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Studies on nucleic acid interactions I. Stabilities of mini-duplexes ($dG_2A_4XA_4G_2 \cdot dC_2T_4YT_4C_2$) and self-complementary d(GGGAAXYTTCCC) containing deoxyinosine and other mismatched bases

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ABSTRACT

The thermal stability of DNA duplexes containing deoxyinosine in a pairing position in turn with each of the four major deoxynucleotides has been investigated by measuring ultraviolet-absorbance at different temperatures. $d(G_2A_4 X A_4G_2)$ and $d(C_2T_4YT_4C_2)$ were prepared by the solid-phase phosphotriester method. When X is deoxyinosine, the Tm values of the duplexes are in the order Y=dC>dA>dG>dT>dU. The Tm of other duplexes containing dG, dA and dT at X were also measured. Self-complementary duplexes d(GGGAAINTTCCC) showed the same order of stability with N being dC, dA, dG and dT. Thermal stabilities of duplexes containing dG instead of dI were compared with other matched and mismatched duplexes. The Tm values of sequence isomers containing purine-pyrimidine combinations were compared. Self-complementary duplexes containing G-C and A-T in the central positions showed Tm values ca. 10° higher than those containing C-G and T-A in the same positions. Thermodynamic parameters and circular dichroism spectra of these oligonucleotides were compared.

INTRODUCTION

The thermodynamic stability of oligonucleotide duplexes are considered to be important information for hybridization of oligonucleotide probes for DNA and RNA. Hydrogen bonds and stacking of bases in oligonucleotides contribute to the stability of the duplex. Homopolymers containing inosine (I) and deoxyinosine (dI) are known to form stable helices with those containing C and dC.[1,2] Inosine is also known to form base pairs with A, C and U in the first position (the wobble position) of the anticodon of tRNA.[3] We have previously reported the use of oligonucleotide probes with deoxyinosine residues at ambiguous points to screen high complexity, genomic DNA libraries.[4,5] To extend the usage of deoxyinosine probes,

fundamental studies on the thermal stability of DNA duplexes containing deoxyinosine are required. In this paper, we report the synthesis and properties of oligodeoxyribonucleotide duplexes containing deoxyinosine in pairing positions with other deoxynucleosides. Duplexes $d(G_2A_4IA_4G_2\ C_2T_4YT_4C_2)$ containing mismatched base pairs were prepared and their stabilities compared by measuring their UV absorbance-temperature profiles. The thermodynamic properties of the self-complementary strand $d(G_3A_2INT_2C_3)$ and some sequence isomers $d(G_3A_2PuPyT_2C_3)$ or $d(G_3A_2PyPuT_2C_3)$ were also studied. The circular dichroism (CD) of these oligonucleotides was also measured. The thermodynamic parameters for double-helix formation for mismatched oligodeoxyribonucleotides[6] and base-pairing involving deoxyinosine[7] have been reported during the course of this study. The sequence dependence of these stabilities will be compared with the available data.

MATERIALS AND METHODS

Oligodeoxyribonucleotides were synthesized by the solid-phase phosphotriester method using dinucleotide blocks[8] on a polystyrene support.[9] Deoxyinosine was protected and phosphorylated as described.[4] Products were purified by reversed phase and anion-exchange high pressure liquid chromatography, and analyzed by the mobility shift method using venom phosphodiesterase[10] (Fig. 1).

Ultraviolet (UV) absorption spectra were measured on a spectrophotometer Shimadzu UV-240 in 0.01 M sodium cacodylate and 0.1 M NaCl (pH 7.0). Tm's were measured on a Beckman DU-8B spectrophotometer in the same buffer as used for measuring the UV absorption at a concentration of 1 A_{260} unless otherwise specified. CD spectra were measured on a JASCO J-500A spectropolarimeter at concentration of 1 A_{260}.

RESULTS

Tm values of heteroduplexes d(GGAAAAXAAAAGG) d(CCTTTTYTTTTCC) (X=I,G,A,T; Y=C,A,G,T,U)

UV-absorbance-temperature profiles of heteroduplexes containing deoxyinosine (X=I, Y=C, A, G, U) were measured.

