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Synthesis and Properties of Hexanucleotides Containing Deoxyinosine¹⁾

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Self-complementary hexanucleotides d(GGINCC) (N=C, A, G, T) were synthesized by the phosphotriester method. The circular dichroism (CD) spectra of these oligonucleotides showed that the oligonucleotides containing I:C or I:A pairs seem to possess a shape similar to deoxyribonucleic acid (DNA) with right-handed stacking.

Keywords—wobble base pair; oligodeoxyribonucleotide; phosphotriester synthesis; reversed-phase chromatography; CD

Oligodeoxyribonucleotides containing deoxyinosine residues at the ambiguous position of codons have been employed as probes for molecular cloning of genes with high complexity.^{2,3)} In model duplexes of short deoxyribonucleic acid (DNA), possible hydrogen bonding between deoxyinosine and deoxycytidine or deoxyadenosine has been suggested on the basis of the thermal and thermodynamic stabilities of these oligonucleotides.^{4,5)} On the other hand, codon–anticodon interaction in the genetic decoding process can occur not only between inosine and adenosine or cytidine but also between inosine and uridine. In order to clarify in detail the structure and properties of paired deoxyinosine in DNA, a large scale synthesis of self-complementary hexadeoxyribonucleotides containing deoxyinosine is necessary. In this report we describe a synthesis of d(GGINCC) (N=C, A, G, T). The circular dichroism (CD) spectra of these oligonucleotides were measured to compare helical structure formation.

Synthesis and Purification of Hexanucleotides

In order to obtain the hexanucleotides in quantity, they were synthesized in solution using dinucleotides and terminally protected d(CpC) as shown in Chart 1. Deoxyinosine was converted to the 5'-dimethoxytrityl derivative using dimethoxytrityl chloride and phosphorylated to give the *o*-chlorophenyl phosphate derivative.²⁾ Four dinucleotides containing protected deoxyinosine were prepared by condensation with 3'-(*o*-chlorophenyl)- β -cyanoethyl phosphate derivatives⁶⁾ of N-protected deoxynucleosides using 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT).⁷⁾ The yields of tetra- and hexanucleo-

TABLE I. Yields of Protected Tetra- and Hexanucleotides

Tetramer	(g)	(mmol)	(%)	Hexamer	(g)	(mmol)	(%)	(A)
d[ICCC]	0.16	0.07	62	d[GGICCC]	0.12	0.04	50	206
d[IACC]	0.30	0.14	68	d[GGIACC]	0.16	0.05	55	168
d[IGCC]	0.15	0.07	34 ^{a)}	d[GGIGCC]	0.11	0.03	51	175
d[ITCC]	0.27	0.13	65	d[GGITCC]	0.13	0.04	46	174

[] Indicate the fully protected oligonucleotide with a terminal phosphodiester. a) Some diastereomers were excluded.

