A Comparative Study of Polydeoxyribonucleotides and Polyribonucleotides by Optical Rotatory Dispersion*

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ABSTRACT: A comparative study of the optical rotatory dispersion (ORD) and absorption spectra of the polynucleotides: polyribocytidylic acid *vs.* polydeoxycytidylic acid, polyribouridylic acid *vs.* polydeoxythymidylic acid, and polyriboadenylic acid *vs.* polydeoxyadenylic acid, indicates a large influence of the 2'-hydroxyl group of ribose on the conformation and the helix–coil transition of these polymers. The differences found between the ribosyl and deoxyribosyl polymers can be explained on the hypothesis that the 2'-hydroxyl group hydrogen bonds with 2-keto group of cytosine and uracil and with the N-3 ring nitrogen of the adenine in the polymer. In addition, comparative studies have been made on the ORD patterns and the helix–coil transition of the alternating copolymer of deoxyadenylic acid–deoxythymdylic acid and the homopolymer complexes: polydeoxyadenylic acid + polydeoxythymidylic acid, polydeoxyadenylic acid + polyribouridylic acid, polyriboadenylic acid + polydeoxythymidylic acid, and polyriboadenylic acid + polyribouridylic acid. The results again indicate that the helical structure of the ribosyl polymers is different from that of the deoxyribosyl polymers. It is postulated that the angle, α, between the transition moments of the neighboring bases in the stack is more oblique in the ribosyl helix than in the deoxyribosyl helix.

Advances in instrumentation and theory on optical rotatory dispersion (ORD) provide a new and powerful approach to the conformation of molecules in solution. One of the unanswered questions concerning nucleic acids is: Do DNA and RNA polymers have the same secondary structure in solution when their primary structures, except the pentose moiety, are identical in base composition and sequence? In other words, does the 2'-hydroxyl group of ribose in RNA exert an influence on the conformation of the polymer? This inquiry is of interest in biology as well as chemistry since the answer may provide new insight into the mechanism of the DNA–RNA transcription process (Chamberlin, 1965). The question could not be answered until polydeoxyribonucleotides and polyribonucleotides of defined base composition and sequence became available.

Samejima and Yang (1964) first reported that the ratio of rotation of the first peak (280–290 mμ) to the second peak (~230 mμ) in the ORD curve is around 1:2 for salmon and thymus DNA in native state, while this ratio is over 500 for liver RNA in 0.14 M salt at room temperature. It appeared, therefore, that an ORD study of the homopolyribonucleotides and -polydeoxyribonucleotides would be of value. In this study ORD of polymers having the bases adenine (rA_n, dA_n),[1] cytosine (rC_n, dC_n), uracil (rU_n), and thymine (dT_n) have been investigated, as well as the homopolymer complexes containing A and U or T. $r(I)_n$ gives a complex and unusual ORD curve (Sarkar and Yang, 1965a), which may be due to the complex ultraviolet absorption spectrum of hypoxanthine resulting from multiple electronic transitions in the 250–280-mμ region. Similar electronic complexities, as well as multiple secondary interactions, complicate studies on polyguanylates.

Our results on the ORD of the ribose polymers are in close agreement with previously published work and the data are reported here for comparison. In some instances we have been able to extend the measurements to lower wavelengths and somewhat different experimental conditions. We have also compared the ORD patterns of double helices of several homopolymer complexes (such as $d(A)_n \cdot d(T)_n$) with those having alternating base sequences (such as $d(A\text{–}T)_n$).

The results indicate that the 2'-hydroxyl group of ribose has a marked influence on the ORD patterns and therefore the conformation and certain structural stabilities of these polymers. Most of the results can be

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[1] All the abbreviations follow the Revised Tentative Rules (1965), IUPAC–IUB combined Commission on Biochemical Nomenclature, published in *Biochemistry 5*, 1445 (1966). Because of the nature of this paper r and d are retained throughout for ribosyl and deoxyribosyl compounds.

TABLE I: Molar Extinction Coefficients (ϵ_{max}) of the Polynucleotides.[a]

Material	Buffer	λ_{max}	ϵ_{max}	Ref
$d(A-T)_n$	pH 7.5	260	6.7	Radding and Kornberg (1962)
$d(A)_n \cdot d(T)_n$	0.1 M Na-PO_4–1 × 10^{-4} M ETDA, pH 7.8	260	6.0	Chamberlin (1965)
	0.001 M Tris–Cl, pH 8.0	260	6.90	Bollum (1966)
r(ApA)	K-PO_4, pH 6.9, $KClO_4$, 0.1 μ	258	13.9	Warshaw and Tinoco (1965)
$d(pA)_2$	0.001 M Tris, pH 8.0	258	11.8	Bollum (1966)
$r(A)_n$	0.1 M NaCl–0.05 M Tris, pH 7.5	257	10.5	Ts'o *et al.* (1962)
$r(A)_n$	0.1 M NaCl–0.1 M sodium acetate, pH 4.85	252	8.6	Ts'o *et al.* (1962)
d(ApA)	0.001 M Tris, pH 8.0	257	13.9	Taken as the same as (ApA)
$d(T)_n$	0.001 M Tris, pH 8.0	264	8.52	Bollum (1966)
$d(pT)_2$	Water	267	9.25	Gilham and Khorana (1958)
$d(pT)_{n=10}$	Water	267	8.52	Jacob and Khorana (1965)
$r(U)_n$	0.1 M NaCl–0.05 M Tris, pH 7.5	260	9.2	Ts'o *et al.* (1962)
$r(C)_n$	0.1 M NaCl–0.05 M Tris, pH 7.5	267	6.5	Ts'o *et al.* (1962)
$r(C)_n$	0.1 M sodium acetate, pH 4.0	275	7.4	Ts'o *et al.* (1962)
$d(C)_n$[b]	pH 6.4, 0.05 M Na^+	274	6 6	Inman (1964)
$d(C)_n$	pH 8.00, 1 × 10^{-3} M Tris	268	7.4	Bollum (1966)

[a] While this work was in progress, Chamberlin (1965) published a list of molar extinction coefficients of polynucleotides. The agreement with the values listed here is within ±5%. [b] Assumed to be the same at pH 5.10, helical form.

explained on the hypothesis that the 2′-hydroxyl group can hydrogen bond with the 2′-keto group of cytosine and uracil and with N-3 of adenine in the polymer. A discussion of the existing data related to such hydrogen bonding possibilities in the nucleosides and nucleotides is also presented.

Materials and Methods

Polyriboadenylic acid, $r(A)_n$, polyribouridylic acid, $r(U)_n$, and polyribocytidylic acid, $r(C)_n$, were purchased from Miles Chemical Co. (Elkhart, Ind.). Polydeoxyadenylic acid, $d(A)_n$, polythymidylic acid, $d(T)_n$, and polydeoxycytidylic acid, $d(C)_n$, were prepared by means of a terminal deoxynucleotidyl transferase isolated from calf thymus gland (Bollum *et al.*, 1964; Bollum, 1966). The average degree of polymerization of the deoxy polymers used is around 200–300. Two types of alternating copolymers of thymidylic and deoxyadenylic acids were investigated. The first type was synthesized by DNA polymerase and was the gift of Professor A. Kornberg, Stanford University. It is denoted as *synthetic* $d(A-T)_n$. The second kind was isolated from *Cancer antennarius* by a mercury-binding procedure (Davidson *et al.*, 1965) and was the gift of Dr. J. Widholm and Professor J. Bonner, California Institute of Technology. It is denoted as *natural* $d(A-T)_n$. The oligonucleotides $d(pT)_2$ and $d(pT)_{3-10}$ were chemically synthesized; they were the gift of Mr. M. Schweizer of our laboratory. The dinucleotide, $d(pA)_2$, was prepared by DNase I digestion of $d(A)_n$ and isolated by chromatography on a DEAE column (Bollum *et al.*, 1964); the dinucleoside monophosphate, d(ApA), was prepared by treating a portion of this compound with bacterial alkaline phosphatase at pH 8 overnight and isolating the product by descending chromatography (*n*-propyl alcohol–ammonia–water, 55:10:35) on acid-washed Whatman No. 3 paper.

Optical densities were determined in 1-cm cells with a Beckman DU or Cary Model 15 spectrophotometer. The absorbance *vs.* temperature profile was obtained from the Beckman DK-1 recording spectrophotometer or from the Cary 15 equipped with thermostated cell holders.

The ORD measurements were made in a 1-cm, water-jacketed cell in a Cary Model 60 spectropolarimeter. Slits were adjusted to give a constant dispersion of 10 A across the exit slit. Response time was varied from 1 to 10 sec to minimize noise and the scanning speed was adjusted correspondingly from 12 to 1.2 mμ/min. Optical densities of the compounds were adjusted to keep the dynode voltage of the instrument below 600 v for all measurements. Haake–Brinkmann thermostated water baths (types NBS and KT-62) were used to control the temperature of the samples to better than 1° of the stated values, and each sample was allowed to equilibrate for 10–15 min before measurement. Instrument stability was checked by running air base lines before and after each experiment, and these agreed to within 0.003° (generally better than 0.002°) in all cases. Frequently samples were rerun once or twice to check the reproducibility of the curves. The instrument has been calibrated with sucrose and

