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CD spectra and some properties of deoxyoligonucleotide duplexes having a C:G terminus (nucleosides and nucleotides. Part 69[1])

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ABSTRACT

In the course of an investigation of the mode of recognition of nucleotide sequences with restriction endonucleases, several deoxyoligonucleotide duplexes having G:C terminal base pairs were synthesized. The oligonucleotides having a 5'-C (3'-G) terminus showed unusual CD spectra with a negative band at the longer wavelength region, when compared to those of the same internal sequences but a 5'-G (3'-C) terminus, which showed a positive band like the B- or A-DNA type. The nature of these CD spectra was compared with those of the Z-DNAs on the effect of salt concentrations, intercalation with ethidium bromide, or ^{31}P-NMR spectra. These unusual spectra may be attributed to the terminal effect of the 5'-C:3'-G pairs.

INTRODUCTION

Current results of studies on the mode of recognition and cleavage of nucleotide sequences by restriction endonucleases have suggested the essential role of the donor and acceptor sites of nucleobases facing on the major or minor grooves of nucleotide double helices.[2,3] We have recently pointed out[4] the significance of the 7-azomethine group of the adenine moiety for the recognition and cleavage of AGATCT sequence by restriction endonucleases Bgl II and Sau 3AI, by the use of 7-deazaadenine in place of adenine. For further investigation, we have synthesized several oligonucleotides having cytosine (C) and guanine (G) terminal sequences. During this study we observed an unusual CD spectral feature of the oligomers with a 5'-CC (hence 3'-GG) terminus. This paper describes the effect of terminal base pairs on the CD spectra of deoxyoligonucleotide duplexes. A preliminary account of this work has appeared.[5]

MATERIALS AND METHODS

General procedures ---- The UV spectra were recorded on a Shimadzu UV-240 or UV-260 spectrophotometer. Melting temperatures (Tm) of oligonucleotides were determined by a Shimadzu UV-240 spectrophotometer attached with a temperature controller SPR-5. The circular dichroism (CD) spectra were measured on a JEOL-J-500A spectropolarimeter at appropriate temperatures and salt concentrations. Prior to the measurements of the CD spcetra of oligonucleotides, all samples were heated to 60°C in solution and allowed to cool slowly to a room temperature. The ^{31}P-NMR was measured on a JEOL FX-90Q spectrometer with ^{1}H-noise decoupling. The starting deoxynucleosides were purchased from Yamasa Shoyu Co., Tokyo.

Synthesis of deoxyoligonucleotides ---- The oligonucleotides were prepared mostly by the solid phase phosphotriester method[6], and others were prepared by the liquid-phase phosphotriester approach[7]. The purity of the nucleotides was checked by HPLC analysis (Gilson, Microsorb C-18 column) and mobility shift analysis[8]. Compounds of purity >99% were used for the experiments. The molar absorbancies of the oligonucleotides were expressed as 10,000 at the absorption maximum in all cases. The oligonucleotides prepared in this study are as follows: CCATGG, CCCGGG, CCGATCGG[4], CAGATCTG, CCCCGGGG, CCAGATCTGG, and CCCGATCGGG as 5'-C (3'-G) self-complementary oligomers; GGATCC, GGGCCC, GAGATCTC, GAGATCTCC[4], AGACCGGTCT, ACCCCGGGGT, TTTAGATCTAAA, and AAAAGATCTTTT as self-complementary oligomers with other terminal base pairs; CCAGATCTTT:AAAGATCTGG, CCAGATCTCC:GGAGATCTGG[4], CCCAGATCTC:GAGATCTGGG, and TTTAGATCTTTT:AAAAGATCTAAA as heteroduplexes.

Digestion with restriction enzymes ---- The method employed is similar to that previously reported[4]. The oligonucleotides were phosphorylated with γ-^{32}P-ATP and T4 polynucleotide kinase to give the 5'-^{32}P-phosphates. These were digested with Bgl II or Sau 3AI. A typical digestion condition with restriction enzymes is as follows: the ^{32}P-nucleotide (10000-30000 cpm) was incubated in a buffer of 10 mM Tris-HCl (pH 7.5), 7 mM $MgCl_2$, 100 mM NaCl, and 7 mM 2-mercaptoethanol, with 72 units of Bgl II (or 50 units of Sau 3AI) in a total volume of 10-20 µl at 20°C for 24 h. The digests were developed by homochromatography and the spots were

excised and counted with toluene scintillator on a Beckman LS-230 liquid scintillation counter.

