CD studies on the conformation of some deoxyoligonucleotides containing adenine and thymine residues

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Received 2 July 1974

ABSTRACT

CD studies of the deoxyoligomer series $d(pT)_n$ and $d(pA)_n$ show increasing CD maxima for oligo (dT)'s with chain length variation from two to seven, while oligo (dA)'s exhibit a decreasing CD maximum. Concentrated solutions of $NaClO_4$ cause a decrease in the CD of longer oligo (dT)'s towards the CD of $d(pT)_2$ which is different from oligo dA's. Probably base-sugar interactions are important in the observed conformational effects. The chemically synthesized oligomers $dpApApTpT$ and $dpTpApTpA$ show deviations in their CD spectra which reflect a dominating conformational effect of $d(pA)_2$ in the former but not in the alternating isomer.

INTRODUCTION

The conformation of the DNA structure is not a geometrically continuous double helix but may change locally with the nucleotide sequence. A number of investigations suggested, that structural studies of oligonucleotides (1-8) may help to explain conformational effects of nucleotide units in the polymeric state of nucleic acids. In view of several evidences which demonstrated that the conformation of DNA varies with extreme different base composition and local sequence differences (9-14) conformational properties of adenine and thymine containing deoxyoligonucleotides are of special interest. Previous studies included extensive ORD or CD measurements with oligoribonucleotides, e.g. oligo (rA) (6,15,16), oligo (rU) (6), oligo (rC) (17,18), oligo (rI) (6,19) and oligomers with mixed bases (20). ORD and CD results were reported in the series of oligo (dA)'s (6,21,22) and some oligo

(dC)'s (13). Conformational differences among deoxyribodinucleoside phosphates containing different bases were reported by Cantor et al. (3,4). The UV spectral properties of oligo (dA)'s and oligo (dT)'s have been compared with the polymeric state of poly (dA) and poly (dT) in the work of Cassani and Bollum (2). CD differences between cyclic and linear oligo (dT)'s of low chain length reflect large variations in torsional angles (5). Though the reported data are scanty a totally different CD behaviour for the oligo (dA) series has been established (6,21).

In this paper an attempt has been made to give an interpretation of the different CD behaviour between oligo (dA)'s and oligo (dT)'s on the basis of a complete oligo dT series not previously reported. Conformational differences of the tetramers dpApApTpT and dpTpApTpA are described.

EXPERIMENTAL

Instrumentation: Condensation reactions were carried out in a glove box using anhydrous phosphorus pentoxide. Separation of the reaction mixtures were performed in an UVICORD II equipped with an Ultro Rac Fraction Collector (LKB) at 4° C. Fractions were concentrated in a rotatory evaporator (VEB Medizintechnik, Leipzig) under vacuum at 20° C. Protected oligonucleotides were stored at -20° C, unprotected oligo's were lyophilized in vials and stored at -5° C.

UV absorption and melting measurements were made in a Hilger spectrophotometer model UVISPEK (Hilger & Watts, Ltd., London). CD spectra were recorded in a Cary 60 spectropolarimeter with 6001 CD attachment using 1 cm cells. The molar ellipticity $[\theta]$ refers to molar residue concentration calculated on a molar phosphorus basis (Table 2) and is expressed in degrees·cm²·decimoles⁻¹.

Extinction coefficients per residue were obtained experimentally as given in Table 2.

Materials: dpT sodium salt was purchased from PWA Pharma, Waldhof, and dpA ammonium salt from Serva, Heidelberg; dicyclohexylcarbodiimide and mesithylene-sulfonylchloride were obtained from Ferak, Berlin, and EGA-Chemie, KG-Keppler & Reif, Steinheim, respectively.

Preparation of deoxyoligoribonucleotides: $d(p^m)_n$; dpT was polymerized according to the method of Khorana and Connors (24) except the isolation of pyridinium-3'-O-acetylthymidine-5'-phosphate which was achieved by ether precipitation (25). Purification of oligo (dpT) with more than six nucleotides following DEAE-cellulose-chromatography has been accomplished by gel-filtration on Sephadex G-25. Elution was done with water.

