Transition from B to Z DNA: Contribution of Internal Fluctuations to the Configurational Entropy Difference

Abstract. *The internal motions of the double-stranded DNA oligomer $(dCdG)_3$ (dC, deoxycytidylate; dG, deoxyguanylate) in the B and Z forms have been calculated in the harmonic approximation. A complete vibrational analysis has been made, and the resulting normal mode frequencies have been used to evaluate the vibrational entropy of B and Z DNA. The greater flexibility of the B DNA hexamer leads to an entropic stabilization relative to the stiffer Z DNA hexamer of 22 calories per mole per kelvin at 300 K. The calculated value is of the same order as that (21 to 27 calories per mole per kelvin) obtained from nuclear magnetic resonance measurements on the methylated duplexes $(m^5dCdG)_3$ and (dCdGm^5dCdGdCdG). This result demonstrates the importance of internal motions, which have been neglected in earlier studies of the transition from B to Z DNA, in the stability of different nucleic acid conformers.*

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Dynamical analyses of macromolecules of biological interest are contributing to our knowledge of their properties (*1*). One area where dynamics is essential is in evaluating the motional contributions to thermodynamic parameters (*2, 3*). This is of particular interest for the entropy contribution to the free energy of different conformers. An ideal case of this type is provided by the transition from B to Z DNA (*4, 5*). Understanding the properties of this transition may be of biological significance if a role for Z DNA in living systems is established.

The relative stability of B and Z DNA in solution depends on the enthalpic and entropic differences. Each of these differences has an internal component and a contribution from the solvent that includes the effects of dissolved ions. Considerable attention has focused on the solvent term since it is known that the Z form is stabilized by high salt. Much less is known about the internal component, which consists of the energy difference between the two structures at their respective potential minima and the thermal motion contribution to the enthalpy and entropy. The thermal motion contribution for each conformer arises from atomic fluctuations in the neighborhood of the minimum energy configuration and can be estimated from molecular or harmonic dynamic approaches (*1–3*). Although the solvent does alter these motions by its effect on the potential of mean force, model calculations suggest that the amplitudes of the motions are less sensitive to the solvent than the time scales (*6*); only the former enter into the thermodynamic properties.

We report here an analysis of one aspect of the thermodynamics of the transition from B to Z DNA, namely, the contribution of internal motion to the enthalpy and entropy differences between the two forms of DNA. We estimate the internal motions from the vibrational normal modes since molecular dynamics simulations and quasi-harmonic estimates of anharmonic contributions (*2, 7*) suggest that a normal mode calculation provides a good first approximation for oligomeric nucleic acid duplexes (*8*) as well as for proteins (*3*). The system studied was the DNA duplex $(dCdG)_3$ (dC, deoxycytidylate; dG, deoxyguanylate), which has been crystallized in the left-handed Z conformation; its x-ray structure was used as the starting point for the calculation (*9*). Since the B form of the $(dCdG)_3$ duplex has not been crystallized, the standard Arnott coordinates (*10*) were energy-minimized to convergence with a molecular mechanics program (*11*). The method and model used have been described (*8*); to represent shielding of the phosphate group by counter ions, a reduced partial charge was used. Once the structure was minimized, the mass-weighted force constant matrix was constructed and the normal modes were determined by diagonalization (*3, 8, 11*). The molecules were treated as composed of a total of 274 atoms (only polar hydrogens are included), so that there are 816 internal modes, in addition to three translations and three rotations. To obtain the motional (vibrational) contribution to the enthalpy and entropy, standard formulas based on the quantum mechanical harmonic oscillator partition function were evaluated (*12*).

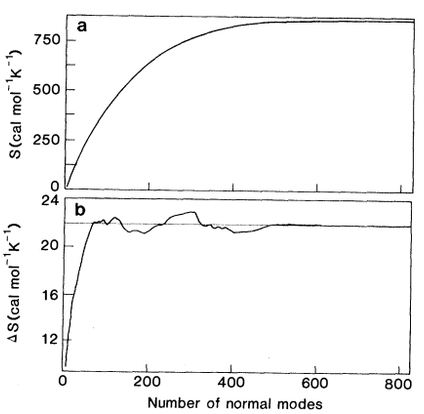

Fig. 1. Vibrational entropy of $(dGdC)_3$ at 300 K obtained from a normal-mode treatment. (a) Convergence of the calculated entropy of the Z conformer as a function of the number of normal modes included; (b) convergence of entropy difference between B and Z DNA as a function of the number of normal modes included. The neglect of the contribution of aliphatic hydrogens is likely to change the absolute entropy somewhat but should have little effect on the entropy difference.

