Oligonucleotide Interactions. II. Differences in Base Stacking in Linear and Cyclic Deoxythymidine Oligonucleotides*

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ABSTRACT: The circular dichroism, ultraviolet absorption, and nuclear magnetic resonance of linear and cyclic thymidine di- and trinucleotides have been studied. In aqueous solution the long-wavelength Cotton effects of the cyclic dinucleotide are opposite in sign to the corresponding bands of the linear compound. Thus cyclic thymidine dinucleotide must have a stacked conformation which is quite different from the base-stacking geometry of any other known oligonucleotide. Nuclear magnetic resonance spectra of the cyclic dimer suggests that the two bases are magnetically equivalent. It is possible to construct a plausible model of the conformation of the cyclic dimer which explains both the optical and magnetic resonance data.

In methanol solution the linear oligothymidylates are unstacked and show optical properties very similar to those of thymidylic acid. Methanol causes substantial changes in the conformation of cyclic thymidine dinucleotide while, in contrast, there is virtually no effect on the cyclic trinucleotide. The vast differences in the base stacking of linear and cyclic oligonucleotides may have implications for the possible structures of loops and hairpins in transfer and ribosomal ribonucleic acids.

There have been a large number of recent studies on the conformation of single-stranded oligonucleotides (Warshaw and Tinoco, 1965; Cantor and Tinoco, 1965; Bush and Brahms, 1967; Brahms *et al.*, 1967). These have led to the general conclusion that at room temperature in neutral aqueous buffer the bases in oligonucleotides are oriented in a vertically stacked conformation. Calculations of the optical properties of oligoribonucleotides suggest that the configuration of the bases is a right-hand helix fairly similar to the structure of a single-stranded DNA (Bush and Tinoco, 1967). The information learned from studies on oligonucleotides is of great assistance in attempts to understand the interactions which stabilize such partially ordered RNA structures as tRNA and 5S rRNA. The current picture of the molecular conformation of both of these naturally occurring biopolymers is a structure consisting of single- and double-stranded helical sections and oligonucleotide loops or hairpins joining two double-helical regions (Madison, 1968). Single-stranded oligonucleotides are good models for the single-stranded regions of a polynucleotide (Cantor *et al.*, 1966). Double-stranded oligonucleotide complexes (Jaskunas *et al.*, 1968) or oligomer–polymer complexes (Cantor and Chin, 1968) can serve as useful models for the conformation of double-helical regions in an RNA. What is needed is a system which will permit the conformation of bases on loops to be studied. Fuller and Hodgson (1967) have proposed that the anticodon loop of a tRNA, which contains seven nucleotide residues, can exist in a conformation which will help to favor interaction of the anticodon with the complementary sequence on the mRNA. In their model five of the seven residues of the loop retain the usual right-hand helical-stacked conformation. To test such an hypothesis it would be useful to have detailed information on the effects of the constraints of loop formation on the conformation of the bases.

Two general types of model compounds exist which can permit this problem to be explored. The first is an oligonucleotide which can form a loop by the construction of intramolecular base pairs. Such loops have been prepared recently from alternating d(A-T) sequences (Scheffler *et al.*, 1968). In all likelihood these loops will have even numbers of residues. Optical measurements could afford information about the conformation of bases on the loop if the optical properties of the double-strand region could be sorted out from the contribution due to the loop. Unfortunately, as work on oligo d(A-T's) has shown, any compound that can form such a loop can also dimerize to form a double strand. An additional complication arises from the fact that a given oligonucleotide sequence may have more than one possible conformation containing different size loops or different numbers of loops. This will complicate attempts to interpret physical measurements on such a system. A second model for a loop is a single-strand covalent oligonucleotide circle. It is this type of system which we shall discuss in the present paper. Here both of the complications discussed above are no longer present. Cyclic oligonucleotides are not completely ideal models for the loops in a tRNA because the constraints due to circle formation are not precisely the same as those due to loop formation. However it seems likely that the types of effects found for oligonucleotide circles are indicative of the phenomena which may also occur in loops.

Materials and Methods

Preparation of Linear and Cyclic Oligothymidines. Thymidine 5′-monophosphate was polymerized using dicyclohexylcarbodiimide in pyridine essentially according to the method of Khorana and Conners (1966). Since cyclic oligonucleotides were the desired product no acetylated thymidine 5′-monophosphate was added to the reaction mixture. Strict precau-

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tions were taken to exclude water from the reaction. The resulting mixture of linear and cyclic oligothymidylic acids was separated on a 4.0 × 60 cm DEAE-cellulose column using an ammonium bicarbonate gradient as described by Khorana and Conners (1966). The resulting elution pattern was so similar to that reported in the literature that the oligonucleotides could be identified by their elution position. The tubes corresponding to each oligonucleotide were pooled and the ammonium bicarbonate was removed by repeated lyophilization.