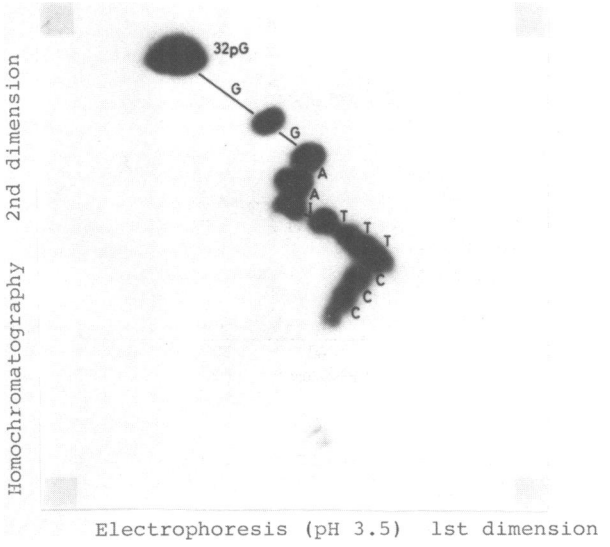

Figure 1.
Mobility shift analysis of d(GGGAAITTTCCC) using Homomix III.[16]

Duplexes containing dG, dA and dT instead of dI were prepared for the measurement of Tm values of duplexes containing only Watson-Crick-type hydrogen bonds. The Tm values of the duplexes are listed in Table I. From these results it can be concluded that normal Watson-Crick-type hydrogen bonds contribute to stabilization of the duplexes. Deoxyinosine-containing duplexes showed lower Tm values when compared to the

Table I Tm values of heteroduplexes

d(GGAAAAXAAAAGG)	d(CCTTTTYTTTTCC)				
	Y=C	Y=A	Y=G	Y=T	Y=U
	Tm=				
X=I	50.9°C	47.0°C	43.8°C	43.4°C	39.7°C
X=G	52.8	43.6	44.0	42.6	40.2
X=A	34.8	38.8	41.7	52.8	51.0
X=T	39.3	49.4	44.6	40.6	39.8

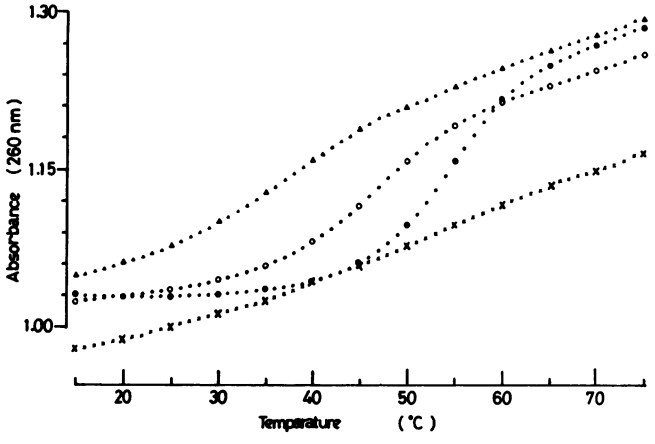

Figure 2.
UV absorbance-temperature profile of self-complementary duplexes d(GGGAAINTTCCC). ● , N=C; ○ , N=A; △ , N=G; × , N=T.

normally-paired ones. The stabilization of deoxyinosine by pairing was in the order dC>dA>dG>dT>dU. This is to be expected from the exclusive incorporation of deoxycytidine phosphate by DNA polymerase using deoxyinosine as a template.[4] It is rather surprising to find that the I:G pair did not destabilize the duplex when compared to the I:T pair. Dependence of the Tm on the sequence of an A-T pair was observed. A:T seems to stabilize the duplex (52.8°C) more than T:A (49.4°C). This agrees well with the result reported by Tinoco and his co-workers.[7] A G:G pair also stabilized the duplex more than G:T. The most unstable purine-containing pair was A:C. Thymidine can pair with deoxyinosine only in skewed position (O^2-N^1 and O^4-N^3) if H-bonding is to exist. The same hydrogen bonding may exist between uridine and inosine in a duplex but with a less hydrophobic nature. Lower Tm values of the dU containing duplex may be explained by a lower hydrophobicity caused by lack of the methyl group.[11] In the anticodon of tRNA, inosine can decode uridine-containing triplets. Precise structures of the base-pairing in ribo-and deoxyribonucleotides involving hypoxanthine should be investigated by nmr or x-ray crystallography.