The convergence of the entropy for the Z form at 300 K as a function of the number of modes of increasing frequency included in the sum is shown in Fig. 1a; on the scale of Fig. 1a, the results for the B form would be virtually identical. At least the 400 lowest vibrational modes are needed to obtain a reasonable approximation to the total entropy in this model (Fig. 1a). Figure 1b shows the entropy difference between the B and Z forms. Here the lower frequencies make the dominant contributions; that is, 95 percent of the entropy difference is obtained from the 50 lowest modes (below 60 cm^{-1}), although some fluctuations in the value of the entropy difference occur up to 450 modes. In general, the lower modes are more delocalized (*8*) and would be expected to be most sensitive to the overall structure of the molecule. The same set of modes is important for the atomic motions that contribute to the x-ray temperature factors (*8, 13*). No individual mode in the low-frequency range dominates the entropy difference. Instead, most of the low-frequency modes in Z DNA have slightly higher frequencies than those in B DNA. This result suggests that Z DNA can be characterized as less flexible than B DNA, with a resulting lower entropy at room temperature.

Comparison of the experimental Raman spectra of the B and Z forms of poly(dGdC) has shown characteristic differences for certain lines in the higher frequency range (500 to 1800 cm^{-1}) (*14*). At 300 K, the contribution to the vibrational entropy difference of the frequency shifts observed in this range is likely to be small; for example, for a vibrational mode at 600 cm^{-1}, a frequency increase of 25 cm^{-1} leads to an entropy change of -0.04 cal mol^{-1} K^{-1}. No data on the low-frequency modes are available.

The values obtained for the vibrational contribution to the enthalpies and entropies for the B and Z forms of duplexed $(dCdG)_3$ and the difference between the two are shown in Table 1. The vibrational contribution to the enthalpy difference is very small, but the configurational entropy difference is important in stabilizing the B form.

Experimental measurements of thermodynamic properties include both the internal and solvent contributions. Nevertheless, we consider the data available for the transition between the B and Z forms. The methylated DNA duplex $(m^5dCdG)_3$ has been studied by two-dimensional nuclear magnetic resonance (NMR) in methanol-water mixtures at 0.1*M* NaCl (*15*). This hexamer crystallizes in the Z form with a structure that differs only slightly from that of $(dCdG)_3$ (*16*). For methanol concentrations between 20 and 40 percent in a temperature range between 285 and 310 K, the transition from Z to B forms for $(m^5dCdG)_3$ is characterized by an enthalpy (ΔH) of 6 to 8 kcal mol^{-1} and an entropy (ΔS) of 21 to 27 cal mol^{-1} K^{-1}.

If the dominant contribution to ΔS did arise from the configurational entropy differences between the two conformers, the agreement between the calculation (see Table 1) and the measurements would be very good. The calculated value of ΔH (12 kcal mol^{-1}) is determined from the energy difference of the two minima and the small vibrational correction in Table 1. The resulting ΔH value is of the same order as the experimental ΔH; however, because of the sensitivity of the theoretical results to the details of the potential function and the importance of solvent effects, the comparison is not very significant. An NMR study (*17*) of the DNA duplex ($dCdGm^5dCdGdCdG$) duplex in 1.8*M* NaCl solution over a temperature range of 10° to 50°C yielded a ΔS value for the transition from Z to B forms of 25 cal mol^{-1} K^{-1}, close to the measurement for $(m^5dCdG)_3$ in methanol. Earlier measurements for poly(dGdC) and poly(dGm^5dC), in which high salt (4*M* to 6*M* LiCl) was used to produce the transition from B to Z forms, indicated that the Z form is more stable at higher temperatures (*5*), although no detailed thermodynamic analysis was made. By contrast, studies of poly(dCdG) in 1*M* to 4.5*M* NaCl indicated that the equilibrium between B and Z forms had a negligible temperature dependence (*4*, *18*). Comparisons of poly(dCdG) with poly(m^5dCdG) show that the transition from B to Z forms occurs at much lower salt concentrations (*19*) in the methylated derivative. Thus, the experimental thermodynamics under the high salt conditions required for the transition from B to Z forms in unmethylated species is unclear. The results suggest the presence of specific salt effects, in addition to possible differences in the binding of water to the two conformers, as well as of more generalized electrostatic contributions (*20*).

That the calculated harmonic entropy change, even if approximate (*2*, *7*), is of the same order as the experimentally measured values demonstrates that the internal motions are significant for the thermodynamics of the transition. A number of independent results suggest the Z conformer is more rigid than the B conformer, in accord with the present calculations; they include an analysis of the Z structure (*9*), its inability to intercalate ethidium bromide (*21*), and its number of slowly exchanging protons (*22*).

Table 1. Vibrational contribution to the enthalpy (H) and entropy (S) of $(dCdG)_3$; T is the absolute temperature. All values are at 300 K; H_v, ΔH_v, and $T\Delta S_v$ have the units kilocalories per mole; S_v and ΔS_v have the units calories per mole per kelvin. The enthalpy includes the zero point contribution.

Conformation	H_v	ΔH_v	S_v	ΔS_v	$T\Delta S_v$
B	1106.3	0.3	891.1	21.8	6.5
Z	1106.0		869.3		

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