RESULTS AND DISCUSSIONS

We have recently reported[4] the synthesis of a self-complementary deoxydecanucleotide GGAGATCTCC (1) and its 7-deazaadenine derivatives in place of A in this sequence and others, and mode of recognition and cleavage of modified nucleotides with restriction endonucleases Bgl II and Sau 3AI. It was disclosed that the presence of the nitrogen-7 (hydrogen bond acceptor) of A in either position in this sequence is essential for the cleavage with Bgl II and not with Sau 3AI. Furthermore, from the experiment of cleavage of heteroduplexes containing 7-deazaadenine in one strand, we proposed a mechanism that an enzyme Bgl II recog-

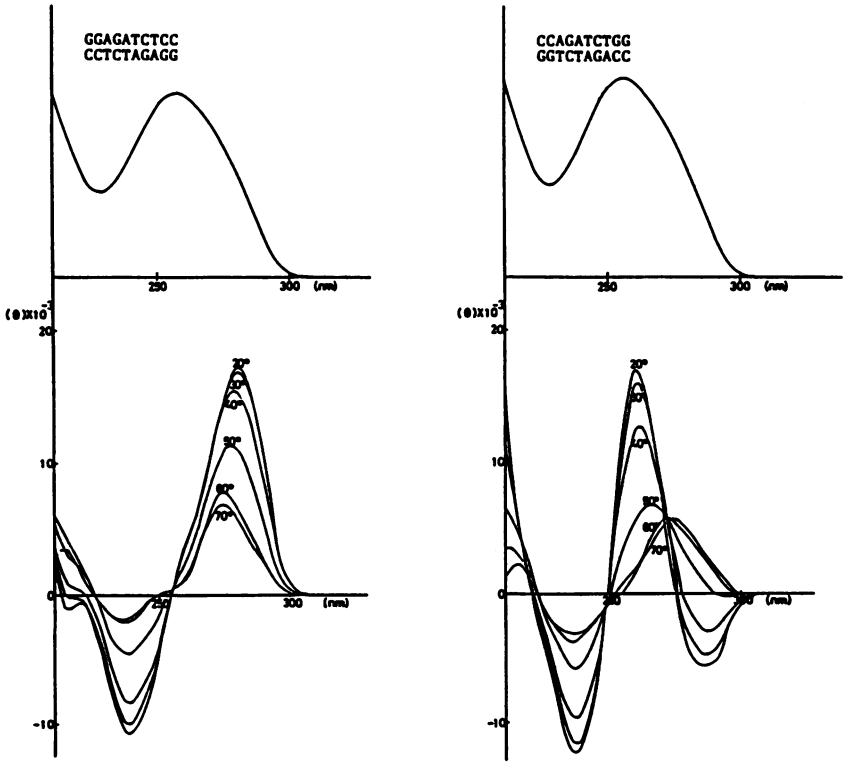

Fig 1 UV and CD spectra of GGAGATCTCC and CCAGATCTGG in 0.01M Na cacodylate, 0.1M NaCl, pH 7.5

Table 1 Summary of CD Bands at Long Wavelength and Melting Temperature(Tm)

- Band		+ Band			
	Tm		Tm		Tm
CCATGG	22-24°	GGATCC	≤18°	TTTAGATCTAAA	32°
CCCGGG	39°	GGGCCC	35°	AAAAGATCTTTT	35°
CAGATCTG	29°	GAGATCTC	26°	AAAAGATCTAAA TTTTCTAGATTT	32°
CCAGATCTGG	45°	GGAGATCTCC	42°		
CCGATCGG	45°	AGACCGGTCT	51°		
CCCCGGGG	50°	ACCCCGGGGT	53°		
CCCGATCGGG	53°	CCAGATCTCC GGTCTAGAGG	43°		
CCAGATCTTT GGTCTAGAAA	38°	CCCAGATCTC GGGTCTAGAG	43°		

All samples were dissolved in 0.01M Na cacodylate buffered at pH 7.5 containing 0.1M NaCl. CD spectra were measured at the appropriate temperatures where the all samples were in a fully ordered conformation.