The structures of cyclic and linear oligothymidylates can be drawn schematically as

structure	pTpT	$p\overset{T}{\underset{T}{}}p$	pTpTpT	$p\,T\,p\,\underset{TpT}{}$
abbreviation	dpTpT	$\widehat{dpTpT}$	dpTpTpT	$\widehat{dpTpTpT}$

Optical Measurements. Circular dichroism measurements were performed mostly at 26° using the circular dichroism attachment to the Cary 60 spectropolarimeter. A 3-sec time constant and a slit program affording a 1-mμ band pass were used for all experiments. Ultraviolet spectra were measured using a Cary 15 spectrophotometer. All results are expressed as residue extinction or residue ellipticity. An extinction coefficient of 9200 per residue at 266 mμ was used for dpTpT (Naylor and Gilham, 1966). The extinction coefficient of dpTpTpT was assumed to be 9150 at 266 mμ, the average of the measured values for $d(pT)_2$ and $d(pT)_4$. It was also assumed that the extinction coefficients of linear and cyclic oligonucleotides were identical. This would not seem to introduce any serious error since the amount of hypochromism found for linear oligothymidylates is extremely small (4% for dpTpT). The solvents used for optical measurements were either 0.1 M $NaClO_4$–0.01 M sodium phosphate (pH 7.2) or reagent grade methanol. Temperature-dependent measurements were performed as described previously (Cantor, 1968). Data reduction was performed as described previously (Cantor and Tinoco, 1965). All measurements were made at concentrations approximately 10^{-4} M in nucleotide residues.

Nuclear Magnetic Resonance. These measurements were performed in D_2O solution using a Varian HA100 spectrometer with a Varian computer of average transients. Depending upon the band, from 15 to 100 scans were used. Aliquots of a D_2O solution of purine were added to the samples of $\widehat{dpTpT}$ to determine the effects of purine binding. All results are expressed in hertz relative to the H_2O lock signal.

Results

Optical Studies. It is well known that optical activity is extremely sensitive to changes in the conformation of oligo- and polynucleotides. Previous studies on the optical rotatory dispersion of dinucleoside phosphate and trinucleoside diphosphates have shown that for most sequences the optical properties of an oligonucleotide are quite different from the sum of the properties of the monomers (Cantor and Tinoco, 1965; Warshaw and Tinoco, 1965). These differences are the result of base stacking. Exactly equivalent differences can be expected for circular dichroism data. The circular dichroism spectra of the cyclic and linear thymidine dinucleotides, $\widehat{dpTpT}$ and dpTpT, and thymidylic acid in aqueous medium at neutral pH are shown in Figure 1. A summary of all of the optical results discussed in this paper is given in Table I. The experimental results for the linear dimer and monomer are in excellent agreement with other values measured in this laboratory for samples of dpTpT prepared from acid degradation of DNA (C. R. Cantor and M. M. Warshaw, unpublished results). There are several significant features in the data presented in Figure 1. The circular dichroism of the monomer shows a strong positive band at 273 mμ which is close to the position of the absorption maximum. This has been assigned to a π–π^* transition analogous to the B_{2u} band of benzene by Miles *et al.* (1967). The shorter wavelength circular dichroism bands visible in the monomer spectrum are thought to be analogous to the B_{1u} and E_{1u} benzene transitions. The circular dichroism of the linear dimer is significantly different from that of the monomer. The two longest wavelength bands are shifted to the red and increased in intensity. There is no corresponding shift of similar magnitude visible in the absorption spectrum of the monomer. Thus it seems likely that the circular dichroism shift and intensification is due to the presence of a stacked conformation in dpTpT. The optical properties of the dimer arise, in part, from interaction of the B_{2u} transitions of the two monomers. There is already available ample experimental evidence to support the idea that the bases in dpTpT or dTpT are stacked (Wacker and Lodemann, 1965; Ts'o *et al.*, 1969).

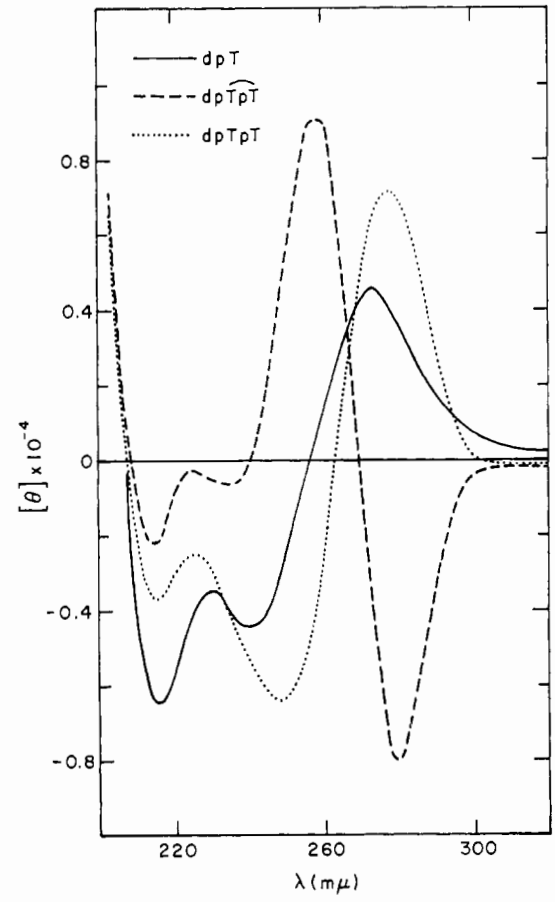

FIGURE 1: Circular dichroism of linear and cyclic thymidine dinucleotides and thymidylic acid in neutral aqueous buffer at 26°. Units are molar ellipticity per residue.

TABLE I: Circular Dichroism of Linear and Cyclic Thymidine Oligonucleotides.

