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Conformation of Oligoinosinates: Chain-Length Dependence and Comparison to Other Oligonucleotides†

Setsuko Tazawa, Ichiro Tazawa, James L. Alderfer, and Paul O. P. Ts'o*

ABSTRACT: The oligomer series of ribosyl inosinates [$(Ip)_{2-14}I$] have been prepared by alkaline hydrolysis of poly(I) and were carefully identified. The optical properties, ultraviolet absorption and circular dichroism (CD), of this series have been studied. These studies were complemented by pmr studies of IpI and $(Ip)_2I$. In addition, using pig liver nuclei ribonuclease, pIpI and $p(Ip)_2I$ were also prepared, carefully characterized, and studied by the above techniques. The CD spectra of oligoinosinates were found to be *strongly* dependent on temperature and salt concentration. These results indicate the sensitivity of CD to electrostatic perturbations exerted on the conformation by the phosphate groups. The proton magnetic resonance (pmr) results indicate that these oligomers have an all anti conformation and the screw axis of the stack is most likely right handed. In contrast to CD, the pmr results show essentially no salt dependence, indicating that all the oligomers have similar conformational properties (*i.e.*, all anti). The uv and CD properties of the oligo(I) series were analyzed together with the published data of rA, dA, dT, rU, rC, and dC oligomers. ϵ *vs.* $1/n$ plots produce two groups: those yielding a straight line (dA, dT, rU) and those having a nonlinear curve (rI, rA, rC, dC). The extinction coefficient of the interior bases (ϵ_{int}) can be deduced for oligomers longer than dimer. Oligomers of dA, dT, and rU have ϵ_{int} which are independent of chain length (n). This observation implies that the nearest-neighbor interaction is the only significant factor in hypochromicity. The chain-length effect on CD spectral parameters is different for each polynucleotide: (1) rA, $[\theta]_{peak}$ and $[\theta]_{trough}$ both increase with n (conservative type); (2) rC, $[\theta]_{trough}$ remains essentially constant with n but $[\theta]_{peak}$ increases with n; (3) dA, $[\theta]_{trough}$ remains essentially constant with n but $[\theta]_{peak}$ decreases with n; (4) rI, $[\theta]_{trough}$ remains essentially constant with n, but $[\theta]_{peak}$ decreases at first and then increases with increasing n; (5) rU, the optical activity is not dependent on n as expected for a nonstacking coil. The conservative CD spectrum derived from base–base interactions is found to be the exception rather than the rule. Also, the chain length necessary for attaining the same physical parameter as the polymer is shorter for uv hypochromicity than for CD spectral parameters.

Much effort, including that from our laboratory, has been devoted to the research on monomeric units of nucleic acids—bases, nucleosides, and nucleotides (see review, Ts'o, 1970). Similarly, dinucleoside monophosphates and dinucleotides have been extensively studied (Brahms *et al.*, 1966, 1967; Davis and Tinoco, 1968; Ts'o *et al.*, 1969a; Chan and Nelson, 1969; Warshaw and Cantor, 1970; Kondo *et al.*, 1970; Tazawa *et al.*, 1970; Fang *et al.*, 1971; Miller *et al.*, 1971; and references cited therein). With this valuable information as backbround, our laboratory has launched a systematic investigation on the properties of oligonucleotides. The first paper in this series concerns the thermodynamic and optical properties of the 1:1 oligoinosinate–polycytidylate complexes (Tazawa *et al.*, 1972).

In this study, the oligomer series of ribosyl inosinate (r-$(I)_{2-14}$) have been carefully prepared and identified. The optical properties—both ultraviolet (uv) absorbance and circular dichroic (CD) spectra—of the oligo(rI) were investigated, and the results complemented by comprehensive proton magnetic resonance (pmr) studies on the dimer and trimer of inosinate. Reports in the literature about the optical properties of oligo(rA), oligo(dA), oligo(rC), oligo(dC), oligo(rU), and oligo(dT) have been analyzed in comparison to those observed in the present studies on oligo(rI).

Experimental Section

Materials. The sources of the chemicals and enzymes used are: poly(I), IDP, and UDP, Miles Laboratory, Elkhart, Ind.; [^{14}C]IDP and [^{3}H]UDP, Schwarz BioResearch, Orangeburg, N. Y.; *Micrococcus luteus* polynucleotide phosphorylase (EC 2.7.7.8), P-L Biochemicals, Inc., Milwaukee, Wis.; pancreatic RNase A (EC 2.7.7.16), Sigma Chemical Co., St. Louis, Mo.; *Escherichia coli* alkaline phosphatase (EC 3.1.3.1), spleen phosphodiesterase (EC 3.1.4.1), and venom phosphodiesterase (EC 3.1.4.1), Worthington Biochemical Corp., Freehold, N. J. The maximum molar extinction coefficient

† From the Department of Radiological Science, The Johns Hopkins University, Baltimore, Maryland 21205. *Received April 21, 1972.* This work was supported in part by a grant from the National Institutes of Health (GM-16066-04), a grant from the National Science Foundation (GB-30725X), and a fellowship from the Jane Coffin Childs Memorial Fund for Medical Research (J. L. A.). This is paper No. 2 in a series entitled: Studies on Oligonucleotides. This paper was presented in part at the 162nd National Meeting of the American Chemical Society, Washington, D. C., 1971.

of poly(I) at pH 6.0, 0.005 M NaOAc, was determined to be 10.1×10^3.

Instrumentation. A Cary 60 spectropolarimeter equipped with the 6001 circular dichroism unit was used for CD studies. A water-jacketed fused quartz window cell of 1-cm path length (Optical Cell Co., Beltsville, Md) was connected to a Haake Brinkmann Model KT-62 constant-temperature circulator. The average temperature of the cell entrance and exit (1–2° difference) was determined. The ultraviolet spectra were obtained on a Cary 15 spectrophotometer. The temperature was controlled by the same unit described above, and measured with a YSI Model 42SC Tele-Thermometer connected to a type 421 probe thermister in contact with the cell. Varian HR-220 and HA-100 nuclear magnetic resonance (nmr) spectrometers were used for pmr studies. A Varian C-1024 computer of average transients was employed to increase the signal-to-noise ratio of the HA-100 instrument. The temperature was regulated by a Varian V-6507 variable-temperature accessory and monitored by methanol and ethylene glycol spectra.

Methods. Optical studies were made at concentrations of 1–2 ODU at 248 nm (about 0.1 mM). Proton magnetic resonance samples were repeatedly dissolved and lyophilized several times in D_2O for exchange purposes; pD was adjusted by 1 M DCl or NaOD. For studies in 1 M NaCl, weighed solid NaCl was added directly to the nmr sample tube, followed by redetermination of pD and concentration.

In both analytical and preparative paper chromatography, the descending technique was used with either Whatman No. 1 or 3MM paper. The solvent systems used were (A) 1-propanol–concentrated ammonia–water (55:10:35, v/v), (B) ethanol–1 M ammonium acetate (pH 7.5) (1:1, v/v), and (C) isobutyric acid–1 M NH_4OH–0.1 M Na_2EDTA (100:60:1.6, v/v).

Maximal extinction coefficients of the oligo(I) were determined by the following procedure of alkaline hydrolysis. Absorbance of oligo(I) (~1 ODU) in NaOAc (0.005 M, pH 6.0) was measured precisely by a Cary 15. This solution (0.7 ml) was weighed, and after addition of 0.3 ml of 1 N NaOH, this sample was incubated at 37° (21 hr). To this solution, 2 N acetic acid (0.2 ml) was added and the total weight of the solution and its absorbance were redetermined. The volumes of the solution before and after hydrolysis to monomer were accurately determined from the weights and the densities of the solution. These data, together with the absorbance measured before and after hydrolysis and the known extinction of the monomer (ϵ_{max}^{248} 12,200), permit the determination of the extinction coefficient of the oligomer in question.

Preparation of Oligo(I). Oligo(I) was prepared by limited alkaline hydrolysis of poly(I), followed by column chromatography. Oligomer chain length was controlled by varying reaction conditions. A preparation yielding heptamer as major product is described. Poly(I) (K^+, 0.5 mmole in P) was dissolved in 0.1 N NaOH (25 ml), incubated at 37° (1 hr), then acidified to pH 1 with HCl (1 N) and left standing at room temperature (4 hr) to cleave 2′, 3′-cyclic phosphate at the 3′ end of oligomers. NH_4OH (concentrated) was added to give pH 8, followed with 3 mg of *E. coli* alkaline phosphatase and incubated at 37° (9.5 hr) to remove terminal 2′- or 3′-phosphate groups. The reaction mixture was then applied to a DEAE-cellulose column (acetate, 2.5 × 75 cm) and eluted by triethylammonium acetate (linear gradient, 0–1.0 M; pH 6.5) in 7 M urea. Fractions of a peak were combined, diluted with a large volume of water and reapplied to a small DEAE-cellulose column (HCO_3^-). This column was washed extensively to remove the urea, and the oligomers were reeluted with triethylammonium bicarbonate buffer (pH 7.5). The buffer was removed by repeated addition and evaporation of water. The residue was dissolved in water (about 1 ml) and passed through a Sephadex G-15 or G-25 column to remove a yellowish contaminant often encountered. Appropriate fractions were combined and lyophilized. The whitish final product was dissolved in water and stored at −70°.

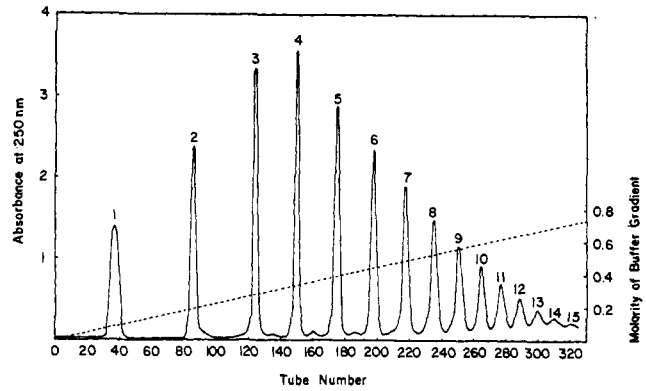

FIGURE 1: DEAE-cellulose elution profile of oligo(I). Linear gradient elution with 0–1 M triethylammonium acetate buffer (pH 6.5) in 7 M urea.

Figure 1 shows the elution profile from the DEAE-cellulose column of a reaction mixture yielding tetramer (I_4) as the major product. Oligomers up to I_{12} were well separated. In another preparation which yielded I_{12} as the major product, I_{15} was obtained with the same resolution as I_{12} shown in Figure 1.

Structure Determination of Oligo(I). From the procedure of preparation described above (alkaline hydrolysis, mild acid, and phosphatase treatments), the materials in the eluted peaks should be a series of oligoinosinates, $(Ip)_nI$. Peak 1 was readily shown to be inosine by paper chromatography and uv spectrum. All other peaks were completely degraded by both spleen and venom phosphodiesterase to give inosine and inosinic acid, indicating that these materials are linear oligoinosinates without terminal or cyclic phosphate groups. The oligomers in peaks 2–8 were shown unambiguously to be dimer to octomer by various techniques, as described in Table I. The chain length of the oligomers in peak 9 and peaks of progressively higher number were safely assigned to be 9, 10, 11, and so on. The R_F values of oligo(I) in paper chromatography are listed in Table II.

In addition to evidence presented in Table I, the chain-length assignments of dimer to pentamer were reconfirmed by another procedure. Dimer, trimer, tetramer, and pentamer were synthesized by another route as outlined in Scheme I. [^{14}C]IDP (617 μmoles) and [^{3}H]UDP (61 μmoles) were copolymerized by polynucleotide phosphorylase (2 mg/ml) in a reaction mixture (16.5 ml) containing Tris-HCl (pH 8.2,

SCHEME I

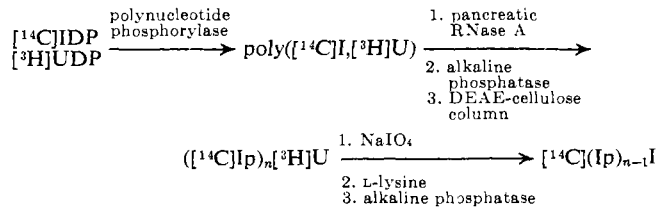

TABLE I: Oligoinosinate Structural Assignments to Column Chromatographic (Figure 1) Peak Positions.