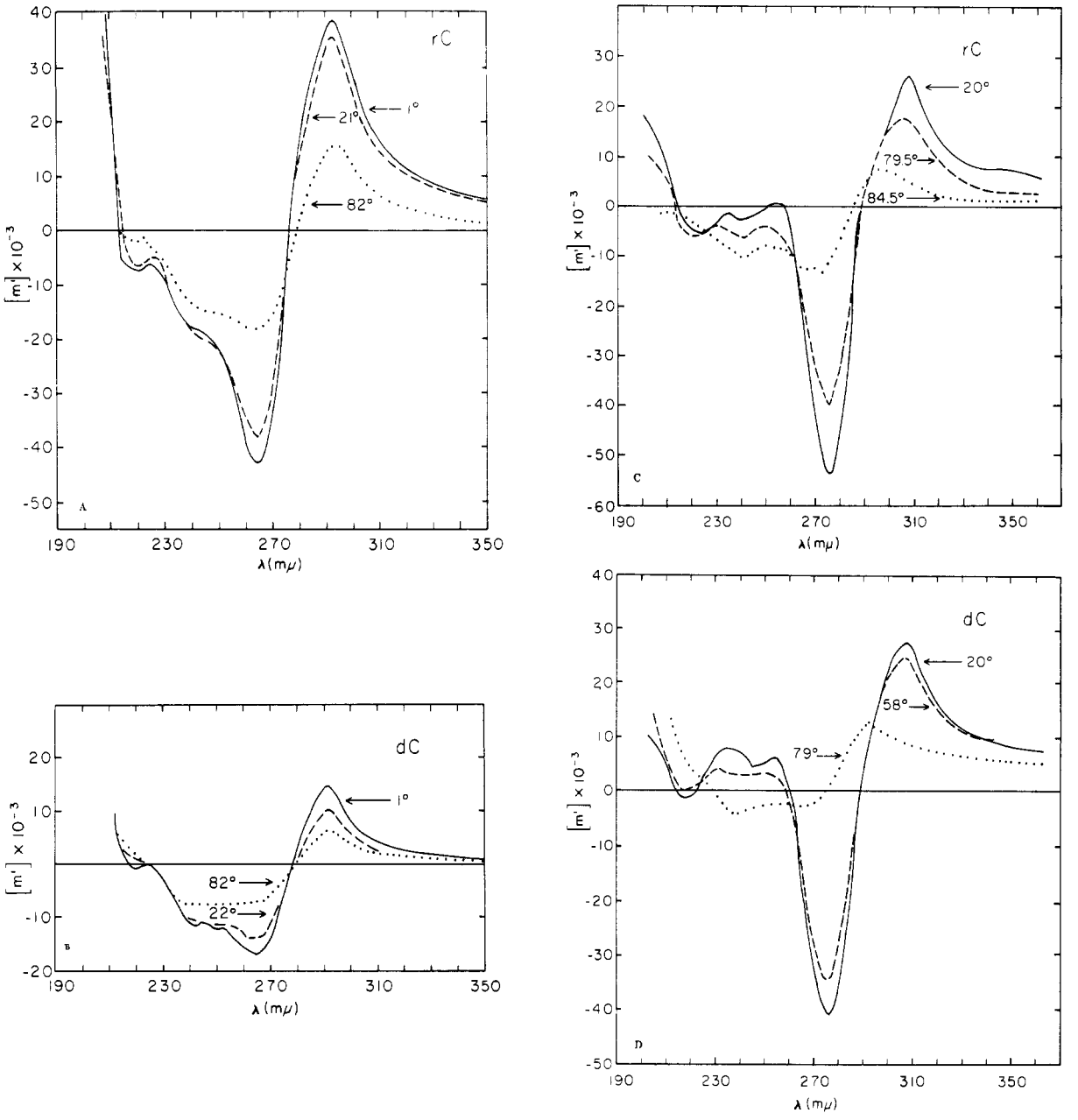


FIGURE 1: ORD curves of $r(C)_n$ and $d(C)_n$. (A) $r(C)_n$ in 0.05 M $Na\text{-}PO_4$, pH 8.4: at 1 (———), 21 (– – – –), and 82° (· · · ·). (B) $d(C)_n$ in 0.05 M $Na\text{-}PO_4$, pH 8.4: at 1 (———), 22 (– – – –), and 82° (· · · ·). (C) $r(C)_n$ in 0.05 M $NaClO_4$, 1×10^{-3} M sodium acetate, pH 4.4: at 20 (———), 79.5 (– – – –), and 84.5° (· · · ·). (D) $d(C)_n$ in 0.05 M $NaClO_4$, 1×10^{-3} M sodium acetate, pH 5.1: at 20 (———), 58 (– – – –), and 79° (· · · ·).

provides results in very close agreement with those reported by Samejima and Yang (1964).

Observed rotations (α) were found by subtracting the rotation of the compound being studied from the value obtained for the solvent under the same conditions and then expressed in terms of the reduced mean residue rotation (m') (Fasman *et al.*, 1964) defined as follows. $(m') = (100\alpha/cL)(3/(n^2 + 2))$, where c = concentration in moles per liter of the base residues, L = path length, 1.00 cm, and n = index of refraction of the solution (assumed to be the same as that of water). No corrections were made from volume variations with temperature.

A major problem in the calculation of (m') is the determination of concentration by absorbance measurements. Reliable data on molar extinction coefficients (ϵ_{max}) are therefore required. Table I lists all the values of ϵ_{max} employed in this paper. The absolute value of (m') is dependent upon the accuracy of these values and is estimated generally within the range of ±5%.

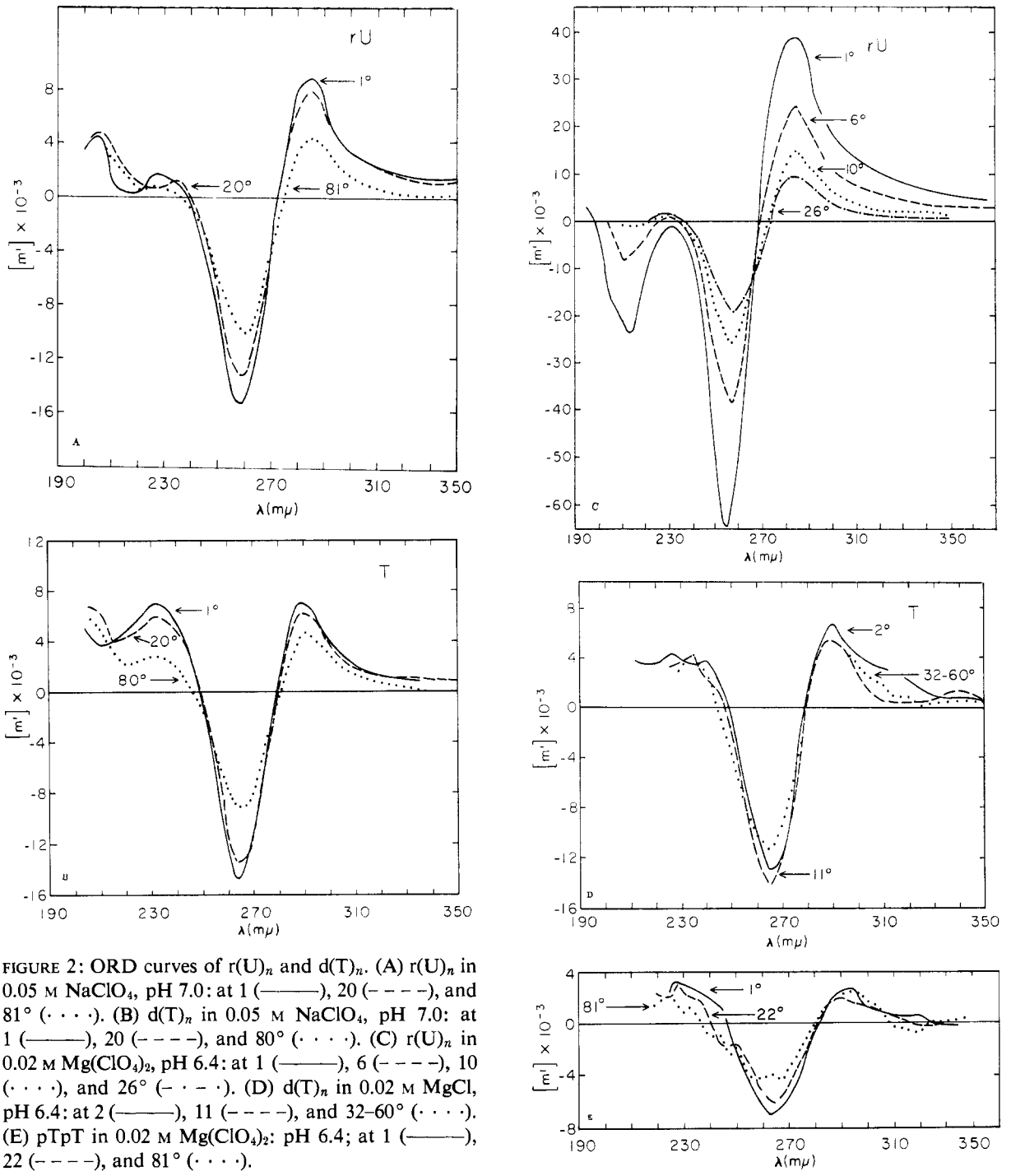


FIGURE 2: ORD curves of $r(U)_n$ and $d(T)_n$. (A) $r(U)_n$ in 0.05 M $NaClO_4$, pH 7.0: at 1 (———), 20 (– – – –), and 81° (· · · ·). (B) $d(T)_n$ in 0.05 M $NaClO_4$, pH 7.0: at 1 (———), 20 (– – – –), and 80° (· · · ·). (C) $r(U)_n$ in 0.02 M $Mg(ClO_4)_2$, pH 6.4: at 1 (———), 6 (– – – –), 10 (· · · ·), and 26° (– · – · –). (D) $d(T)_n$ in 0.02 M MgCl, pH 6.4: at 2 (———), 11 (– – – –), and 32–60° (· · · ·). (E) pTpT in 0.02 M $Mg(ClO_4)_2$: pH 6.4; at 1 (———), 22 (– – – –), and 81° (· · · ·).

The ϵ_{max} of $d(A)_n$ is not included in Table I and will be reported in later portions of the paper.

Results

Studies on Homopolymers: $r(C)_n$ and $d(C)_n$

The ORD curves of both $r(C)_n$ and $d(C)_n$ at pH 8.4 are given in Figure 1A,B at several temperatures. The shapes of these curves are quite similar to each other, but the absolute rotation value of the $d(C)_n$ curve is only about 30–40% that of $r(C)_n$.

The ORD curves of $r(C)_n$ at pH 4.4 and $d(C)_n$ at pH 5.1 at several temperatures are given in Figure 1C,D. Here both the shape and the magnitude of the curves are similar except in the region of 230–260 mμ (the region of the second peak) where the rotation of $d(C)_n$ is positive and the rotation of $r(C)_n$ is negative.

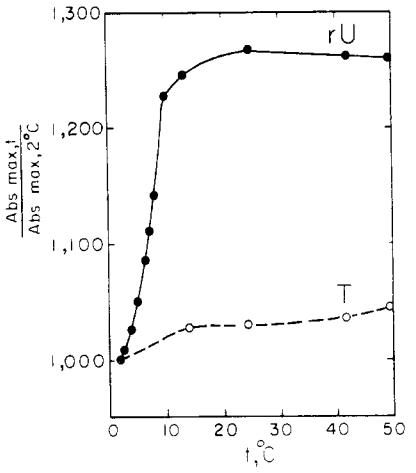
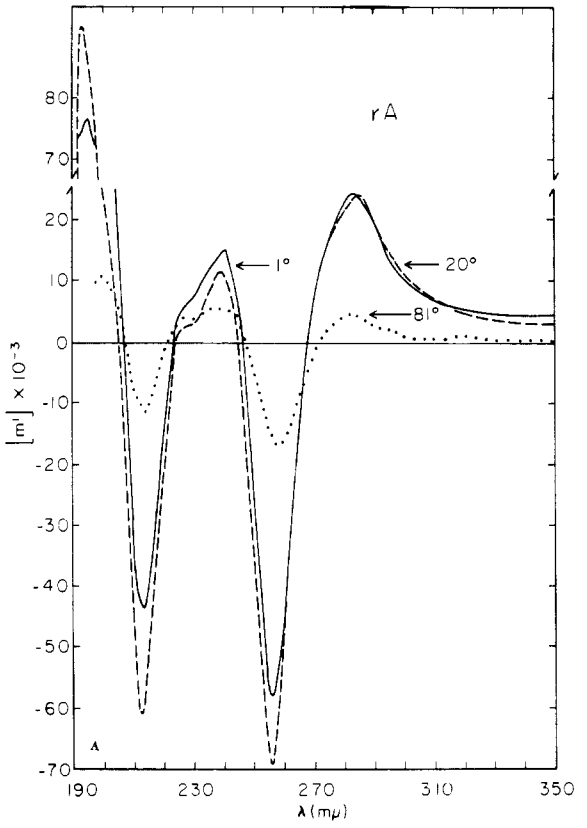


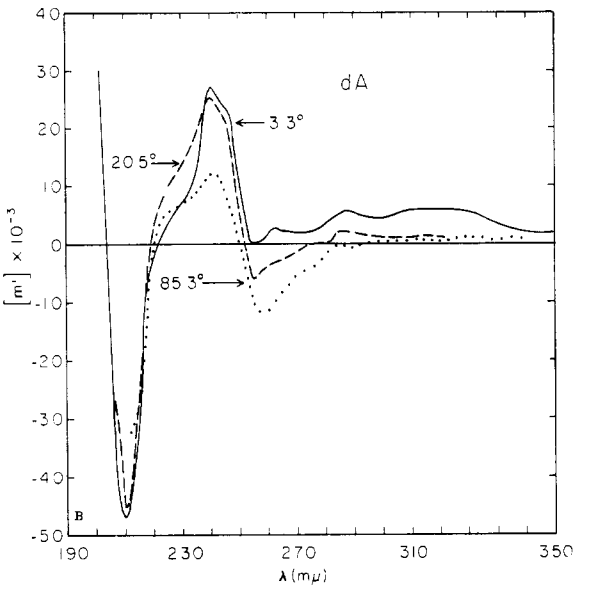
FIGURE 3: The absorbance *vs.* temperature profile of $r(U)_n$ and $d(T)_n$ at 260 mμ in 0.02 M $Mg(ClO_4)_2$, pH 6.4.

