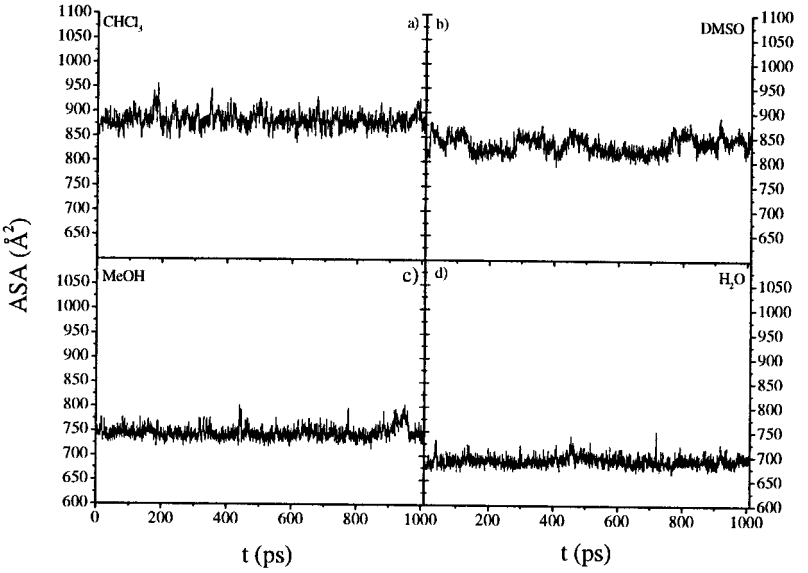

FIGURE 9 Accessible surface area versus time for the ribodinucleoside monophosphate rAprA in (*a*) $CHCl_3$, (*b*) DMSO, (*c*) MeOH, and (*d*) aqueous solution.

both of the sugar moieties of rAprA in the different solutions (Fig. 11). In RNA the sugar puckering is in the $C_{3'}$-*endo* mode, in which the pseudorotation phase angle varies from 0° to 36° (Saenger, 1988). The only change in the pseudorotation phase angle occurred in the period from 882 ps to 923 ps in MeOH. Otherwise the pseudorotation phase angle in both of the sugar moieties of rAprA was stable in aqueous and organic solutions. The maximum out-of-plane pucker for rAprA was in the range of 40.3° to 43.3° in all of the solvents.

For dTpdT we observed a number of changes in the pseudorotation phase angle of both the sugar moieties in all solvents (Fig. 12). Two main sugar puckering modes are observed in DNA: the $C_{3'}$-*endo*, 0°-36°, and $C_{3'}$-*exo*, 180°-216°, modes (Saenger, 1988). The number of changes was found to be higher in the aqueous solution than in the organic solutions. For the 5′ sugar moiety, the maximum out-of-plane pucker varied from 41.5° to 43.4° and from 38.5° to 42.2° for the 3′ moiety. For rAprA and dTpdT, the fluctuations of the maximum out-of-plane pucker were 4°-7° in all of the solvents.

The interactions between the different solvents and the rAprA and dTpdT molecules were also analyzed (Table 2). In $CHCl_3$ and DMSO the van der Waals energy dominated, whereas in MeOH and water the major contribution to the total solute-solvent interaction energy was from the electrostatic energy. Similar solute-solvent interaction energies were obtained for rAprA and dTpdT, except that the electrostatic energy was smaller in MeOH and aqueous solution for dTpdT than for rAprA. The relations between the van der Waals and electrostatic energies in the different solvents were found to agree with the preference for base stacking in the various solvents, as seen in the PMF curves (Figs. 1 and 2).