Figure 1 Peak Position	Inosinate:Inosine from Hydrolysis[a]	Other Procedure for Assignment	Chain-Length Assignment
2	1.05 (spleen phosphodiesterase)[b] 1.03 (venom phosphodiesterase)[b]	Identical with IpI synthesized chemically from ApA[d]	IpI
3	1.83 (KOH)[c]		$(Ip)_2I$
4		Starting oligomer, I_3, I_2, pI, and I were formed after limited hydrolysis by spleen phosphodiesterase[e]	$(Ip)_3I$
5		Identical with I_5 synthesized from I_5U ([^{14}C]I,[^{3}H]U)[f]	$(Ip)_4I$
6	5.33 (KOH)[c]	Complete phosphorolysis with polynucleotide phosphorylase gave I_2 and IDP in 1:4.2 ratio[g]	$(Ip)_5I$
7	6.01 (KOH)[c]		$(Ip)_6I$
8		I_5 was obtained after three successive removals of the 3′-terminal residue[h]	$(Ip)_7I$

[a] After hydrolysis, the products were applied to paper chromatography (mostly solvent B). Only two spots of inosine and inosinic acid were observed in all cases. These spots and appropriate blanks were cut and extracted with water, and their uv spectra were measured to yield the inosine:inosinate ratio. [b] Conditions are the same as those previously published (Tazawa *et al.*, 1970). [c] Incubation is at 37° (24 hr) in KOH (0.3 N). [d] IpI was synthesized from ApA by HNO_2 deamination. ApA (free acid, *ca.* 12 mg, OD (260 nm) = 560) was suspended in 2 N acetic acid (1 ml) with the addition of $NaNO_2$ (180 mg) in water. The clear, viscous solution was stirred in the dark for 4 hr and was diluted with water. The nucleotidic materials were first adsorbed and then eluted from charcoal for the removal of inorganic salt. The eluate was concentrated, and chromatographed on Whatman No. 3MM paper. Development in 2-propanol–concentrated ammonia–water (7:1:2, v/v) revealed two bands. The major band (70% yield, OD (248.5 nm) = 336) was shown to be IpI by its: (1) uv spectrum ($\lambda_{max}^{pH\ 6.0}$, 248.5 nm); (2) mobility in paper electrophoresis (0.45 relative to pI, 0.05 M triethylammonium bicarbonate buffer, pH 7.5); (3) enzymic degradation products (both spleen and venom phosphodiesterase treatment yielded inosine and inosinate in equal amounts). The minor band was not identified but is likely to be $I_{2'}p_{5'}I$. [e] Oligomer in peak 4 (OD (248.5 nm) ≈ 2) dissolved in 40 ml of water and 5 μl of NH_4OAc (1 M, pH 6.5) was incubated with spleen phosphodiesterase (0.04 unit) at 37° (2 min). The products developed in paper chromatography (solvent B) revealed spots corresponding to inosine, pI, IpI, and IpIpI in addition to the starting material. [f] See Experimental Section for the synthesis of $(Ip)_5U$ by degradation of poly([^{14}C]I,[^{3}H]U). [g] Oligomer from peak 6 (OD (248.5 nm) = 4) in 0.4 ml of solution containing Tris-HCl (0.1 M, pH 8.2), $MgCl_2$ (5 mM), Na_2EDTA (0.3 mM), and K_2HPO_4 (10 mM) was incubated at 37° (4 hr) with polynucleotide phosphorylase (type 15, 0.4 unit). The hydrolytic products analyzed by paper chromatography (solvent A) revealed the presence of IpI and IDP in 1:4.2 ratio. [h] Oligomer in peak 8 was treated successively with $NaIO_4$, L-lysine, and alkaline phosphatase according to a procedure for the removal of the terminal nucleotide (Neu and Heppel, 1964). Analysis of the products by paper chromatography (solvent A) shows the presence of hypoxanthine and the slow-moving oligoinosinate. The oligomer $(I)_{n-1}$ was then eluted and treated again for the removal of the second residue. The resulting oligomer $(I)_{n-2}$ was reisolated again and treated for the third time. The product $(I)_{n-3}$ was shown to be I_5 by paper chromatography; therefore, the original oligomer, $(I)_n$, in peak 8 is proven to be I_8 by this procedure.

0.15 M), $MgCl_2$ (10 mM), and EDTA (0.4 mM). After incubation at 37° (4.5 hr), the reaction mixture was chilled. Cold water (3.5 ml), 5% sodium dodecyl sulfate (2 ml), and 10% phenol (2 ml) were successively added. The mixture was shaken for 10 min and phenol was added (4.5 g) with continued shaking of the mixture. Following separation (by centrifugation) and isolation of the aqueous layer, phenol (5 g) was again added with more shaking of the mixture. On reisolating the aqueous layer, 0.01 M NaOAc (pH 5, 5.5 ml), absolute ethanol (100 ml), and cold acetone (100 ml) were sequentially added. A white precipitate was then collected by filtration and dissolved in water (~50 ml). This solution was dialyzed at 5° against 0.05 M NaCl + 5 mM EDTA, 5 mM NaCl + 0.5 mM EDTA, and finally against water. The yield of the polymer was about 2600 ODU (249 nm). The poly([^{14}C]I[^{3}H]U) (86 ODU) was then hydrolyzed by RNase A (50 μg) and alkaline phosphatase (200 μg) in 0.05 M Tris-HCl (pH 7.5, 0.5 ml) containing EDTA (1 mM). After incubation at 37° (3 hr), the mixture was diluted to 5 ml with water and treated with 5% sodium dodecyl sulfate, 10% phenol, and solid phenol, as described above. Applying the aqueous layer to a DEAE-cellulose column (HCO_3^-, 1.5 × 54 cm) and then eluting from the column (linear gradient, 0–0.8 M triethylammonium bicarbonate; pH 7.5), a series of well-resolved peaks were obtained. The I:U ratio of these peaks was determined from the ^{14}C and ^{3}H radioactivities and the specific activities of [^{14}C]IDP and [^{3}H]UDP used for the polymerization. With this information and the known hydrolytic specificity of RNase A, the structure of the oligomer $(Ip)_nU$ in these peaks can be assigned. Peak 1 was found

TABLE II: Paper Chromatographic Properties of Oligoinosinates.[a]

Compound	Solvent A		Solvent B		Solvent C	
	R_F	R_{I_4}	R_F	R_{pI}	R_F	R_{I_5}
I	0.54		0.67		0.55	
pI	0.27		0.40	1.00	0.29	
I_2	0.35		0.48	1.20	0.29	
I_3	0.23		0.34	0.86	0.14	
I_4	0.13	1.00	0.21	0.51	0.07	
I_5	0.07	0.54		0.26	0.03	1.00
I_6	0.04	0.31		0.12		0.58
I_7		0.22				0.31
I_8		0.12				0.14
I_9		0.07				0.09
I_{10}						0.05
I_{11}						0.03

[a] Descending technique was used with Whatman No. 1 paper. For composition of solvent systems, see Methods.

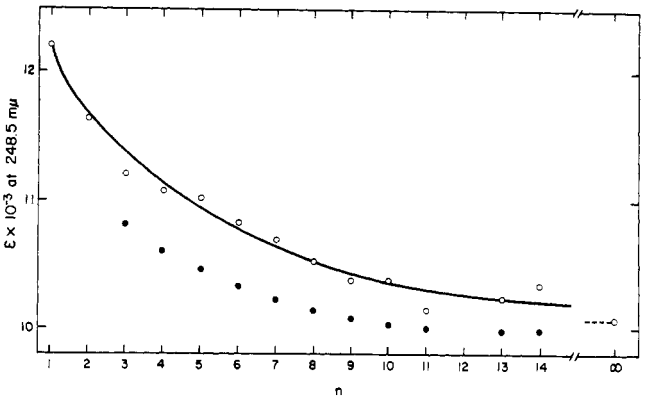

FIGURE 2: Molar extinction coefficients (O) of oligo(I) as a function of chain length, at 24°, 0.005 M NaOAc (pH 6.0). Solid line is the best-fit curve through experimental values. Solid circles are the calculated internal extinction coefficients using eq 2.

to be uridine. Peak 2 was assigned to be IpU (I:U = 1.04); peak 3, $(Ip)_2U$ (I:U = 2.13); peak 4, $(Ip)_3U$ (I:U = 3.17); peak 5, $(Ip)_4U$ (I:U = 4.21); peak 6, $(Ip)_5U$ (I:U = 4.74). The oligomers in peaks 2, 3, 4, and 6 were then treated with $NaIO_4$, L-lysine, and alkaline phosphatase in succession (Neu and Heppel, 1964) for the removal of the terminal uridylic acid residue. The R_F values of the oligomers in peaks 2, 3, 4, and 6 in solvent A were 0.36, 0.24, 0.14, and 0.04, respectively, before treatment; after treatment, they became 0.54, 0.34, 0.23, and 0.07, respectively, with uracil (R_F 0.59) consistently obtained as another reaction product. The R_F values of the oligomers in peaks 2, 3, 4, and 6 after treatment are identical to those of inosine, IpI, $(Ip)_2I$, and $(Ip)_4I$, respectively (Table II). This agreement indicates that the oligomer chain-length assignments of the alkaline hydrolysis preparation are indeed correct.

Preparation of $(pI)_2$ and $(pI)_3$. The 5′-phosphorylated dimer and trimer were prepared from hydrolysis of poly(I) using a pig liver nuclei ribonuclease. This enzyme was prepared and used according to the procedure of Heppel (1966). The incubation mixture was applied to Whatman No. 3 MM paper, and developed for about 18 hr in solvent A. Four bands were detected, and their respective R_F values (relative to pI as 1.00) are 0.83 (A band, unknown), 0.67 (B band, $(pI)_2$), 0.53 (C band, $(pI)_3$), and 0.36 (D band, $(pI)_4$). Materials in B band and C band were eluted from paper and treated with spleen phosphodiesterase to remove any contaminating oligomers not phosphorylated at the 5′ terminus. After treatment, material in B band was applied to a DEAE-cellulose column (bicarbonate, 1.5 × 51 cm), and eluted with ammonium bicarbonate buffer. From the two well-resolved peaks, the first peak was shown to be the desired product, $(pI)_2$. Material in C band was similarly purified from a DEAE-cellulose column which yielded a single peak, $(pI)_3$. Both dimer and trimer were further purified by passing through Sephadex G-50 and Chelex-100 columns.

The compound, $(pI)_2$, isolated from B band was shown to have an R_F value lower than that of IpI in paper chromatography in solvent A. Upon treatment of alkaline phosphatase, this compound yielded a product which now had an R_F value identical to IpI. Treatment of $(pI)_2$ with: (1) spleen phosphodiesterase had no effect on the R_F value as anticipated; (2) venom phosphodiesterase yielded a single spot in chromatography with the R_F value identical with that of 5′-IMP; and (3) alkaline phosphatase followed by venom phosphodiesterase gave two spots of equal uv absorbance having R_F values identical with those of inosine and 5′-IMP. These procedures fully established this dimer to be $(pI)_2$.

The compound, $(pI)_3$, eluted from C band had an R_F value lower than $(Ip)_2I$, and is converted by alkaline phosphatase treatment to a single compound with an R_F value identical to $(Ip)_2I$. Treatment of $(pI)_3$ with spleen and venom phosphodiesterase produced the same results described above for $(pI)_2$, but alkaline phosphatase followed by venom phosphodiesterase treatment yielded two spots with R_F values identical with those of inosine and 5′-IMP, and with a ratio of uv absorbance of 1:2, respectively. These characterizations also have firmly established the trimer to be $(pI)_3$.