Sample	Solvent	Cotton Effects in Order of Decreasing Wavelength							
		λ (mμ)	[θ]	λ (mμ)	[θ]	λ (mμ)	[θ]	λ (mμ)	[θ]
dpT	Aqueous buffer	273	+4,500	241	−4,400	217.5	−6,400		
dpTpT	Aqueous buffer	278	+7,100	247.5	−6,400	215.5	−3,700		
$\overset{\frown}{\text{dpTpT}}$	Aqueous buffer	280	−8,000	258.5	+9,000	235	−600	215	−2,200
dpTpTpT	Aqueous buffer	277.5	+9,900	249	−7,700	214	−2,100		
$\overset{\frown}{\text{dpTpTpT}}$	Aqueous buffer	291	+350	260.5	+5,200	223	+9,600	207	−9,200
dpT	Methanol	273	+7,900	242	−6,100	218	−8,900		
dpTpT	Methanol	273	+7,900	240	−4,200	217.5	−6,400		
$\overset{\frown}{\text{dpTpT}}$	Methanol	262.5	+11,400	236.5	−1,500	217.5	−1,900		
dpTpTpT	Methanol	273	+6,600	241	−4,200	217.5	−7,200		
$\overset{\frown}{\text{dpTpTpT}}$	Methanol			261	+5,600	223.5	+13,800	210	−6,600

More striking is the remarkable difference between the circular dichroism of the linear and cyclic thymidine dinucleotides. The results at long wavelength can be described simply as a change in sign of the circular dichroism bands arising from the B_{2u} transition of thymidine. This cannot be due to the presence of a double charged phosphate on the linear dinucleotide since other results in this laboratory have shown that there is virtually no effect at all of terminal phosphates on the circular dichroism of deoxypyrimidine oligonucleotides (C. R. Cantor and M. M. Warshaw, unpublished results). The reversal in sign of the circular dichroism must be due to a different ordered conformation of the bases in the cyclic dimer. It is worth noting that all linear ribo- and deoxypyrimidine oligonucleotides for which circular dichroism or optical rotatory dispersion measurements are available show a positive long-wavelength Cotton effect. This is also true for all dinucleotides containing one thymidine or uridine and one purine. All of these linear dinucleotides are believed to have a right-handed helical conformation. Thus the orientation of the bases in $\overset{\frown}{\text{dpTpT}}$ must be quite different indeed. The unlikely possibility that the anomalous circular dichroism of the cyclic dimer arises from distortion of the thymidine rings similar to the effects found for paracyclophanes can be discounted by the fact that the ultraviolet absorption spectra of $\overset{\frown}{\text{dpTpT}}$ and dpTpT are extremely similar. The spectra of these two compounds are shown in Figure 2. No large spectral shifts between linear and cyclic compound are in evidence, nor is there any change in band shape. Both of these effects are noticeable in paracyclophanes (Cram *et al.*, 1954).

The optical results presented above suggest that the bases in cyclic dithymidylic acid have an ordered structure. It was of interest to see if this structure was rigid and arose strictly from the constraints of forming a circle on the phosphodiester backbone.

The simplest way to test this is to study the circular dichroism as a function of temperature (Davis and Tinoco, 1968). Two wavelengths, 245 and 275 mμ, were chosen to attempt to maximize any observed changes. The temperature dependence of the circular dichroism of the monomer and the two dimers at these wavelengths is shown in Figures 3 and 4. First consider the results for the linear dimer and the monomer. The circular dichroism of dpT is temperature sensitive at 275 mμ but not at 245 mμ. The origin of this temperature dependence is not well understood. At both wavelengths, however, the circular dichroism of the linear dimer changes in such a way that by 80° it has approached fairly near to that of the monomer. This is evidence that at high temperatures the bases of the dimer have become unstacked. Similar results have been observed for a whole series of linear ribonucleoside diphosphates (Davis and Tinoco, 1968) and for several deoxydinucleoside phosphates (M. M. Warshaw and C. R. Cantor, unpublished results). Contrast these results with data for the cyclic dimer presented in Figures 3 and 4. Here, while the circular dichroism is temperature dependent, there is no indication that the optical properties are converging to those of the monomer as the temperature is raised. Thus although the conformation of $\overset{\frown}{\text{dpTpT}}$ can be altered by changes in temperature, the bases cannot, even at high temperature, assume orientations sufficiently distant and uncorrelated to eliminate strong optical interactions. Experiments of Davis (1966) have shown that alcohols are effective denaturing agents for the stacked conformation of oligoribonucleotides. If base stacking stabilizes the conformation of cyclic dithymidylic acid the circular dichroism should be very sensitive to the solvent. Circular dichroism spectra of dpTpT, dpT, and $\overset{\frown}{\text{dpTpT}}$ in methanol are shown in Figure 5. In this solvent the circular dichroism spectra of linear and cyclic dimers are fairly similar in shape and have the same sign for all bands. This is just the opposite of what was found in water. The spectrum of the linear dimer in methanol is virtually the same as the monomer. The cyclic dimer has drastically different circular dichroism in methanol than in water. In neither solvent, however, does it resemble the spectrum of either the monomer or the linear dimer. This supports the conclusion drawn earlier from studies of the temperature dependence that the orientation of the bases in $\overset{\frown}{\text{dpTpT}}$ is sensitive to the environment. The conformation of this compound cannot, under the denaturing conditions we