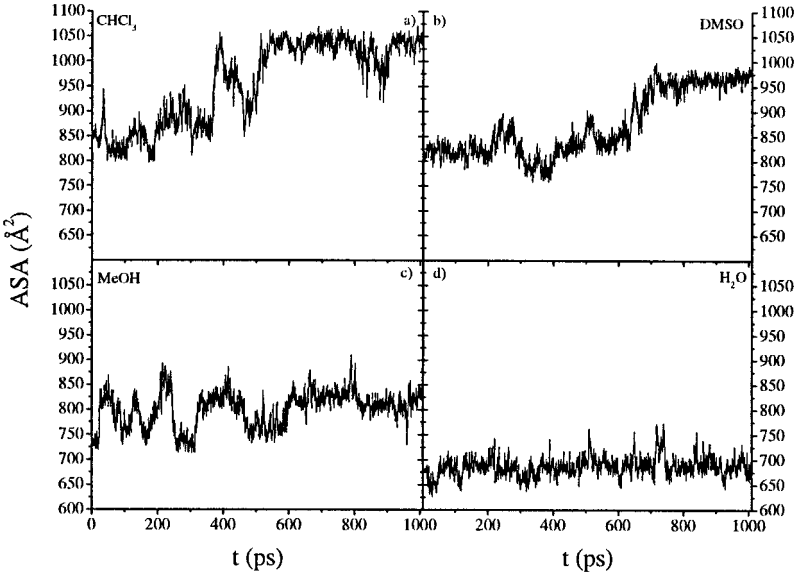

FIGURE 10 Accessible surface area versus time for the deoxyribodinucleoside monophosphate dTpdT in (*a*) $CHCl_3$, (*b*) DMSO, (*c*) MeOH, and (*d*) aqueous solution.

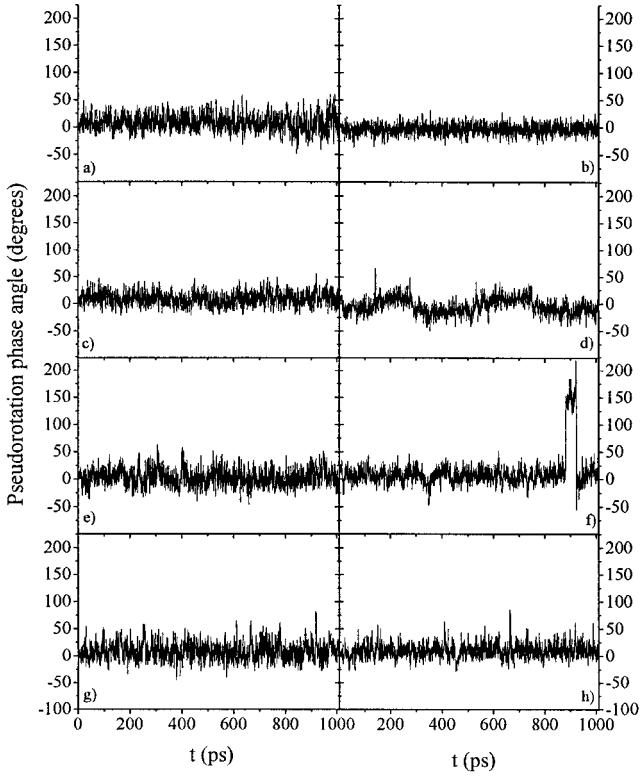

FIGURE 11 Pseudorotation phase angle as a function of time for the ribodinucleoside monophosphate rAprA in $CHCl_3$ for (*a*) 5′ moiety and (*b*) 3′ moiety; in DMSO for (*c*) 5′ moiety and (*d*) 3′ moiety; in MeOH for (*e*) 5′ moiety and (*f*) 3′ moiety; and in H_2O for (*g*) 5′ moiety and (*h*) 3′ moiety.

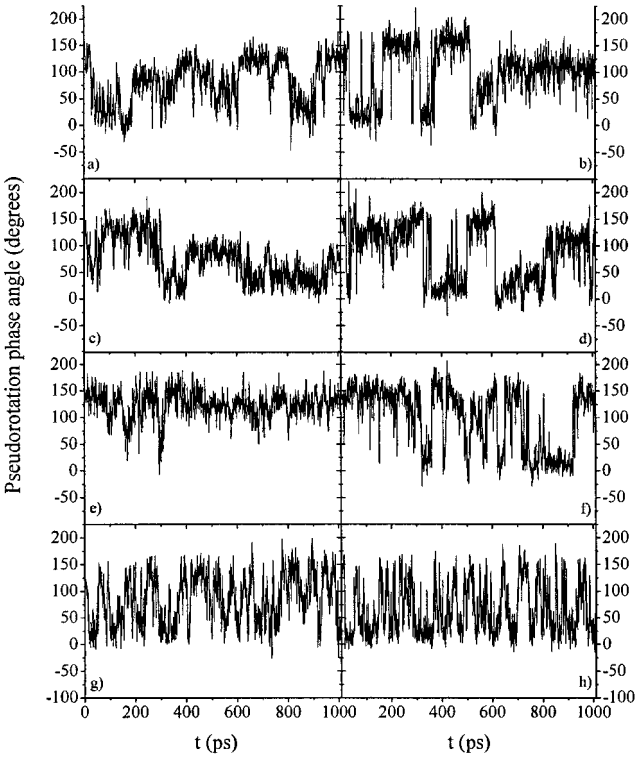

FIGURE 12 Pseudorotation phase angle as a function of time for the deoxyribodinucleoside monophosphate dTpdT in $CHCl_3$ for (*a*) 5′ moiety and (*b*) 3′ moiety; in DMSO for (*c*) 5′ moiety and (*d*) 3′ moiety; in MeOH for (*e*) 5′ moiety and (*f*) 3′ moiety; and in H_2O for (*g*) 5′ moiety and (*h*) 3′ moiety.