$d(pA)_n$: For polymerization of dpA the method of Ralph and Khorana (26) was used. A modification of Narang and Dheer (27) was applied in the synthesis of $dpbza$ to remove benzoic acid by gel filtration. In the synthesis of N,3'-O-Diacetyl-deoxyadenosine-5'-phosphate pyridiniumacetate was removed by ether precipitation of the nucleotide (25). The polymerization mixture was held at low temperature and neutral pH throughout all separation procedures to avoid terminal dephosphorylation. Resolved fractions of the DEAE-cellulose chromatography were purified by gel filtration on Sephadex G-25 (superfine) using 0.1 M TEAB as eluant. The resulting elution pattern gave positions of fractions according to the chain length, the structure of which was identified by enzymatic cleavage.

$d(pTpApTpA)$: Synthesis of $d(pTpApTpA)$ was carried out by the procedure of Weimann et al. (28). Preparation of the protected compounds $d(CNEt)pT$ and $dpbza (Ac)$, condensation reaction and introduction of protective groups into the resulting dinucleotide have been achieved by procedures described by Khorana and co-workers (29,30). Protected compounds were freshly synthesized before condensation to avoid decreased yields. Mesitylene-sulfonyl-chloride was used to generate condensation reaction. The mixture of compounds resulting from condensation between $d(CNEt)pTpzbA$ and $dpTpzbA (Ac)$ were separated by gel filtration due to differences in molecular weights of the final products and reactants (Table 1). After removal of protecting groups pure $d(TpApTpA)$ was obtained by DEAE-cellulose chromatography. Yield of $d(pTpzbApTpzbA)$ was 20 % related to $d(CNEt)pTpzbA$.

$d(pApApTpT)$: The protected dimer $d(CNEt)pbzApbza$ was prepared according to the method of Smirnow (31); its purification as $dpbApbza$ has been achieved using DEAE-cellulose column chromatography, the elution pattern of which is shown in Fig. 1. Oligonucleotide chains with more than two residues were repeatedly

Table 1 Gel-chromatography of deoxyoligomers on Sephadex G-25 (superfine)

	K_{av}
dTpA	0.42
d(pTpA) ₂	0.28
d(pbzA) ₃	0.29
d(pbzA) ₄	0.176
d(pT) ₂	0.36
d(pbzApbzApTpT)	0.165
d(pApApTpT)	0.198

Elution with 0.1 M triethylammonium bicarbonate, pH 7.5.

Fig. 1 Chromatography of the reaction mixture obtained in the synthesis of dpbzApbzA on a DEAE-cellulose column (4.2 x 60 cm, bicarbonate form). Elution was performed with a linear gradient of TEAB in 10 % ethanol. Fractions of 14.5 ml were collected every 10 min. Peak II contained dpbzA, III: dpbzApbzA, IV: dpbzApbzApbzA, V: dpbzApbzApbzApbzA.

chromatographed on Sephadex G-25 (superfine); the characteristic K_{av} values are given in Table 1.

Acetylation of dpTpT was carried out according to Khorana (30).

Condensation reaction of d(CNEt)pbzApbzA and dpTpT (Ac) was performed in presence of mesitylene-sulfonylchloride using a special anaerobic apparatus (23). Following treatment with acetic anhydride-pyridine to cleave pyrophosphate (32) separation on Sephadex G-50 (superfine) gave successful results (Fig. 2).

Fig. 2 Separation of reaction products in the condensation of dpbzApbzApTpT on Sephadex G-50 (superfine) (column 2.5 x 80 cm), eluted with 0.1 M TEAB. Fractions of 3.7 ml/10 min were collected.

Peak II contained the tetranucleotide, III dpTpT and dpbzApbzA.

Absorbance at 265 nm -x-x-, 285 nm -o-o- .

After removal of protecting groups the reaction products were finally chromatographed on Sephadex G-25. The final product yielded 28.5 % related to d(CNEt)pbzApbzA; acidic hydrolysis showed a ratio of adenine:thymine = 1.00:1.03.