nizes one strand and cleaves the other strand, whereas Sau 3AI recognizes and cleaves the same strand. In order to study the mode of action of various restriction enzymes, a variety of

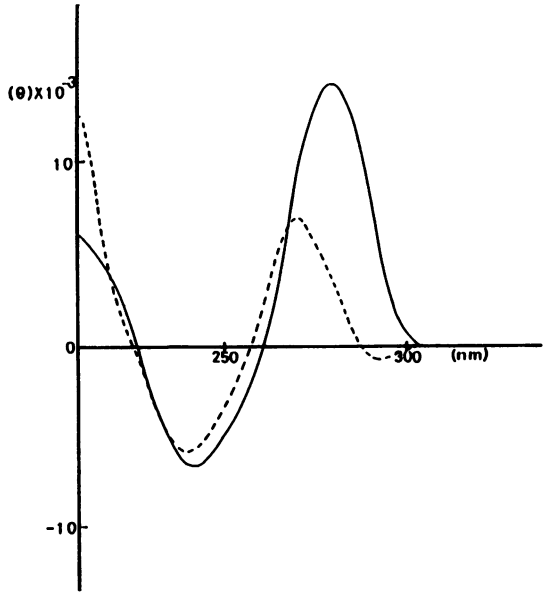

Fig 2 CD spectra of GAGATCTC(——) and CAGATCTG (---) in 0.01M Na cacodylate, 0.1M NaCl, pH 7.5 at 15°C

synthetic oligonucleotides are required. In addition, it seems necessary to take into account the effect of nucleotide sequences close to the recognition sequence.

Therefore, we first prepared a decanucleotide having the recognition sequence of Bgl II and Sau 3AI with a reversed terminal sequence, that is CCAGATCTGG (2). It turned out that this nucleotide was cleaved by both enzymes but the rates were significantly slower in both cases. This result led us to investigate some physicochemical differences of the two terminal sequence isomers. In addition, oligonucleotides with various chain lengths and terminal base pairs have been prepared for comparison.

CD spectra of oligonucleotides

While the UV spectra of GGAGATCTCC (1) and CCAGATCTGG (2) are almost identical, there is a distinct difference in the CD spectra (Fig. 1) measured at 20°C in 0.1 M NaCl at pH 7.5 (Tm of 1 and 2 are 42°C and 45°C, respectively), irrespective of the presence or the absence of ethylenediaminetetraacetic acid. The CD spectrum of 1 is of the regular B-type DNA. In contrast, the negative CD band of 2 at the 285 nm region is remarkable. These spectra were temperature-dependent and at 70°C both showed almost identical regular spectra for the single-stranded oligomers. In order to investigate whether the generality of this interesting CD pattern of 1 is found for the self-complementary oligomers having 5'-CC (3'-GG) sequence or not, several oligomers were synthesized and their CD spectra compared.

The result, including the Tm's, is summarized in Table I. It is apparent that all oligomers having a 5'-CC terminus showed a similar pattern of negative-positive-negative bands. The magnitudes of the negative bands at the longer wavelength region are decreased as the chain length is increased. In addition, 5'-capping with only one C also showed weak but distinct negative band as is shown in the case of CAGATCTG when compared to that of GAGATCTC (Fig. 2). Furthermore, capping of the 5'-CC terminus of CCCCGGGG with a base other than C, resulted in an almost complete loss of the negative band as shown, for example, in the case of ACCCCGGGGT (Fig. 3).

Therefore, it can be stated that the self-complementary oligonucleotides having 5'-C (or CC) termini exhibit negative CD