The long-range electrostatic interactions are important for nucleic acid structure and dynamics, and need to be properly treated to achieve stable nanosecond simulations of nucleic acids. In one study that presents accurate crystal molecular dynamics simulations of two RNA dinucleotides using the particle mesh Ewald method (Lee et al., 1995), a 400-ps MD simulation in aqueous solution of the double-stranded riboguanylyl-3′,5′-ribocytidine is also reported. First a 20-ps NVT (constant volume and temperature) MD simulation was performed with the RNA molecule and the two sodium ions fixed. Thereafter a 50-ps NVT MD simulation without any constraints was carried out, followed by a 400 ps NPT (constant pressure and temperature) MD simulation resulting in a rmsd of 1.02 Å for all of the heavy atoms (Lee et al., 1995). The single-stranded riboguanylyl-3′,5′-ribouridine has earlier been simulated for 1.0 ns under different conditions, and stable trajectories were obtained in aqueous solution (Norberg and Nilsson, 1995a). In this study united- and all-atom parameter sets were used, and different water models and boundary conditions were applied. For the dimer, stable trajectories with rmsd from 0.80 Å to 1.27 Å were obtained in the NVE (constant volume and energy), NVT, and NPT ensembles (Norberg and Nilsson, 1995a). In another investigation the riboguanylyl-3′,5′-ribouridine was shown to stack as well as the riboguanylyl-3′,5′-ribocytidine (Norberg and Nilsson, 1995c). The rms deviations of the RNA molecule were observed to vary from 0.80 Å to 1.27 Å (Norberg and Nilsson, 1995a). This study showed that stable nanosecond MD simulations could be achieved by treating the nonbonded interactions with a shifting function (Brooks et al., 1983), smoothly shifting the nonbonded interaction energies to zero at a cutoff of 8.5 Å, a truncation scheme with a stronger influence at short distances than the shifting of forces and energies to zero at 11.5 Å used here; the electrostatic force at 4-Å separation is changed by 30% and 13%, respectively.

The present investigation has confirmed these results and clearly demonstrated that stable MD trajectories can be obtained for rAprA and dTpdT in aqueous solution by using

TABLE 2 Solute-solvent interaction energies

Solute	Solvent	E_{tot} (kcal/mol)	E_{ele} (kcal/mol)	E_{vdW} (kcal/mol)
rAprA	$CHCl_3$	−50.8 (13.2)	−6.2 (4.3)	−44.6 (10.7)
	DMSO	−93.1 (7.5)	−25.9 (7.0)	−67.2 (4.0)
	MeOH	−153.7 (17.7)	−116.6 (15.2)	−37.2 (6.6)
	H_2O	−154.5 (27.7)	−138.5 (24.9)	−15.9 (6.5)
dTpdT	$CHCl_3$	−51.6 (13.1)	−10.0 (4.9)	−41.6 (10.4)
	DMSO	−96.3 (8.2)	−25.9 (8.1)	−70.5 (4.5)
	MeOH	−123.7 (27.6)	−88.4 (24.9)	−35.3 (6.2)
	H_2O	−107.3 (43.2)	−93.7 (40.2)	−13.6 (6.4)

the atom-based force-shift method (Brooks et al., 1985). This has also been shown for a pentamer and a hexamer (Norberg and Nilsson, 1996d) and DNA and RNA dodecamers (MacKerell, 1997; Norberg and Nilsson, 1996b). Stable trajectories, as judged by rmsd, can be obtained with the same accuracy as with the particle mesh method, by using other methods to truncate the long-range nonbonded interactions, even in a highly charged nucleic acid system in a low dielectric organic solvent. It should be noted that this has not been achieved by overly restricting the mobility of the solute molecules, as evidenced by the fluctuations in the rmsd (Figs. 3 and 4) and in the structural parameters discussed above.

