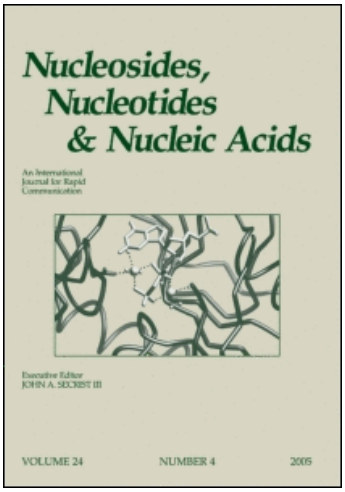

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APPLICATION OF THE THERMODYNAMIC PARAMETERS OF DNA STABILITY PREDICTION TO DOUBLE-HELIX FORMATION OF DEOXYRIBOOLIGONUCLEOTIDES †

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ABSTRACT: The energetics of a double helix formation of deoxyribooligonucleotides has been studied by measuring optical melting curves and calculating thermodynamic values based on the nearest neighbor model and parameters. The results show that, though the model is valid, the values of the nearest-neighbor thermodynamic parameters reported previously should be improved for some base pairs in order to predict more precisely the stability of the double helix of the oligomers by the parameters.

INTRODUCTION

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 has dissociated into its two single strands, the rule of Wallace *et al.*[1] has been used. Current predictions of the structures of nucleic acids, especially deoxyribooligonucleotides and ribooligonucleotides,[2,3] by the thermodynamic parameters for the formation of a base pairing depend largely on the nearest neighbor model.[4,5] This model assumes that structure formations of the nucleic acids are driven by interaction between

†This paper is dedicated to Dr. Morio Ikehara on the occasion of his 70th birthday.

nearest neighbor base pairs in a DNA or an RNA. It has been known that the method can predict the stability of the oligonucleotide double helices with perfect base pairs[6] and was applied to the studies of RNAs.[7-9] However, the method has not been applied directly to the prediction of the stability of *deoxy*ribooligonucleotide double helices and then it has not been tested whether the values of the thermodynamic parameters for each base pairing of a *DNA* are valid.

In this work, we have studied energetic behaviors of a self-complementary double-helix formation of *deoxy*ribooligonucleotides with identical nearest neighbors by measuring optical melting curves and calculating thermodynamic parameters. The pairs of the self-complementary oligomers with *identical nearest neighbors* are [1] d(GCCGGC) and d(GGCGCC), [2] d(CCGCGG) and d(CGGCCG), [3] d(TCATGA) and d(TGATCA), and [4] d(TCTATAGA) and d(TAGATCTA). The study can provide insights into whether the nearest-neighbor model and parameters in the thermodynamics are valid to predict the stability of the double helix of the DNA oligomers by the parameters.

EXPERIMENTAL

Materials. The deoxyribooligonucleotides were synthesized chemically on solid support with a phosphoramidite method[6] and purified with a high-performance liquid chromatography (HPLC) after deblocking. The oligomers were further purified 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.[7] Single-strand extinction coefficients were calculated from extinction coefficients of dinucleotide monophosphates and nucleotides.[10] The buffer was 1 mol dm^{-3} NaCl, 10 mmol dm^{-3} Na_2HPO_4, and 1 mmol 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. For each oligomer, 9 or 10 profiles of the optical melting were measured over a 50-fold range in

strand concentration. The melting temperature, T_m, was determined as described previously.[11]

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;[11]

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

where R is the gas constant. (2) Enthalpy and entropy changes were derived from fitting individual melting curves to the calculation curves with sloping base lines[12] in order to confirm the results obtained with the method (1) described above.

Nearest-Neighbor Calculation. According to the nearest-neighbor model,[2-4] 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.*[2] for helix initiation and propagation.

RESULTS AND DISCUSSION

Each pairs of the duplexes of the oligomers consist of identical nearest neighbors as shown in Fig. 1.

Typical melting curves of d(TCATGA) and d(TGATCA) at the same concentration of the oligomers were shown in Fig. 2. Plots of T_m^{-1} vs. $\log(C_t)$ for both oligomers were shown in Fig. 3. The shapes of the melting curves of these oligomers forming the B-type double-helices[13] are very similar each other as shown in Fig. 2. The values of T_m are very close, 22.4 and 21.5 °C for 0.12 mmol dm^{-3} d(TCATGA) and d(TGATCA), respectively. These results suggest that the deoxyribooligonucleotides with identical nearest neighbors show similar melting behavior. In Fig. 3, the plots of eq. 1 for these oligomers are also very similar, and the linearity of the plots suggests the melting is the two-state transition, that is, double-helix to single-strand transition.[12]

Thermodynamic parameters for double-helix formation were obtained by eq. 1 in the above section and eq. 2:

$$\Delta G^o_{37} = \Delta H^o - T\Delta S^o \quad (2)$$

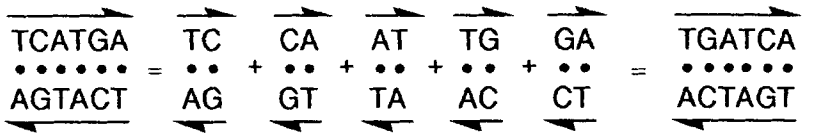

Fig. 1. The schematic illustration of the self-complementary duplexes of d(TCATGA) and d(TGATCA) and the sum of their identical nearest neighbors.