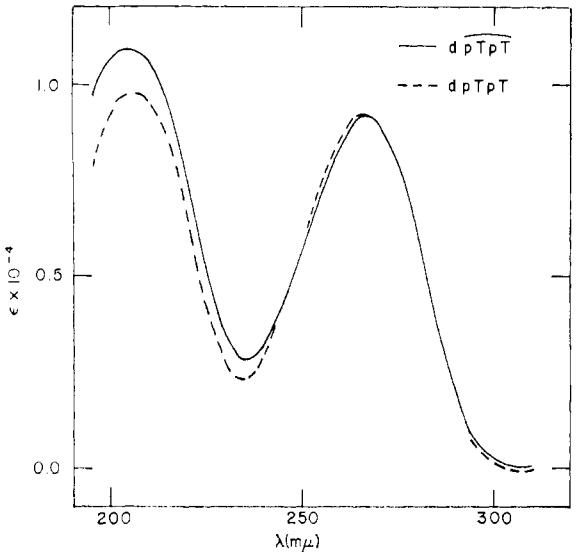

FIGURE 2: Ultraviolet absorption spectrum of linear and cyclic thymidine dinucleotides in aqueous solution. Units are molar extinction per residue. The extinction coefficients of dpTpT and dpTpT have been assumed to be the same at 266 mμ.

have used, become sufficiently randomized to eliminate strong optical interactions between the bases.

Comparative studies on trinucleotides also show striking differences between the conformation of linear and cyclic compounds. The circular dichroism spectra of dpTpTpT and dpTpTpT are given in Figure 6. The linear trimer has a circular dichroism quite similar to that of the linear dimer. The spectrum of the former can be accurately estimated from the experimentally observed linear dimer and monomer spectra

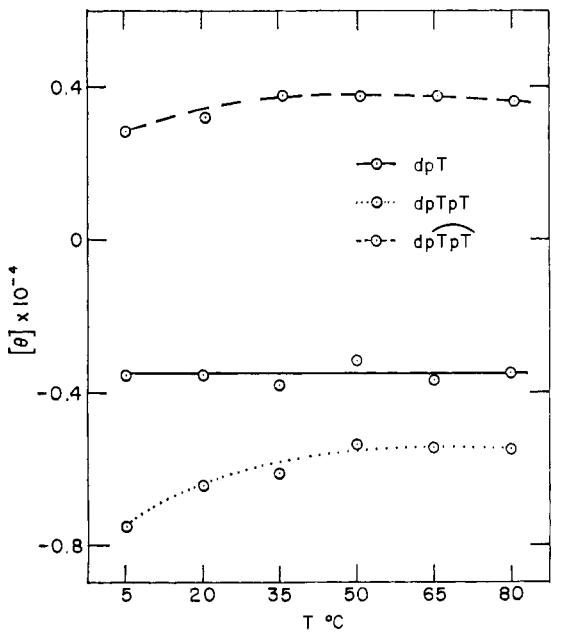

FIGURE 3: Temperature dependence of the circular dichroism of thymidylic acid and linear and cyclic thymidine dinucleotides in aqueous solution at 245 mμ.

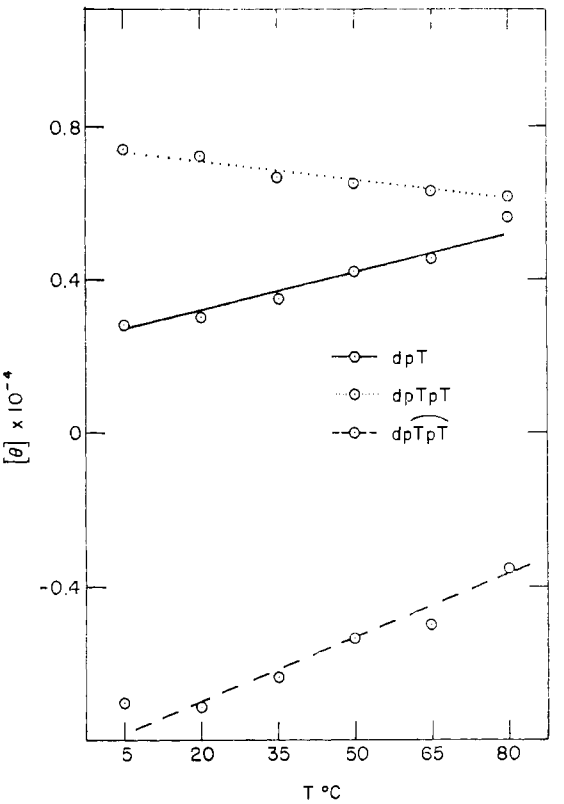

FIGURE 4: Temperature dependence of the circular dichroism of thymidylic acid and linear and cyclic thymidine dinuclotides in aqueous solution at 275 mμ.

using nearest-neighbor methods (Cantor and Tinoco, 1965). This implies that the conformation of neighboring bases in linear dimers and trimers is quite similar. In contrast the circular dichroism of the cyclic trimer resembles neither that of the linear trimer nor that of the cyclic dimer. At long wavelengths the circular dichroism of dpTpTpT shows a weak single Cotton effect centered at 260 mμ very reminiscent of the monomer spectrum but shifted to the blue. An additional very weak circular dichroism band appears at 290 mμ. The intensity of this band is just barely large enough to permit detection. That some sort of ordered conformation of bases exists in the cyclic trimer is shown by a new intense double Cotton effect at 215.5 mμ which is absent in all other known oligomers containing thymidine. The circular dichroism of the linear and cyclic trinucleotides was measured in methanol to assess the relative importance of base-stacking interactions and backbone constraints in stabilizing the molecular conformation. These results are shown in Figure 7. In methanol the circular dichroism of dpTpTpT closely resembles the monomer in magnitude and shape. Thus the base-stacking interactions have been weakened to the point where the conformation is essentially random. In contrast, the circular dichroism of the cyclic trimer in methanol is virtually the same as that in aqueous solution. Apparently the conformation of this compound is determined predominantly by the backbone. This makes it an ideal candidate for attempts to calculate, *a priori*, a most stable conformation since solvent effects can probably be ignored.