CONCLUSIONS

We have demonstrated that the stacking free energy is favored in aqueous solution for both the rAprA and dTpdT molecules. The rms deviations from the initial, stacked conformations were found to be higher in organic solutions than in aqueous solution, and the base-base orientation of rAprA was parallel in aqueous solution and found to be nearly perpendicular in $CHCl_3$. The preference for base stacking follows the order high dielectric (water) $>$ intermediate dielectric solvents (methanol or dimethyl sulfoxide) $>$ low dielectric (chloroform). This order also correlates well with the van der Waals and electrostatic energies from the solute-solvent interactions, and thus also with the enthalpic nature of stacking interactions. The increased flexibility of the bases that we observe in the low-dielectric solvents also contributes to the reduction of the free energy cost of unstacking as seen in the PMF calculations, especially for the smaller pyrimidine bases in dTpdT, by reducing the difference in configurational entropy between stacked and unstacked states.

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REFERENCES

Aida, M. 1988. An ab initio molecular orbital study on the sequence-dependency of DNA conformation: an evaluation of intra- and inter-strand stacking interaction energy. *J. Theor. Biol.* 130:327–335.

Alden, C. J., and S.-H. Kim. 1979. Solvent-accessible surfaces of nucleic acids. *J. Mol. Biol.* 132:411–434.

Altona, C., and M. Sundaralingam. 1972. Conformational analysis of the sugar ring in nucleosides and nucleotides. A new description using the concept of pseudorotation. *J. Am. Chem. Soc.* 94:8205–8212.

Arnott, S., P. J. C. Smith, and R. Chandrasekaran. 1976. Atomic coordinates and molecular conformations for DNA-DNA, RNA-RNA, and DNA-RNA helices. *In* CRC Handbook of Biochemistry and Molecular Biology: Nucleic Acids, Vol. 2, 3rd Ed. G. D. Fasman, editor. CRC Press, Cleveland, OH. 411–422.

Beveridge, D. L., and F. M. DiCapua. 1989. Free energy via molecular simulation: applications to chemical and biomolecular systems. *Annu. Rev. Biophys. Biophys. Chem.* 18:431–492.

Boczko, E. M., and C. L. Brooks, III. 1993. Constant-temperature free energy surfaces for physical and chemical processes. *J. Phys. Chem.* 97:4509–4513.

Brooks, B. R., R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan, and M. Karplus. 1983. CHARMM: a program for macromolecular energy, minimizations, and dynamics calculations. *J. Comp. Chem.* 4:187–217.

Brooks, C. L., III, B. M. Pettitt, and M. Karplus. 1985. Structural and energetic effects of truncating long ranged interactions in ionic and polar fluids. *J. Chem. Phys.* 83:5897–5908.

Broyde, S. B., R. M. Wartell, S. D. Stellman, and B. Hingerty. 1978. Minimum energy conformations of DNA dimeric subunits: potential energy calculations for dGpdC, dApdA, dCpdC, dGpdG, and dTpdT. *Biopolymers.* 17:1485–1506.

Cantor, R. C., and P. R. Schimmel. 1980. Biophysical Chemistry. W. H. Freeman, San Francisco.

Chachaty, C., B. Perly, A. Forchioni, and G. Langlet. 1980. *Syn-anti* equilibrium of purine bases in dinucleoside monophophates as studied by proton longitudinal relaxation. *Biopolymers.* 19:1211–1239.

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.

Davis, J., and I. Tinoco, Jr. 1968. Temperature-dependent properties of dinucleoside phosphates. *Biopolymers.* 6:223–242.

Drew, H. R., R. M. Wing, T. Takano, C. Broka, S. Tanaka, K. Itakura, and R. E. Dickerson. 1981. Structure of a B-DNA dodecamer: conformation and dynamics. *Proc. Natl. Acad. Sci. USA.* 78:2179–2183.

Fogolari, F., A. H. Elcock, G. Esposito, P. Viglino, J. M. Briggs, and J. A. McCammon. 1997. Electrostatic effects in homeodomain-DNA interactions. *J. Mol. Biol.* 267:368–381.

Frechet, D., R. Ehrlich, P. Remy, and J. Gabarro-Arpa. 1979. Thermal perturbation differential spectra of ribonucleic acids. II. Nearest neighbour interactions. *Nucleic Acids Res.* 7:1981–2001.

Guàrdia, E., G. Sesé, and J. A. Padró. 1994. On the hydrogen bonding effects in liquid methanol: a molecular dynamics simulation study. *J. Mol. Liq.* 62:1–16.

