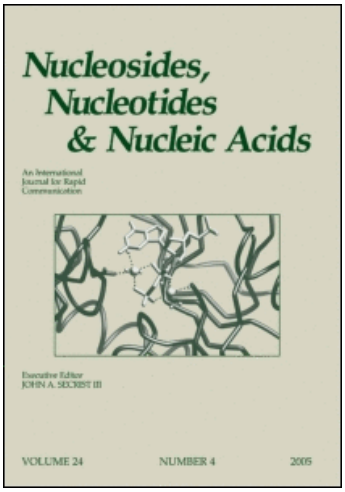

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Melting Behavior and Stability of $(dA)_8 \cdot (dT)_8$ Double Helix

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MELTING BEHAVIOR AND STABILITY OF (dA)8•(dT)8 DOUBLE HELIX†

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ABSTRACT: Melting behavior and stability of double helix of octadeoxyribonucleotides, (dA)8•(dT)8, have been studied by a UV measurement and a calculation of nearest-neighbor model. The helix of (dA)8•(dT)8 exhibited the thermodynamic parameters similar to those of B-form DNA.

INTRODUCTION

Structure and function of nucleic acids are well known to be sequence dependent.[1] It should be relatively easy to understand the molecular basis of functions by nucleic acids if the secondary and tertiary structures of the nucleic acids can be predicted.

In order to estimate the melting temperature, T_m, at which 50% of the double strand (the secondary structures) has dissociated into its two single strands, the rule of Wallace *et al.*[2] has been used for a DNA helix. The rule is based on the rough assumption that the T_m value increases 2 °C per dA:dT base pair and 4°C per dG:dC base pair. Current prediction methods of the structures of nucleic acids, especially deoxyribooligonucleotides[3,4] and ribooligonucleotides,[5,6] by the thermodynamic parameters for the formation of a base pairing depend largely on the nearest-neighbor model.[7] It has been known that the method can predict the stability of the

†This paper is dedicated to the late Professor Tohru Ueda, Hokkaido University.

ribooligonucleotide double helices with perfect base pairs[8,9] and was applied to the studies of RNAs.[10-12] However, the method has not been applied directly to the prediction of the stability of *deoxy*ribooligonucleotide double helices.

In this work, we have studied melting behavior and stability of octa*deoxy*ribonucleotide double-helix consisting of dA:dT base pairs, $(dA)_8 \cdot (dT)_8$, by a UV measurement and the improved nearest-neighbor calculation.[4] This study can provide insights into the dependence of the behavior of $(dA)_8 \cdot (dT)_8$ on temperature and whether the values of the thermodynamic parameters for each base pairing of a DNA are valid.

EXPERIMENTAL

Materials. The octadeoxyribooligonucleotides, $(dA)_8$ and $(dT)_8$, were obtained from Pharmacia and purified with high-performance liquid chromatography (HPLC) and desalted with a C-18 Sep-Pak cartridge. Final purity of the oligomer checked by HPLC was greater than 99 %. Oligonucleotide concentrations (C_t) as strand concentrations were calculated from the high-temperature absorbance.[13] Single-strand extinction coefficients were calculated from extinction coefficients of dinucleotide monophosphates and nucleotides.[14] The buffer was 1 mol dm^{-3} NaCl, 10^{-2} mol dm^{-3} Na_2HPO_4, and 10^{-3} mol dm^{-3} Na_2EDTA, pH 7.0. Prior to dilution of the oligonuclotides, the buffer was degassed by heating to 90°C for 10 min.

UV Measurement. UV melting curves (absorbance vs. temperature curves) were measured at 260 nm on Hitachi U-3200 and U-3210 spectrophotometers. The heating rate was 0.5 or 1.0 °C/min regulated by Hitachi SPR-7 and SPR-10 temperature-controllers. Nine melting curves were measured over a 50-fold range in strand concentration.

Thermodynamic Parameters. Thermodynamic parameters for double-helix formation were obtained by two methods. (1) Reciprocal melting temperature, T_m^{-1}, was plotted against $\log(C_t/4)$ to give enthalpy and entropy changes (ΔH^o and ΔS^o) with eq. 1;[15]

$$T_m^{-1} = (2.303R/\Delta H^o) \log(C_t/4) + (\Delta S^o/\Delta H^o) \qquad (1)$$

where R is the gas constant. (2) Enthalpy and entropy changes were derived from fitting individual melting curves to the calculation curves with sloping

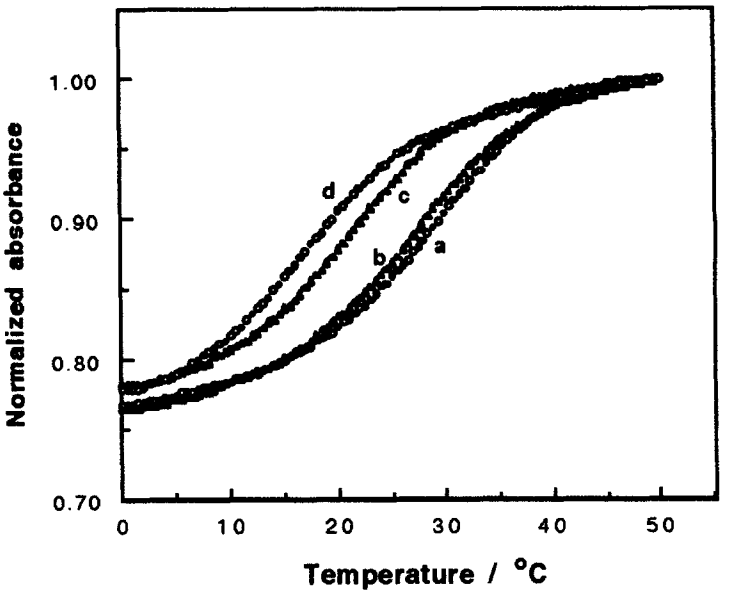

FIG. 1. Melting curves of (a) 143, (b) 67.1, (c) 10.8, and (d) 3.33 μmol dm^{-3} (dA)8•(dT)8 in 1 mol dm^{-3} NaCl buffer.

base lines[16] in order to confirm the results obtained with the method (1) described above.