Results and Discussion

Ultraviolet Absorbance Studies. The maximal molar extinction coefficients of oligo(I) (from IpI to $(Ip)_{13}I$) and poly(I) at room temperature in 0.005 M sodium acetate (pH 6.0) are shown in Figure 2. The smooth, decreasing values of ϵ_{max} of oligomers as a function of chain length can be further analyzed with respect to the nature of base–base interaction. We can formally classify the physicochemical studies on the changes in properties from an extension of dimer to oligomer series into two separate considerations: (1) the dependence of the measurable parameters on effects beyond the nearest-neighbor interaction—a consideration concerning the problem of measurement, and (2) the influence of chain length on the base stacking between the nearest neighbors—a consideration concerning the intrinsic properties of individual oligomers. For instance, if the hypochromic effect is derived only from the interaction of the nearest-neighbor chromophores and if the chain length of the oligomer does not enhance base stacking in a dimeric segment, we might expect to find a decrease in extinction coefficient ($\Delta\epsilon$) proportional to the number of pairs of residues. Such a relationship can be expressed as

$$\epsilon_n = (\epsilon_0 - \Delta\epsilon) + \Delta\epsilon/n \quad (1)$$

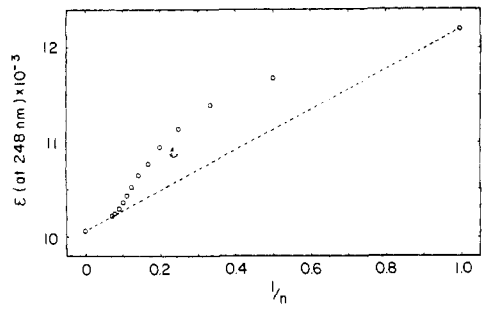

FIGURE 3: Molar extinction coefficients of oligo(I) as a function of inverse chain length. The plotted ϵ's are obtained from the best-fit curve in Figure 2.

where ϵ_0 is the extinction coefficient of the isolated residue (or the monomer), ϵ_n is the observed, mean residue extinction coefficient of the oligomer, and n is the chain length (Simpkins and Richards, 1967). A plot of ϵ_n *vs.* $1/n$ would be linear, with the slope equal to $\Delta\epsilon$ and with the intercept at $1/n = 0$ of $(\epsilon_0 - \Delta\epsilon)$, corresponding to the extinction coefficient of the polymer. Simpkins and Richards (1967) found that this equation described satisfactorily the extinction coefficient of oligo(U) ($(Up)_2$ to $(Up)_{11}$) and poly(U). In Figure 3, the plot of ϵ_n of oligo(I) *vs.* $1/n$ shows that the hypochromicity data of the oligo(I) does not follow all of the basic assumptions of this equation. These results indicate that the base–base interaction in the oligo(I) cannot be described only in terms of pairwise, nearest-neighbor interaction.

The hypochromicity data of the oligo(I) perhaps can be analyzed in another manner. We shall divide the bases in the oligomer into two types: those located externally (at the ends) and those located internally. We shall further assume that the extinction coefficient of the external bases at the end is the same as that of the dimer (ϵ_{dimer}). Thus

$$\epsilon_n = f_{ext}\epsilon_{dimer} + f_{int}\epsilon_{int} \qquad (2)$$

$$= 2/n\epsilon_{dimer} + (1 - 2/n)\epsilon_{int}$$

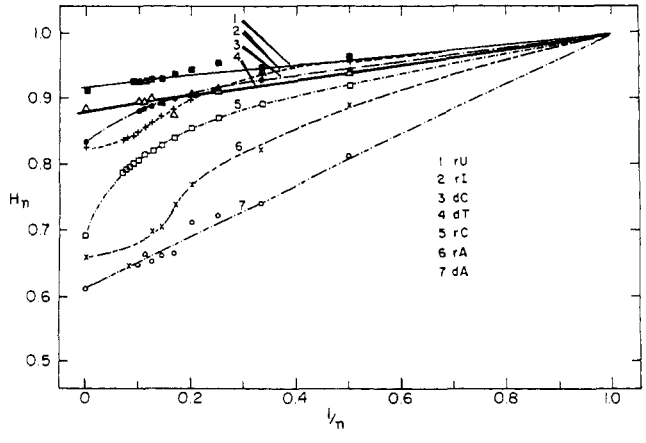

FIGURE 4: Monomer normalized ϵ_n as a function of inverse chain length. Data were obtained from the following sources. $(Ip)_{n-1}I$ (+): this work, conditions 0.005 M NaOAc, pH 6.0. $(Up)_n$ (■): Simpkins and Richards (1967), conditions 0.1 M NaCl + 0.02 M Tris, pH 7.0; $(pdA)_n$ (○) and $(pdT)_n$ (Δ): Cassani and Bollum (1969), conditions 1 mM Tris (pH 8.0). $(Cp)_{n-1}C$ (□) and $(dCp)_{n-1}dC$ (●): Adler *et al.* (1967), conditions 0.08 M NaCl + 0.02 M Tris (pH 8.5); $(Ap)_{n-1}A$ (×): Brahms *et al.* (1966), conditions 0.1 M NaCl–0.01 M Tris (pH 7.4).

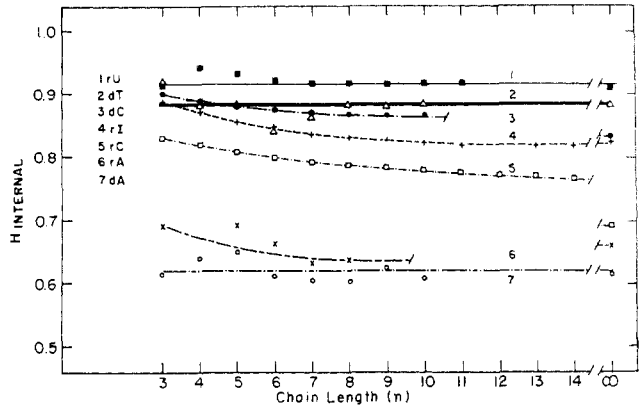

FIGURE 5: Monomer normalized $\epsilon_{internal}$ of oligomers and polymers as a function of chain length. Data and conditions are same as Figure 4.

where f_{ext} is the fraction of the external bases of the oligomer ($f_{ext} = 2/n$), and f_{int} is the fraction of the internal bases ($f_{int} = 1 - 2/n$). When the physicochemical measurements of the oligomer series are describable by the assumptions involved in eq 1, then these two equations (eq 1 and 2) are basically the same. However, when the data in a ϵ_n *vs.* $1/n$ plot cannot be fitted to a straight line demanded by eq 1, then the analysis using eq 2 graphically illustrates the dependence of the properties of internal bases as a function of chain length. As shown in Figure 2, when the chain length of the oligomers is as large as ~10 ($n \approx 10$), the internal bases of such an oligomer will have the same extinction coefficient (or same degree of hypochromicity) as the polymer which can be considered as containing only internal bases.

Comparative Ultraviolet Absorbance Studies—Oligomer Series Containing Uridine, Thymidine, Cytidine, Deoxycytidine, Adenosine, and Deoxyadenosine. The hypochromicity data of the oligo(I) series were shown in the preceding section not to be describable by eq 1 (Figures 2 and 3). It is of importance to know whether the inadequacy of the first assumption, or of the second assumption, or both, is the cause of this failure. To this end, the hypochromicity data in uv absorbance of six oligomer series reported in the literature were analyzed by eq 1 and 2 in comparison to those from oligo(I).

The data of these seven oligomer series can be divided into two classes (Figures 4 and 5). The $(Up)_{2-11}$ series (Simpkins and Richards, 1967), the $(pdT)_{2-10}$ series (Cassani and Bollum, 1969), and the $(pdA)_{2-10}$ series (Cassani and Bollum, 1969) all belong to the first class; the data of these three oligomer series can be described by eq 1. Within experimental error, the hypochromicity data of these oligomer series can be fitted to a straight line (Figure 4) in the H_n *vs.* $1/n$ plot (hypochromicity, $H_n = \epsilon_n/\epsilon_{monomer}$);[1] and the hypochromicity of the first internal base in the trimer is the same as the internal bases of the polymers (Figure 5). This apparent compliance of the data of the oligo(U), oligo(T), and oligo(dA) series to eq 1 is significant. The data—especially that of $(pdA)_{2-10}$—reveal that the hypochromic effect in the oligonucleotides rests only on the interaction of the nearest-neighbor chromophores, and that the interactions among chromophores farther away than the two nearest neighbors are of little consequence. This is the requirement for the first assumption. As for the

[1] In conforming to the format of eq 1, the term hypochromicity in this paper is defined as $\epsilon_n/\epsilon_{monomer}$ instead of conventionally as $1 - \epsilon_n/\epsilon_{monomer}$.

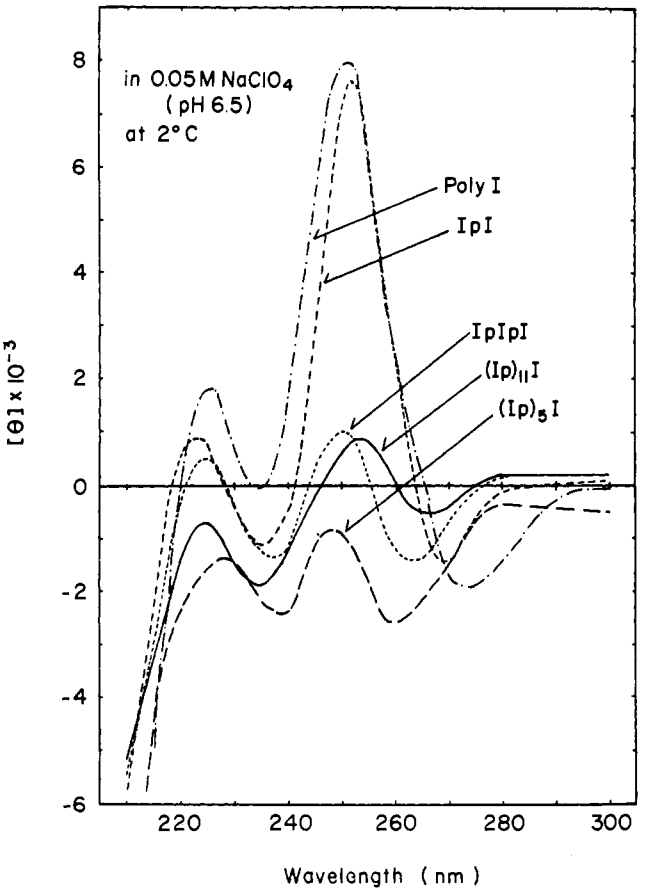

FIGURE 6: Circular dichorism spectra of oligoinosinates and poly(I) at 2°, 0.05 M $NaClO_4$ (pH 6.5).

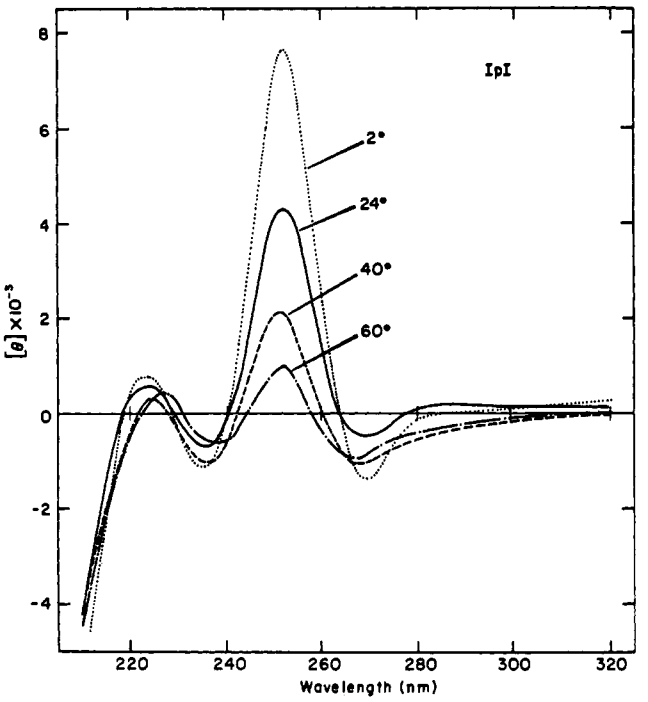

FIGURE 7: Circular dichroism spectra of IpI as a function of temperature in 0.05 M $NaClO_4$ (pH 6.5).

second assumption, there are two ways to satisfy its requirement. First, when the bases of a particular oligomer have very little tendency to stack, the increase in chain length of the oligomer would have no effect on the stacking of the bases in the oligomer. Thus, owing to the minimal tendency in stacking, the extent of base stacking in the trimer under this condition can be essentially the same as that of the polymer. This is likely the explanation for the compliance of the data from the oligo(U) series and the oligo(T) series to the requirement of the second assumption. Second, when the bases of other oligomers have a great tendency to stack, then the increase in chain length of the oligomer would have little effect on the stacking of the bases in the oligomers, if a *maximal* degree of stacking has also been reached. Thus, owing to the maximal tendency in stacking, the extent of base stacking in the trimer under this condition can be the same as that of the polymer. This could be the reason for the compliance of the data of the oligo(dA) series to the requirement of the second assumption. Proton magnetic resonance studies from our laboratory (Fang *et al.*, 1971) indeed indicate the extensive stacking of the dimer, dApdA.