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

Herskovits, T. T., S. S. Singer, and E. P. Geiduschek. 1961. Nonaqueous solution of DNA: denaturation by urea and its methyl derivatives. *Arch. Biochem. Biophys.* 94:99–114.

Johnson, N. P., and T. Schleich. 1974. Circular dichroism studies of the conformational stability of dinucleoside phosphates and related compounds in aqueous neutral salt solutions. *Biochemistry.* 13:981–987.

Jorgensen, W. L. 1986. Optimized intermolecular potential functions for liquid alcohols. *J. Phys. Chem.* 90:1276–1284.

Jorgensen, W. L., J. M. Briggs, and M. L. Contreras. 1990. Relative partition coefficients for organic solutes from fluid simulations. *J. Phys. Chem.* 94:1683–1686.

Jorgensen, W. L., J. Chandrasekhar, J. D. Madura, R. W. Impey, and M. L. Klein. 1983. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* 79:926–935.

Kang, H., P.-J. Chou, W. C. Johnson, Jr., D. Weller, S.-B. Huang, and J. E. Summerton. 1992. Stacking interactions of ApA analogues with modified backbones. *Biopolymers.* 32:1351–1363.

Kondo, N. S., and S. S. Danyluk. 1976. Conformational properties of adenylyl-3′ leads to 5′-adenosine in aqueous solution. *Biochemistry.* 15:756–768.

Kovacs, H., A. E. Mark, J. Johansson, and W. F. van Gunsteren. 1995. The effect of environment on the stability of an integral membrane helix: molecular dynamics simulations of surfactant protein C in chloroform, methanol and water. *J. Mol. Biol.* 247:808–822.

Kumar, S., D. Bouzida, R. H. Swendsen, P. A. Kollman, and J. M. Rosenberg. 1992. The weighted histogram analysis method for free-energy calculations on biomolecules. I. The method. *J. Comp. Chem* 13:1011–1021.

Lee, B., and F. M. Richards. 1971. The interpretation of protein structures: estimation of static accessibility. *J. Mol. Biol.* 55:379–400.

Lee, C. H., and I. Tinoco, Jr. 1977. Studies of the conformation of modified dinucleoside phosphates containing 1,N^6-ethenoadenosine and 2′-*O*-methylcytidine by 360-MHz ^{1}H nuclear magnetic resonance spectroscopy. Investigation of the solution conformations of dinucleoside phosphates. *Biochemistry.* 16:5403–5414.

Lee, H., T. Darden, and L. Pedersen. 1995. Accurate crystal molecular dynamics simulations using particle-mesh-Ewald: RNA dinucleotides—ApU and GpC. *Chem. Phys. Lett.* 243:229–235.

Lide, D. R. 1993. Handbook of Chemistry and Physics. CRC Press, Boca Raton, FL.

Lowe, M. J., and J. A. Schellman. 1972. Solvent effects on dinucleotide conformation. *J. Mol. Biol.* 65:91–109.

MacKerell, A. D., Jr. 1997. Influence of magnesium ions on duplex DNA structural, dynamic, and solvation properties. *J. Phys. Chem.* 101: 646–650.

MacKerell, A. D., Jr., J. Wiórkiewics-Kuczera, and M. Karplus. 1995. An all-atom empirical energy function for the simulation of nucleic acids. *J. Am. Chem. Soc.* 117:11946–11975.

McCammon, J. A., and S. C. Harvey. 1987. Dynamics of Proteins and Nucleic Acids. Cambridge University Press, Cambridge.

Mierke, D. F., and H. Kessler. 1993. Improved molecular dynamics simulations for the determination of peptide structures. *Biopolymers.* 33: 1003–1017.

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

Norberg, J. 1995. A biophysical perspective of dynamics, structure, and thermodynamics of nucleic acids. Molecular dynamics simulations of nucleic acids. Ph.D. thesis. Karolinska Institutet, Stockholm, Sweden.

Norberg, J., and L. Nilsson. 1994a. High-pressure molecular dynamics of a nucleic acid fragment. *Chem. Phys. Lett.* 224:219–224.

Norberg, J., and L. Nilsson. 1994b. Stacking-unstacking of the dinucleoside monophosphate guanylyl-3′,5′-uridine studied with molecular dynamics. *Biophys. J.* 67:812–824.

