Analysis of oligonucleotide structure using hyperchromism measurements at long wavelengths

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Oligonucleotide structural information can be obtained by observing heat-induced changes in UV absorption at wavelengths above 280 nm. The experimental method is simple, requiring two absorption measurements on the same sample, which are used to calculate the percentage hyperchromicity for the helix to coil conversion Y_λ , (where $Y_\lambda = 100[(\epsilon_\lambda \text{ at } T_2^\circ\text{C})/(\epsilon_\lambda \text{ at } T_1^\circ\text{C})] - 100$; T_1 and T_2 are temperatures at which essentially all molecules of the oligomer are in their structured form or in their random coil form, respectively). We find that, at long wavelengths, Y_λ is very sensitive to the type of stacking present in the structured form of the oligonucleotide; for example, the B-helical form of the duplex d(CGCGCG) has a strong positive value ($Y_{296} = 14$), while its Z-helical form has a strong negative value ($Y_{296} = -37$). These values are for oligomer solutions containing 0.1 M Na^+ and 4.0 M Na^+ , respectively, the conditions under which the B and Z forms are known to predominate (1).

Long wavelength hyperchromism analysis can also be applied to oligomer hairpins. For example, a spectrophotometric study at 260 nm performed on d(CGCGAGTGACGCG) shows that the tridecamer assumes a hairpin structure. In the presence of 0.01 M Na^+ , this oligomer has $Y_{296} = 5$, suggesting a B-like arrangement, whereas in solutions of 4.0 M Na^+ , it has $Y_{296} = -34$, indicating a preference for a Z-like structure. Furthermore, a plot of Y_{296} v. $[\text{Na}^+]$ (Figure 1) shows that this hairpin maintains its Z-like conformation even below concentrations of 0.8 M Na^+ , an unexpected result in view of the known cases of Z helices involving non-modified nucleotides in aqueous solution, which require Na^+ concentrations in excess of 3 M for stability. Similarly, the hairpin formed by d(CGCG-AGTTTGACGCG) has Y_{296} values of -28 and 0 for Na^+ concentrations of 4.0 M and 0.1 M, and the forms predominating in these solutions are assigned Z and B conformations, respectively. By contrast, the hairpin d(ATCCTAGTTTGAA-GGAT) has Y_{296} values of 2 (4.0 M Na^+) and 5 (0.1 M Na^+), indicating that the B-like conformation is preferred at both salt concentrations. All of the above assignments are supported by CD and ^{31}P -NMR analyses using published methods (2, 3). Long wavelength Y values are not simply a response to helix type-two RNA duplexes, U(UA)₆A and AGUCACCA, which exist in the A-helical form, have Y_{296} (1.0 M Na^+) values of -55 and 0 , respectively. The absorption and CD analyses of the above-mentioned molecules were carried out at concentrations of 5 AU₂₆₀/ml in solutions of 10 mM sodium phosphate (pH 7.0), containing appropriate amounts of NaCl, with T_1 and T_2

set at 22 and 70, respectively. ^{31}P -NMR analyses were performed on strand concentrations of 2.5 mM in D₂O solutions of 1 mM EDTA – 10 mM sodium cacodylate (pH 7.0) containing appropriate amounts of NaCl.

The observed differences in long wavelength Y values can be explained by assuming the presence of $n-\pi^*$ transitions. Such transitions have been considered before as contributors to the spectra of polynucleotides; for example, it has been suggested that they are responsible for the long wavelength shoulder in the spectrum of the mixture poly(A) + poly(U) (4). Similar shoulders appear in the spectra of the duplexes poly(dGdC) (2) and d(CGCGCG) (1), when they are in their Z-helical forms. The $n-\pi^*$ transition dipoles in polynucleotide helices are polarized parallel to the helix axis and are expected to result in absorbance increases during random coil to helix conversion (4, 5). We suggest that the large differences in the Y values given above can be understood in terms of the effects of special stacking arrangements on the $n-\pi^*$ transitions. Inspection of the crystal structures of duplexes d(CGCGCG) (6) and U(UA)₆A (7) reveals that, in the Z-helix of the former, stacking occurs between the N3 of the C in each CG dinucleotide and the N3 of the C on the other strand that pairs with the G, while in the case of the latter duplex, stacking occurs between the N1 of the A in each UA dinucleotide and the N1 of the A on the other strand that pairs with the U. We propose that the *collinear* head-to-tail alignments of the $n-\pi^*$ transition dipoles associated with these stacked azine nitrogens are responsible for the large increase in long wavelength absorbance upon formation of these types of helices. This is consistent with the observation that the B form of the duplex d(CGCGCG), which lacks this particular N3 stacking arrangement, does not exhibit a large negative Y_{296} value. Instead, its Y value is positive, presumably due to the predominant hyperchromic effect of the $\pi-\pi^*$ transitions at this wavelength.

It has been noted that transitions of the $n-\pi^*$ type have the potential of providing new structural information because they are more localized in the heterocyclic ring than $\pi-\pi^*$ transitions (8). The above results indicate that the former are certainly more sensitive to changes in the oligomer base-stacking arrangements. For example, the difference in Y values at 296 nm for the B and Z forms of duplex d(CGCGCG) ($\Delta Y_{296} = 51$) far exceeds the difference at 260 nm ($\Delta Y_{260} = 5$). Moreover, it should be possible to exploit $n-\pi^*$ transitions for observing helix-coil equilibria during the heat-induced denaturation of oligomers. Indeed, a plot of A_{296} v. T for a structure lacking a large

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negative Y_{296} value contains a hypochromic region that reflects the two-state helix-coil conversion, and, although this region is weak compared with the analogous hyperchromic curve in the plot of A_{260} v. T , thermodynamic data obtained from the two plots are similar (Fig. 2). The fact that the helix-coil conversion is not seen in the A_{296} v. T plots for molecules with large negative Y_{296} values is probably due to the precipitous change in absorption associated with disruption of the collinear dipole arrangements.

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Figure 1. Plots of percentage hyperchromicities v. sodium ion concentrations for the hairpin d(CGCGAGTGACGCG) (A) and the duplex d(CGCGCG) (B).

Figure 2. Melting curves obtained for the duplex d(CCACTTCG) at a concentration of 1.0 AU₂₆₀/ml in a solution of 1.0 M NaCl–0.05 M sodium phosphate, pH 7.0. Curve A (absorbance read at 260 nm) and Curve B (absorbance read at 296 nm) were normalized by setting the absorbance obtained at 95°C in each case to 1.0. Values for thermodynamic parameters ΔH° and ΔS° obtained from Curve A are $-53 \text{ kcal.mol}^{-1}$ and -141 eu , respectively; while those obtained from Curve B are $-55 \text{ kcal.mol}^{-1}$ and -151 eu , respectively.