RESULTS AND DISCUSSION

Oligo(dT) and Oligo(dA): The CD spectra and characteristic maxima of the complete oligo(dT) series up to eleven residues are presented in Fig. 3 and in Table 2. Both CD maxima, located at 277 nm and around 250 nm, are not shifted with increasing chain length but their amplitudes become substantially increased from two to seven residues. A corresponding plot of the CD data indicates that further chain length prolongation does not cause an additional enhancement of these maxima, except in the linear increase around 220 nm (Fig. 4). In contrast to the series of

Fig. 3 CD spectra of the oligo(dT) series measured in 0.02 M NaCl.

$d(pT)_n$ the positive CD maximum in the oligo(dA) series decreases and is displaced from 270 nm to 276 nm with increasing chain length up to $d(pA)_7$ (Table 2, Fig. 4). This behaviour agrees with that first reported by Bush and Scheraga (21) for $d(pA)_2$, $d(pA)_6$ and poly(dA). The appearance of a defined second band within the broad CD maximum of $d(pA)_6$ or $d(pA)_7$ as found in this previous work (21) we could not observe in our CD spectra. However, the

long wavelength shift (Table 2) and the displacement of the intercept from 250 to 253 nm of $d(pA)_n$ was quite obvious in our spectra.

Table 2 UV and CD spectral data of deoxyoligonucleotides in 0.02 M NaCl

Oligomer	$\epsilon_{\max} \cdot 10^{-3}$ ($\text{cm}^{-1} \cdot \text{molar}^{-1}$)	CD $\max_1$ (nm)	$[\theta] \cdot 10^{-4}$	CD $\max_2$ (nm)	$[\theta] \cdot 10^{-4}$
$d(pA)_2$	13.6	270	0.88	250	-1.54
$d(pA)_3$	12.8	272	0.68	250	-1.70
$d(pA)_4$	11.9	274	0.58	250	-1.68
$d(pA)_5$	11.2	275	0.54	250	-1.58
$d(pA)_6$	10.8	275	0.51	250	-1.59
$d(pA)_7$	10.1	276	0.51	250	-1.48
$d(pT)_2$	8.87	277	0.72	247	-0.49
$d(pT)_3$	9.3	276	0.91	247	-0.60
$d(pT)_4$	8.85	277	0.98	248	-0.64
$d(pT)_5$	9.1	276	1.05	250	-0.72
$d(pT)_6$	8.9	277	0.95	250	-0.69
$d(pT)_7$	8.8	277	1.10	249	-0.76
$d(pT)_8$	8.2	277	1.09	250	-0.68
$d(pT)_9$	8.4	277	1.11	250	-0.71
$d(pT)_{10}$	9.2 (8.7) ^a	277	1.14	250	-0.67
$d(pT)_{11}$	9.2 (8.5) ^a	277	1.14	250	-0.69
$dpTpA$	11.5 ^b	273	0.74	252	-1.44
$dpTpApTpA$	10.8 ^b	273	0.78	250	-1.20
$dpApApTpT$	11.3 ^b	270	1.07	250	-1.28

$\epsilon_{\max}$ values are based on phosphorus analysis related to the monomer.

^a Values from Cassani and Bollum (2);

^b Values determined by phosphorus analysis and from hyperchromic changes on venom diesterase digestion.

The CD band near 280 nm in $d(pA)_6$ and poly dA has been assigned as $n \rightarrow \pi^*$ transition (21). Despite some controversial conclusions (33,34) more recent data show the importance of the rotational strength of an $n \rightarrow \pi^*$ band at longer wavelength (34,35). According to Bush and Scheraga interior residues of the deoxy oligomer exhibit the 280 nm band (due to the shift of the maximum towards 276 nm, Table 2), but not the dimer. In comparing the conform-

Fig. 4 Variation of CD maxima with increasing chain length of oligo dT and oligo dA.