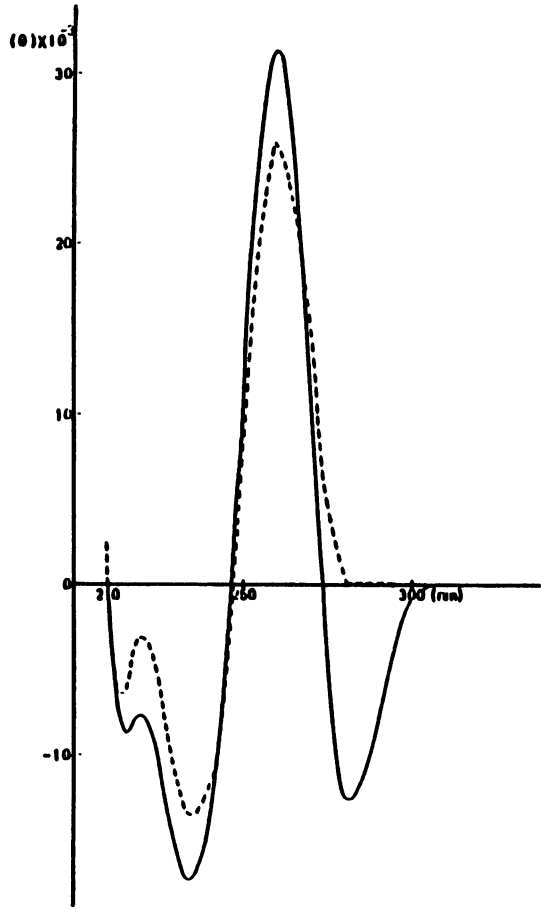

Fig 3 CD spectra of CCCCGGGG(——) and ACCCCGGGGT(---) in 0.01M Na cacodylate, 0.1M NaCl, pH 7.5 at 20°C

bands at the longer wavelength region. As is well known, the Z-DNAs are characteristic in showing negative bands at the longer wavelength region.[9] However, this pattern is different and the salt effects for the 5'-CC oligomers are again different from those of Z-DNA. For example, CCATGG reached a maximum of negative magnitude at 1 M NaCl concentration, but decreased at higher salt concentrations, 3-5 M (Fig. 4), unlike that of Z-DNA. The same salt effect was observed in the case of 2. Although the structu-

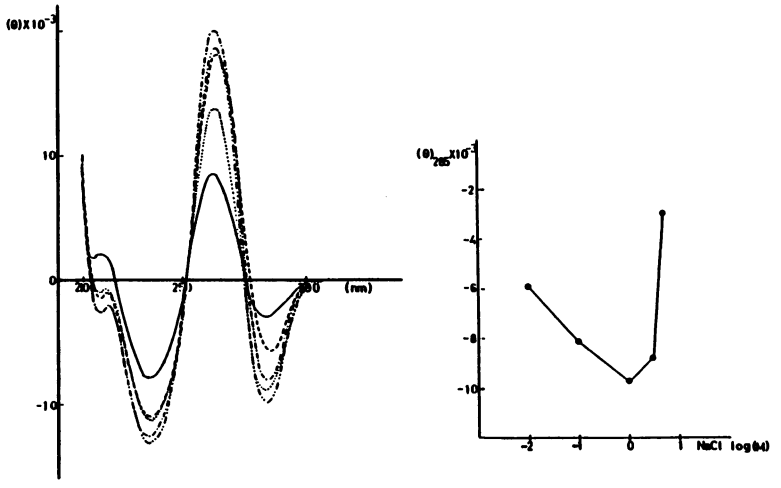

Fig 4 CD spectra of CCATGG as a function of NaCl concentration; 0.01M(-----), 0.1M(-·-·-), 1M(-··-··), 3M(·····), 5M(——) in 0.01M Na cacodylate, pH 7.5 at 4°C

ral basis for this interesting CD pattern is not clear, this seems to be a reflection of some conformational difference between 5'-CC:3'-GG terminal base pairs and 5'-GG:3'-CC and other base pairs.

The CD spectra of a heteroduplex CCAGATCTCC:GGAGATCTGG (3), showed a rather regular B-DNA type CD spectra (Fig. 5). However, the molar ellipticity of the positive band of 3 was rather small when compared to those of 1 or 2. This can be explained by the assumption that 3 is a hybrid of 1 and 2 in terms of the terminal sequences. The duplex of 2 has two 5'-CC:3'-GG and 1 has two 5'-GG:3'-CC termini, while 3 has one of each. Since all these duplexes have the same 6 base-pairs (AGATCT:AGATCT) with identical sequence in both directions, the CD spectra of 3 should be the mean of 1 and 2. In fact, the synthetic spectra of 1 and 2 are almost identical with the observed spectra of 3 (Fig. 5). Therefore the negative band due to the 5'-CC:3'-GG terminus in 3 is cancelled out by the strong positive band due to the other end. A similar result was obtained when the CD spectra of oligomers in similar situations (for example, AAAAGATCTTTT, TTTAGATCTAAA and a heteroduplex AAAAGATCTAAA:TTTAGATCTTTT, Fig. 6) were compared. In

