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Triple-Helix and Double-Helix Formations of Octamers of Deoxyriboadenylic and Deoxyribothymidylic Acids

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Triple-helix and double-helix formations of octamers of deoxyriboadenylic and deoxyribothymidylic acids (dA_8 and dT_8) have been studied spectrophotometrically. The free-energy changes in 50 mmol dm^{-3} $MgCl_2$ buffer at 25 °C were -21.2 kJ mol^{-1} for the triplex formation and -29.3 kJ mol^{-1} for the duplex formation, respectively. The other results by UV and CD spectroscopies were also reported.

The triple-helix form of nucleic acids known to contain homopurine and homopyrimidine sequences has generated interest in its biological significance and chemotherapeutic application.[1-3] Furthermore, the recent works of Dervan's and Helene's groups[4-7] on the sequence-specific DNA cleavage and ligation based on triple-helix formations have led to great interest as DNA reactions in nonenzymatic systems. Recently, the structural analysis of the triple helix consisting of decamers of deoxyriboadenylic and deoxyribothymidylic acids, $(dA)_{10}\cdot 2(dT)_{10}$, by NMR has shown that both Watson-Crick and Hoogsteen base pairings are in the triplex form and $MgCl_2$ stabilizes the triplex structure.[8] However, little is currently known about the thermodynamic property of the unusual structure in spite of its importance.

In this work, we have studied quantitatively the double-helix and triple-helix formations (Eqs. 1 and 2) of octamers of deoxyriboadenylic and deoxyribothymidylic acids (dA_8 and dT_8) in a phosphate buffer containing 100 mmol dm^{-3} NaCl or 50 mmol dm^{-3} $MgCl_2$ by UV and CD spectroscopies. This is the first report about the thermodynamic parameters for the formation of the triple helix by using a "melting" method which is being developed rapidly due to current interest.[9-12]

$$dA_8 + dT_8 \rightleftharpoons dA_8\cdot dT_8 \text{ duplex} \quad (1)$$

$$dA_8\cdot dT_8 \text{ duplex} + dT_8 \rightleftharpoons (dA)_8\cdot 2(dT)_8 \text{ triplex} \quad (2)$$

Oligonucleotide concentrations (C_t), strand concentrations, were calculated from the high-temperature absorbance at 260 nm. Single-strand extinction coefficients were calculated from extinction coefficients of dinucleotide monophosphates and nucleotide.[13] The buffers were 10 mmol dm^{-3} sodium phosphate (pH 7.1) and either 100 mmol dm^{-3} NaCl or 50 mmol dm^{-3} $MgCl_2$. Equimolar solutions of dA_8 and dT_8 were combined to give mixtures with final stoichiometric ratios of either 1:1 or 1:2 dA_8 to dT_8.

The CD spectroscopy confirmed the presence of the triplex in a solution containing a 1:2 molar ratio of dA_8:dT_8 and 50 mmol dm^{-3} $MgCl_2$, which was consistent with the result of dA_{10} and dT_{10}.[8] Figure 1 shows the CD spectra of a $(dA)_8+2(dT)_8$ solution containing dA_8 and dT_8 in a 1:2 molar ratio at 5 °C compared with the normalized sum of the CD spectra of a $dA_8 \cdot dT_8$ plus dT_8 solutions. In Fig. 1a, the CD spectrum of the $(dA)_8+2(dT)_8$ solution is very similar to the sum of the CD spectra of the $dA_8 \cdot dT_8$ plus dT_8 solutions, suggesting there is not the triplex formation in 100 mmol dm^{-3} NaCl buffer. On the other hand, in Fig. 1b, the CD spectrum of the $(dA)_8+2(dT)_8$ solution exhibits differences relative to the sum of the CD spectra of the $dA_8 \cdot dT_8$ plus dT_8 solutions, which include a substantial amplitude decrease of the positive band at 280 nm, and blue-shifts of the positive band at 259 nm and of the negative band at 248 nm. The result is consistent with that of the $(dA)_{10} \cdot 2(dT)_{10}$ solution in which there is a triplex formation.[8]