Nuclear Magnetic Resonance Studies. There is a large amount of evidence that stacking of oligonucleotide bases pro-

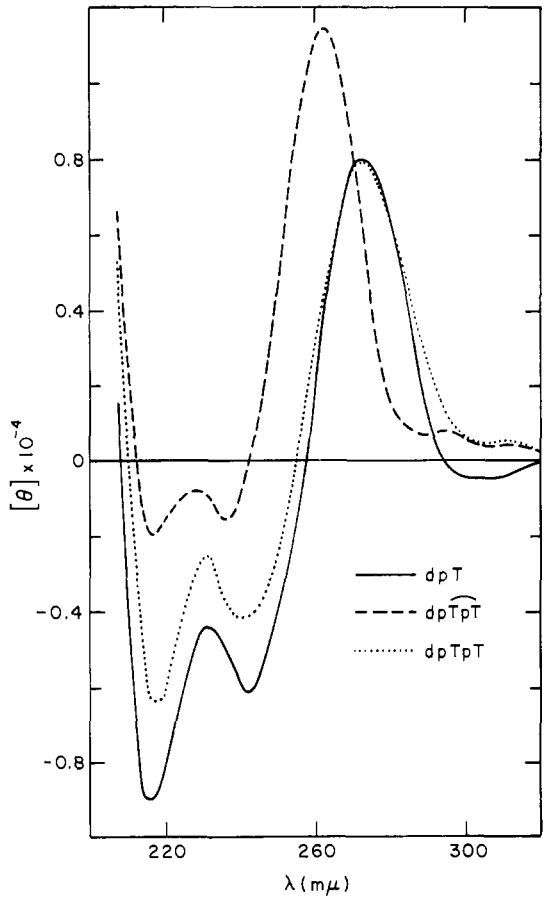

FIGURE 5: Comparison of the circular dichroism of linear and cyclic thymidine dinucleotides and thymidylic acid in methanol.

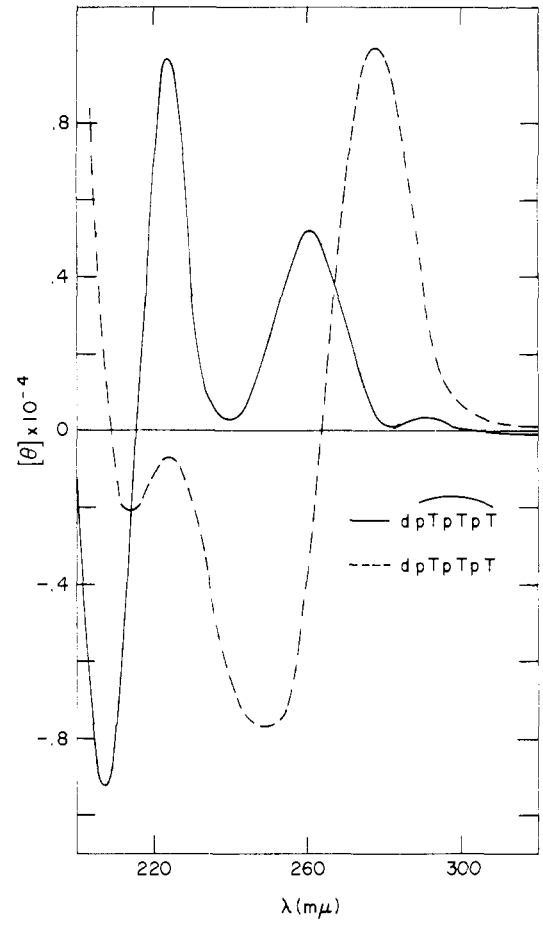

FIGURE 6: Circular dichroism of linear and cyclic thymidine trinucleotides in neutral aqueous buffer at 26°.

foundly influences their nuclear magnetic resonance spectrum. This is primarily due to the effect of the ring current of one base on the chemical shifts of the aromatic protons of neighboring bases. There are also effects due to the diamagnetic shifts of nearby phosphate residues (Schweizer *et al.*, 1968). Nuclear magnetic resonance studies have provided some of the most conclusive evidence for base stacking of free nucleosides in concentrated aqueous solution (Broom *et al.*, 1967) and bases in ribo- and deoxyribodinucleoside phosphates (Ts'o *et al.*, 1969; McDonald *et al.*, 1967). In linear homodinucleotides the two bases are known to be magnetically inequivalent. This is due to the asymmetric helical structure of the stacked array. The ring current expected for thymine will be smaller than that of the other, more aromatic bases. Chan *et al.* (1966) have studied the nuclear magnetic resonance of dTpT in D_2O using a 60-MHz spectrometer. They observed that the spectra of H_6 and methyl protons of dTpT and dT were quite similar except for small shifts in the positions of the methyl resonance. The only evidence of possible magnetic inequivalence of the ring protons was a noticeable broadening of the H_6 proton resonance of the dimer. This result suggested that the two bases might be almost magnetically indistinguishable. When purine was added to a solution of dTpT large upfield shifts were observed, indicative of purine stacking in between the two thymine bases. The methyl and H_6 resonances were split into unresolved multiplets separated by about 5 Hz. This was interpreted as an asymmetric intercalation of purine between the bases.