Table II Tm values of self-complementary duplexes

d(GGGAAXYTTCCC) SC-XY	0.25 A (260nm)	0.50 A	1.0 A	2.0 A	3.0 A
	Tm=				
SC-IC	48.5°C	51.1°C	52.6°C	55.0°C	55.8°C
IA	42.5	44.7	45.8	48.0	49.0
IG	—	35.0	36.5	38.3	39.7
IT	—	—	—	—	—
II	—	—	—	—	—
SC-GC	56.5°C	59.2°C	60.7°C	62.8°C	63.5°C
GA	42.0	44.1	45.9	48.5	50.3
GG	—	33.2	36.7	38.4	40.8
GT	—	—	—	—	—
SC-AT	51.6°C	54.8°C	57.0°C	58.0°C	58.8°C
TA	40.6	42.3	43.9	45.2	45.9
CG	50.4	51.0	52.2	55.5	56.2
AC	—	—	—	—	—
CT	—	—	—	—	—

Thermal stabilities of the self-complementary dodecanucleotides d(GGGAAXYTTCCC)

In order to observe an enhanced effect of deoxyinosine in pairing with other deoxynucleosides, self-complementary dodecanucleotides were synthesized and UV-absorbance-temperature profiles were measured as shown in Fig. 2. Tm values at several concentrations of these five strands containing deoxyinosine and nine duplexes containing matched or mismatched base pairs are shown in Table II. Duplexes containing I-T, I-I, G-T, A-C and C-T at the X-Y positions did not show cooperative melting. However, at higher concentration ($> 1A_{260}$) the I-T and G-T oligomers showed sigmoidal curves. Thus it is unlikely that hairpin structures exist. Using Tm values of the nine self-complementary (SC) duplexes shown in Table II, van't Hoff plots are shown in Fig. 3. The G-G containing duplex (SC-GG) showed the most unstable

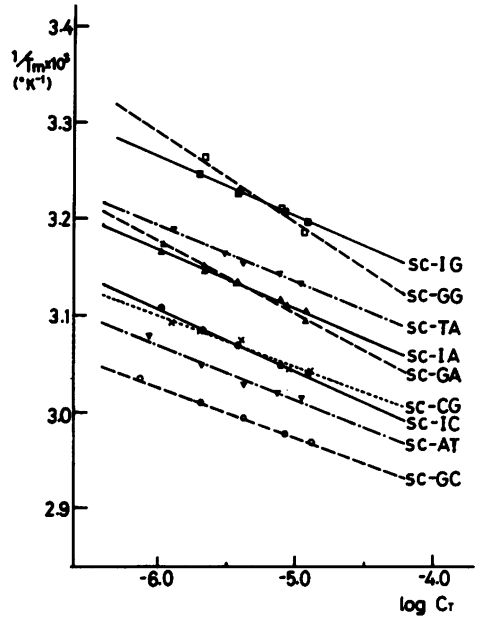

Figure 3.
van't Hoff plots of 1/Tm vs. log C_T for self-complementary duplexes d(GGGAAXYTTCCC).

Table III Thermodynamic values for self-complementary duplexes formation

d(GGGAAXYTTCCC) SC-XY	ΔG° (kcal/mol) 25°C	50°C	ΔH° (kcal/mol)	ΔS° (cal/deg·mol)
SC-IC	-13.4	- 8.6	-70.8	-192
IA	-12.4	- 7.0	-75.9	-213
IG	-10.1	- 4.8	-73.5	-212
SC-GC	-16.8	-10.8	-88.2	-240
GA	-11.3	- 7.3	-59.5	-161
GG	- 9.0	- 5.9	-46.8	-126
SC-AT	-14.6	- 9.4	-77.2	-210
TA	-11.9	- 6.4	-77.1	-218
CG	-13.7	- 8.2	-79.1	-220