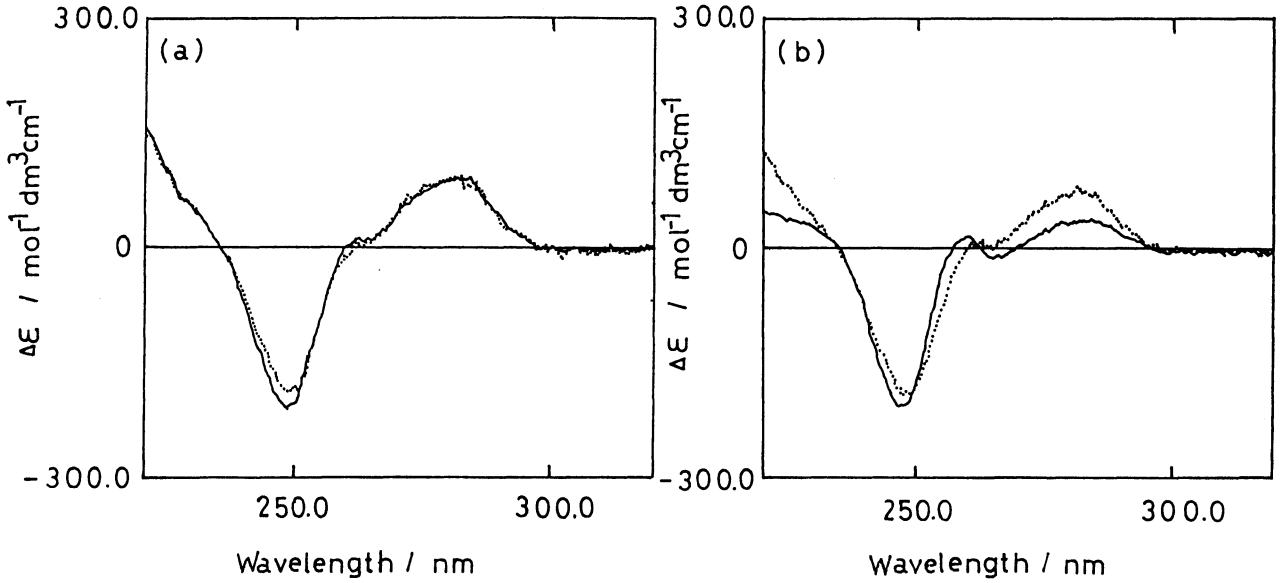

Fig. 1. CD spectra of a $(dA)_8+2(dT)_8$ solution in (a) 100 mmol dm^{-3} NaCl and (b) 50 mmol dm^{-3} $MgCl_2$ buffers at 5 °C compared with the normalized sum of the CD spectra of a $dA_8 \cdot dT_8$ plus dT_8 solutions (1 unit of $(dA)_8$ or $(dT)_8$; 0.03 mmol dm^{-3}). The solid and dashed lines show the CD spectra of the $(dA)_8+2(dT)_8$ solution and the $dA_8 \cdot dT_8$ plus dT_8 solutions, respectively.

Absorbance vs. temperature curves (melting curves) on a Hitachi U-3200 programable spectrophotometer were measured at 260 and 284 nm at which the melting of the duplex and triplex can be detected respectively. The heating rate was 0.2 or 0.5 °C/min, regulated with a Hitachi SPR-7 temperature controller. The cuvette-holding chamber was flushed with dry N_2 gas for the duration of the runs. Prior to dilution of the oligonucleotides for experiments, buffers were degassed by heating at 90 °C for 10 min. Figure 2 shows the melting curves at 260 and 284 nm from the $dA_8 \cdot dT_8$ duplex to the single strands and from the $(dA)_8 \cdot 2(dT)_8$ triplex to the $dA_8 \cdot dT_8$ duplex plus dT_8 strand (Eqs. 1 and 2).