We have performed similar experiments with linear and cyclic dithymidylic acid. Spectra were obtained at 100 MHz to improve the resolution. This enabled a clear magnetic inequivalence of the two bases in dpTpT to be observed. In the linear dimer the H_6 resonance appears as two multiplets split by 6 Hz. Ts'o *et al.* observed a similar smaller split of the H_6 resonance of dTpT at 100 MHz (1969). They attribute the larger splitting in dpTpT to specific deshielding by the 5′-phosphate. No splitting is observed for the methyl protons; some evidence for asymmetry appears in the H_1' triplet. These results are shown in Figure 8 along with the nmr spectrum of cyclic dithymidylic acid. There is no evidence for inequivalence of the two bases in $\overset{\frown}{\text{dpTpT}}$. The methyl resonance appears as a sharp doublet, split by 1.2 Hz; the H_6 proton shows a poorly resolved narrow quadruplet; and the H_1' proton appears as a triplet. This last observation also shows that the two sugars of $\overset{\frown}{\text{dpTpT}}$ are equivalent since in dTpT Chan *et al.* could observe a H_1' resonance which was a composite of two triplets (1966). Purine was added to our D_2O solution of the cyclic dimer to see if any asymmetric mode of binding was possible. Upfield shifts in the positions of methyl, H_6, and H_1' resonances were observed which were quite analogous to those

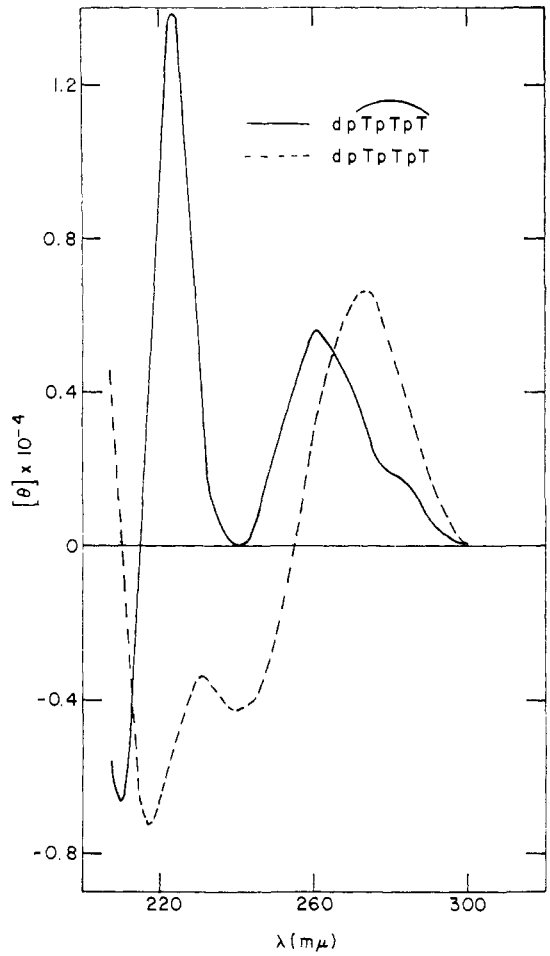

FIGURE 7: Circular dichroism of linear and cyclic thymidine trinucleotides in methanol.

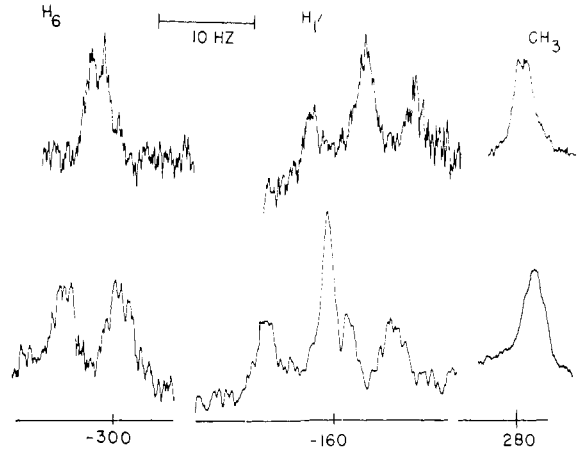

FIGURE 8: Nuclear magnetic resonance spectra of $\overset{\frown}{dpTpT}$ (upper set of curves) and dpTpT (lower set of curves) in D_2O. Chemical shifts are measured in Hz relative to the H_2O lock signal. The spectrometer gain is different for each peak because different numbers of computer of average transients scans were used.

found for dTpT (Chan *et al.*, 1966), but no additional splitting could be noticed. Thus it seems that the binding of purine is either symmetric or proceeds on a time scale sufficiently fast to obliterate any differences in the effect on the two thymine bases.

The implications of the optical and magnetic resonance studies described above can be summarized simply. The two bases of $\overset{\frown}{dpTpT}$ cannot be related by a plane or point of symmetry since this would lead to no optical activity in excess of that found for the individual mononucleosides. However, the nuclear magnetic resonance results suggest that they are related by an axis. This would be a C_2 axis.