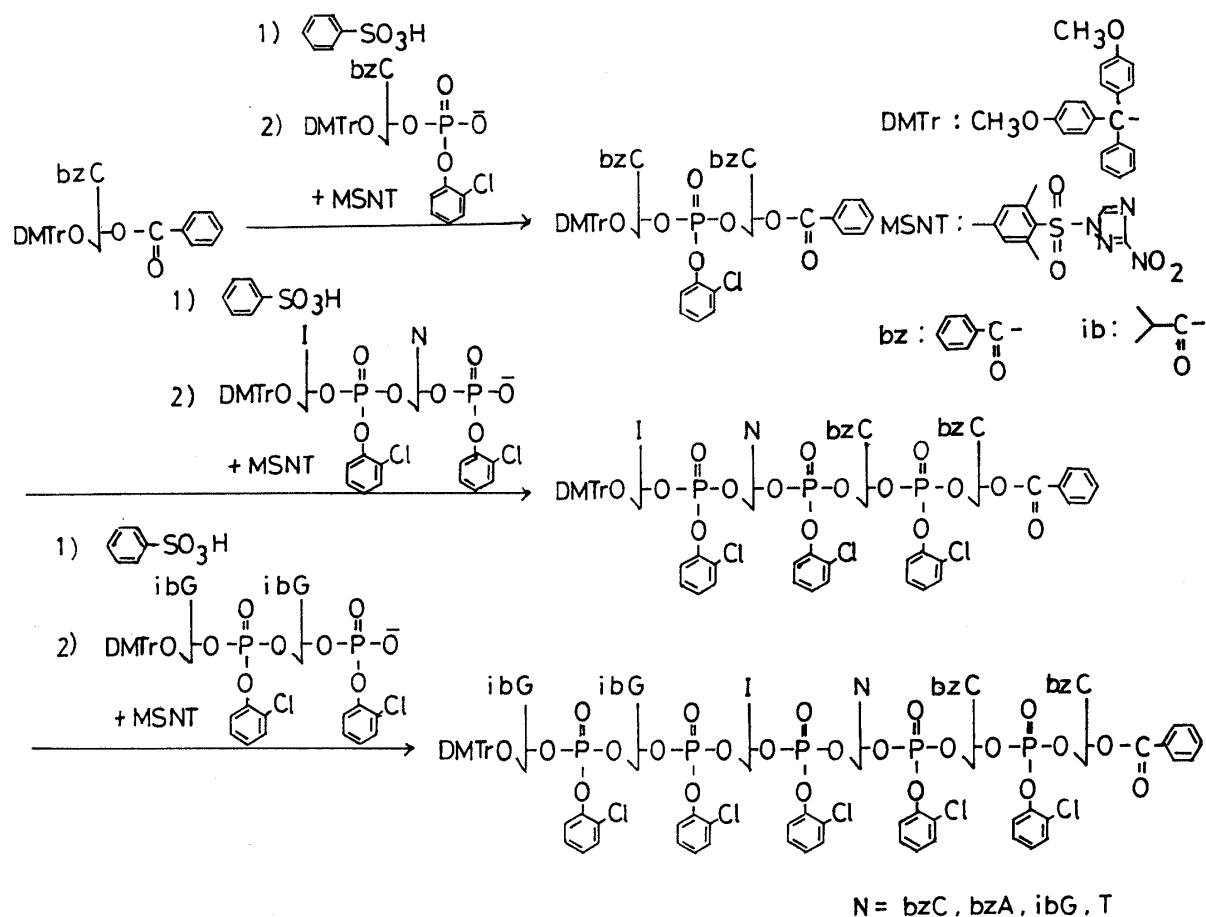


Chart 1

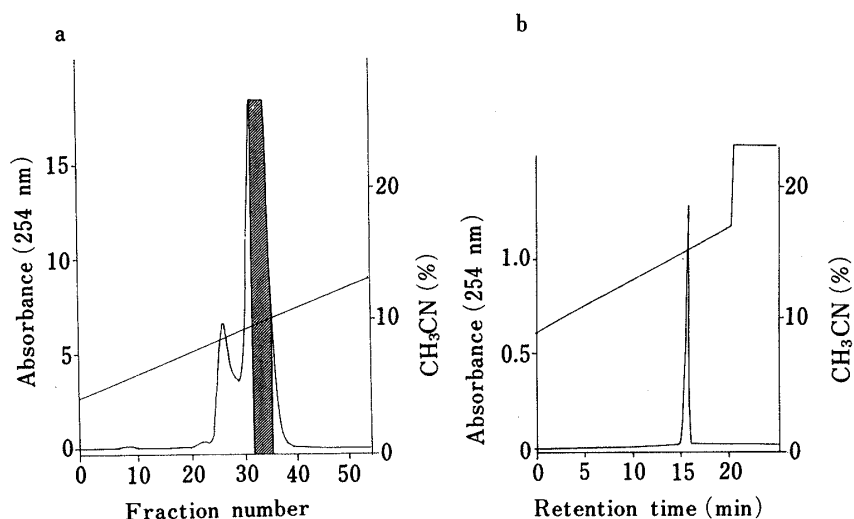


Fig. 1. Reversed-Phase Chromatography of d(GGICCC)

a) On a column (1.5 × 22 cm) of C-18 silica gel (Waters, 35—105 μm) using a gradient of acetonitrile (5—16%) in 50 mM triethylammonium bicarbonate. Fractions indicated by hatching were combined.