The data of the oligo(I), oligo(rA), oligo(rC), and oligo(dC) series belong to the second class. The data in this class do not give a linear line in H_n *vs.* $1/n$ plot (Figure 4) and therefore cannot be described by eq 1. This failure can be due to the noncompliance to the requirements of either or both of the two assumptions. However, since it has been concluded in the preceding paragraph that the phenomenon of uv hypochromicity in oligonucleotides rests on the interaction of the nearest-neighbor chromophores only, the inadequacy in eq 1 must be due to the requirement in the second assumption. In other words, the chain length of these oligomers does exert an influence on the degree of stacking of the bases in this class of oligomers. It is interesting to note that while the maximal extent of hypochromicity of oligo(I) (~0.82) is less than that of oligo(rA) (~0.64), the dependence of the hypochromicity on chain length is similar in both series; the hypochromicity of the internal bases becomes equivalent to that of the polymer at n about 7–8 for oligo(rA) series and at n about 10 for the oligo(I) series. The situation for oligo(rC) series and oligo(dC) series is apparently different. The hypochromicity of the polymer is still much larger than that of the internal bases at chain length of 10 in the oligo(dC) series and at chain length of 14 in the oligo(rC) series. Apparently, the effect of chain length on the degree of base stacking in the cytosine oligomer series is more gradual than that of oligo(I) or oligo(rA) series.

Circular Dichroism Studies. The CD spectra of IpI, $(Ip)_2I$, $(Ip)_5I$, $(Ip)_{11}I$, and poly(I) in 0.05 M $NaClO_4$ (pH 6.5) at 2° are shown in Figure 6. The spectra of IpI and poly(I) are quantitatively similar to those published by Formoso and Tinoco (1971) measured under somewhat different conditions. The dependence of CD spectra on chain length displayed in Figure 6 is unusual. Such an unusual property of the oligo(I) series was also observed by Pochon and Michelson (1970); their proposed explanation concerning the change of nucleosidyl conformation involving syn–anti equilibrium will be discussed in later paragraphs.

The temperature effects on the CD spectrum of IpI and $(Ip)_2I$ are shown in Figures 7 and 8, respectively. At elevated temperature, the spectrum of IpI becomes increasingly similar to that of $(Ip)_2I$, while at the same temperature elevation, $(Ip)_2I$ nearly resembles the monomer, inosinic acid (Figure 9). The temperature effect on the CD spectrum of IpI shown in Figure 7 is also in accord with that reported by Formoso and Tinoco (1971).

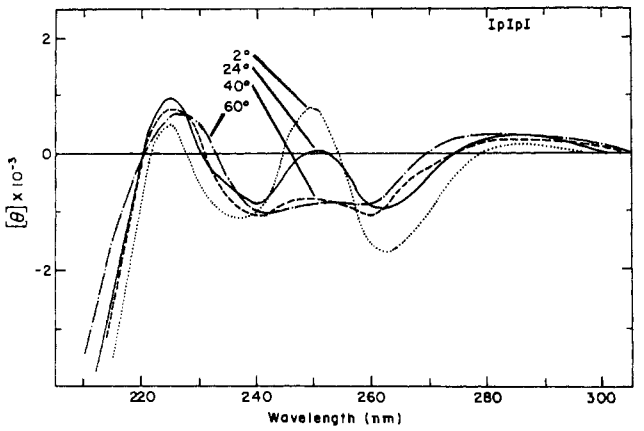

FIGURE 8: Circular dichroism spectra of IpIpI as a function of temperature in 0.05 M $NaClO_4$ (pH 6.5).

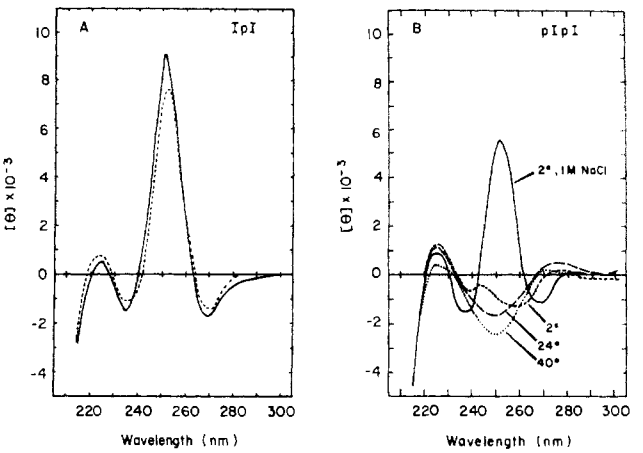

FIGURE 10: Salt and temperature dependence on the circular dichroism spectra of IpI and pIpI in 0.05 M $NaClO_4$ (pH 6.5). Conditions 2° (– – – –), 24° (— —), 40° (· · · · · ·), and 2° in 1 M NaCl (——).

The extension from a dinucleoside monophosphate (IpI) to a trinucleoside diphosphate (IpIpI) involves the addition of one nucleoside (inosine) and one phosphate. It would be of interest to compare the effect of the addition of just the phosphate group. In Figure 9, the comparison between the CD spectra of inosine, 3′-IMP, and 5′-IMP is shown. The negative band at 245 nm is hardly changed upon addition of a phosphate group to the nucleoside, though there may exist some difference at the 225-nm region between the spectrum of 5′-IMP and the spectrum of 3′-IMP, which is practically identical with that of inosine. On the other hand, addition of a 5′-phosphate to IpI causes a dramatic change in its CD spectrum. In 0.05 M $NaClO_4$, the spectrum of pIpI at 2° (Figure 10B) closely resembles the spectrum of $(Ip)_2I$ between 24 and 40° (Figure 11A) and is not similar at all to the spectrum of IpI at 2° (Figure 10A). At 24–40°, the spectrum of pIpI (Figure 10B) now is nearly equivalent to that of the 5′-IMP (Figure 9). Similarly, addition of a 5′-phosphate group to $(Ip)_2I$ has a substantial effect on the CD spectrum. At 0.05 M $NaClO_4$, the CD spectrum of $p(Ip)_2I$ at 2° (Figure 11B) is about the same as that of $(Ip)_2I$ at 24° (Figure 8), but not that of $(Ip)_2I$ at 2°. At about 60°, the CD spectrum of $p(Ip)_2I$ resembles that of 5′-IMP.

The existence of two phosphate groups in pIpI may introduce electrostatic repulsion. If so, such a phenomenon should be highly dependent on salt concentrations. As shown in Figure 10A,B, while the change of solvent from 0.05 M $NaClO_4$ to 1 M NaCl solution has little effect on the CD spectrum of IpI at 2°, this change has an immense effect on the CD spectra of pIpI. Similarly, the change to 1 M NaCl has a very large effect on the CD spectra of $(Ip)_2I$ and $p(Ip)_2I$ (Figure 11A,B). In 0.05 M $NaClO_4$–1 M NaCl, and at 2°, the CD spectra of IpI, pIpI, $(Ip)_2I$, and $p(Ip)_2I$ all become very similar to each other; the $[\theta]$ value of the peak at ~250 nm, however, is largest for $p(Ip)_2I$ (about 14×10^3) and smallest for pIpI (about 6×10^3). In Figure 12, the CD spectrum of $(Ip)_2I$ in high salt (1 M NaCl), the spectrum of $(Ip)_2I$ in low salt (0.05 M $NaClO_4$) and the spectrum of IpI in low salt are compared, all after *subtraction* of the CD spectrum of 5′-IMP. In comparison of these *difference* spectra, that of $(Ip)_2I$ in high salt is practically identical with that of IpI in low salt, except a slight blue shift of the CD band from 252 to 248 nm; while the intensity of the CD band at 248 nm

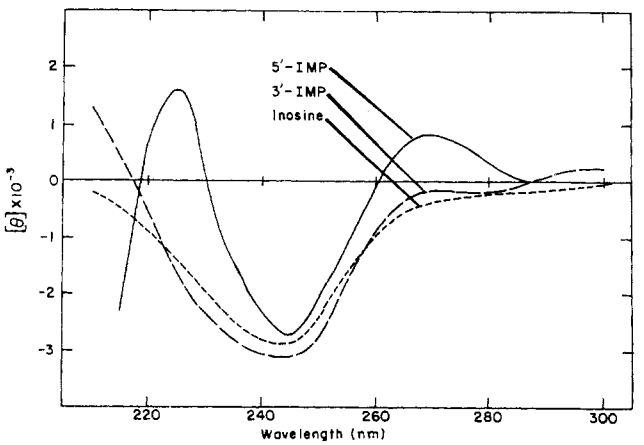

FIGURE 9: Circular dichroism spectra of inosine, 3′-IMP, and 5′-IMP at 2° in 0.05 M $NaClO_4$ (pH 6.5).

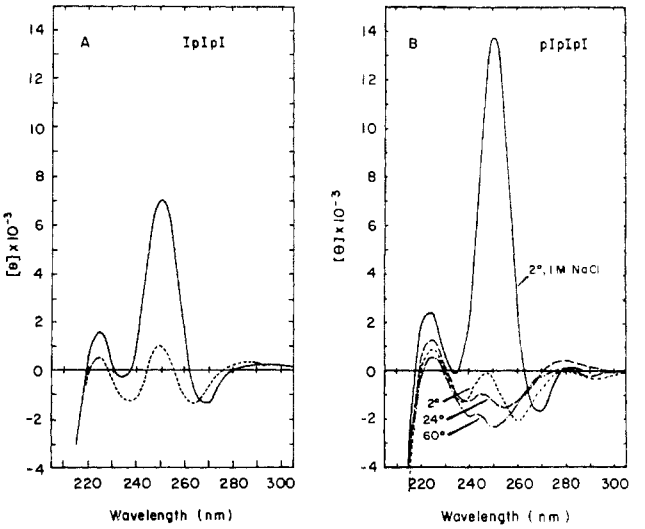

FIGURE 11: Salt and temperature dependence on the circular dichroism spectra of IpIpI and pIpIpI in 0.05 M $NaClO_4$ (pH 6.5). Conditions 2° (– – – –), 24° (— —), 60° (· — · —), and 2° in 1 M NaCl (——).

of the $(Ip)_2I$ in low salt is definitely much smaller. It should be clearly noted that there is little change in the negative CD band at 265–270 nm, which may correspond to the shoulder of the uv absorption spectrum of $(Ip)_2I$ (shown in the insert of Figure 12); all the change takes places only at the 248- to 252-nm CD band, corresponding to the main uv absorption band of $(Ip)_2I$ at 249 nm.