It is instructive to compare the proton-induced coil-to-helix transition of $r(C)_n$ and $d(C)_n$ at room temperature. In both cases, the first maximum and the minimum are shifted to longer wavelengths in accordance with the red shift of the absorption spectra. The minimum is also increased in magnitude for both polymers, especially in the case of $d(C)_n$. However, in the case of the first peak there is a threefold increase for $d(C)_n$ in this transition but a 25–30% decrease for $r(C)_n$.

The data of $r(C)_n$ in Figure 1 are in close agreement with those previously published by Fasman *et al.* (1964) and by Sarkar and Yang (1965a). In Figure 1, however, the measurements have been extended to lower wavelengths and to lower temperature (1°).

Ultraviolet absorption spectrum *vs.* temperature studies has been studied for $r(C)_n$ and $d(C)_n$ at pH 8.4, 0.05 M sodium phosphate. For $r(C)_n$, a 15–16%





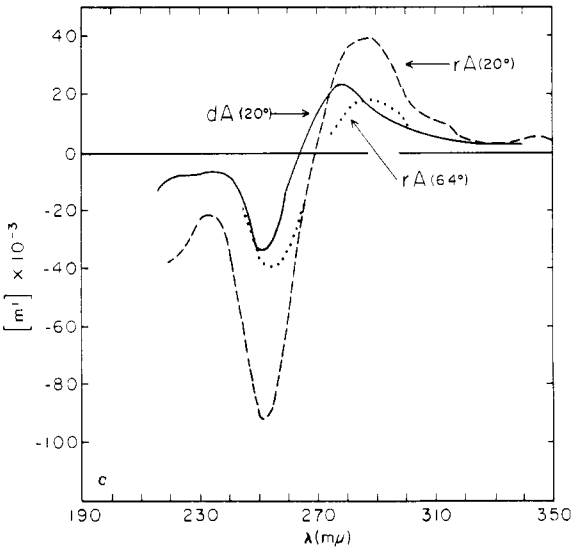


FIGURE 4: ORD curves of $r(A)_n$ and $d(A)_n$. (A) $r(A)_n$ in 0.05 M $NaClO_4$, pH 7.0: at 1 (———), 20 (– – – –), and 81° (· · · ·). (B) $d(A)_n$ in 0.05 M $NaClO_4$, pH 7.35: at 3.3 (———), 20 (– – – –), and 85.3° (· · · ·). (C) $d(A)_n$ and $r(A)_n$ in acidic solutions: $d(A)_n$ at 20°, 1×10^{-3} acetic acid, pH 3.4 (———); $r(A)_n$ at 20° (– – – –), and at 64° (· · · ·), pH 5.0, in 0.05 $NaClO_4$, 1×10^{-3} M HAc–NaAc.

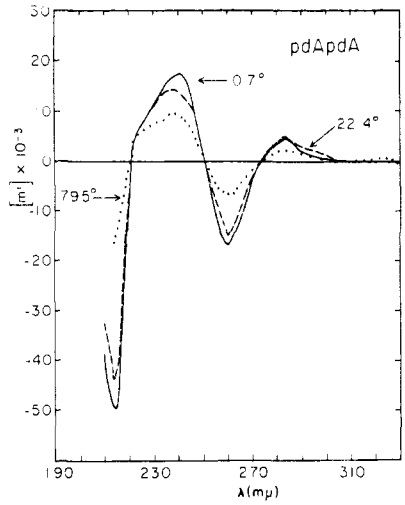


FIGURE 5: ORD curves of pdApdA in 0.05 M $NaClO_4$, pH 7.3: at 0.7 (———), 22 (– – – –), and 79.5° (· · · ·).

hyperchromicity was observed between 23 and 90°, in agreement with previous results (Ts'o *et al.*, 1962). In the same temperature range, no hyperchromicity was observed for $d(C)_n$ at the absorption maximum (267 mμ) and about 3% hyperchromicity was seen at the broad absorption minimum (240 mμ). In accordance with this finding, when both polymers are unprotonated at pH 7.5–8.0, the ϵ_{max} of $d(C)_n$ at 268 mμ at room temperature is about 15% higher than ϵ_{max} of $r(C)_n$ (Table I).

$r(U)_n$ and $d(T)_n$

The ORD curves of $r(U)_n$ and $d(T)_n$ in 0.05 M $NaClO_4$ are given in Figure 2A,B. The magnitude and shape are similar except in the region of 230 mμ. The magnitude of the second peak of the $d(T)_n$ is larger than that of the $r(U)_n$. Both curves are relatively insensitive to temperature suggesting that these polymers are random coils (Richards *et al.*, 1963).

The ORD curves of $r(U)_n$, $d(T)_n$, and $d(pT)_2$ in 0.02 M Mg^{2+} are given in Figure 2C–E. In this ionic environment, $r(U)_n$ is known to exist in an ordered state (Lipsett, 1960) below 5°. The ORD curve of $r(U)_n$ under these conditions is sensitive to temperature as shown by the progressive diminution of the large first peak (285 mμ) as temperature is increased until it becomes similar to that observed in 0.05 M sodium ions. On the other hand, the $d(T)_n$ curve in Mg^{2+} solution is insensitive to temperature and similar to that observed in 0.05 M sodium ions. The $d(T)_n$ curve is also similar to the curve of $d(pT)_2$ (Figure 2E) but twofold greater in magnitude (peaks and trough) as expected from semiempirical calculations (Cantor and Tinoco, 1965). The absorbance *vs.* temperature profiles of $r(U)_n$ and $d(T)_n$ in Mg^{2+} solution (Figure 3) clearly indicate that there is a transition for $r(U)_n$ and not for $d(T)_n$. The ORD curves of $d(pT)_{8-10}$ obtained in Mg^{2+} and Na^+ solutions are equivalent to those of $d(T)_n$.

The data in Figure 2A,C are qualitatively similar to those reported by Sarkar and Yang (1965b) and by Lamborg *et al.* (1965) even though the experimental conditions are different in all cases.

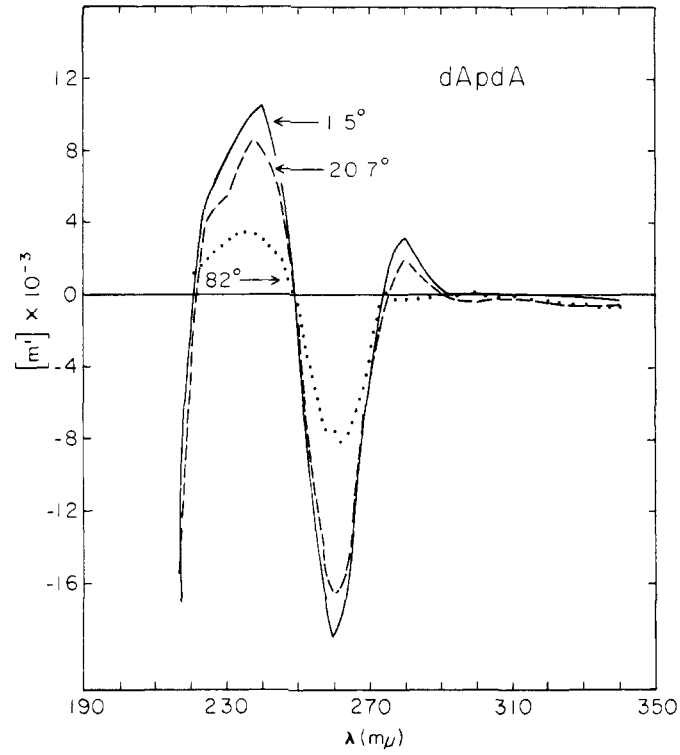


FIGURE 6: ORD curves of dApdA in 0.05 M $NaClO_4$, pH 7.3: at 1.5 (———), 20.7 (– – – –), and 82° (· · · ·).

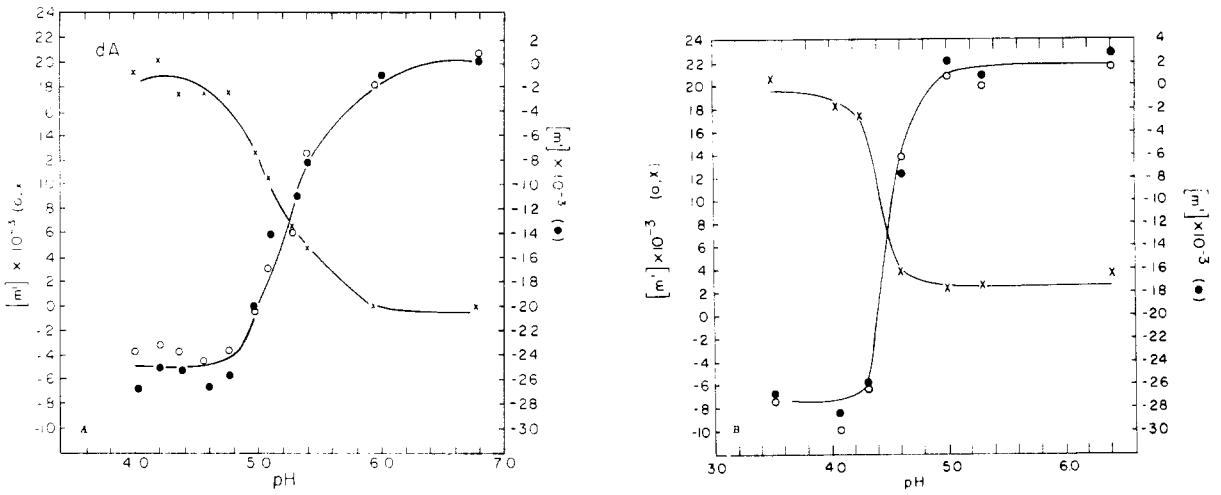


FIGURE 7: The (m') *vs.* pH profile of d(A)$_n$. (A) In 1 × 10^{-3} M sodium acetate, titration starting from pH 4.0 with addition of small amount of NaOH: (x-x-x-x), at 279 mμ (left scale); (●-●-●-●), at 252.5 mμ (right scale); at o-o-o-o 240 mμ (left scale); temperature at 22°. (B) In 0.2 M $NaClO_4$, 0.02 M sodium acetate. Samples were prepared individually at different pH; (x-x-x-x), at 279 mμ (left scale); (●-●-●-●), at 252 mμ (right scale); and (o-o-o-o) at 240 mμ (left scale); temperature at 20°.

r(A)$_n$ and d(A)$_n$

The ORD curves of r(A)$_n$ and d(A)$_n$ at pH 7.0–7.35, 0.05 M salt, and at varying temperatures are given in Figure 4A,B. The difference in the region from 320 to 250 mμ is very striking. The prominent peak (282 mμ) and trough (256 mμ) in the ORD of r(A)$_n$ is much reduced in the ORD of d(A)$_n$. From 250 to 200 mμ the shapes of the ORD curves of r(A)$_n$ and d(A)$_n$ are similar, although the curve of r(A)$_n$ has a lower peak and deeper trough, or generally a more negative value in rotation than d(A)$_n$.

In Figure 4C, the ORD of the r(A)$_n$ and d(A)$_n$ at acidic pH are given. The shape of the ORD curve of d(A)$_n$ is similar to that of the r(A)$_n$ at 20°, and the magnitude of the peak and the trough is similar to that of the r(A)$_n$ at 64°, half-way through the melting curve at this pH (5.0) and ionic strength (0.05 M). In comparison to r(A)$_n$ the first peak of d(A)$_n$ is shifted by 10 mμ toward shorter wavelengths, and the shoulder at wavelength 230–240 mμ is more positive in value.