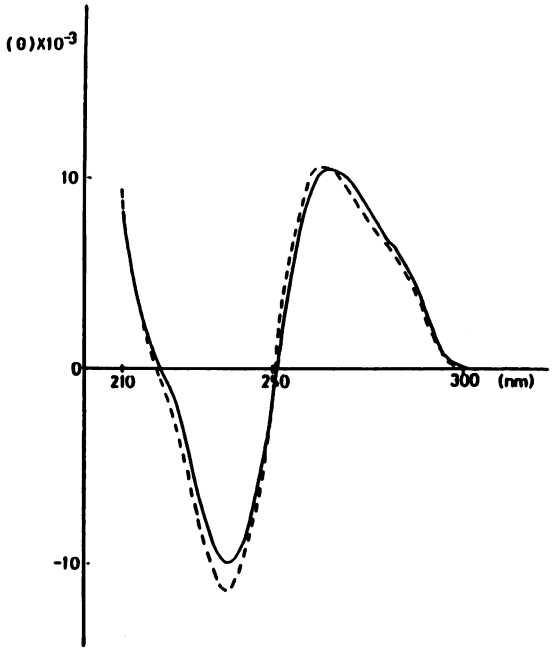

Fig 5 Comparison between observed(——) and calculated(---) CD spectra of CCAGATCTCC:GGAGATCTGG duplex in 0.01M Na cacodylate, 0.1M NaCl, pH 7.5 at 20°C

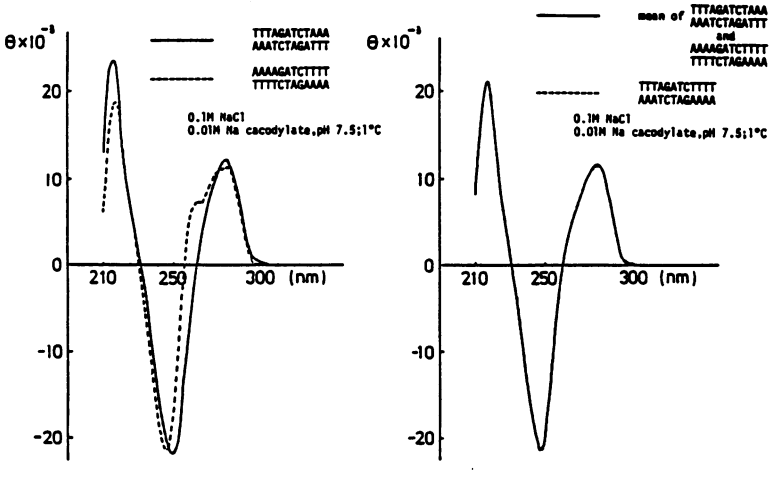

Fig 6 Comparison between observed and calculated CD spectra of the AAAAGATCTAAA:TTTAGATCTTTT duplex

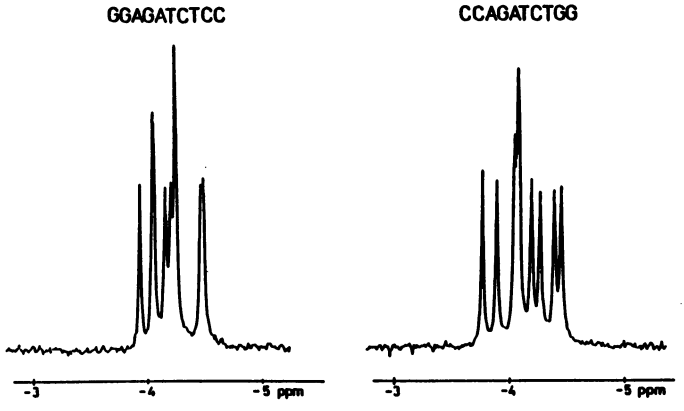

Fig 7 Proton noise-decoupled P-NMR spectra of CCAGATCTGG and GGAGATCTCC in 0.01M Na cacodylate, 0.1M NaCl, 1mM EDTA, pD 7.1 at 32°C.
The chemical shifts were measured relative to an internal standard of trimethyl phosphate.