Carefully reviewing the information in Figures 6–12, we are led to two important conclusions. (1) The effect on the CD spectra by change in temperature, the introduction of phosphate group and the nucleoside residue into the chain (pI *vs.* pIpI *vs.* IpIpI *vs.* pIpIpI), and addition of salt (1 M NaCl) are all directly interrelated. Starting from the monomeric inosine, addition of a phosphate for the formation of nucleotide has little effect on its CD spectrum, even though the influence from the 5′-phosphate group (formation of 5′-IMP) appears to be larger than that of the 3′-phosphate (formation of 3′-IMP) indicating a possible small influence of the phosphate substitution on pentose-base conformation (Figure 9). Introduction of an inosine residue to pI for the formation of IpI has a significant effect on the CD of pI (or Ip), indicating the interaction of two linked inosine residues (Figures 7, 9, and 12). In dilute salt solution (0.05 M), addition of a 5′-phosphate group to IpI and $(Ip)_2I$ to form pIpI and pIpIpI has the effect on the CD spectra of IpI and $(Ip)_2I$ of a temperature elevation of ~58° (2 to 60°) and ~22° (2 to 24°), respectively (Figures 7, 8, 10, and 11). Addition of 1 M NaCl to the solution counteracts the introduction of the phosphate group, while the addition of 1 M NaCl has little effect on the CD spectra of IpI (Figure 10A). In strong salt condition, the spectra of IpI, pIpIp, IpIpI, and pIpIpI now all closely resemble each other. In dilute salt solution and at elevated temperature, the spectrum of pIpIpI can be converted to one resembling 5′-IMP. These studies conclusively demonstrate the electrostatic effect of the phosphate group on the conformation of oligo(I) as revealed by their CD spectra; the intricate, counterbalancing interaction between the phosphate and the base, as related to their influence on the oligo(I) conformation, is also well demonstrated in these studies. (2) The effect on the CD spectra of these oligo(I) by change in temperature, in phosphate to base ratio, and in salt concentration all takes place exclusively in the 245- to 255-nm CD bands. The temperature effect on IpI and $(Ip)_2I$ is illustrated in Figures 7 and 8; the effect of introduction of phosphate to IpI is shown in Figure 10A,B; the effect of salt (1 M NaCl) on pIpI, $p(Ip)_2I$, and $(Ip)_2I$ is demonstrated in Figures 10, 11, and particularly 12. In every case, these alterations bring about a significant change of the CD band at the 245- to 255-nm region only without major variation in other spectral areas. The CD band at 245–255 nm can be changed from a negative value (monomer, -3×10^3; pIpI in 0.05 M salt at 40°, -2×10^3) to a positive value (IpI at 2°, 8×10^3; $p(Ip)_2I$ at 2° in 1 M NaCl–0.05 M $NaClO_4$, 14×10^3). In the uv absorption spectrum of the inosine derivative (see insert, Figure 12), the 245- to 255-nm region is the location of the main absorption band and has been identified as the B_{1u} band of the $\pi \rightarrow \pi^*$ transition by magnetic CD studies (Voelter *et al.*, 1968). The CD spectra of the dimer and trimer also show a negative CD band at ~270-nm region, corresponding to the shoulder of the uv absorption spectrum which has been classified as the B_{2u} band of the $\pi \rightarrow \pi^*$ transition by the magnetic CD studies. This CD band is little affected by all these perturbations (temperature, phosphate: base ratio, salt, etc.) directly. This observation, together with the *difference* spectra shown in Figure 12, indicate convincingly

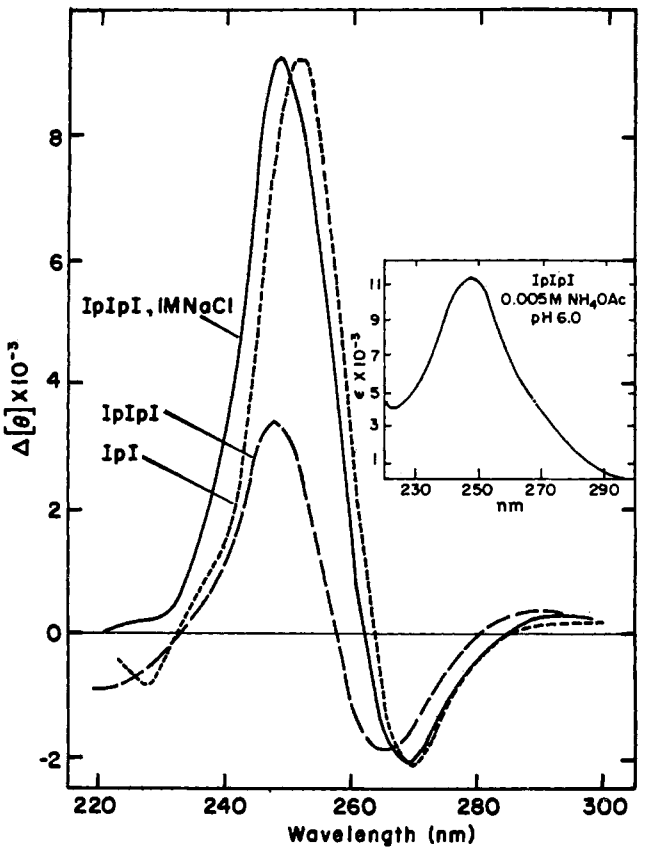

FIGURE 12: Circular dichroism difference spectra (minus pI) of IpI and IpIpI at 2° in 0.05 M $NaClO_4$ (pH 6.5) and of IpIpI at 2° in 1 M NaCl. Inset is uv absorbance spectrum.

that the optical activity derived from the interaction of the chromophores in IpI and $(Ip)_2I$ is *not* the "conservative" type due to the coupled oscillator splitting of the $\pi \rightarrow \pi^*$ transition as in the case of ApA. The optical activity of IpI due to interaction is rather small and does not possess positive and negative bands of nearly equal magnitude.

The effects of the addition of the phosphate group on the optical activities of the dinucleoside monophosphates have been previously noticed. As reported by Bush and Scheraga (1969), the peak and the trough of the CD spectrum of ApAp ($[\theta]_{peak} = 1.00 \times 10^4$; $[\theta]_{trough} = -1.81 \times 10^4$) is substantially smaller than that of ApA ($[\theta]_{peak} = 2.48 \times 10^4$; $[\theta]_{trough} = -2.4 \times 10^4$). The same conclusion was reached by Inoue and Satoh (1969) in their comparative optical rotatory dispersion (ORD) study on the phosphate effect on ApA, CpC, etc. They found that the presence of a 3′-phosphate group (and to a lesser extent, also a 2′-phosphate group) at ApAp(3′) reduces the first Cotton effect by about 42%, as compared to that of ApA, and that this reduction effect can be practically removed by the methylation of the 3′-terminal phosphate. Similarly, the rotation of CpCp(3′) appears to be smaller than CpC at neuteral pH. Inoue and Satoh (1969) had also observed a noticeable change of ORD spectrum of IpIp(3′) from solution of 0.1 ionic strength to solution of 1.0 ionic strength. Comparison of the CD curve of dApdA (Miller *et al.*, 1971) and that of pdApdA (Bush and Scheraga, 1969) again reveals a significant decrease in amplitude (33%). On the other hand, Cantor *et al.* (1970) reported that dTpdT, pdTpdT, and pdTpdTp all have similar CD spectra which are insensitive to salt concentration. This could be due

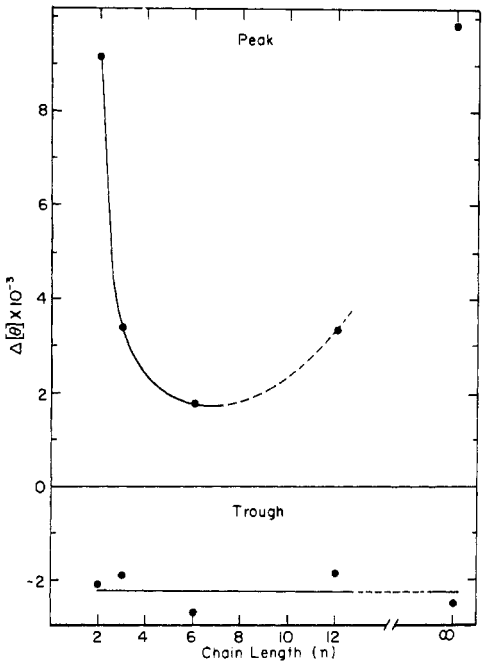

FIGURE 13: Monomer corrected $[\theta]_{peak}$ and $[\theta]_{trough}$ of oligoinosinates at 2° in 0.05 M $NaClO_4$ (pH 6.5).

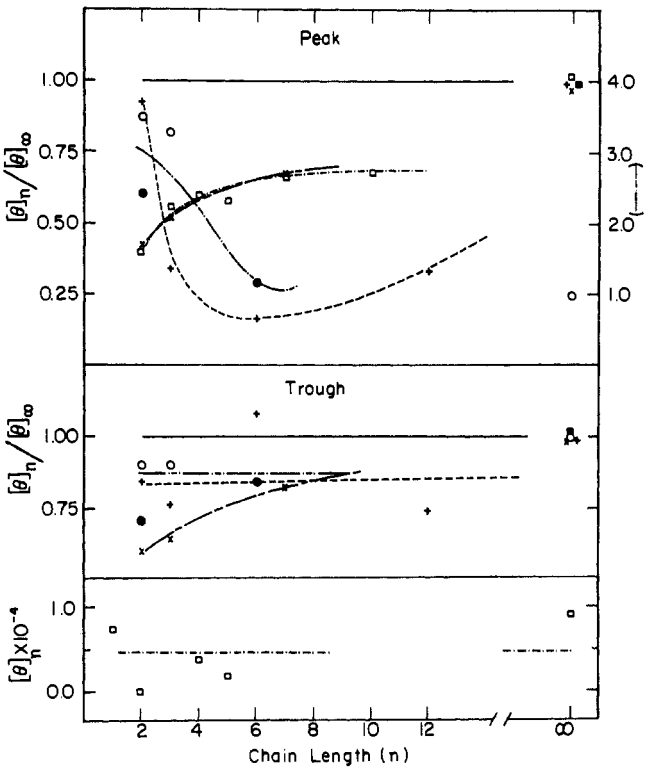

FIGURE 14: Polymer normalized $[\theta]_{peak}$ and $[\theta]_{trough}$ (monomer corrected) of oligonucleotides as a function of chain length. The oligonucleotides are the same as in Figure 4: $(Ip)_{n-1}I$ (+), $(Up)_n$ (■), $(dA)_{n-1}dA$ (○), $(pdA)_n$ (●), $(Cp)_{n-1}C$ (□), and $(Ap)_{n-1}A$ (×). The raw values for $[\theta]_{trough}$ of rC are plotted in the bottom frame.

to the low level of base stacking of the oligo(T); however, they also reported that the CD spectra of dApdG and dApdGp are similar.

Comparative Circular Dichroism Studies—Oligomer Series Containing Various Nucleosides. The unusual dependence of the CD spectra of the oligo(I) on chain length discussed in the preceding section is summarily presented in Figure 13. After subtracting the values from the 5′-IMP, the *remaining* intensities of the peak (248–252 nm) and the trough (270 nm) of the dimer, trimer, hexamer, dodecamer, and polymer at 2°, 0.05 M $NaClO_4$ (pH 6.5), are plotted as a function of chain length (Figure 13). While the details of these curves cannot be certain, the general tendency is clear. The differential intensity of the peak in CD decreases initially and significantly from the dimer to the trimer stage; then at some stage between the hexamer and dodecamer, it begins to increase again. At the polymer level, the differential peak intensity becomes about the same or slightly larger than that of the dimer. The differential intensity of the trough, on the other hand, remains practically the same from dimer to polymer (Figure 13).

For a better understanding of the data on oligo(I) illustrated in Figure 13, a comparison to four other oligomer series (rU, rA, dA, and rC) is shown in Figure 14. In this figure, the intensity values at the peak and at the trough of the first CD band of the five series are all corrected for the monomer and normalized with respect to those of the corresponding polymers except in one instance. The results on the oligo(U) series in Figure 14 (in solid lines) are actually derived from the ORD measurements which showed that $(Up)_2$, oligo(U), and poly(U) all have practically the same ORD spectra (Simpkins and Richards, 1967); therefore, there is no chain length effect on the CD spectra of $(Up)_2$ through poly(U). For the oligo(rA) series (Brahms *et al.*, 1966), both the intensities at the peak position and at the trough position increase with increasing chain length in a more or less parallel fashion (Figure 14). This type of dependence on chain length fulfills the expectation of a "conservative Cotton effect" derived from coupled oscillator splitting of the strong $\pi \rightarrow \pi^*$ transition, which gives positive and negative bands of nearly equal magnitude. However, the series of oligo(dA) gave a totally different type of CD dependence (Miller *et al.*, 1971; Bush and Scheraga, 1969; K. N. Fang, L. S. Kan, P. S. Miller, and P. O. P. Ts'o, unpublished results). Though the available data are scanty, it is sufficient to show that the intensity of the first CD peak at the 265- to 280-nm region decreases from dimer to trimer, and to hexamer, and remains small even at the polymer stage, while the intensity of the first trough at the 250-nm region stays about the same throughout the series (Figure 14). As for the oligo (rC) series, the intensity of the first CD peak at the 280-nm region (Brahms *et al.*, 1967) increases at longer chain length, similar to the situation of the rA series; however, the intensity of the trough (or shoulder) region again remains practically the same from dimer, through oligomer, to polymer. (In Figure 14, the CD value of 5′-CMP was not substracted from those of the dimer–oligomer–polymer series. The reason for this exception is that the actual values are very close to zero and change between positive and negative, and thus cannot be normalized.)