The ORD curves of d(pA)$_2$ and d(ApA) are given in Figures 5 and 6. The shape and the magnitude of these curves are similar to that of r(ApA), reported by Warshaw *et al.* (1965). Upon close examination, the magnitudes of the curves of d(ApA) appears to be slightly less than that of r(ApA), especially the first peak. The presence of a 5′-phosphoryl group, as in d(pA)$_2$, increases the magnitude of the peak and the trough at 240–210 mμ.

The ORD of poly dA at about 20° was followed at varying pH. The effect of pH on the secondary structure in 1 × 10^{-3} M buffer is shown in Figure 7A, in which the rotation at 279, 252.5 (the maximum and the minimum of the first peak and trough of the acidic form), and at 240 mμ (the maximum of the largest peak of the neutral form) are plotted. The figure shows a helix–coil transition phenomenon with a midpoint around pH 5.3. In 0.22 M salt, the midpoint of the transition is shifted to about pH 4.4.

Hyperchromicity measurement of d(A)$_n$ in 0.2 M NaCl–0.001 M Tris, pH 7.5, gave a value of 19% increase of ϵ_{max} (257 mμ) at 90° *vs.* initial measurement at 24°. The increase is gradual over the whole temperature range. This value is about the same as r(A)$_n$ under similar conditions (Ts'o *et al.*, 1962). The ϵ_{max} (257 mμ) per mole of base or phosphorus in 1 mM Tris, pH 8.0, was found to be 10 ± 0.2 × 10^{-3} based on careful phosphorus analysis on several samples. In 1 mM Tris–0.1 M NaCl, ϵ_{max} was found to be 9.8 ± 0.1 based on analysis of phosphorus and adenine content. The estimation of the adenine was done spectrophotometrically after hydrolysis in 0.1 N HCl at 40° for 50 hr. After this reaction period, the optical density of the solution in the acid was constant. This value of ϵ_{max} for d(A)$_n$ is essentially identical with that of r(A)$_n$ (10.1–10.5 × 10^{-3}) under similar conditions (Holcomb and Tinoco, 1965; Ts'o *et al.*, 1962).

Figure 8 shows the ultraviolet spectra of d(A)$_n$ at three different pH values in 1 mM buffer and pH 4.07 in 0.22 M salt. The effect of salt concentration and pH on the λ_{max} of d(A)$_n$ was carefully analyzed as shown in Figure 9. The λ_{max} shifts from 257 to 260 mμ when the pH of the solution is lowered to pH 5. Upon further decrease of pH to 4 or lower, λ_{max} now shifts to 255–253 mμ. The exact pH for the transition is influenced by salt concentration. In 1 mM buffer, the transition takes place at higher pH while in 0.22 M salt–buffer concentration, the transition occurs at lower pH values. The effect of pH on λ_{max} is cor-

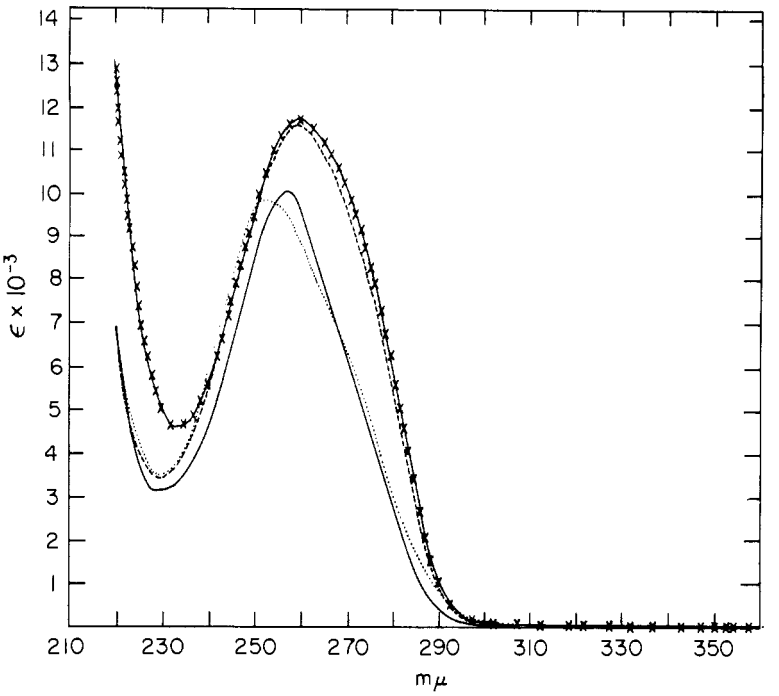


FIGURE 8: The ultraviolet absorption spectra of $d(A)_n$: (———), pH 8.0, 1×10^{-3} M Tris; (– – – –), pH 5.25, 1×10^{-3} M acetate; (x-x-x-x), pH 4.07, 0.2 M $NaClO_4$, 0.02 M NaAc; (· · · ·), pH 3.23, 1×10^{-3} M acetate.

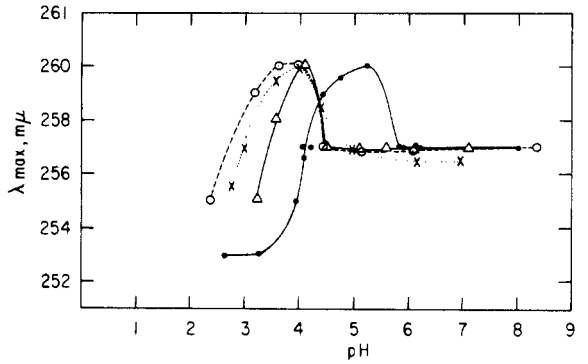


FIGURE 9: The effect of salt concentration and pH on the λ_{max} of $d(A)_n$ ultraviolet absorption spectra: (-●-●-●-), 10^{-3} M buffers (Tris or acetate); (-Δ-Δ-Δ-Δ-), 0.02 M $Na-PO_4$ or acetate buffer plus 0.03 M $NaClO_4$; (---x---x---), 0.02 M $Na-PO_4$ or acetate buffer plus 0.09 M $NaClO_4$; (---⊙---⊙---), 0.02 M $Na-PO_4$ or acetate buffer plus 0.2 M $NaClO_4$.

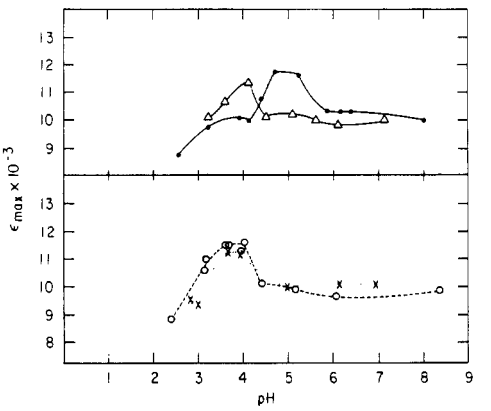


FIGURE 10: ORD effect of salt concentration and pH on the ϵ_{max} of $d(A)_n$ ultraviolet absorption spectra. Symbols are the same as Figure 9.

related with the change of ϵ_{max} (Figure 10). It can be concluded that at low degree of protonation there is a bathochromic shift of λ_{max} from 257 to 260 mμ with a concomitant increase in ϵ_{max} to 11.6–11.8 $\times 10^{-3}$. At higher levels of protonation, a gradual hypsochromic shift is accompanied by a decrease in ϵ_{max}. The limit for the downward shift of λ_{max} appears to be 253 mμ. Examination of the ORD *vs.* pH plots of Figure 7A,B shows that $d(A)_n$ exhibiting a λ_{max} at 260 mμ is substantially in the helical form.

There are considerable technical difficulties in working with $d(A)_n$ in acidic solution. At pH 3.80, 0.01 M acetate (λ_{max} at 255 mμ), the ultraviolet absorption curve of the $d(A)_n$ solution was constant at room temperature for 20 hr and was not reduced by two centrifugations, each for 10 min, at 10,000*g*. At pH 3.2, however, centrifugation reduced the optical density about 10%. The optical density of the supernatant solution is stable for 20 hr at room temperature, indicating that no further aggregation occurs. The ORD curve in Figure 4C was obtained from

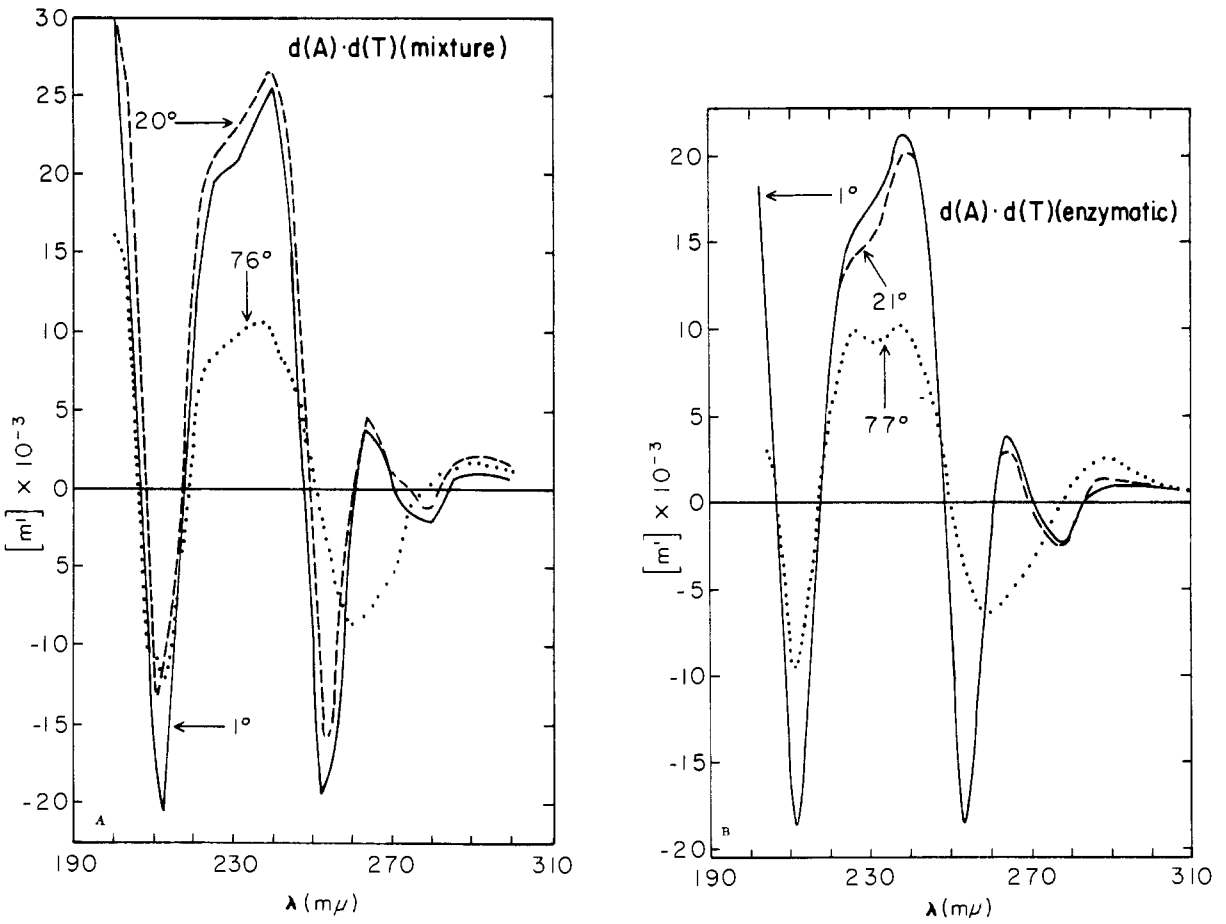


FIGURE 11: ORD curves of $d(A)_n \cdot d(A)_n$. (A) 1:1 mixture of $d(A)_n$ in 0.05 M $NaClO_4$, pH 7.4: at 1 (———), 20 (– – – –), and 76° (· · · ·). (B) Synthesized enzymatically with $d(A)_n$ template and TTP; in 0.05 M $NaClO_4$, pH 7.4: at 1 (———), 21 (– – – –), and 77° (· · · ·).