Possible Structure of Cyclic Thymidine Oligonucleotides. The results discussed above strongly argue for a structure of $\overset{\frown}{dpTpT}$ and $\overset{\frown}{dpTpTpT}$ that is quite different from the corresponding linear oligomers. From the circular dichroism and nuclear magnetic resonance data, it is possible to construct a plausible model for the conformation of the bases in these two oligomers. The lowest energy circular dichroism bands of thymidine oligomers arise from a π–π^* transition in thymidine. The direction of the transition moment of this band is shown in Figure 9. It was determined through single crystal studies by Stewart and Davidson (1964). The long-wavelength circular dichroism of linear and cyclic thymidine dinucleotides is approximately conservative. This suggests that the major term which contributes to the circular dichroism in this region is an exciton coupling between the two chromophores (Tinoco, 1968). Using such an assumption Bush and Tinoco have calculated the circular dichroism of UpU by approximating the transition moment direction of U to be in the same direction as T (1967). They find quite reasonable agreement with experiment. Let us first outline this calculation for a homodinucleotide. A single absorption band in the monomeric chromophore will be split into two circular dichroism bands of opposite signs in a dimer. The rotational strengths of these bands are given by (Tinoco, 1963)

$$R_{oA\pm} = \mp \nu_a \mathbf{R}_{12} \cdot \boldsymbol{\mu}_{1oa} \times \boldsymbol{\mu}_{2oa}$$

where ν_a is the frequency of the electronic transition in the monomer, $\boldsymbol{\mu}_{1oa}$ and $\boldsymbol{\mu}_{2oa}$ are, respectively, the electric transition moments of chromophores 1 and 2, and $\mathbf{R}_{12}$ is the distance between the two chromophores. The two bands will be split by a

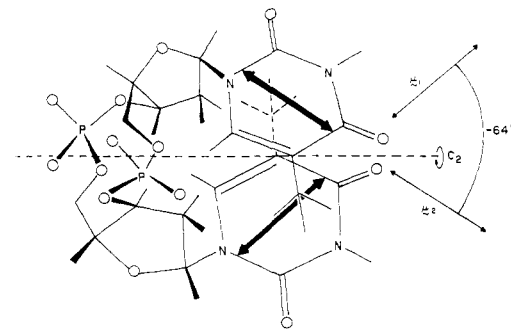

FIGURE 9: Schematic drawing of a possible model for the conformation of $\overset{\frown}{dpTpT}$. The arrangement of atoms in the phosphate–ribose backbone has been distorted to allow all of the atoms to be visible. The thick double arrows in the planes of the bases show the known orientation of the transition moment of the 266-mμ absorption band of thymine.

frequency $\nu_{a\pm} = \nu_a \pm {}^1/_2 V_{12}$. V_{12} arises from the electronic interaction between the chromophores. This can be approximated by a dipole–dipole term

$$V_{12} = \frac{1}{|\mathbf{R}_{12}|^3}(\boldsymbol{\mu}_{1oa} \cdot \boldsymbol{\mu}_{2oa} - 3\boldsymbol{\mu}(_{1oa} \cdot \mathbf{R}_{12})(\boldsymbol{\mu}_{2oa} \cdot \mathbf{R}_{12})/|\mathbf{R}_{12}|^2$$

If the bases are assumed to be in parallel planes, the above expressions simplify as follows

$$R_{oA\pm} = \mp \nu_a |\mathbf{R}_{12}| |\boldsymbol{\mu}_{1oa}| |\boldsymbol{\mu}_{2oa}| \sin \theta_{12}$$

$$V_{12} = \frac{1}{|\mathbf{R}_{12}|^3} |\boldsymbol{\mu}_{1oa}| |\boldsymbol{\mu}_{2oa}| \cos \theta_{12}$$

where θ_{12} is the angle between the transition moments of the two bases. In linear pTpT the configuration of the bases probably resembles that of the B form of DNA. This implies that θ_{12} is approximately 36°. Thus V_{12} is positive, ν_{a-} is the long-wavelength band, and R_{oA-} is positive. This leads to the prediction that the sign of the long-wavelength circular dichroism band of the linear dimer is positive, in agreement with experiment. Since a negative long-wavelength circular dichroism band was observed for dpTpT we must search for a possible conformation with θ between 90 and 180° or between 0 and −90°. At the same time the nuclear magnetic resonance results suggest that in this conformation the two bases are related by a C_2 axis of symmetry. Various conformations were tested by using Corey–Pauling–Koltun models. There is a surprising lack of plausible stacked configurations of bases which can be constructed for dpTpT with space-filling models. The one conformation which we have been able to find which fits the two requirements stated above is shown in Figure 9. The configuration of the backbone of the dimer has been distorted in the figure to allow all of the atoms to be visible. The arrangement of bases in the structure in Figure 9 is a "left-handed helix" optically since the angle between the transition moment of the two thymidines is −64°. Since a C_2 axis is present the structure is, of course, not actually a helix. If this structure proves to be correct it will be the first time that an oligonucleotide has been found in a conformation other than one closely related to the right-handed helical DNA.

It should be noted that changes in the sign of circular dichroism bands can also result simply when the conformation of the bases relative to the sugar changes from *anti* to *syn* (Miles *et al.*, 1969). Although this possibility cannot completely be ruled out by the evidence presented here, it seems unlikely for the following reasons. Miles *et al.* (1969) have shown that the circular dichroism change accompanied by an *anti* to *syn* transformation is essentially just a change in sign. The circular dichroism of dpTpT shows bands which do not coincide at all well with positions of monomer circular dichroism bands. Thus much of the circular dichroism must arise from interaction between the two thymine chromophores. Suppose, for example, that the backbone of the model shown in Figure 9 is kept constant while the bases are rotated to the *syn* conformation. The transition moments of the two bases will now be parallel. This will lead to no optical activity except for the monomer spectrum reversed in sign. This is contrary to our experimental findings.