The survey in Figure 14 shows a considerable diversity among the five oligomer series in their dependence of CD properties on chain length. The CD properties of the oligo(U) series show no dependence on chain length, reflecting a lack of base–base interaction in this series. The peak intensity of the first CD band in the oligo(rA) series and oligo(rC) series increases with longer chain length, while the peak intensity in the oligo(dA) series decreases with longer chain length, and the peak intensity in the oligo(rI) series first decreases and then increases with increasing chain length. As for the trough intensity of the first CD band, only that of the oligo(rA) series

TABLE III: Chemical Shifts of Base and H-1′ Protons of Monomers, Dimers, and Trimers of Inosinate[a] (Parts per Million from Tetramethylsilane Capillary).

Compound	pD	Temp (°C)		Chemical Shifts, δ (Neg) H-8	H-2	H-1′
3′-IMP	6.0	5		8.77	8.62	6.54
		30		8.83	8.695	6.60
		60		8.82	8.70	6.60
5′-IMP	6.0	5		8.87	8.60	6.545
		30		8.92	8.68	6.61
		60		8.90	8.69	6.61
IpI	6.0	5	Ip–	8.64	8.53	6.34
			–pI	8.73	8.56	6.43
	6.0	30	Ip–	8.71	8.62	6.43
			–pI	8.81	8.64	6.53
	6.0	60	Ip–	8.73	8.64	6.47
			–pI	8.82	8.65	6.55
pIpI[b]	6.0	5	pIp–	8.80	8.53	6.38
			–pI	8.75	8.555	6.46
	6.0	30	pIp–	8.85	8.63	6.48
			–pI	8.82	8.64	6.54
	7.0	30	pIp–	8.91	8.63	6.505
			–pI	8.83	8.64	6.55
IpIpI	6.0	5	Ip–	8.59	8.50	6.39
			–pIp–	8.69	8.49	6.34
			–pI	8.69	8.53	6.40
	6.0	30	Ip–	8.68	8.59	6.48
			–pIp–	8.78	8.59	6.42
			–pI	8.79	8.62	6.51
	6.0	60	Ip–	8.69	8.62	6.51
			–pIp–	8.80	8.62	6.44
			–pI	8.81	8.63	6.54
pIpIpI[c]	6.0	5	pIp–	8.71	8.49	6.41
			–pIp–	8.685	8.49	6.34
			–pI	8.685	8.53	6.42
	6.0	30	pIp–	8.80	8.59	6.49
			–pIp–	8.80	8.59	6.43
			–pI	8.80	8.61	6.51
	7.0	30	pIp–	8.85	8.59	6.49
			–pIp–	8.79	8.58	6.43
			–pI	8.80	8.61	6.515

[a] All extrapolated to infinite dilution unless specified. [b] Concentration at 0.014 M (dimer) for experiments at 30°. [c] Concentration at 0.014 M (trimer) for all experiments.

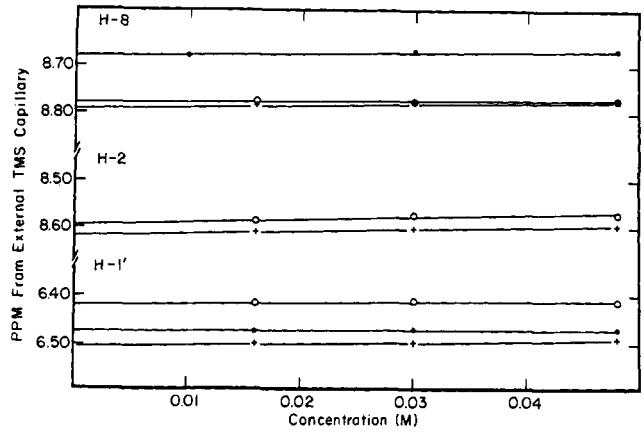

FIGURE 15: Concentration dependence of pmr chemical shifts of IpIpI at 30°, pD 6.0. Ip– (●), –pIp– (○), and –pI (+). The values of H-2 for the Ip– and –pIp– residues are coincident.

increases with longer chain length; in all the other three series (rI, dA, and rC), the trough intensity shows little dependence on chain length. This survey reveals no general rule in governing the CD dependence on chain length for these oligomer series, and the conservative Cotton effect appears to be an exceptional case rather than a common example. The geometry of the stacking pattern is known to be different between rAprA *vs.* dApdA and between poly(rA) *vs.* poly(dA) (Fang *et al.*, 1971; Kondo *et al.*, 1972; Alderfer and Smith, 1971; Alderfer *et al.*, 1971). The adenine bases are arranged in a more oblique manner in the ribosyl derivatives than the adenine bases in the deoxyribosyl derivatives which are arranged in a more parallel manner. This consideration has been proposed previously as the explanation for the reduction of the conservative Cotton effect due to the reduction in the angle between the transition moments in a dipole approximation (Kondo *et al.*, 1970, 1972). Bush and Scheraga (1969) proposed that a $n \rightarrow \pi^*$ band at the 280-nm region is responsible for the optical activity at this wavelength; this band could be shifted to a shorter wavelength in the monomer due to solvation and reappears in the polymer spectrum when the bases become interior and are kept from contacting the solvent. A small difference between the magnetic CD spectrum of adenine at pH 2 *vs.* that at pH 7 did suggest the possibility of a $n \rightarrow \pi^*$ transition in this region (Voelter *et al.*, 1968). As for the cytosine chromophores, the broad band at the 280-nm region consists of both B_{1u} and B_{2u} transitions; and the 230-nm absorption band is an $n \rightarrow \pi^*$ transition (Voelter *et al.*, 1968). Though the assignments of these bands provide us with more insight about the optical properties of the isolated chromophores, a considerable amount of both theoretical and experimental work is needed for a better understanding of the optical activities derived from the interaction of these chromophores in the oligonucleotides.

Proton Magnetic Resonance Studies. Four compounds, IpI, pIpI, $(Ip)_2I$, and $p(Ip)_2I$, have been investigated by pmr methods as a supplement to the optical studies. One major objective of this study is to ascertain the conformation of nucleosidyl units in the dimers and the trimers. The concentration dependence of the chemical shifts of the nine protons (three of each H-8, H-2, and H-1′) of $(Ip)_2I$ at 30° is shown in Figure 15. The dependence is very slight and a similar situation was also observed for the concentration dependence of IpI. Unless specified, the data reported have been extrapolated to infinite dilution to avoid the complication of intermolecular interaction. Since the concentration dependence of the chemical shifts is so slight and the line width of the resonances is narrow at all these concentrations (about 1 Hz), these oligomers are unlikely to associate to a significant extent even at 0.04 M.

The chemical shifts and the assignments of these resonance lines at 5, 30, and 60° from 3′-IMP, 5′-IMP, IpI, pIpI, $(Ip)_2I$, and $p(Ip)_2I$ are reported in Table III. The assignments of the resonances from the dimers and trimers have been established by the following procedures: (1) deuteron exchange of H-8

at elevated temperature with D_2O (Schweizer *et al.*, 1964; Ts'o *et al.*, 1969a,b); (2) specific line broadening of H-8 and H-1′ due to Mn^{2+} binding (Chan and Nelson, 1969; Fang *et al.*, 1971); (3) consideration of the conformational model. By the deuteron-exchange procedure, the H-8 resonances are all easily separated from the H-2 resonances, while the H-1′ resonances are doublets located at the region of 6.0–6.6 ppm. Comparison of the chemical shifts of the dimers and the trimers to their respective monomeric units yields the value of dimerization shifts or trimerization shifts, *i.e.*, $\Delta\delta_D$ (or $\Delta\delta_T$) = δ_{dimer} (or δ_{trimer}) − $\delta_{monomer}$. $\Delta\delta_D$ or $\Delta\delta_T$ (positive) are indexes of neighboring interaction between the residues in a dimer or trimer.

The assignments of the resonances and their implication for the conformation of IpI and pIpI are described below. In the presence of Mn^{2+}, the H-8 at the lower field in the IpI spectrum was broadened in the same manner as the H-8 of 5′-IMP. Thus, the H-8 (8.81, 30°, Table III) at the lower field was identified as the H-8 of the –pI portion of IpI. The H-8 of a 5′-purine nucleotide is known to be deshielded specifically by the 5′-phosphate group as compared to that of a nucleoside (or of 3′-nucleotides), when the nucleosidyl residue is in an anti conformation. This identification of the low-field H-8 as the H-8 of the –pI residue in IpI is in accord with the expectation of this specific deshielding phenomenon of the 5′-phosphate group. The remaining H-8 resonance at higher field (8.71, 30°, Table III) was then assigned to the H-8 of the Ip– residue in IpI. The Mn^{2+}-broadening phenomenon and this assignment of the low-field H-8 to the –pI residue also indicate that the –pI residue in IpI is in an anti conformation predominantly. In the presence of Mn^{2+}, the H-1′ located at higher field (6.43, 30°, Table III) was broadened in the same fashion as the H-1′ of 3′-IMP. This high-field H-1′ resonance was then assigned to the H-1′ of Ip– residue and the remaining low-field H-1′ line (6.53, 30°, Table III) was assigned to the H-1′ of –pI residue in IpI. As for the H-2 protons, one resonance (8.62, 30°, Table III) is located at a field slightly higher than the other (8.64, 30°, Table III). From the consideration of the conformation model, this observation suggests that the nucleosidyl unit in Ip– residue is also mainly in an anti conformation and the high-field H-2 resonance belongs to the H-2 proton of the Ip– residue in IpI, since the anti conformation of –pI residue has already been established. If the Ip– residue is in a syn conformation instead, the two H-2 resonances should have nearly identical spectral positions. This argument is strongly supported by the study on pIpI. All the H-2 resonances and H-1′ resonances of pIpI are nearly identical with those of IpI; however, one H-8 resonance (8.71, 30°, Table III) in IpI was shifted to a lower field position (8.85, 30°, Table III) in pIpI. This shift is expected from a change of an Ip– residue in IpI to a pIp– residue in pIpI due to the specific deshielding effect of a 5′-phosphate group mentioned above. This reasoning was confirmed by the observation that this H-8 resonance (8.85, 30°) in pIpI is the only resonance in Table III which is sensitive to the change of pD 6.0 to 7.0, and is lowered to 8.91 ppm. This change of pD should increase the ionization of the secondary phosphate group in pIp– residue, and it has been demonstrated that the increase in 5′-phosphate ionization enhances the specific shielding effect of the phosphate group when the nucleosidyl unit is in anti conformation (Schweizer *et al.*, 1968; Danyluk and Hruska, 1968). The above observations on pIpI reveal that both nucleotidyl residues (pIp– and –pI) are in anti conformation. Since the chemical shifts of pIpI and IpI are nearly identical except for the H-8 of pIp– discussed above, it is virtually certain that the IpI also has both residues (Ip– and –pI) in anti conformation. This study also confirms all the assignments of the resonances for IpI and pIpI in Table III. As for the handedness of the screw axis of IpI and pIpI, the location of the H-1′ proton of Ip– residue (or pIp– residue) at higher field (6.43, 30°, Table III) and the relatively small difference (0.09 ppm) between the H-1′ of Ip– residue *vs.* the H-1′ of –pI residue can be considered as the demonstration of a predominantly right-handed stack according to the consideration of the conformational model.