$d(A)_n$ solution after centrifugation. At pH 3.0 or below, centrifugation in a Servall or clinical centrifuge brought about loss in optical density from the solution although the λ_{max} was unchanged for the remaining supernatant solution. Without centrifugation the optical density remained constant upon standing overnight. It is tentatively concluded that aggregation of $d(A)_n$ at pH above 3.5 is not sufficiently advanced for the polymers to be sedimented out of solution by low-speed centrifugation, even though analytical ultracentrifugation of $d(A)_n$ solutions at pH 7.0 and at pH 3.8 indicates that the S value of the $d(A)_n$ has been substantially increased already at pH 3.8. At pH values below 3.5, the degree of aggregation is such that the polymer can be sedimented by low-speed centrifugation but not by gravity. In acidic solution, elevated temperature probably caused hydrolysis of $d(A)_n$ as indicated by a partial irreversibility of ORD melting curves of $d(A)_n$ at pH 4.0.

Studies of $d(A)_n \cdot d(T)_n$, Synthetic $d(A\text{–}T)_n$ and Natural $d(A\text{–}T)_n$

The ORD of $d(A)_n \cdot d(T)_n$ is shown in Figure 11. The physical hybrid formed by a 1:1 mixture of $d(A)_n$ and $d(T)_n$ is seen in Figure 11A, and $d(A)_n \cdot d(T)_n$ formed enzymatically by polymerization of dTTP on a $d(A)_n$ template (Bollum, 1966; F. J. Bollum, private communication) is shown in Figure 11B. Each sample is shown at three temperatures. The similarity of these curves indicates that the stoichiometry and secondary structure is quite similar in these two homopolymer complexes regardless of the mode of preparation.

The ORD of $d(A\text{–}T)_n$, the alternating copolymer, obtained from two different sources is shown in Figure 12A, B. Each sample is shown at three different temperatures. The *natural* $d(A\text{–}T)_n$ was isolated from *C. antennarius* by a mercury-binding procedure (Davidson *et al.*, 1965) and the *synthetic* $d(A\text{–}T)_n$ was synthesized by DNA polymerase (Schachman *et al.*, 1960). The curves in Figure 12B are similar to those previously reported by Samejima and Yang (1965) but the measurement has been extended to 200 mμ to include a third peak located at 212 mμ. The most interesting difference between the synthetic $d(A\text{–}T)_n$ and natural $d(A\text{–}T)_n$ is the effect of temperature be-

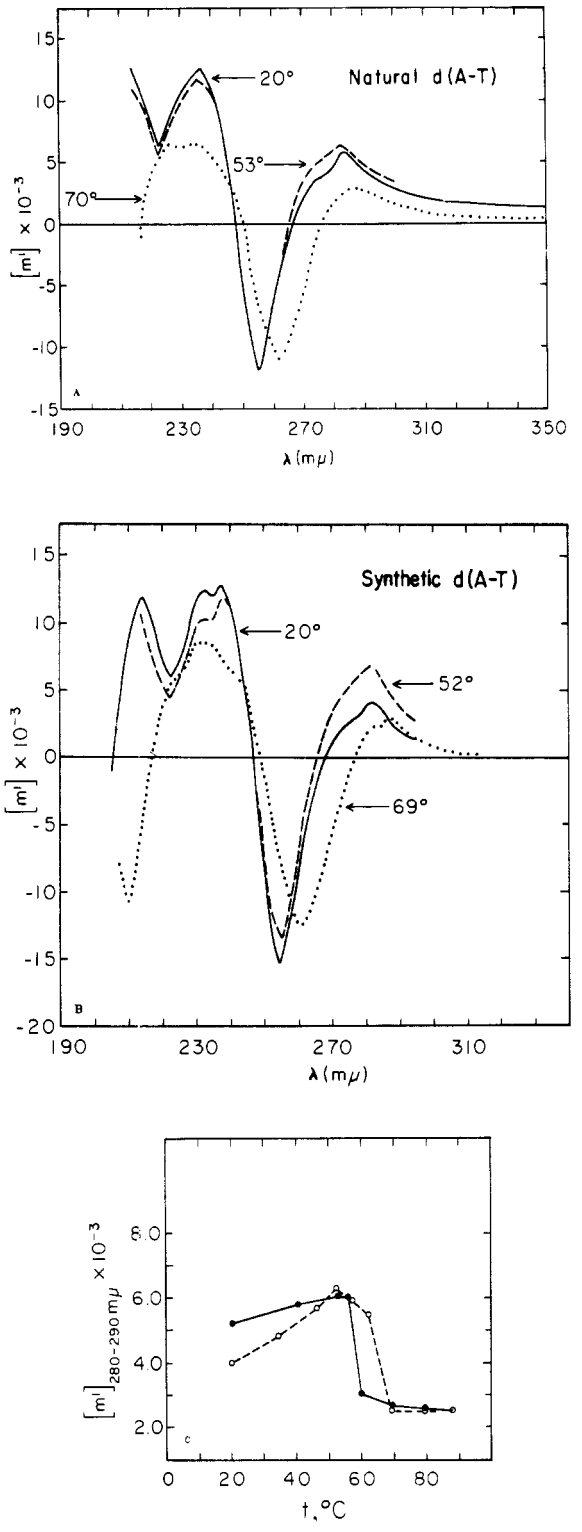


FIGURE 12: ORD curves of d(A–T)$_n$. (A) Natural d(A-T)$_n$ isolated from crab in 0.05 M $NaClO_4$, pH 7.4: at 20 (———), 53 (– – – –), and 70° (· · · ·). (B) Synthetic d(A-T)$_n$ prepared from DNA polymerase in 0.05 M $NaClO_4$, pH 7.4: at 20 (———), 52 (– – – –), and at 69° (· · · ·). (C) The $(m')_{280-290m\mu}$ *vs.* temperature profile of natural d(A-T)$_n$ (solid line) and synthetic d(A-T)$_n$ (dotted line) in 0.05 M $NaClO_4$.

tween 20 and 50° on the height of the first peak located at 292 mμ. The rotation of *natural* d(A–T)$_n$ is not sensitive to temperature variation from 20 to 53°, just before the onset of the melting. On the other hand, the rotation (m') of *synthetic* d(A–T)$_n$ is sensitive to temperature changes. In particular, m' of the first peak at 282 mμ increases from 4×10^{-3} (at 20°) to 6×10^{3} (at 50°) as shown in Figure 12C. Just before the helix–coil transition, the value of m' at 282 mμ of *natural* d(A–T)$_n$ is now about the same as that of the *synthetic* d(A–T)$_n$. This phenomenon is probably related to some difference in the secondary structure of these polymers at 20° and is discussed more fully later. The difference in T_m of the melting curves in Figure 12C may be due to a small difference in salt concentration and should not be taken seriously. Because of the shortage of materials the samples were not dialyzed; therefore, their ionic environments were not rigorously controlled.

A comparison of the homopolymer complex, d(A)$_n$·d(T)$_n$, and the copolymer complexes, d(A–T)$_n$, is shown in Figure 13A,B. Above 70° the ORD of d(A–T)$_n$ and d(A)$_n$·d (T)$_n$ is rather similar (Figure 13B). At 20°, however, the curves differ markedly, especially at 260–300 mμ (Figure 13A).

Studies on the 1:1 Mixtures of r(A)$_n$, d(A)$_n$, r(U)$_n$, and d(T)$_n$

The four permutative pairs of 1:1 mixtures of these homopolymers all give sharp, one-step melting curves as measured by ultraviolet absorption. The values of the T_m for these complexes in 0.05 M $NaClO_4$, pH 7.0, are 61.5, 59, 51, and 41.5°, respectively, for d(A)$_n$·d(T)$_n$, r(A)$_n$·d(T)$_n$, r(A)$_n$·r(U)$_n$, and d(A)$_n$·r(U)$_n$. These values are in agreement with recently published data of Chamberlin (1965) in 0.1 M sodium phosphate, pH 7.8 (68.5, 64.1, 56.8, and 45°, respectively). The hyperchromicity observed was about 40–50% in all cases.

The ORD curves of the four permutative pairs in the helical form (0.05 M $NaClO_4$, 20°) are shown in Figure 14. The troughs and the peaks of these curves are roughly the same but the magnitude of the two peaks (around 285 and 230–240 mμ) is quite different. The d(A)$_n$·d(T)$_n$ also has multiple peaks at 260–290-mμ region.

The ORD of the d(A)$_n$·(U)$_n$, r(A)$_n$·d(T)$_n$, and r(A)$_n$ (U)$_n$ in 0.05 M $NaClO_4$, pH 7.0, at various temperatures is shown in Figures 15–17. The curve in Figure 17 of poly r(A)$_n$·(U)$_n$ is similar to that previously reported by Sarkar and Yang (1965b). It is shown here for the sake of comparison and discussion.

Discussion

Data presented above demonstrate the pronounced effect of the 2′-hydroxyl group of the pentose on the conformation and stability of the polynucleotides. We shall review the chemistry of these compounds systematically starting from the monomers.

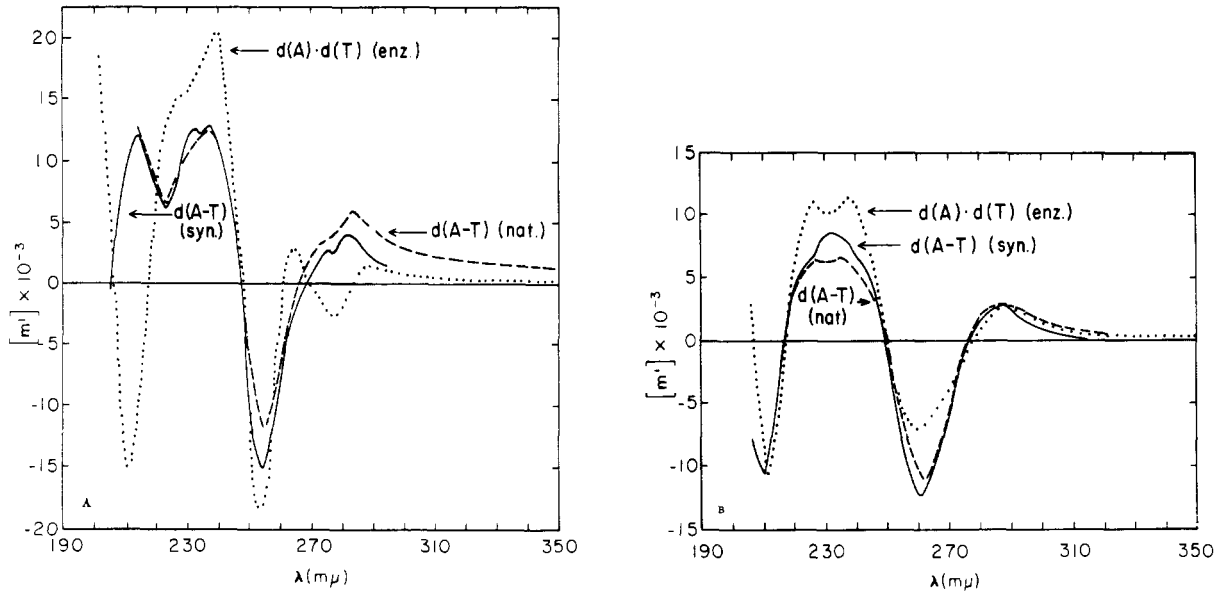


FIGURE 13: Comparison of the ORD curves of synthetic $d(A\text{-}T)_n$ (· · · ·), natural $d(A\text{-}T)_n$ (– – – –), and $d(A)_n \cdot d(T)_n$ (———) (enzymatic) in 0.05 M $NaClO_4$. (A) At 20°. (B) At 70° for both $d(A\text{-}T)_n$ and 77° for $d(A)_n \cdot d(T)_n$.