general, self-complementary oligonucleotides exhibit CD spectra of doubled molar ellipticities due to the symmetry of the two fold axis in the form of the duplex.[10]

Further studies for elucidation of conformational characteristics of the oligonucleotides having 5'-CC:3'-GG termini will be required by other means in addition to the CD measurements. As a preliminary experiment, ^{31}P-NMR spectra of 1 and 2 were compared. These showed no distinct differences in the backbone structure of the phosphoester linkage, since the ^{31}P-chemical shift differences are within 0.7 ppm (Fig. 7) as found in the regular B-DNA and not like that of Z-DNA where the difference is

$$1 \quad \begin{matrix} GG & AG & AT & CT & CC \\ CC & TC & TA & GA & GG \end{matrix} \quad n = 2.1$$

$$2 \quad \begin{matrix} C & CA & GA & TC & TG & G \\ G & GT & CT & AG & AC & C \end{matrix} \quad n = 2.6$$

Fig 8 The intercalation sites of ethidium bromide to the synthetic oligonucleotides 1 and 2
"n" denotes the maximum number of ethidium bromide bound per oligonucleotide determined from a Scatchard plot.

Table 2 Cleavage of Synthetic Oligonucleotides by Restriction Endonuclease Bgl II and Sau 3AI

	Rate of Hydrolysis(%)	
	Bgl II	Sau 3AI
1 pGGAGATCTCC CCTCTAGAGGp	77	96
2 pCCAGATCTGG GGTCTAGACCp	39	51
3* pGGAGATCTGG CCTCTAGACC	8	94
4* GGAGATCTGG CCTCTAGACCp	24	75
5* pAAAGATCTGG TTTCTAGACC	4	70
6* AAAGATCTGG TTTCTAGACCp	20	86

The rate of hydrolysis was expressed by the ratio of counts of released ^{32}P-trinucleotides and intact deca-nucleotides. "p" denotes the labelled phosphate.
*The 5'-labelled oligonucleotides were digested in the presence of 1.0 OD unit of the same unlabelled oligo-nucleotides.

1.5 ppm.[11] The extent of intercalation of ethidium bromide[12] to 1 and 2 was next examined which showed some differences in the maximum number of binding (n = 2.1 for 1 and 2.6 for 2). This means that 4 molecules of ethidium bromide will bind to a duplex of 1, while 5 molecules will bind to that of 2. This may be rationalized if one assume that the site of intercalation of ethidium bromide is as shown in Fig. 8. This may be a reflection of a difference of the degree of stacking of the GG:CC pairs at the different termini.

Recognition of 1, 2, and 3 by restriction enzymes

Nucleotide 1, its terminal sequence isomer 2, and a hybrid duplex 3 were 5'-^{32}P-phosphorylated and subjected to Bgl II and Sau 3AI digestions. The result is summarized in Table II. It is evident that 1, having regular CD spectra for a B-DNA type, was cleaved faster with both enzymes than the isomer 2 (entry 1 and 2). In the case of heteroduplex 3, which was labelled on one strand, the strand having the 5'-CC sequence was cleaved faster than the other one (entry 3 and 4) in Bgl II digestion. This is again interesting and consistent with the assumption[4] that Bgl II recognizes one strand and cleaves the other strand. In the case of Sau 3AI cleavage, there is no essential difference in the rate, although the 5'-GG strand was cleaved a little faster. For

comparison, the cleavage of a heteroduplex AAAGATCTGG:CCAGATCTTT is shown (entry 5 and 6). The strand having the 5'-CC sequence was again cleaved faster, as expected.

It is worth emphasizing the finding that an oligomer CCAAGCTTGG, containing an AAGCTT recognition sequence of Hind III, was not cleaved by this enzyme.[13] The 3'-CC (5'-GG) capping would also have affected the cleavage.

In conclusion, we have found that self-complementary oligonucleotides having a 5'-cytosine (hence 3'-guanine) terminus showed a negative CD spectra, which is not due to a Z-DNA structure. This terminal base-pair effect may also reflect the stacking of intercalator to the helices and in the rate of cleavage by restriction enzymes.

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