The procedure for assignments of resonances in IpIpI and pIpIpI is the same as that for the dimer; however, there is an additional problem. In the presence of $MnCl_2$, two H-8 resonances and two H-1′ resonances in the spectrum of $(Ip)_2I$ are broadened, rather than only one of each as in the case of the dimer. This clearly indicates that the *unbroadened* H-8 resonance (8.68, 30°, Table III) belongs to the H-8 in the Ip– residue, and the *unbroadened* H-1′ resonance (6.51, 30°, Table III) belongs to the H-1′ in the –pI residue of IpIpI. The two remaining H-8 resonances and H-1′ resonances are assigned in accordance with the justifiable assumption that the protons located in the interior residue –pIp– are more shielded than those located in the exterior residues (Ip– or –pI) in IpIpI. In this manner, resonances at 8.78 and 6.42 ppm (Table III) were assigned respectively to the H-8 and H-1′ of –pIp– residue, and resonances at 8.79 and 6.48 ppm were assigned respectively to H-8 of –pI residue and the H-1′ of Ip– residue. The fact that the H-8 protons of both –pIp– and –pI residues were broadened by Mn^{2+} ions can be taken as evidence that these two nucleotidyl units are predominantly in anti conformation. Comparison between the chemical shifts of IpIpI and pIpIpI (Table III) indicates that all the resonances are similar except that one H-8 resonance was moved downfield in pIpIpI; the resonance of this H-8 was found to be sensitive to the change of pD from 6.0 to 7.0. Following the reasoning analogous to the dimer, we may conclude that this resonance belongs to the H-8 proton of the pIp– residue and all the residues in pIpIpI are in an anti conformation. Since the chemical shifts of the other eight protons (or the trimerization shifts) of IpIpI and pIpIpI (Table III) are nearly identical, all the residues in IpIpI most likely also have an anti conformation similar to that of pIpIpI. The H-2 resonances of the trimers can now be assigned in a manner analogous to the dimer (Table III), with the assumption that the most shielded proton is located in the interior –pIp– residue and with the consideration of the conformational model. In view of the fact that the chemical shifts (Table III) and trimerization shifts (Table V) of the H-1′ resonances in the trimer are so close to the corresponding values of H-1′ resonances in the dimer, the screw axis of the trimer most likely is also right handed as in the case of the dimer.

The coupling constants, $J_{H-1'-H-2'}$, of IMP, dimer, and trimer are shown in Table IV. These J values from the inosinyl dimers and trimers are significantly lower than the respective values in monomers at low temperature; they become larger, approaching the values of the monomers, at higher temperature. In this respect, the ribosylinosinyl dimers and trimers are similar to the ribosyladenylyl dimers and trimers, and not to the deoxyadenylyl dimers and trimers (Fang *et al.*, 1971; Kondo *et al.*, 1972). This indicates that upon base stacking in IpI and $(Ip)_2I$, the conformation of the ribose residue in the dimers and trimers is being compressed (see discussion in Kondo *et al.*, 1972).

In view of the significant effect of salt (1 M NaCl) on the CD spectra of pIpI described in the preceding section, the

TABLE IV: Coupling Constant, $J_{H-1',H-2'}$,[a] of Monomers, Dimers, and Trimers of Inosinate.

Compound	Temp (°C)	Hz (p)Ip–	Hz –pIp–	Hz –pI
3′-IMP[b]		5.6		
5′-IMP[b]				5.6
IpI	5	4.7		4.0
	30	5.0		4.7
	60	5.3		4.9
IpI, 1 M NaCl	5	4.6		3.8
pIpI	5	4.1		4.1
pIpI, 1 M NaCl	5	4.0		4.0
IpIpI	5	4.2[c]	4.6	3.8[c]
	30	5.0	5.2	4.8
	60	5.2	5.2	5.0

[a] Error in measurement the order of 0.2 Hz. [b] Value is temperature independent at concentrations studied (<0.02 M). [c] Due to peak overlap, error somewhat larger, ~0.4 Hz.

TABLE V: Chemical Shifts[a] of Monomers and Dimers of Inosinate in 1 M NaCl at 5° (Parts per Million from Tetramethylsilane Capillary).

Compound	pD		Chemical Shifts (Neg) H-8	H-2	H-1′
3′-IMP	6.0		8.84	8.705	6.60
5′-IMP	6.0		8.95	8.69	6.62
	4.0[b]		8.91[b]	8.69[b]	6.62[b]
IpI	6.0	Ip–	8.72	8.60	6.40
		–pI	8.76	8.65	6.49
pIpI	6.0	pIp–	8.83	8.565	6.44
		–pI	8.74	8.64	6.50

[a] All extrapolated to infinite dilution unless specified. [b] At 0.005 M concentration.

TABLE VI: Dimerization and Trimerization Shifts ($\Delta\delta$)[a] of IpI, pIpI, IpIpI, and pIpIpI.[b]

Compound	Temp (°C)		H-8	H-2	H-1′
IpI	5	Ip–	0.13	0.09	0.20
		–pI	0.14	0.05	0.11
	30	Ip–	0.11	0.07	0.17
		–pI	0.11	0.04	0.08
	60	Ip–	0.09	0.05	0.13
		–pI	0.08	0.04	0.05
IpI, 1 M NaCl	5	Ip–	0.12	0.11	0.195
		–pI	0.19	0.04	0.12
pIpI	5	pIp–	0.10	0.08	0.20
		–pI	0.115	0.06	0.09
	30[c]	pIp–	0.105	0.07	0.16
		–pI	0.10	0.04	0.06
pIpI, 1 M NaCl	5	pIp–	0.14	0.14	0.205
		–pI	0.21	0.055	0.11
IpIpI	5	Ip–	0.18	0.12	0.15
		–pIp–	0.21	0.13	0.24
		–pI	0.18	0.07	0.14
	30	Ip–	0.15	0.10	0.12
		–pIp–	0.17	0.10	0.22
		–pI	0.13	0.06	0.10
	60	Ip–	0.13	0.08	0.09
		–pIp–	0.14	0.08	0.20
		–pI	0.095	0.06	0.07
pIpIpI[c]	5	Ip–	0.185	0.13	0.175
		–pIp–	0.21	0.13	0.24
		–pI	0.18	0.08	0.13
	30	Ip–	0.15	0.11	0.155
		–pIp–	0.15	0.11	0.21
		–pI	0.12	0.07	0.095

[a] In parts per million, $\Delta\delta_{D \text{ or } T} = \delta_{dimer}$ (or δ_{trimer}) $- \delta_{monomer}$. [b] All values obtained from chemical shifts extrapolated to infinite dilution unless otherwise specified. [c] Concentration at 0.014 M (dimer or trimer).

pmr properties of IMP, IpI, and pIpI in 1 M NaCl at 5° were investigated (Table V). The chemical shifts are slightly lowered owing to the change of the magnetic susceptibility and the use of external reference.

Dimerization and trimerization shifts ($\Delta\delta$, Table VI) are the parameters derived from pmr studies which are the direct indication of the neighboring interaction (mostly base–base interaction) of the residues in the dimer and the trimer. Three major conclusions concerning the $\Delta\delta$ data in Table VI are discussed below. (1) Within experimental error (±0.02 ppm), the $\Delta\delta_D$ values of IpI and pIpI are essentially the same, and similarly, the $\Delta\delta_T$ values of IpIpI and pIpIpI are nearly identical. Thus, the pmr data do not indicate a *significant* change of conformation upon addition of a 5′-phosphate group to the dimer or trimer; for instance, the change of an anti to syn conformation of one or all nucleosidyl units can be safely excluded. This observation is in contrast to the data from CD studies described above. (2) Addition of 1 M NaCl also does not noticeably change the $\Delta\delta_D$ values of IpI, except that of the H-8 in –pI residues to the extent of 0.05 ppm, barely above the level of experimental error. This result may be attributable to a variety of factors: a small increase in base stacking (since $\Delta\delta_D$ of the H-2 of Ip– is also slightly increased, 0.03 ppm); a slight change in the nucleosidyl conformation (to a conformation closer to syn form); or a minute reduction in charge of the phosphate group in IpI due to Na^+ binding, etc. Addition of 1 M NaCl to pIpI does cause a change of $\Delta\delta_D$ values of H-8 of –pI (0.1 ppm), H-8 of pIp– (0.04 ppm, barely significant) and H-2 in pIp– (0.06 ppm) of pIpI. This observation suggests that a small increase in base stacking probably does take place for pIpI in 1 M NaCl; the increase in $\Delta\delta_D$ of H-2 in pIp– is important in this respect, since the $\Delta\delta_D$ value of this proton is mainly sensitive to stacking in a dimer with both nucleotidyl units in anti conformation. (3) As described previously relating to the problem of assignment of resonances, the $\Delta\delta_D$ values of IpI and pIpI are in complete agreement for a conformation of right-handed, anti,anti stack.

TABLE VII: Comparison between the Dimerization Shifts ($\Delta\delta_D$) of IpI and the Trimerization Shifts ($\Delta\delta_T$) of IpIpI (in Parts per Million).[a]

			$\Delta\delta_T$[a]	$\Delta\delta_D$[a]	$\Delta\delta_{dif}$[b]
5°	H-1′	Ip–	0.15	0.20	−0.05
		–pIp–	0.24 (0.29)	(0.31)	−0.07
		–pI	0.14	0.11	+0.03
	H-2	Ip–	0.12	0.09	+0.03
		–pIp–	0.13 (0.19)	(0.14)	−0.01
		–pI	0.07	0.05	+0.02
	H-8	Ip–	0.18	0.13	+0.05
		–pIp–	0.21 (0.36)	(0.27)	−0.06
		–pI	0.18	0.14	+0.04
30°	H-1′	Ip–	0.12	0.17	−0.05
		–pIp–	0.22 (0.22)	(0.25)	−0.03
		–pI	0.10	0.08	+0.02
	H-2	Ip–	0.10	0.07	+0.03
		–pIp–	0.10 (0.16)	(0.11)	−.001
		–pI	0.06	0.04	+0.02
	H-8	Ip–	0.15	0.11	+0.04
		–pIp–	0.17 (0.28)	(0.22)	−0.05
		–pI	0.13	0.11	+0.02
60°	H-1′	Ip–	0.09	0.13)	−0.04
		–pIp–	0.20 (0.16)	(0.18)	+0.02
		–pI	0.07	0.05	+0.02
	H-2	Ip–	0.08	0.05	+0.03
		–pIp–	0.08 (0.14)	(0.09)	−0.01
		–pI	0.06	0.04	+0.02
	H-8	Ip–	0.13	0.09	+0.04
		–pIp–	0.14 (0.23)	(0.17)	−0.03
		–pI	0.09	0.08	+0.01

[a] The values in parentheses are obtained from the summation of $\Delta\delta$ values from Ip– and –pI residues. [b] $\Delta\delta_{dif} = \Delta\delta_T - \Delta\delta_D$.

Comparison between the Dimerization Shifts of IpI vs. the Trimerization Shifts of IpIpI—Dependence of Chemical Shifts on Chain Length. The simplest expectation in the comparison between the $\Delta\delta_D$ of IpI and the $\Delta\delta_T$ of IpIpI is that the observed values for the Ip– residues and for the –pI residues are the same in both dimer and trimer, and the observed values for the –pIp– residue is the summation of the values for Ip– and for –pI residues. This expectation is based on two assumptions. (1) The conformation of the trimer is constructed from an exact extension of the dimer—the time-averaged geometrical relationship between the first and the second residue in the trimer is exactly the same as that between the second and third residue, as well as exactly the same as that in the dimer. (2) The magnetic field effect of the ring-current anisotropy or any other through-space field effects do not extend beyond the nearest-neighbor residue—the chemical shifts of the first residue are not influenced by the third residue and *vice versa*. Various considerations do suggest that these assumptions are unlikely to hold. For instance, the dynamic process of right-handed stack to left-handed stack interconversion occurs to a significant extent in the dimer (Tazawa *et al.*, 1970), but probably takes place hardly at all in a trimer. This situation will affect the chemical shifts of the H-1′ protons. The rotational freedom of the nucleosidyl unit of the interior residue (the middle residue) around the glycosyl linkage (or ϕ_{cn}) is likely to be more restricted, since this interior base is sandwiched between the two outside bases. If the H-8 proton of the interior base is kept at a close distance to the 5′-phosphate group of the –pIp– residue, this proton can be more deshielded. Finally, besides the possible increase in the extent of base stacking, the possible magnetic field effects between the first and the third residues cannot be discounted on *a priori* grounds.