Fig. 5 Plots of CD maxima as a function of chain length in $d(pT)_n$ and $d(pA)_n$ expressed in terms of $(N-1)/N$ according to equation (1).

ational effects in the series of $d(pT)_n$ and $d(pA)_n$ assignments of the $n \rightarrow \pi^*$ transition are yet difficult. On the other hand a possible contribution of the sugar-base interaction in the CD of the present data enable us to interpret some effects of the sugar-base torsional angle in the deoxy oligomers. On the basis of a recent method proposed by Bush and Brahms (34) an approach to separate CD data due to sugar-base effects from base-base interaction can be made using equation (1), where

$$[\theta](N)/N = \Delta[\theta](S-B) + (N-1)/N [\theta](nn) \quad (1)$$

$[\theta](N)/N$ is CD amplitude per residue, $\Delta[\theta](S-B)$ represents sugar-base interaction and $\Delta[\theta](nn)$ the nearest neighbour interaction between bases, which both are assumed to be constant for a discrete oligomer series (34). The corresponding plots of the CD maxima of oligo(dA)'s and oligo(dT)'s show straight lines in accordance with those obtained by Bush and Brahms (34) for some ribo oligomers. Extrapolation of the positive and negative CD maximum to the $[\theta]$ value of the monomer at $(N-1)/N = 0$ indicate CD bands of $[\theta] \sim 3 \times 10^3$ at 277 nm and $[\theta] \sim -1 \times 10^3$ at 250 nm. For the free deoxy monomer $[\theta]_{274}$ is approximately 4×10^3 and in the region 240 to 250 $[\theta]$ is 2 to 4×10^3 (3). This shows that the torsional angles of the monomer are different in the oligomer due to a variation in sugar-base interaction. The lack of hyperchromicity on heating of $d(pT)_9$ (Fig. 6) and poly dT (2,6) indicates the absence of base-base interactions. Thus the chain length dependence of CD in the oligo dT series we attribute entirely to sugar-base effects. Regarding the Cotton effect associated with the conformation of the monomer unit (36) the orientation of the sugar relative to the transition dipole moment of the optical transition of the base is important. Different from the conformation of linear oligo(dT), cyclic oligo(dT)'s may adopt stacked conformation (57). A very different type of CD dependence of the oligo(dA) series is seen in Fig. 5 for the positive maximum, while the negative CD band shows no distinct dependence (Table 2). The CD extrapolated for the monomer has a large amplitude of $[\theta] 1.4 \times 10^4$ at this wavelength when the torsional angle is those of the oligomer. This is strikingly different from the free deoxy monomer which has a maximum value of $[\theta] = -0.2 \times 10^4$ at 264 nm (3). Thus it appears that a large

Fig. 6 Heating curves of deoxy oligomers in 0.02 M NaCl. 1, $d(pA)_7$; 2, $d(pT)_9$; 3, $dpTpApTpA$; 4, $dpApApTpT$; 5, $d(pA)_2$.

Fig. 7 CD spectra of $d(pA)_7$ and $d(pT)_9$ in the presence of high concentrations of various salts. 1: 0.02 M NaCl; 2: 5 M NaCl; 3: 4.8 M Na acetate; 3a: 7.5 M LiCl; 4: 7.2 M $NaClO_4$; 4a: 5 M $NaClO_4$; 5: 5.4 M Na trifluoroacetate; 6: $d(pT)_2$ in 0.02 M NaCl.

sugar-base effect is involved in addition to the base-base interactions already demonstrated by other investigators (6,21,22). In Fig. 6 as an example the thermal melting of $d(pA)_7$ indicates substantial base stacking in contrast to $d(pT)_9$, which exhibits almost no hyperchromic change.

Effect of ionic strength: The dependence of torsional effects on chain length should be sensitive to certain variations of the environment. Cantor et al. (3) reported no influence of the ionic strength on the CD of $dplpTpTp$ up to the highest salt concentration of 0.1 M $NaClO_4$ employed in their studies. We recorded CD spectra of $d(pT)_9$ and $d(pA)_7$ in the presence of different concentrated salts. The results in Fig. 7 show that 5 M $NaCl$ and 7.5 M $LiCl$ do not substantially affect the CD of $d(pA)_7$. A significant depression of the positive CD maximum appears, however, at 5 M $NaClO_4$. Similarly $d(pT)_9$ shows almost no changes from 0.02 M to 5 M $NaCl$ (curve 1 and 2), a slight CD decrease at 4.8 M $NaAc$ (curve 3), but a large lowering of the positive and negative CD amplitude in concentrated perchlorate or trifluoroacetate solution (curves 4a and 5). In these two cases the CD spectra coincide with those of $d(pT)_2$ indicating a variation of base-sugar interaction due to the action of the anion. The CD decreases monotonously with increasing $NaClO_4$ concentration (Table 3). Strong dehydrating salts such as 7.5 M