Norberg, J., and L. Nilsson. 1995a. NMR relaxation times, dynamics, and hydration of a nucleic acid fragment from molecular dynamics simulations. *J. Phys. Chem.* 99:14876–14884.

Norberg, J., and L. Nilsson. 1995b. Potential of mean force calculations of the stacking-unstacking process in single-stranded deoxyribodinucleoside monophosphates. *Biophys. J.* 69:2277–2285.

Norberg, J., and L. Nilsson. 1995c. Stacking free energy profiles for all 16 natural ribonucleoside monophosphates in aqueous solution. *J. Am. Chem. Soc.* 117:10832–10840.

Norberg, J., and L. Nilsson. 1995d. Temperature dependence of the stacking propensity of adenylyl-3′,5′-adenosine. *J. Phys. Chem.* 99: 13056–13058.

Norberg, J., and L. Nilsson. 1996a. A conformational free energy landscape of ApApA from molecular dynamics simulations. *J. Phys. Chem.* 100:2550–2554.

Norberg, J., and L. Nilsson. 1996b. Constant pressure molecular dynamics simulations of the dodecamers: d(GCGCGCGCGCGC)$_2$ and r(GCGCGCGCGCGC)$_2$. *J. Chem. Phys.* 104:6052–6057.

Norberg, J., and L. Nilsson. 1996c. Influence of adjacent bases on the stacking-unstacking process of single-stranded oligonucleotides. *Biopolymers.* 39:765–768.

Norberg, J., and L. Nilsson. 1996d. Internal mobility of the oligonucleotide duplexes d(TCGCG)$_2$ and d(CGCGCG)$_2$ in aqueous solution from molecular dynamics simulations. *J. Biomol. NMR.* 7:305–314.

Ogasawara, N., and Y. Inoue. 1976. Titration and temperature-dependent properties of homodinucleoside monophosphates. Evaluation of stacking equilibrium quotients for neutral and half-ionized ApA, CpC, GpG, and UpU. *J. Am. Chem. Soc.* 98:7054–7060.

Pohorille, A., S. K. Burt, and R. D. MacElroy. 1984. Monte Carlo simulation of the influence of solvent on nucleic acid base associations. *J. Am. Chem. Soc.* 106:402–409.

Poland, D., J. M. Vournakis, and H. N. Scheraga. 1966. Cooperative interactions in single-strand oligomers of adenylic acid. *Biopolymers.* 4:223–235.

Powell, J. T., E. G. Richards, and W. B. Gratzer. 1972. The nature of stacking equilibria in polynucleotides. *Biopolymers.* 11:235–250.

Ryckaert, J.-P., G. Ciccotti, and H. J. C. Berendsen. 1977. Numerical integration of the Cartesian equations of motion of a system with constraints: molecular dynamics of *n*-alkanes. *J. Comp. Phys.* 23: 327–341.

Saenger, W. 1988. Principles of Nucleic Acid Structure. Spinger Verlag, New York.

Straatsma, T. P., and J. A. McCammon. 1992. Computational alchemy. *Annu. Rev. Phys. Chem.* 43:407–435.

Thiyagarajan, P., and P. K. Ponnuswamy. 1978. Conformational characteristics of dApdA, dApdT, dTpdA, and dTpdT from energy minimization studies. *Biopolymers.* 17:533–553.

Ts'o, P. O. P., I. S. Melvin, and A. C. Olson. 1963. Interaction and association of bases and nucleosides in aqueous solution. *J. Am. Chem. Soc.* 85:1289–1296.

van Gunsteren, W. F., and H. J. C. Berendsen. 1990. Computer simulation of molecular dynamics: methodology, applications, and prespectives in chemistry. *Angew. Chem. Int. Ed. Engl.* 29:992–1023.

van Holde, K. E., J. Brahms, and A. M. Michelson. 1965. Base interactions of nucleotide polymers in aqueous solution. *J. Mol. Biol.* 12:726–739.

Verlet, L. 1967. Computer "experiments" on classical fluids. I. Thermodynamical properties of Lennard-Jones molecules. *Phys. Rev.* 159: 98–103.

Watts, M. T., and I. Tinoco, Jr. 1978. Role of hypermodified bases in transfer RNA. Solution properties of dinucleoside monophosphates. *Biochemistry.* 17:2455–2463.

Zheng, Y.-J., and R. L. Ornstein. 1996. A molecular dynamics and quantum mechanics analysis of the effect of DMSO on enzyme structure and dynamics: subtilisin. *J. Am. Chem. Soc.* 118:4175–4180.