b) On a column (4.6 × 250 mm) of TSK-gel ODS-120A using a gradient of acetonitrile in 0.1 M triethylammonium acetate. Solvent A and solvent B contained 5% and 25% acetonitrile, respectively.

Gradient: 20—60%. Flow rate: 1 ml/min.

tides are listed in Table I.

An aliquot of the hexanucleotide was deblocked and purified by reversed-phase chromatography. As an example, the elution profile of d(GGICCC) in a preparative run is shown in Fig. 1a. The purity of the hexamer was analyzed by high-pressure liquid chromatography (HPLC) as shown in Fig. 1b.

Properties of d(GGINCC)

Temperature-absorbance profiles of d(GGINCC) (N = C, A, G, T) were measured in the presence of 0.01 M cacodylate and 0.1 M NaCl, as shown in Fig. 2. Hexamers containing I : C or I : A pairs showed co-operative melting at the strand concentration of *ca.* $2 A_{260}$ ($T_m = 18^\circ\text{C}$). On the other hand, no distinct co-operative melting was observed in the case of I : G or I : T hexamer.

CD spectra of these four hexamers are shown in Fig. 3. The I : C and I : A hexamers (Fig. 3a) showed rather non-conservative spectra. The spectra for I : G and I : T hexamers (Fig. 3b) had smaller molecular ellipticities compared to those for I : C and I : A hexamers, which seemed to form hydrogen bondings at all six nucleotides.

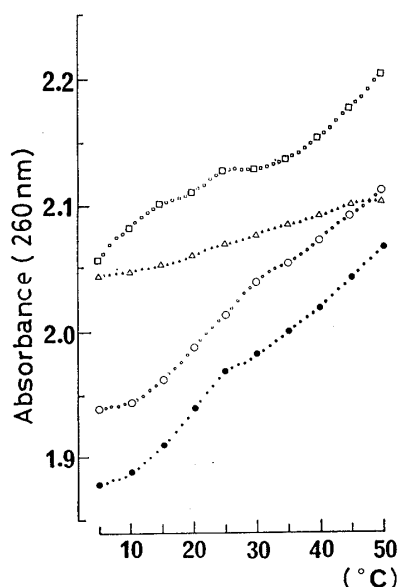


Fig. 2. UV Absorbance-Temperature Profiles of Self-Complementary Duplexes d(GGINCC)
●●●●●, N = C; ○○○○○, N = A; □□□□□, N = G; △△△△△, N = T.

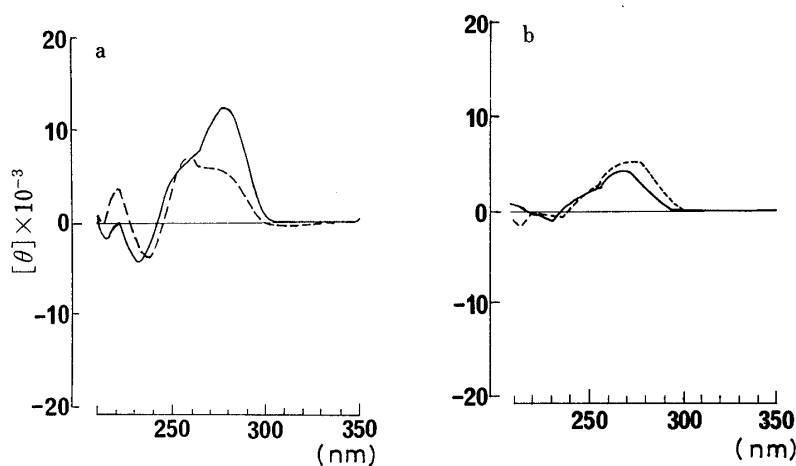


Fig. 3. CD Spectra of d(GGINCC)

a) —, N = C; ---, N = A.
b) —, N = G; ---, N = T.