Table 3 Variation of CD maxima of $d(pT)_9$ with increasing $NaClO_4$ concentration

$NaClO_4$ M	0.02	1.35	2.70	5.00	7.20	7.5 M $LiCl$
$[\theta]_{276-77} \cdot 10^{-4}$	1.10	0.98	0.90	0.87	0.79	0.94
$[\theta]_{250} \cdot 10^{-4}$	-0.75	-0.58	-	-0.44	-0.42	-0.61

$LiCl$ also reduce the CD amplitude, but 7.2 M $NaClO_4$ is most effective. Contrary to $d(pT)_9$ the CD amplitude of $d(pA)_7$ further decreases and is not displaced towards the CD of the dimer. This could have reasons in the variation of the base stacking. The same is valid for oligo (dT) series with shorter chain length, e.g. for the deoxy trimer (Table 4). Lowering of the negative and positive CD amplitudes are caused in 5 M $NaClO_4$ and on deprotonation (pH 11) of thymine residues, but not in 5 M $NaCl$ solution. CD_{max} values reach that of the dimer (Table 4). Our results also testify the validity of the method of Bush and Brahms (34).

Table 4 Comparison of CD maxima of $d(pT)_3$ and $d(pA)_3$ in concentrated salt solution

Oligomer	Salt	$[\theta]_{\max_1} \cdot 10^{-4}$	$[\theta]_{\max_2} \cdot 10^{-4}$
$d(pT)_2$	0.01 M NaCl	0.72	-0.50
$d(pT)_3$	0.01 M NaCl	0.93	-0.64
	0.01 M NaCl, pH 11	0.73 ^a	-0.53 ^b
	5 M NaCl	1.07	-0.85
	5 M NaClO ₄	0.73 ^c	-0.42
$d(pA)_3$	0.01 M NaCl	0.68	-1.84
	5 M NaCl	0.69	-1.90
	5 M NaClO ₄	0.58	-1.54

^a CD_{max₁} 270 nm, ^b CD_{max₂} 233 nm, ^c CD_{max₁} 273 nm.

The observed effects show that the torsional angles in the deoxy oligomer may be affected by salts known as denaturing agents or strong salting in agents, but no substantial changes in the sugar-base interaction occur through shielding of phosphate sites on decreasing the water activity (5 M NaCl, activity coefficient of water $f_{H_2O} = 0.976$). Greater lowering of the water activity (Table 3, 7.5 M LiCl, $f_{H_2O} = 0.817$) may also produce a small effect. On the basis of available data it is possible to suggest that anions such as perchlorate may induce the observed CD changes by an influence on the base or on the deoxyribose group (37). The results also account for the interpretation of changes in torsional angles as observed in salt effects on CD of DNA (38-40).

$d(pTpApTpA)$ and $d(pApApTpT)$: The CD spectra of two sequence isomers containing adenine and thymine are shown in Fig. 8. There is no significant difference in the CD of $dpTpA$ and the alternating tetramer while $dpApApTpT$ shows larger amplitudes of the positive CD bands. The CD maximum at 270 nm and the intercept at 260 nm closely resembles that of $dpApA$ (Table 2). The CD maximum of the alternating isomer is intermediate between the oligo(dT) and oligo(dA) series. Thus the result suggests a dominating role in the conformation of adjacent adenines in the tetramer, probably due to higher degree of base stacking [see

Fig. 8 CD spectra of d(pApApTpT), d(pTpApTpA) and d(pTpA) in 0.02 M NaCl.

also hyperchromicity in thermal melting (Fig. 6)] which can affect the sugar-base interaction in d(pT)₂ adjacent to d(pA)₂.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. H. Venner for generous support of this work and Dr. G. Luck for helpful discussion. We thank Miss Ch. Radtke for valuable technical assistance and Dr. H. Reinert for enzymatic detection of deoxy oligomers.

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