Monomers

Pyrimidine Nucleosides and Nucleotides. The existence of intramolecular hydrogen bonding between the 2-carbonyl group of the base and the 2′-hydroxyl group of the pentose is supported by the following evidence. (a) Ultraviolet spectrophotometric titration studies were done by Fox and co-workers (1952, 1957, 1958). (b) Research on rate of hydrolysis of different ribose dinucleotides and nucleic acids. Witzel (1963) concluded from his results that hydrogen bonding of the keto group in pyrimidines must take place in order to provide a mechanism for the observed hydrolytic reaction. (c) Recent proton magnetic resonance studies of the 5′ nucleotides done in our laboratory (M. P. Schweizer, A. D. Broom, D. P. Hollis, and P. O. P. Ts'o, to be published). We observe that the doubly ionized phosphate of the 5′ nucleotide exerts a specific deshielding effect on the H-6 proton. This effect is significantly reduced in the deoxynucleotide. We interpret this to mean that the hydrogen bonding of the carbonyl group and 2′-hydroxyl group provides a more favorable "*anti*" configuration for the phosphate to be in proximity of the H-6 proton of the ring. (d) Infrared studies on the nucleosides and derivatives in chloroform. Pitha *et al.* (1963) concluded that there is hydrogen bonding between the sugar OH group and the heterocyclic part of the nucleosides.

This intramolecular hydrogen bonding should have the following effects. (i) It should reduce the freedom of rotation between the glycosyl linkage of the N_1–C'_1 bond between the base and the pentose (Michelson, 1963). (ii) It should significantly hinder intermolecular hydrogen bond formation by the carbonyl group. (iii) The pK_a of the nucleosides and nucleotides should be affected. This has been found to be especially true for cytosine derivatives, in which a lowering of 0.15–0.2 pH unit for the pK_a values were found for the intramolecularly hydrogen bonded (ribose) compounds (Fox and Shugar, 1952; Fox *et al.*, 1957). In this case, the partial protonation at the carbonyl group by the intramolecular hydrogen bond can increase the positive charge of the ring through the readily delocalized electronic system. The pK_a of the uridine (9.25) is, however, quite close in comparison to that of deoxyuridine (9.30), both determined spectrophotometrically (Fox and Shugar, 1952). It is not immediately apparent why the difference is smaller than that found for the cytosine derivatives. The pK_a value of uridine determined titrimetrically was reported to be 9.17 by Levene and Bass (1931).

Purine Nucleosides and Nucleotides. The existence of intramolecular hydrogen bonding between the N-3 and the 2′-hydroxyl group is supported by the following evidence. (a) A comprehensive nmr study recently done in our laboratories (A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o, to be published) indicated that at infinite dilution in D_2O, 25°, the H-2 proton and the H-8 proton of deoxyadenosine and 2′-*O*-methyladenosine are upfield by 0.08–0.1 ppm over the H-2 and H-8 protons of adenosine. These data are interpreted to mean that the intramolecular hydrogen bonding of the 2′-hydroxyl group to the adenine N-3 shifts the protons of the heterocyclic ring downfield. This shift is similar to that observed upon protonation of adenine N-1 in acidic solution. (b) Spectrophotometric titration of the pK_a of guanosine and deoxyguanosine give values of 2.15 and 2.80, respectively (Grossman *et al.*, 1961). Grossman *et al.* interpreted these data as indicative of intramolecular hydrogen bonding of the 2-amino group of the base in the guanosine.

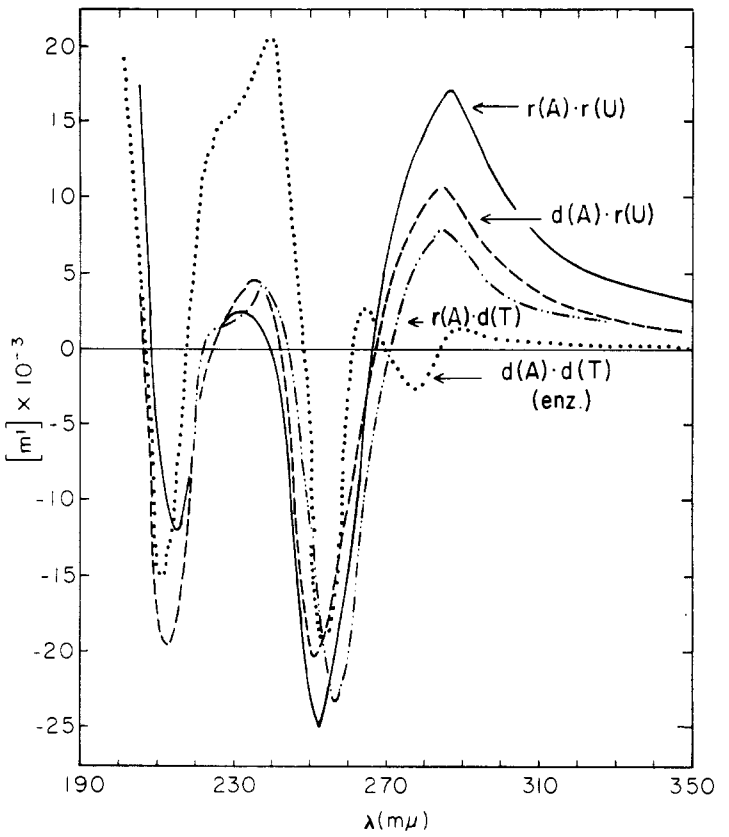


FIGURE 14: The ORD curves at 20° of $r(A)_n \cdot r(U)_n$ (———), $d(A)_n \cdot (U)_n$ (– – – –), $d(A)_n \cdot d(T)_n$ (· · · ·) (enzymatic), and $r(A)_n \cdot d(T)_n$ (– · · – · · –), at 0.05 M $NaClO_4$.

Their interpretation is based on the assumption that protonation of the base takes place at the 2-amino group. It is now known, however, that protonation of guanosine takes place at N-7 (Tsuboi *et al.*, 1962; Jones and Robins, 1963). It is difficult to see how hydrogen bonding with the 2-amino group could affect the basicity of N-7. On the other hand, hydrogen bonding of the 2′-hydroxyl group with N-3 can readily affect the N-7 nitrogen through the delocalized electronic system. This hydrogen bonding system, involving N-3, may still give the stereochemical hindrance observed for the formaldehyde reaction (Grossman *et al.*, 1961). Preliminary results (P. O. P. Ts'o, unpublished) on the potentiometric titration of adenosine and deoxyadenosine (0.05 M, in 0.15 NaCl at 25°), indicate that the pK_a of deoxyadenosine is about 0.1 pH unit higher than that of adenosine. This observation also supports the suggestion of intramolecular hydrogen bonding of adenosine. (c) Infrared studies on the adenosine derivatives in chloroform. Pitha *et al.* (1963) concluded that intramolecular hydrogen bonding occurs in adenosine as well as in pyrimidine nucleosides. An earlier review by Michelson (1961) mentioned unpublished infrared studies that indicate intramolecular hydrogen bonding of the 2′-OH group in adenosine derivatives. (d) Witzel (1963) also drew conclusions about hydrogen bonding between the 2′-OH group and the N-3 of purine. In support of his conclusion, he found that the absorption minimum of pA and pG is shifted to longer wavelength upon ionization of the 2′-OH group in the ribose (Witzel, 1960). He considered, however, this bond to be weaker in purine nucleosides than in pyrimidine nucleosides.

The net effect of this intramolecular hydrogen bonding in purine nucleosides should be similar to pyrimidine nucleosides, *i.e.*, reduction in freedom of rotation about the glycosyl linkage and lowering the pK_a of the bases. The N-3 position, however, is not expected to be involved in the intermolecular hydrogen bonding scheme and should produce no effect in this regard.

Homopolymers

The considerations on the intramolecular hydrogen bonding in the ribose mononucleotides mentioned above provides an introduction to our interpretation of the ORD results on the homopolymers.

$d(C)_n$ vs. $r(C)_n$. Three conclusions may be derived from the experimental observations. (1) In neutral or slightly alkaline medium (pH 8.4, 0.05 M sodium phosphate), $d(C)_n$ has much less stacking interaction than $r(C)_n$. This conclusion is arrived at as follows. (a) Between 23 and 90°, $r(C)_n$ exhibits 15–16% hyperchromicity and

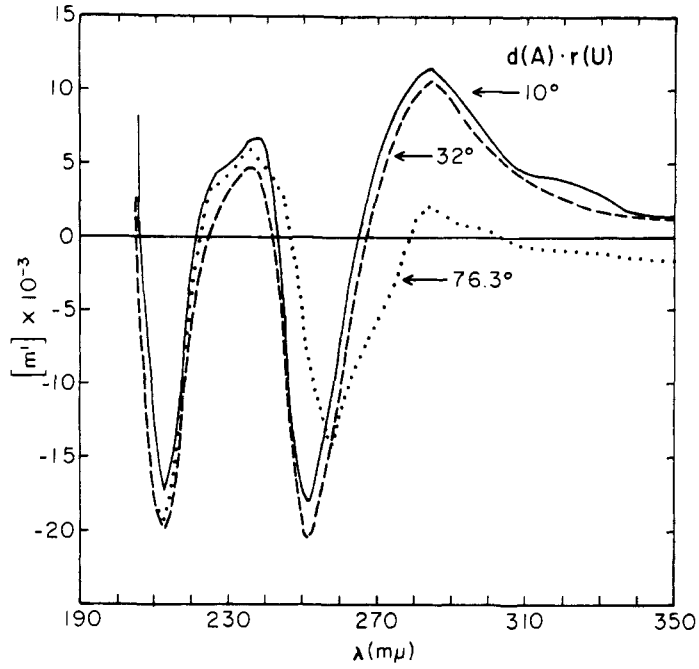


FIGURE 15: The ORD curve of $d(A)_n \cdot r(U)_n$ in 0.05 M $NaClO_4$ at 10 (———), 32 (– – – –), and 76.5° (· · · ·).

less than 1% was observed for $d(C)_n$ (see Results). The molar extinction coefficient of $d(C)_n$ is about 15% higher than that of the $r(C)_n$ at 23° (Table I). (b) The ORD curves of $d(C)_n$ and $r(C)_n$ are generally similar to each other, but the absolute rotation value of the $d(C)_n$ curve at 1° is about the same as that of the $r(C)_n$ at 82° (Figure 1A,B), indicating a higher degree of secondary structure arising from stacking interaction in $r(C)_n$. (2) In acidic solution $d(C)_n$ and $r(C)_n$ form similar structures, as indicated by identical ORD curves (Figures 1C and 14) and by a common requirement for protonation for helix formation. There is a major difference in the stabilities of the acid forms of $d(C)_n$ and $r(C)_n$. Titration studies at 25° have indicated that the transition pH of $d(C)_n$ in 0.05 M sodium ions is at pH 7.2 (Inman, 1964), and the transition pH of $r(C)_n$ in 0.1 M sodium ions is at pH 5.7 (Hartman and Rich, 1965). Thus, at room temperature helix formation in $r(C)_n$ requires more protons in solution (1.5 pH units) than does $d(C)_n$. (3) The hydrogen-binding scheme of the acid form of $r(C)_n$ has been shown to involve two pairs of interchain hydrogen bonds from the 2-carbonyl to 4-amino group, with a proton shared by two N-3 ring nitrogens from both chains (Akinrimisi *et al.*, 1963; Langridge and Rich, 1963; Hartman and Rich, 1965). The hydrogen bonding scheme may be the same for $d(C)_n$.