In conclusion, deoxyinosine-containing hexanucleotides could form duplexes when the complementary strands contained deoxycytidine or deoxyadenosine at the paired position. The stabilization of the duplex may arise from the hydrogen bonding between hypoxanthine and cytosine or adenine. Although the stacking effect of hypoxanthine is not excluded, it is unlikely that guanine has less stacking effect than cytosine. The nuclear magnetic resonance (NMR) spectra of the imino protons between dI and dC or dA in these hexamers also suggested formation of hydrogen bonding involving in the H1 imino proton of the dI residue.⁸⁾

Experimental

General Methods—Thin layer chromatography (TLC) was performed on precoated Silica gel 60F₂₅₄ (Merck) using chloroform and methanol. For reversed-phase TLC, silanized silica gel (Merck) was used. For column chromatography, Wakogel C-300 (Wako Pure Chemicals) and C-18 silica gel (35—100 μ m, Waters) were used. Protected dinucleotides were prepared and the β -cyanoethyl moiety was removed as described.⁹⁾ Other general methods for purification and analysis of oligonucleotides were as described.^{10,11)}

Ultraviolet (UV) temperature profiles were recorded with a Beckman DU-8B spectrophotometer and CD spectra were measured with a JASCO 500A spectrophotometer.

d(bzCpbzC(Bz))—d((MeO)₂Tr-bzCp) (1.23 g, 1.5 mmol) and d(bzC(Bz)) (0.52 g, 1.2 mmol) were dried by evaporation with pyridine. The mixture was dissolved in pyridine (4 ml) and treated with MSNT (9.89 g, 3 mmol) at room temperature for 20 min. Aqueous pyridine (30%) was added and the product was applied to a column of silica gel (30 g) after extraction with chloroform and washing with 0.1 M triethylammonium bicarbonate. The dimethoxytrityl moiety of the dimer was removed by treatment with benzenesulfonic acid (12 ml, 7.5 mmol) in chloroform (28 ml) in an ice bath for 10 min. The mixture was washed with saturated sodium bicarbonate and water, then applied to the same column. The yield was 0.83 g, 0.91 mmol (76%).

Hexanucleotides—As an example, d(GGICCC) was synthesized by condensation of d(IpbzCpbzCpbzC(Bz)) (0.16 g, 0.07 mmol) and d((MeO)₂Tr-ibGpibGp) (0.12 g, 0.077 mmol) using MSNT (0.07 g, 0.16 mmol) at room temperature for 20 min. The reaction was checked by TLC and the product was isolated by silica gel chromatography as above. Fractions containing the protected hexamer were combined and the product was precipitated with hexane. Fractions contaminated with the tetramer were purified by chromatography on a column of silica gel. The yield was 0.12 g, 0.035 mmol (50%).

The protected hexamer (50 mg) was dissolved in 1 M tetramethylguanidinium 2-pyridinealdoximate⁷⁾ in dioxane (4.4 ml) and diluted with water (4.4 ml). The mixture was kept at 30 °C overnight and concentrated. The residue was dissolved in pyridine (1.5 ml) and heated with concentrated ammonia (10 ml) at 60 °C for 5 h. The dimethoxytritylated hexamer was concentrated and subjected to gel filtration on Sephadex G-25 (2.0 $\times$ 44 cm) in 0.1 M triethylammonium bicarbonate. Fractions containing the product were concentrated, desalted by evaporation with water and treated with 80% acetic acid (2 ml) for 30 min. Acetic acid was removed by evaporation and the aqueous solution was washed with ethyl acetate to remove dimethoxytrityl alcohol, then subjected to reversed-phase chromatography as shown in Fig. 1a. The product was analyzed by reversed-phase HPLC as shown in Fig. 1b.

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