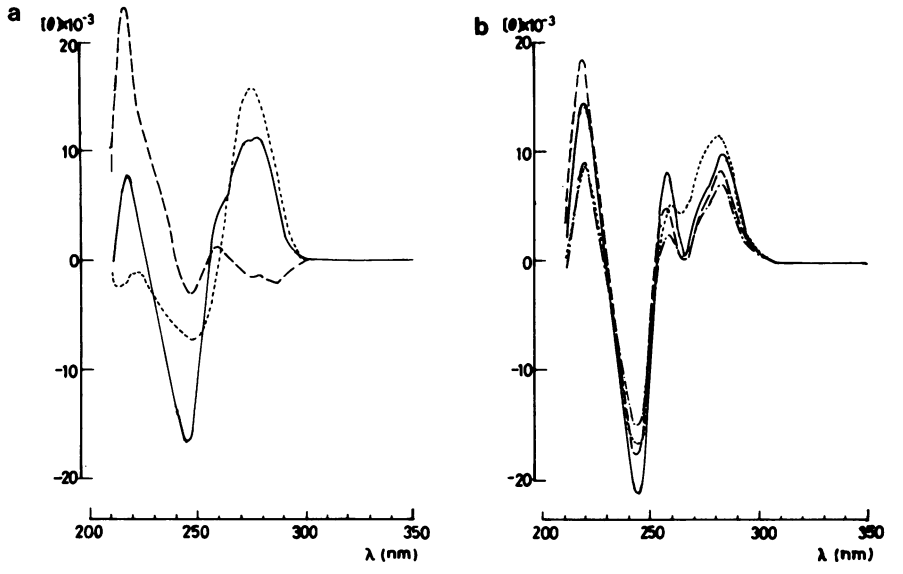

Figure 4.
a) CD spectra for d(GGAAAAGAAAAGG) (——),d(CCTTTTUTTTTCC) (----) and their duplex (——).
b) CD spectra for heteroduplexes $\frac{d(GGAAAAIAAAAGG)}{(CCTTTTNTTTTCC)d}$: ——, N=C; ——, N=A; —·—, N=G; ----, N=T.

thermodynamical behavior. Thermodynamic values for duplex formation were obtained according to a two-state model[12] and are listed in Table III. The values of $\Delta G°$, $\Delta H°$ and $\Delta S°$ are calculated using the conditions in Fig. 3. At higher strand concentrations, SC-GG showed higher Tm values than SC-IG (Table II). However, as indicated by the $\Delta G°$ values in Table III, SC-GG were thermodynamically more unstable than SC-IG at 25°C. Similar results were observed when the thermodynamic stability of SC-IA and SC-GA were compared. Although SC-GA had almost the same Tm value as SC-IA and hydrogen bonding between dA and dG has been suggested by nmr[13] and x-ray[14] data,[13] $\Delta G°$ for SC-GA was larger than that for SC-IA. SC-GC showed the highest stability and was 3.1 Kcal/mol more stable than the isomer SC-CG. This indicates that the sequence of purine-pyrimidine is favored in double strand formation due to a base stacking. Duplex formation of SC-AT is more favored than SC-CG and SC-TA.

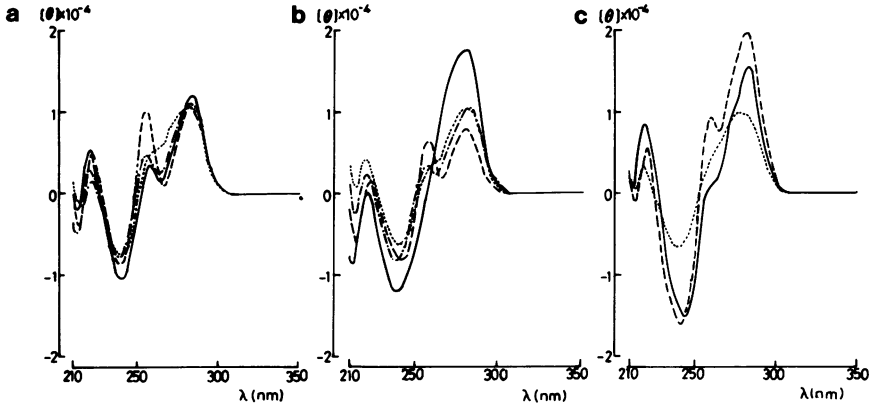

Figure 5.
CD spectra for d(GGGAAXYTTCCC): a) ——, SC-IC; ——, SC-IA; —·—, SC-IG; ----, SC-IT. b) ——, SC-GC; ——, SC-GA; —·—, SC-GG; ----, SC-GT. c) ——, SC-AT; ——, SC-TA; ----, SC-CT.