Nearest-Neighbor Calculation. According to the nearest-neighbor model,[3-5] a free-energy change (ΔG^o) of helix formation for non-self-complementary sequences consists of two terms: (1) a free-energy change for helix initiation associated with forming the first base pair in the duplex, and (2) a sum of propagation free energies for forming each subsequent base pair. Therefore, the stability of the deoxyribooligonucleotide double helices can be calculated with the parameters of Breslauer *et al* [3] for helix initiation and propagation. However, their parameters, though are generally good, are not complete at some nearest-neighbor base pairs, and then the predicted stability of some sequences is not consistent with the measured value.[17] In this work, therefore, the improved nearest-neighbor parameters[4] were used to calculate the stability of the double helices.

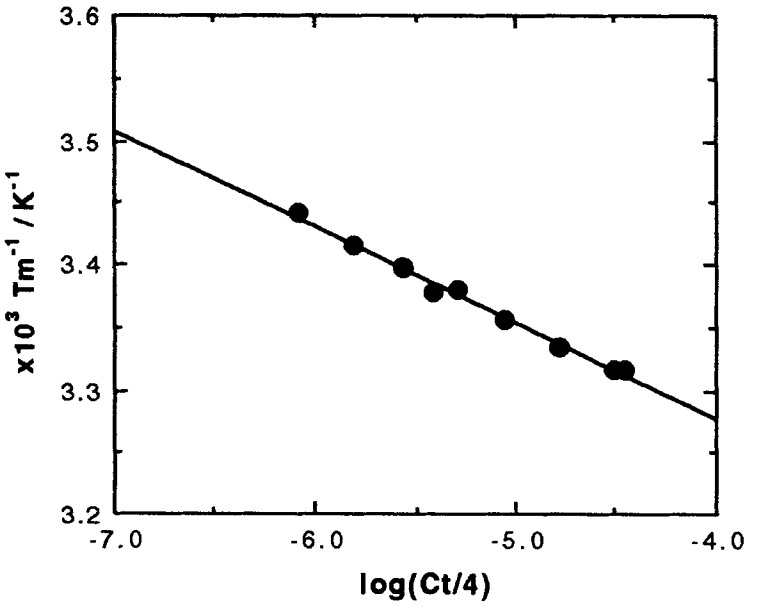

FIG. 2. Plot of T_m^{-1} vs. log(C_t/4) for (dA)8•(dT)8 .

TABLE 1. Calculated Thermodynamic Parameters for Double-Helix Formation of (dA)8•(dT)8 [a]

oligomer	$-\Delta H^o$ kcal mol^{-1}	$-\Delta S^o$ cal mol^{-1}K^{-1}	$-\Delta G^o_{25}$ kcal mol^{-1}	T_m [b] °C
(dA)8•(dT)8	62.7	188	6.65	26.8
	(59.8)	(178)	(6.73)	(27.7)

a) The values in the parentheses were obtained from the plot for (dA)8•(dT)8 in Fig. 2. Estimated errors are ±4% in ΔH^o, ±4% in ΔS^o, ±8% in ΔG^o_{25}, and ±4% in T_m, respectively. b) The melting temperature is for 10^{-4} mol dm^{-3} (dA)8•(dT)8.

RESULTS AND DISCUSSION

Melting Behavior of (dA)8•(dT)8. Figure 1 shows the typical melting curves of (dA)8•(dT)8 which were normalized by the absorbance at 50 °C. In Fig. 1, the continued increase in the absorbance at 260 nm with temperature above 40 °C is due to the unstacking of single stranded (dA)8 since (dT)8 does not show this behavior. The value of T_m increases with increasing concentration of (dA)8•(dT)8. Doubling the concentration of (dT)8 in the solution, [dA]:[dT]=1:2, did not alter the magnitude of the absorbance change at 260 nm, indicating triple helices which existed in 50 mmol dm^{-3} $MgCl_2$ buffer at a low temperature range[18] were not formed under the present condition.

The Predicted and Observed Thermodynamic Parameters. Plot of T_m^{-1} vs. $\log(C_t/4)$ (dA)8•(dT)8 for is shown in Fig. 2. If the melting is a two-state transition, that is, double-helix to single strand transition, the plot should be linear. The result shows that the melting of (dA)8•(dT)8 is a two-state transition.

The values of ΔH^o, ΔS^o, ΔG^o_{25}, the free-energy change at 25 °C, and T_m calculated with the improved nearest-neighbor parameters are listed in Table 1 with the values obtained from the plot for (dA)8•(dT)8 in Fig. 2.

The ΔH^o, ΔS^o, ΔG^o_{25}, and T_m of -59.8 kcal mol^{-1}, -178 cal mol^{-1} K^{-1}, -6.73 kcal mol^{-1}, and 27.7 °C, respectively, measured for (dA)8•(dT)8 in this work are close to the predicted values as shown in Table 1, suggesting that the double helix has thermodynamic properties expected for B-form DNA. The results also suggest that the double helix exists not as slipping duplexes with 5' and 3' dangling ends[20] but as the duplex with fullmatch dA:dT base pairs, because the slipping duplexes have larger predicted values of ΔG^o_{25} than the measured values in Table 1, though those duplexes may exist transiently.[4]

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