In neutral or slightly alkaline solution, the higher degree of secondary structure in $r(C)_n$ (discussion section) can be explained on the ground that the intramolecular hydrogen bonding of the 2′-hydroxyl group to the 2-carbonyl group greatly reduces rotational freedom around the N_1–C'_1 bond (discussion section). This may enhance the stacking of bases along the chain. The lower stability of the helix of $r(C)_n$ (discussion section) can be explained by the following reasons: (a) that the intramolecular hydrogen bonds greatly hinder participation of the 2-carbonyl group in interchain hydrogen bonding (discussion section), (b) and that the pK_a of the ribosyl cytosine group is lowered by intramolecular hydrogen bonding. (discussion section). Both of these effects will tend to lower the transition pH of $r(C)_n$ as compared to $d(C)_n$.

$d(T)_n$ vs. $r(U)_n$ and $r(T)_n$. The ORD patterns (Figure 2A,B) of the $d(T)_n$ and the $r(U)_n$ are much the same at room temperature and in the absence of Mg^{2+}. In the presence of Mg^{2+} ions (0.1–0.2 M) and at low temperature (0.10°) $r(U)_n$ acquires an ordered structure (Figure 2C) having a T_m of about 8° (Figure 3; Lipsett, 1960; Shugar and Szer, 1962). $r(T)_n$ in 0.01 M $MgCl_2$ was found to have a T_m of 36° (Shugar and Szer, 1962) and the higher T_m can be explained generally on the basis of increase of hydrophobic stacking interaction since thymidine associates to a greater extent than uridine in water (Ts'o *et al.*, 1962; Solie, 1965). On the other hand, both optical density *vs.* temperature profile (Figure 3) and the ORD patterns *vs.* temperature studies (Figure 2D) indicate that $d(T)_n$ has very little stacking interaction and secondary structure even at 1°, 0.02 M Mg^{2+}. It appears that the presence of 2′-hydroxyl groups in the polymer contributes a major stabilizing influence on secondary structure. This effect is opposite to the effect observed in $r(C)_n$, where the presence of 2′-hydroxyl groups destabilizes the helical structure of $r(C)_n$.

The hydrogen bonding scheme of the ordered forms of $r(U)_n$ or $r(T)_n$ has not been established. The N-3

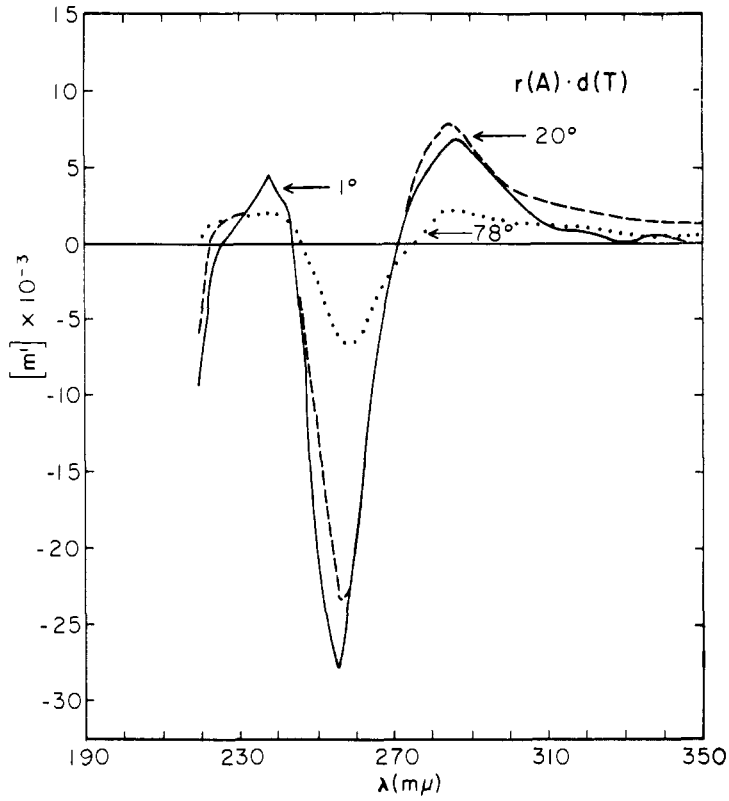


FIGURE 16: The ORD curves of $(A)_n \cdot d(T)_n$ in 0.05 M $NaClO_4$ at 1 (———), 20 (– – – –), and 78° (· · · ·).

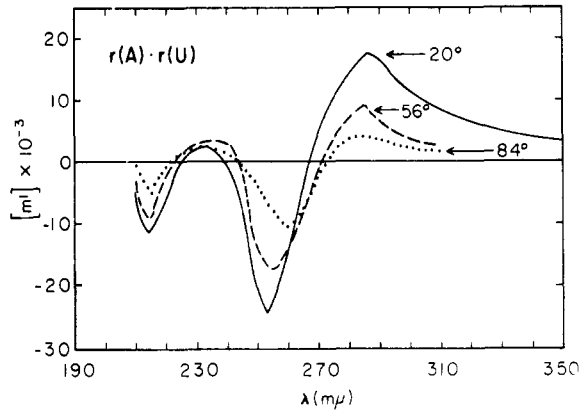


FIGURE 17: The ORD curves of $r(A)_n \cdot r(U)_n$ in 0.5 M $NaClO_4$: at 20 (———), 56 (– – – –), and 84° (· · · ·).

position may be involved since Szer and Shugar (1961) reported that N-3 methylation eliminates the secondary structure of $r(U)_n$. The most plausible hydrogen bonding scheme proposed (Donohue, 1956; Green *et al.* (1962) is a double-stranded helix with the two interchain hydrogen bonds formed between the N-3 (donor) from one chain and the O-4 (acceptor) from the other.

If this hydrogen bonding scheme for $r(U)_n$ or $r(T)_n$ is accepted then the effect of the 2′-hydroxyl group can be readily understood. Since the 2-carbonyl group does not participate in this hydrogen bonding scheme, the intramolecular hydrogen bonding of the carbonyl group to the 2-hydroxyl group does not hinder the helix formation (discussion section). A reduction of the rotational freedom along the axis of N_1–C'_1 bond (discussion section) may enhance base stacking and increase stability of the helix. Thus, we postulate that the helical form of $r(U)_n$ favors the hydrogen bonding of the 2′-hydroxyl group to the 2-keto group more than the coil form of $r(U)_n$, thereby gaining extra energy for stabilization. Therefore, the opposite effect of intramolecular hydrogen bonding on the stability of the helix or $r(C)_n$ *vs.* $d(C)_n$ is because here the 2-carbonyl group participates in the hydrogen bonding scheme of the helical $r(C)_n$. The requirement of protonation in the C polymers also contributes to this difference.

$d(A)_n$ vs. $r(A)_n$. The differences between these two polymers can be discussed in three different aspects. (a) At neutral pH $d(A)_n$ and $r(A)_n$ have the same ultraviolet hyperchromicity upon heating. This would seem to indicate that the two polymers have the same degree of stacking interaction. The ORD patterns of $d(A)_n$ and $r(A)_n$ are, however, vastly different. The prominent peak and trough in the region of 250–210 mμ of $r(A)_n$ (Figure 4A) is absent in the ORD pattern

of $d(A)_n$ (Figure 4B). According to exciton theory the rotational strength (R_k) is a trigonometric function of the angle between a given transition moment in one base and the corresponding moment in the neighboring base along the polymer. This angle is called $\sim$ in the notation of Van Holde *et al.* (1965) and Brahms *et al.* (1966) (denoted as $2\pi/P$ in the general theory of Bradley *et al.* (1963), where P is the number of residues per turn in a regular helix) in their calculation of the circular dichroism of the oligomers of riboadenylate. If α is 0 ($P = 1$, straight stack) or 180° ($P = 2$, alternating stack), the rotational strength arising from the nearest neighbor interaction is zero, and no optical activity will be observed. The difference in ORD of $r(A)_n$ and $d(A)_n$ at 240–310 mμ suggest that the angle between this transition moment must neither be 0 nor 180° in the case of $r(A)_n$ and very close to 0 or 180° in the case of $r(A)_n$. The blue shifts of the polymer absorption maximum, 257 mμ as compared to 259 mμ for the monomer, indicate that the bases of the $d(A)_n$ are probably in a straight stack ($\alpha = 0$). Since these two polymers are identical in their primary structures, except for the 2′-OH group, it appears that an intramolecular hydrogen bond from the 2′-OH to N-3 of adenine may cause the angle, α, formed between the neighboring bases in the stacks of $r(A)_n$ to be more oblique. It should be noted that the optical activity at 240–310 mμ of dA dimer (Figure 5 and 6) is not greatly different from rA dimer. If the above explanation is valid, then the parallelness of the base stacks must increase from dimer to polymer. (b) At acid pH the ORD $d(A)_n$ is similar to that of $r(A)_n$ indicating resemblance of over-all structure and intermolecular bonding in the helical form (Rich *et al.*, 1961). The magnitude of the peak and the trough of $d(A)_n$ is less than that of the $r(A)_n$ in full helical form (Figure 4C, results section). This information suggests a slightly different arrangement of the bases in the helical $d(A)_n$ as compared to $r(A)_n$.

The spectrum of protonated $d(A)_n$ (Figure 8–10) is greatly different from that of $r(A)_n$ (Helmkamp and Ts'o, 1962). Partial protonation (pH 5.0 or below) results in a 3-mμ bathochromic shift and a 17% increase in ϵ_{max} in $d(A)_n$ while $r(A)_n$ exhibits a hypsochromic shift of 4 mμ and a lowering of 18% in ϵ_{max}. ORD shows $d(A)_n$ to be in the helical form at this pH (comparison of Figure 7A,B with Figure 10). This remarkable difference between the spectra of the acidic forms of $r(A)_n$ and $d(A)_n$ suggests that all the protons may not all go to the N-1 position as generally expected for the $r(A)_n$. The ultraviolet spectrum of protonated, 3-substituted cyclic adenine nucleoside (2′,3′-*O*-isopropylidene-3,5′-adenosine cyclonucleoside *p*-tolysulfonate) has a λ_{max} of 272 mμ and ϵ_{max} 16.3×10^3 (Clark *et al.*, 1951) which is comparable to the spectrum of the protonated 3-ethyladenine, *i.e.*, λ_{max} 274 mμ and ϵ_{max} 18.4×10^3 (Lawley and Brookes, 1964), or the spectrum of protonated 3-methyladenine, *i.e.*, λ_{max} 274 mμ and ϵ_{max} 15.9×10^3 (Brookes and Lawley, 1960). On the other hand, the spectrum of the protonated adenine or adenosine is similar to the spectrum of the protonated 1-methyladenine which as a λ_{max} at 259 mμ and ϵ_{max} 11.7×10^3 (Brookes and Lawley, 1960), to the spectrum of protonated 1-methyladenosine which has a λ_{max} at 256.5 mμ and ϵ_{max} 13.6×10^3 (Jones and Robins, 1963), or to the spectrum of the protonated 1,9-dibenzyladenine which has a λ_{max} at 261.5 mμ and ϵ_{max} 14.5×10^{-3} in 0.1 N HCl–95% ethanol (Leonard *et al.*, 1965). This information supports the general notion that protonation of adenine and adenosine usually takes place at N-1. (It should be noted, however, that protonation of adenosine causes its λ_{max} to have a hysochromic shift of 2 mμ while the protonation of adenine causes its λ_{max} to have a bathochromic shift of 2 mμ. The explanation of this difference is not apparent at present.) But the red shift of λ_{max} and enhancement of ϵ_{max} in the spectrum of $d(A)_n$ upon protonation, together with the spectral information about the N-3 substituted adenines, suggest that some of the protons may go to N-3 instead of N-1 in the case of $d(A)_n$. The possibility of protonation at N-7 position is considered unlikely for the following two reasons. (i) If we accept the premise that the hydrogen bonding scheme of the $d(A)_n$ helix is essentially that of the $r(A)_n$ helix, then the N-7 is in the middle of the helix, hydrogen bonded to the 6-amino group. Protonation at this nitrogen will not allow the helix to form. (ii) Methylation studies show that the order of N-reactivity for adenine in deoxyadenylic acid is N-1 > N-7 > N-3, but for the adenine in DNA N-3 > N-1 > N-7 (Lawley and Brookes, 1963, 1964). This is an indication that reactivity of various nitrogen atoms can be influenced greatly by the conformation of the polymer. In summary, though at present we cannot quantitatively assess the proportion of the protons going to the N-3 *vs.* that going to N-1 in $d(A)_n$, all this information thus indicates that upon partial protonation the proportion of the protons going to N-3 in $d(A)_n$ is higher than that in $r(A)_n$.