The $\Delta G°$ values at 50°C were compared with those of 25°C , since hybridization was often performed at 50°C. At this temperature the I-G pairs seem to be disadvantageous compared to the G-G pairs. The $\Delta G°$ values are -4.8 and -5.9 Kcal/mol. This may be due to a larger entropy value for SC-GG. SC-IC and SC-IA showed reasonably stable thermodynamic behavior.

CD spectra for heteroduplexes and self-complementary duplexes

CD spectra of 4 purine tridecamers and 5 pyrimidine tridecamers as well as their combinations shown in Table I were measured at 20 °C. Fig. 4a shows the CD spectra for two complementary single strands containing G and U, respectively. Characteristic changes are obserbed when strands form a duplex. Double-stranded tridecamers containing deoxyinosine showed similar profiles to each other as shown in Fig. 4b. However, stable duplexes containing I:C and I:A had a distinct positive peak at 260 nm. Fig. 5 shows the CD spectra for self-complementary dodecanucleotides containing deoxyinosine (a), deoxyguanosine (b) and thymidine (c). All spectra were close to the conservative shape. However dodecamers containing deoxyinosine and mismatched deoxyguanosine showed an extra Cotton effect near 260 nm.

DISCUSSION

Heteroduplexes containing deoxyinosine were designed so that hypoxanthine could pair with each of four major bases and the single strands would not be self-complementary. The results shown in the first line in Table I indicate that I:C and I:A pairs favor the stability of duplexes of this type, as expected. The I:T pair in the tridecamer on the other hand, did not seem to stabilize the duplex. This result is consistent with the thermodynamic values for helix formation of deoxyinosine-containing $dCA_3XA_3G + dCT_3YT_3G$.[7]

Thermodynamic parameters for the self-complementary duplexes dGGGAAXYTTCCC shown in Table III clarified the order of thermodynamic stability at 25°C for double strand formation ; G:C>A:T>C:G>I:C>I:A>T:A>G:A>I:G>G:G. These results contribute to understanding of neighboring effects in the double strand formation of oligodeoxyribonucleotides. The difference in stability in strands containing purine-pyrimidine isomers may be explained by stacking interactions of base residues. Possible hydrogen bonding schemes for pairing involving deoxyinosine have been shown by Martin et al.[7] and previous nmr studies on G-containing pairs[13,14] compare and correlate the number of hydrogen bonds with the stability of these duplexes. The high Tm and stability shown by the self-complementary duplex SC-GA indicates for base pairing of the type observed by Kennard and coworkers,[15] in the crystal structure of an oligonucleotide containing G-A pairs is probably present. Thus this stability is probably anomalously high. Indeed, it has been found (Kawase et al., unpublished results) that 5 mismatches in 23 base-pairs, the G-A mismatches cause almost complete loss in stability and base stacking of the strands whereas I-A pairs are accommodated quite well.

CD spectra were shown to have drastic differences in shape for the duplexes. Duplexes containing I:C, I:A, G:A, and T:A seem to have an extra transition near 260 nm besides a positive Cotton effect. From these conserved patterns, these duplexes are believed to have right-handed B-form helices under the conditions measured. For more detailed studies on the nature of helicity, short duplexes containing deoxyinosine have

been synthesized for measurement of imino protons by nmr spectroscopy.

To investigate hybridization of deoxyinosine-containing oligonucleotides on nitrocellulose filters, model duplexes containing the sequence for a naturally-occurring peptide gene will be required. Studies along this line will be reported shortly.

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This paper is dedicated to Professor Morio Ikehara on the occasion of his retirement from Osaka University in March 1986.

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