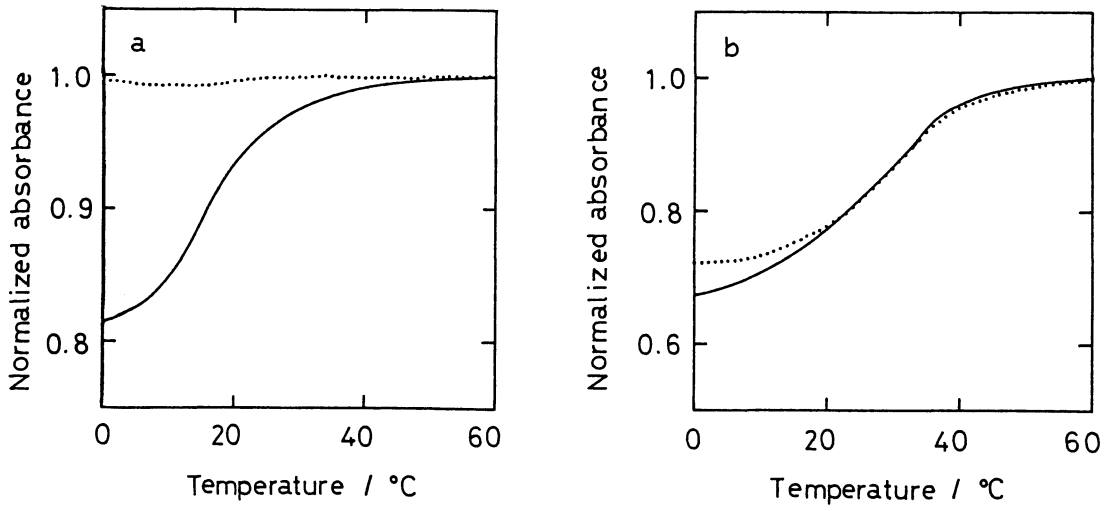

Fig. 2. Melting curves at (a) 284 nm and (b) 260 nm for the $(dA)_8 \cdot 2(dT)_8$ triplex (C_t; 0.15 mmol dm^{-3}) and the $dA_8 \cdot dT_8$ duplex (C_t; 0.10 mmol dm^{-3}) in 50 mmol dm^{-3} $MgCl_2$ buffer. The solid and dashed lines show the meltings of the $(dA)_8 \cdot 2(dT)_8$ triplex and the $dA_8 \cdot dT_8$ duplex, respectively.

In Fig. 2a, melting of the third strand, dT_8, from the underlying duplex, $dA_8 \cdot dT_8$, is accompanied by a hyperchromic absorbance change at 284 nm, whereas the melting of the $dA_8 \cdot dT_8$ duplex is not. In Fig. 2b, the slight difference between these curves at a low-temperature range is due to the melting of the third strand. The melting curve of the underlying duplex after melting of the third strand in the figure is very similar to that of the $dA_8 \cdot dT_8$ duplex at a high-temperature range.

The melting curves were measured over a 50-fold range in the oligonucleotide concentration and the melting temperature, T_m, on each run was determined as described previously.[10] Plots of the reciprocal melting temperatures, T_m^{-1}, at 284 and 260 nm vs. $\log(C_t/4)$ are shown in Fig. 3. The linearities of T_m^{-1} vs. $\log(C_t/4)$ in Fig. 3 show a two-state transition from the $(dA)_8 \cdot 2(dT)_8$ triplex to the $dA_8 \cdot dT_8$ duplex plus the third strand in Eq. 2 and from the $dA_8 \cdot dT_8$ duplex to the single strands in Eq. 1.

The free energy changes at 25 °C, $\Delta G°_{25}$, of the duplex formation in Eq. 1 and the triplex formation in Eq. 2 were determined by Eqs. 3 and 5, and Eqs. 4 and 5:[14]

$$T_m^{-1} = (2.30R/\Delta H°) \log(C_t/4) + (\Delta S°/\Delta H°) \quad (3)$$

$$T_m^{-1} = (2.30R/\Delta H°) \log(C_t/6) + (\Delta S°/\Delta H°) \quad (4)$$

$$\Delta G°_{25} = \Delta H° - 298 \times \Delta S° \quad (5)$$

where $\Delta H°$ and $\Delta S°$ are the enthalpy and entropy changes, respectively, and R is the gas constant. The $\Delta G°_{25}$ value of -29.3 kJ mol^{-1} for the $dA_8 \cdot dT_8$ duplex formation is very similar to -28.6kJ mol^{-1} obtained by the melting behavior of the underlying duplex after melting of the third strand. The value shows the stability of the duplex formation between dA_8 and dT_8 strands by the Watson-Crick base pairings. On the other hand, -21.2 kJ mol^{-1} was obtained as the value of the triplex formation between the underlying duplex and the third strand. The value shows the stability of the triplex formation between $dA_8 \cdot dT_8$ and dT_8 by the Hoogsteen base pairing.[8] The results suggest that the stability of the Watson-Crick base pair in the DNA duplex is different from that of the Hoogsteen base pair in the DNA triplex.

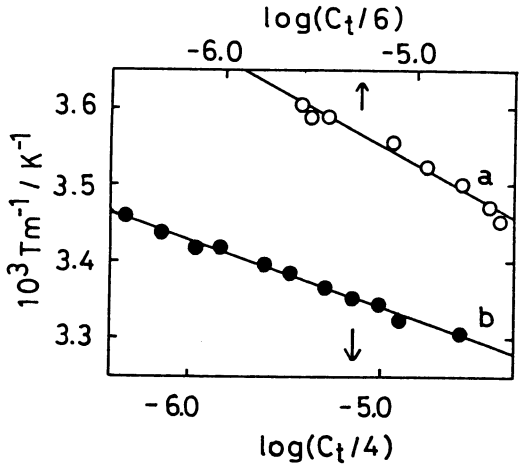

Fig. 3. Plots of T_m^{-1} vs. $\log(C_t)$ for (a) $dA_8 \cdot 2(dT)_8$ at 284 nm and (b) $dA_8 \cdot dT_8$ at 260 nm.

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