The major question which remains at present is the conformation of dpTpT in methanol. Manipulations of Corey–Pauling–Koltun models show clearly that there are a number of plausible unstacked or partially unstacked conformations which can be reached without breaking covalent bonds. Additional physical and chemical studies are in progress to obtain more detailed information about the conformation of dpTpT and larger cyclic oligonucleotides.

Studies with molecular models show that there are a fairly large number of possible stacked and partially stacked configurations for the cyclic trinucleotide dpTpTpT. One possible structure which would explain the near-ultraviolet circular dichroism of this compound is a linear stack in which the angle between the transition moments of bases 1 and 2 is opposite in sign to the angle between bases 2 and 3. This would lead to a cancelling of the two nearest-neighbor coupled oscillator terms. It is not clear from the model how to account for the intense short-wavelength band observed in the cyclic trimer.

Discussion

The conformation of cyclic di- and trinucleotides appears to be strikingly different from that of the corresponding linear compounds. There are several important implications of this result. Since cyclic oligomers can still form stacked conformations, it is conceivable that linear oligonucleotides can exist in a mixture of several stacked forms. This might help to explain difficulties in simultaneously fitting more than one optical property to a two-state stacked–unstacked model (Davis and Tinoco, 1968; Glaubiger *et al.*, 1968). Some very indirect evidence for the existence of more than one stacked configuration for dTpT arises from the sterospecificity of the formation of the photodimer. There are four possible geometric isomers for the thymine dimer. It is known that the quantum efficiency of thymine dimer formation in dTpT can be correlated with the ability of the molecule to stack in different solvents (Wacker and Lodemann, 1965). Weinblum and Johns have found that there are two products produced by ultraviolet irradiation of dTpT (1966). The major isomeric dimer arises from a reaction which would be favored by a stacked conformation equivalent to a single strand of right-hand DNA helix. In addition, a second product is found which would be expected to occur if the dimer were stacked in a conformation similar to that shown in Figure 6 for the cyclic dimer. Studies on the photochemistry of cyclic deoxythymidine oligonucleotides are now in progress in our laboratory.

The conformational constraints introduced by forming a short cyclic phosphodiester backbone may be quite similar to the effects of looping out a short stretch of single-stranded residues from a double-stranded helix. In this case the types of conformations we have found for cyclic oligomers cannot be excluded as possibilities for the structures of loops and hairpins postulated to occur in such molecules as tRNA. This might have profound implications for the conformational stability and biochemical specificity of these systems. If the bases on the loops and hairpins of tRNA and 5S rRNA were unable to stack, there would be a considerable enthalpy barrier to the formation of loops. The stability of loops might strongly

depend upon the base sequence. Previous discussions of possible models for tRNA (Cantor *et al.*, 1966) and 5S rRNA (Cantor, 1967) have noted that the base uracil which exhibits the weakest stacking is very frequently located on loops. Fuller and Hodgson have unstacked two pyrimidine bases in their model for the anticodon loop of tRNA (1967). One of the loops of almost all of the tRNAs of known sequence has several dihydrouridines. This base is likely to exhibit no base stacking whatsoever. The results presented here suggest that it is possible that the bases on loops are mostly stacked although not all of them in the right-hand helical array found for linear oligonucleotides. If it should turn out that the stacking of any appreciable fraction of the bases of a tRNA resembles that found for cyclic oligomers, the semiempirical methods used for estimating the types of secondary structure in tRNA would have to be modified considerably (Cantor *et al.*, 1966).

The recent, elegant work of Clark *et al.* (1968) demonstrated that a nonadecanucleotide isolated from *Escherichia coli* $tRNA^{fMet}$ containing the complete anticodon loop and adjacent double-stranded region will bind to the ribosomes in the presence of ApUpG. An undecanucleotide containing the anticodon loop sequence but not constrained into a loop by a double-stranded region will not bind to the ribosome. This suggests that the particular geometry of bases constrained on a loop may assist codon–anticodon recognition. At the present time it would be premature to conclude that the conformation of loops of length seven such as those found on tRNA need have any similarity to the conformation of the small circles we have studied. What is needed is studies on larger cyclic oligonucleotodes which would clearly be better models for tRNA loops.

The forces responsible for stabilizing nucleic acid conformations have not yet been elucidated in sufficient detail to permit accurate estimates of the most stable conformation of even dinucleoside phosphates to be attempted. It is clear that studies on natural and synthetic oligonucleotides will be of help in determining some of the parameters necessary for conformational calculations. Experiments in which the orientation of the bases and sugars can be altered should be especially useful (Adler *et al.*, 1968). At present there has been only a limited start at computer calculations of the most stable oligonucleotide conformations (Scott, 1968; Sasisekharan *et al.*, 1967). Such computations are more difficult than the corresponding peptide work because of the much larger number of degrees of freedom in the phosphodiester bond. Once such calculations become practical, however, cyclic oligonucleotides may well serve as an important test case much as small cyclic peptides have been used to check the accuracy of protein calculations (Scott *et al.*, 1967).

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