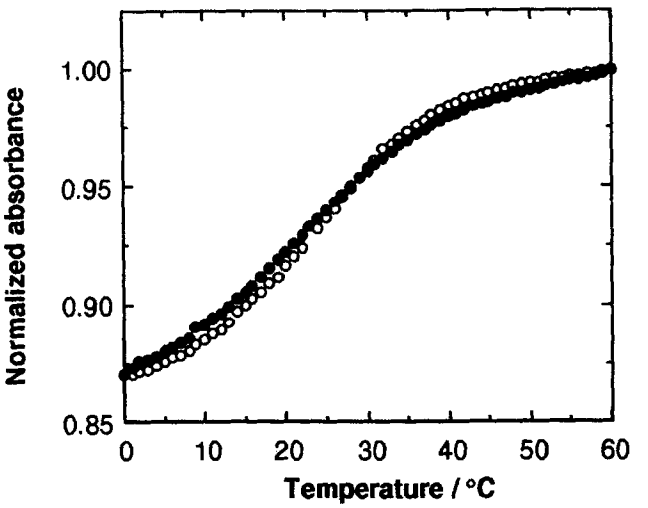

Fig. 2. Optical melting curves of 0.12 mmol dm^{-3} d(TCATGA) (O) and d(TGATCA) (●) at 1.02 mol dm^{-3} Na^+.

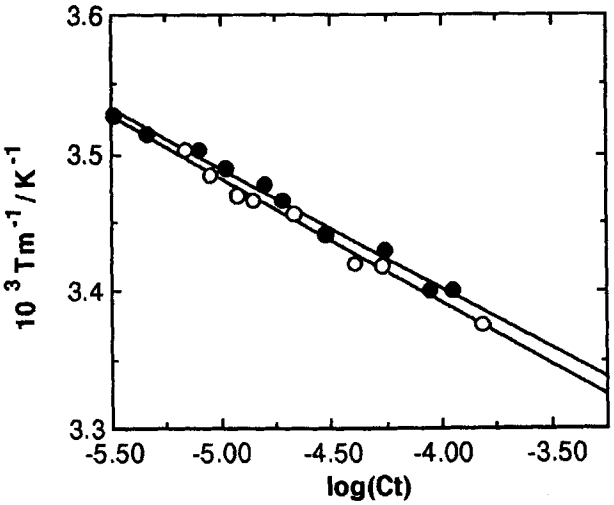

Fig. 3. Plots of T_m^{-1} vs. log(C_t) for d(TCATGA) (O) and d(TGATCA) (●) at 1.02 mol dm^{-3} Na^+.

Table 1. Theremodynamic Parameters for the Double-Helix Formation of the Self-Complementary Deoxyribooligonucleotides at 1.02 mol dm^{-3} Na^{+}[a,b]

oligomer	$-\Delta H^{o}$ kJ mol^{-1}	$-\Delta S^{o}$ J $mol^{-1}K^{-1}$	$-\Delta G^{o}37$ kJ mol^{-1}	T_m[c] °C
d(GCCGGC)	189	495	35.7	57.9
d(GGCGCC)	182	480	33.5	54.5
			(36.8)	(55.3)
d(CCGCGG)	173	451	33.3	55.0
d(CGGCCG)	162	410	34.9	60.0
			(38.8)	(57.9)
d(TCATGA)	211	636	12.9	21.8
d(TGATCA)	220	672	11.7	20.9
			(3.95)	(-3.6)
d(TCTATAGA)	191	558	17.6	27.3
d(TAGATCTA)	206	595	21.0	32.9
			(6.53)	(12.2)

a) Estimated errors are within ±5% in ΔH^{o}, ±5% in ΔS^{o}, ±10% in $\Delta G^{o}37$, and ±8% in T_m, respectively. b) The values in the parentheses were calculated from nearest-neighbor parameters of a DNA. c) The melting temperatures are for the oligomers of 0.1 mmol dm^{-3}.

where ΔH^{o}, ΔS^{o}, and $\Delta G^{o}37$ are the enthalpy, entropy, and free-energy changes for double-helix formation, respectively, R is the gas constant, and T is 310 K. The values of ΔH^{o}, ΔS^{o}, and $\Delta G^{o}37$ obtained from the plots of T_m^{-1} vs. $\log(C_t)$ like Fig. 3 were listed in Table 1 with those of T_m. Table 1 also shows the thermodynamic parameters predicted from the nearest-neighbor parameters of a DNA[2] according to the method described previously.[9]

In Table 1, for sequences with identical nearest neighbors, [1] d(GCCGGC) and d(GGCGCC), [2] d(CCGCGG) and d(CGGCCG), [3] d(TCATGA) and d(TGATCA), and [4] d(TCTATAGA) and d(TAGATCTA),

the average differences in the observed values of ΔH°, ΔS°, $\Delta G^{\circ}37$, and T_m are 3, 3, 5, and 4%, respectively, and these are within experimental errors. The differences between the predicted and observed values of $\Delta G^{\circ}37$ and T_m are also relatively small for the pairs [1] and [2]. However, for the pairs [3] and [4], the differences of the values are much larger than would be expected if they were due to experimental error: The observed values of $-\Delta G^{\circ}37$ are about three times larger than the predicted values for the pairs [3] and [4]. It suggests the present nearest-neighbor parameters[2] for the prediction of a DNA duplex may not be perfect, especially for AT base pairs of which values were obtained by fitting a small number of data sets of *poly*mers. Therefore, from these results, it is concluded that the deoxyribooligonucleotides with identical nearest neighbors have similar stabilities, that is, *the nearest neighbor model is good in a DNA as well as an RNA, but the thermodynamic parameters for the nearest neighbors should be improved in order to predict more precisely the stability of a DNA double helix.*

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