When the pH of the solution is lowered further the spectrum exhibits a hypsochromic shift of λ_{max} and lowering of ϵ_{max}. The ORD pattern is essentially unchanged. Although there is aggregation at this pH we do not believe these observations to be optical artifacts of aggregation (see Results). If our reasoning to this point is valid, then this is an indication that upon further increase in (H^+) more protons now go to the N-1 position of $d(A)_n$. The reason for this is not immediately apparent.

Why is it that during early stages of protonation the proton tends to go to N-3 in the $d(A)_n$ helix, but go to N-1 in the $r(A)_n$ helix? We propose that in the $r(A)_n$ helix there is a degree of hydrogen bonding between N-3 and the 2′-OH which cannot occur in the $d(A)_n$ helix. Support for this notion may be taken from alkylation studies on nucleic acids (Lawley and Brookes, 1963). No 3-methyladenine is found in RNA after the reaction (most of the alkylation takes place at N-1), but 3-methyladenine is the *major* product from the alkylation of native DNA, and is a *minor* product for heat-denatured DNA.

(c) The pH for the helix–coil transition at 20°

examined by ORD indicates that in 0.001 M salt this transition is at pH 5.3 and in 0.22 M salt it is at pH 4.4 (Figure 7A,B). Similar results are also obtained from the ultraviolet spectrum (Figures 9 and 10). The effect of salt on the pH transition for $d(A)_n$ is similar to $r(A)_n$ as studied by potentiometric titration. This confirms the suggestion that the over-all structure of the two helices are the same. The transition pH of $r(A)_n$ in 0.001 M KCl at 20° is 6.8 and in 0.15 M KCl it is 6.0 (D. M. Holcomb, unpublished results). In 0.01 M KCl at 26° Steiner and Beers (1957) found 6.4 and in 0.1 M KCl at 26° it is 6.0. Under similar conditions $d(A)_n$ apparently requires a pH about 1.5 units lower than that required by the $r(A)_n$ to go into the helical form. The greater number of protons required for the formation of $d(A)_n$ helix indicates that the $r(A)_n$ helix is more stable. The greater stability of the $r(A)_n$ helix may be ascribed to the intramolecular hydrogen bonding of the 2'-OH group to the base.

The ϵ_{max} of $d(A)_n$ at neutral pH reported in this paper (10×10^3) is at variance with the value of 8.6×10^3 reported by Chamberlin (1965) and 8.7×10^3 reported by Bollum *et al.* (1964). The source of these descrepancies is not yet certain. The complex spectral properties of $d(A)_n$ were overlooked in the original study claiming observation of the acid form of $d(A)_n$ (Bollum *et al.*, 1964).

Homopolymer Complexes and Alternating Copolymer Complexes

Comparison of the temperature effect on the ORD curves of *natural* $d(A\text{–}T)_n$ and *synthetic* $d(A\text{–}T)_n$ reveals an interesting detail. Around the 285-mμ peak, rotation of the *natural* $d(A\text{–}T)_n$ is not affected by temperature until T_m is reached (Figure 12C). Rotation of the *synthetic* $d(A\text{–}T)_n$ is low (only 60% of the rotation of *natural* $d(A\text{–}T)_n$) but increases with temperature up to level of natural $r(A\text{–}T)_n$ until the T_m is reached (Figure 12C). The *natural* $d(A\text{–}T)_n$ was isolated by means of Hg complexing without the use of heating and has been shown to be a straight-chain double helix with no branching or hair-pin structures (Davidson *et al.*, 1965). This polymer also contains 3% G–C in its base composition which, however, is unlikely to have an effect on the secondary structure noticeably by ORD. The *synthetic* $d(A\text{–}T)_n$, on the other hand, has been shown to have branches and hair-pin-like structures resulting from base pairing within a single chain (Inman and Baldwin, 1962; Inman *et al.*, 1965). It is tempting to attribute the difference in temperature response (below 60°) of the 285-mμ peak of these polymers to the dissimilarity in structure as discussed above. Increase of rotation in the visual region of thymus DNA before melting had been previously observed (Doty *et al.*, 1959; Helmkamp and Ts'o, 1961). This may be related to renaturation of local denaturation in the DNA. It would be of interest to study this property of *synthetic* $d(A\text{–}T)_n$ after annealing the polymer about 10° below T_m. If the above argument turns out to be valid then the troughs, the second and third peaks of the ORD curves, are not affected by the branching and hair-pin structures.

Recent studies indicated that in a 1:1 mixture of $d(A)_n$ and $d(T)_n$, a 1:1 double helix is formed (Chamberlin, 1965; Bollum, private communication, 1965). The ORD curves of $d(A)_n \cdot d(T)_n$ and the synthetic $d(A\text{–}T)_n$ at temperatures above their T_m (around 70°) show much similarity. The ORD curves of these two kinds of polymers are distinctly different at room temperature (Figure 13A). This implies that the helical structures of $d(A\text{–}T)_n$ and of $d(A)_n \cdot d(T)_n$ are not the same. Recent studies by X-ray diffraction on fibers indicate that the structure of the synthetic $d(A\text{–}T)_n$ is similar to that of DNA (Davies and Baldwin, 1963) but the structure of the $d(A)_n \cdot d(T)_n$ is unlike that of DNA (Langridge, 1966). The T_m of $d(A\text{–}T)_n$ is 5° lower than $d(A)_n \cdot d(T)_n$ and buoyant density of the alternating copolymer is greater by 0.04 g ml^{-1} (Bollum, 1966; F. J. Bollum, private communication). However, the difference in ORD may also be due to the dissimilarity of the exciton splitting of the absorption bands because of the difference in the sequence of the constituent chains. At present, these two possible interpretations cannot be clearly evaluated and determined.

The differences between the ORD curves of DNA and RNA was first reported by Samejima and Yang (1964). They stated " . . . . ., the second peak of DNA is always much stronger than its first peak, whereas the opposite is true for RNA . . . . ." (Samejima and Yang, 1965). This observation is supported by circular dichroism measurements (Brahms and Mommaerts, 1964). We have reexamined this problem under model conditions by comparing the ORD of the helices formed by 1:1 mixture of the homopolymers of $r(A)_n$, $d(A)_n$, $r(U)_n$, and $d(T)_n$ (Figure 14). The sequence the composition (except T and U) of all the helices formed by these pairs are all alike. The only difference is the presence of the 2'-OH. In 0.05 M sodium ions and all these pairs form a 1:1 complexes (Bollum, 1966; Chamberlin, 1965; Stevens and Felsenfeld, 1964) except the mixture of $d(A)_n$ and $r(U)_n$ mixture which may be $d(A)_n \cdot 2r(U)_n$ (Chamberlin, 1965; Riley *et al.*, 1966). Thus, the ORD of the 1:1 mixture of $d(A)_n$ and $r(U)_n$ cannot be interpreted without reservation. The ORD of $d(A)_n \cdot d(T)_n$ resembles that of DNA in having a second peak much greater than the first. The ORD curve of $r(A)_n \cdot r(U)_n$ resembles that of the RNA, with the second peak much less than the first (Figure 14). The 1:1 mixture of $r(A)_n \cdot d(T)_n$ and $d(A)_n$ brings an increase in the first peak as compared to the original polydeoxynucleotide complex and decrease in the second peak as compared to original polyribonucleotide complex, resulting in the contribution from both peaks being nearly equivalent. This phenomenon probably cannot be due to a difference in the optical contribution of ribose and deoxyribose since essentially identical ORD curves can be obtained from helical $d(C)_n$ and $r(C)_n$ (Figure 1C,D) as well as from helical $d(A)_n$ and $r(A)_n$ (Figure 4C). We feel that these substantial differences in ORD pattern are a clear reflection of different helical structures, because here sequence and base composition are identi-

cal. It may be noted also that the hyperchromicity of the melting of all these four pairs are about the same, 40–50% (Results). Following the previous argument, presented in the discussion on $r(A)_n$ and $d(A)_n$, we venture to speculate that the angle, α, between the transition moments of the neighboring bases in the deoxyribosyl helix is closer to 0 or π as compared to the α of the ribosyl helix which is more away from 0 or π. In other words, the stack of the bases of the ribosyl helix is more oblique and the stack of the deoxyribosyl helix is more parallel. Further advances in X-ray diffraction studies (Langridge, 1966; Sasisekharan and Sigler, 1965; Sato *et al.*, 1966) may provide more knowledge about these structural differences. It would also be useful to compare the circular dichroism of these polymers.

The thermal stability of the four pairs does not correlate in any simple way with the ORD patterns. The T_m of $d(A)_n \cdot d(T)_n$ (61.5°) and the T_m of $r(A)_n \cdot d(T)_n$ (59°) are close, but their ORD patterns before melting are quite different. It is interesting to note that the melting of $r(A)_n \cdot d(T)_n$ (Figure 16) causes a large reduction (80%) at the trough and a slight red shift (5 mμ) of the minimum. This is similar to the melting of $r(A)_n \cdot r(U)_n$ (Figure 17). The melting of $d(A)_n \cdot r(U)_n$ (Figure 15) causes a smaller reduction (30–50%) at the trough and a much greater red shift (9 mμ) of the minimum, similar to the melting of $d(A)_n \cdot d(T)_n$ (Figure 11A) complexes. The influence of the methyl group on the stability of these complexes is clearly indicated. The exchange of $d(T)_n$ with $r(U)_n$ brings about a substantial lowering in T_m regardless of the partner chain in the complex. This was demonstrated directly by Shugar and Szer (1962) by showing that T_m of $r(A)_n \cdot r(T)_n$ is 20° higher than $r(A)_n \cdot r(U)_n$. Differences in stability of the helices of I and C in ribose and deoxyribose polymers have also been reported (Chamberlin and Patterson, 1965).

Speculation about the role of the 2′-hydroxyl group in hydrogen bonding to the oxygen atom of the adjacent phosphate group has been advanced by crystallographers (Spencer *et al.*, 1962; Langridge and Gamatos, 1963). This type of bonding is of importance in the mechanism of hydrolysis (formation of the cyclic nucleotide intermediates) of RNA (Witzel, 1963; Brown and Todd, 1953). Infrared circular dichroism studies (Sato *et al.*, 1966) suggest that the orientation of the PO_2 group and perhaps even the mode and the strength of interaction of the PO_2 group in RNA and $r(A)_n \cdot 2(U)_n$ is quite different from that in DNA.

In conclusion, the polydeoxyribonucleotides and the polyribonucleotides do have markedly different properties and conformation. We propose that intramolecular hydrogen bonding of the 2′-OH group to the 2-keto of pyrimidines and to the N-3 of the adenine may take place in the ribopolymers. Most of the differences in the physical properties of these two types of polymer can be explained on the basis of this proposal.

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