The comparison between the dimerization shifts ($\Delta\delta_D$) of IpI and the trimerization shifts ($\Delta\delta_T$) of IpIpI are shown in Table VII. With one exception, all the $\Delta\delta_D$ values of the Ip– and –pI residues are smaller than the corresponding $\Delta\delta_T$ values. This could reflect an increase in base stacking in the trimer, or in the magnetic field effect between the first and the third residues (simply termed as distant-neighbor effect), or both. There are two suggestive pieces of evidence to indicate the existence of the distant-neighbor effect, one of which will be discussed in a later paragraph. The other one is that the difference ($\Delta\delta_{dif}$, Table VII) between the $\Delta\delta_D$ and $\Delta\delta_T$ for the H-2 and H-1′ protons in both Ip– (+0.03 ppm) and –pI (+0.02 ppm) is temperature independent, *i.e.*, the same $\Delta\delta_{dif}$ values from 5 to 60°. Within experimental error, the same holds true for H-8 protons from 30 to 60°; only at 5° do the $\Delta\delta_{dif}$ values appear to be slightly larger. The temperature insensitivity of the $\Delta\delta_{dif}$ values suggests that the origin of these values may not be due to additional base stacking in the trimer which should have a different response to temperature from that in the dimer, but may be due to a temperature-independent intrinsic factor. The distant-neighbor effect could be such a factor, since certain considerations of its origin suggest that it may not be sensitive to temperature. The exception noted above concerns a negative $\Delta\delta_{dif}$ value (Table VII), which is that from the H-1′ of Ip– residue. The $\Delta\delta_D$ of this H-1′ in the dimer is larger than the $\Delta\delta_T$ of this same proton in the trimer to about the same extent from 5 to 60°. While no definitive explanation can be offered at present why this H-1′ of the Ip– residue is more shielded in the dimer than in the trimer, one reasonable suggestion is that this finding may be related to the absence of right-handed to left-handed interconversion in the trimer. From the consideration of the conformational model, a left-handed stack would cause a significant increase in shielding of H-1′ of the Ip– residue.

The $\Delta\delta_T$ values of the protons in the interior residue (–pIp–) deserve a more thorough discussion. Except in two cases to be discussed later, the $\Delta\delta_T$ values of the protons in the –pIp– are *smaller* than those derived from the summation (the bracketed values in Table VII) of the $\Delta\delta_T$ values of the Ip– and the –pI residues. This observation could be an indication of the distant-neighbor effect which the Ip– and –pI residues receive. If so, together with the assumption that the conformations of the dimer and trimer are essentially similar, the observed $\Delta\delta_T$ values for the –pIp– residue and the summed $\Delta\delta_D$ values (the bracketed values under the column of $\Delta\delta_D$ in Table VII) from the $\Delta\delta_D$ values of Ip– and –pI can be compared. Such a procedure cannot be used for the H-1′ protons, since the $\Delta\delta_D$ of H-1′ in the Ip– residue is larger than the $\Delta\delta_T$ of the same proton for the reason already discussed, *i.e.*, possibly due to the absence of right-handed to left-handed conversion in the trimer. Such a procedure was found to be applicable for the H-2 protons. Indeed, within the experimental error, the $\Delta\delta_T$ values of H-2 protons in the –pIp– residue of the trimer are the same as the summation of the $\Delta\delta_D$ values from Ip– and –pI residues in the dimer. This is the second observation which supports the existence of the distant-neighbor

effect. However, for the H-8, the $\Delta\delta_T$ values of the -pIp- residue in the trimer are consistently smaller (0.03–0.06 ppm less) than the summed values from the dimer. We propose that the H-8 proton of the -pIp- residue in the trimer is held at an averaged distance closer to the 5′-phosphate group than the H-8 of the -pI residue in the dimer because the nucleosidyl unit in the -pIp- is sandwiched between the two outside bases. The H-1′ protons present another interesting situation which includes the two exceptions mentioned above. In comparing the observed $\Delta\delta_T$ values of H-1′ in the -pIp- residue to the summed values (in parentheses, Table VII) from the $\Delta\delta_T$ values of H-1′ in Ip- and -pI residues of the trimer, an interesting trend was observed. At 5°, the observed $\Delta\delta_T$ is smaller than the summed value as may be expected due to the existence of distant-neighbor effects at the two terminal residues; at 30°, the observed $\Delta\delta_T$ value is equal to the summed value, and at 60° the observed $\Delta\delta_T$ value is larger than the summed value. The data reveal that the observed $\Delta\delta_T$ of the H-1′ in -pIp- is relatively insensitive to temperature change (a reduction by about 20% from 5 to 60°), while the summed value is decreased by about 45% from 5 to 60°. Obviously, the H-1′ proton in the -pIp- residue is receiving a larger shielding effect from two terminal residues than that exerted by the -pIp- residue in return to the two H-1′ protons in the two terminal residues. One possible explanation for such a phenomenon is that the dynamic geometrical relationship among the three residues in the trimer is not symmetrical, *i.e.*, the relationship between the first residue and the second residue is not identical to that between the second and the third residues.

In summary, the comparison between the dimerization shifts of IpI and the trimerization shifts of IpIpI reveal the following information: (1) a possible existence of the distant-neighbor field effect between the two terminal residues in the trimer; (2) the absence of right-handed to left-handed interconversion in the trimer; (3) a reduction of the rotational freedom of the nucleosidyl unit in the interior residue in the trimer (more rigidly fixed in an anti conformation); (4) a possible asymmetry in the geometrical relationship among the residues in the trimer. It should be noted that the polymerization shifts ($\Delta\delta_{polymer} - \Delta\delta_{monomer}$) of poly(rI) at 30° are 0.24 ppm for H-1′, 0.15 ppm for H-2, and 0.21 ppm for H-8 (Alderfer *et al.*, 1972). The corresponding observed trimerization shifts of the interior residue (Table VII) are 0.22 ppm for H-1′, 0.10 ppm for H-2, and 0.17 ppm for H-8. Therefore, the trimerization shift of the H-1′ is almost that of the polymerization shift already and the others are about 60–80%. This analysis suggests that the distant-neighbor effect cannot be very extensive along the chain, and that the level of shielding of the interior residue in the trimer is close but not equal to that in the polymer.

Correlation among the Studies on Ultraviolet Hypochromicity, Circular Dichroism, and Proton Magnetic Resonance. In a comparison between the data in Figure 5 and those in Figure 14, no apparent correlation can yet be established between the chain-length dependence on uv hypochromicity and the chain-length dependence on CD properties. For instance, in the rI series, the uv hypochromicity of the interior bases in I_{10} is about the same as that in the poly(I), but the CD spectrum (the original spectrum (Figure 6), the subtracted spectral properties (Figure 13), or the normalized spectral properties (Figure 14)) of I_{12} is still very different from that of poly(I). In the rA series, again the hypochromicity of the interior bases in rA_{7-8} is about the same as that in poly(A) (Figure 5), but the CD amplitude of rA_{10} is still much smaller than that of the poly(rA) (Figure 14). In the dA series, the interior base of the trimer already has the same degree of hypochromicity as the poly(dA); as for the CD properties, not until the level of hexamer (Figure 14, Bush and Scheraga, 1969) does the CD spectrum of poly(dA) resemble that of the oligomer, $(pdA)_6$. The series of rC cannot be evaluated because of the lack of data on the oligomer with n larger than 15. As for the series of rU and dT, the optical properties of these compounds are not chain-length dependent above the dimer stage. We may conclude that in general, the requirement in chain length for the oligomer to attain the same observed degree of residue–residue interaction as the polymer for these two techniques is much shorter for the uv hypochromicity than for the CD. The chain-length effect on uv hypochromicity is more abrupt, while that on CD is more gradual.

The present study on the influence of chain length on the pmr properties of oligonucleotides is only at the beginning stage. Besides the comparison between IpI and IpIpI, another important aspect of the pmr study is the comparison between IpI *vs.* pIpI, as well as the comparison between IpIpI *vs.* pIpIpI, in the absence or the presence of 1 M NaCl. All these alterations (dimer to trimer, addition of 5′-phosphate group to the terminal residue, and addition of 1 M NaCl) cause a dramatic change of the CD spectra. However, these alterations do not change the basic conformational model as far as the pmr measurements are concerned. The most outstanding example is the comparison between the CD spectra of IpI *vs.* pIpI (Figure 10) on the one hand, and the comparison between the dimerization shifts of IpI *vs.* pIpI (Table VI) on the other. While the difference in CD is large, the difference in dimerization shifts is minimal. Similarly, the effect of addition of 1 M NaCl to the CD spectra of pIpI is great, while the effect of addition of NaCl to the dimerization shifts of pIpI is small. The pmr data do not indicate that the nucleosidyl units in these oligomers have been changed from the anti conformation to the syn conformation—both residues if pIpI clearly are in anti conformation. Therefore, further studies are needed in order to understand the real nature of conformational changes which have such a significant effect on the CD spectra of the oligo(I).

Concluding Remarks

The uv absorbance and the CD spectra of the oligomer series of ribosyl inosinate ($r(I)_{2-14}$) have been carefully investigated, and the results have been complemented by comprehensive pmr studies on the dimer and the trimer of inosinate. Reports in the literature about the optical properties of oligo(rA), oligo(dA), oligo(rC), oligo(dC), oligo(rU), and oligo(dT) have been analyzed in comparison to those observed in the present studies on oligo(rI). The uv hypochromicity data of these oligomer series can be analyzed by a formal approach which is self-consistent. The results indicate that the hypochromic effect in the oligonucleotides depends mainly on the interaction of the nearest-neighbor chromophores, and that the interactions among chromophores farther away than the two nearest neighbors are of little significance. The pmr studies indicate that the nucleosidyl units in the dimer, IpI, or pIpI are in anti conformation and the screw axis of the stack is most likely right-handed. Addition of a 5′-phosphate at the terminal residue and addition of strong salt cause little change in the conformational model as reflected by the pmr properties of the dimer or trimer; these changes, however, have a very significant effect on the CD

spectra of the dimer and trimer. The pmr properties of the trimer can be understood from the pmr properties and the conformational model of the dimer, though various interpretations are still tentative and need to be confirmed. The pmr studies indicate a possible existence of the distant-neighbor field effect between the two terminal residues in the trimer and other conformational changes due to the presence of an interior residue. As for the CD results, more work, both in theoretical and experimental aspects, is urgently needed for a better understanding. The optical activities derived from the interaction of the chromophores of oligo inosinates cannot be described by a conservative Cotton effect which is originated from the coupled oscillator splitting of the strong $\pi \rightarrow \pi^*$ transition in giving positive and negative bands of nearly equal magnitude. In fact, in examining the optical activities of these oligomer series, only the CD spectra of oligo(rA) can be interpreted by the conservative Cotton effect, which appears to be an exceptional case rather than a common example. The chain-length dependence of the CD properties of the oligo(rI) is unusual: the peak intensity first decreases significantly and then increases again with the increasing chain length approaching that of the dimer, IpI. The CD spectra are sensitive to the addition of the 5'-phosphate group and salt concentration, indicating that it is influenced strongly by electrostatic forces exerted by the phosphate groups. The real nature of the conformational change, which is reflected mainly in the CD but not in uv absorbance or pmr data, remains to be investigated. However, it can be concluded generally that the requirement in chain length for the oligomer to attain the same measurement of residue–residue interaction as the polymer is much shorter for the uv hypochromicity than for the CD.

Each measurement—the uv absorbance (hypochromicity), CD spectrum, and pmr spectrum—has a different degree of dependence or sensitivity to various aspects of the conformation of the dimer and oligomers. Only when all these measurements (and perhaps others as well) can be correlated and unified in a satisfactory manner can we have a full understanding about the conformation of these oligonucleotides as well as the measurements themselves.

Acknowledgment

The authors thank Dr. Gerrit DeBoer for helpful discussions in preparing this paper.

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