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# Minor Nucleosides in RNA: Optical Studies of Dinucleoside Phosphates Containing Inosine

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## Synopsis

From the study of circular dichroism (CD) spectra and hypochromism we conclude that the dinucleoside phosphates IpA, ApI, and IpI stack, while IpU stacks very little. Studies with various concentrations of IpI in low salt and 1*M* NaCl indicate that the stacking geometry of this compound is sensitive to the ionic strength of solution. The CD of poly I is presented and compared to the data for IpI. Little change was found in the CD of poly (G,I) (1:1) with change of salt concentration, and we conclude that, unlike poly I, there is no major structural change. From the CD of poly (G,I), IpI, and GpG, the CD of IpG plus GpI is calculated by using the nearest-neighbor approximation. From the calculated spectrum, we tentatively conclude that there is stacking in either IpG or GpI or both.

## INTRODUCTION

In addition to the usual four RNA bases, minor bases are found in chromosomal RNA, ribosomal RNA and transfer RNA. The minor bases are modified forms of the normal bases; for example hypoxanthine, dihydrouracil, thiouracil, and methylated bases. A complete compilation of known minor bases has been made.[1]

All known tRNA's contain minor bases. Thus it appears justified to conclude that minor bases are necessary to the function of tRNA. This leads to the expectation that each minor base should have some unique biological property differing from the corresponding property of any normal base. If any of the normal bases, adenine, guanine, uracil, or cytosine could replace a minor base and not disrupt the functions of tRNA, there would be an evolutional advantage for this happening. For the appearance of a normal base, only one DNA base pair need be expressed, while for the appearance of a minor base many more than one must be expressed for the synthesis of the necessary enzymes which convert the appropriate normal base. If there were nothing special about the minor base, organisms which could use adenine, guanine, uracil or cytosine instead would be selectively favored.

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While this argument may or may not be valid, there is some support gained by considering the methylated bases, particularly *N*,*N*-dimethylguanine, *N*,*N*-dimethyladenine, 1-methylguanine, etc. These have the obvious property of being unable to base pair in the Watson-Crick configuration and thus have a decisive effect on the secondary structure of a tRNA molecule. The proposed structures for tRNA[2–4] all show these methylated bases in single strand regions.

The specificity of each tRNA interacting with the correct amino-acyl synthetase and with the ribosome–mRNA complex must be related to its structure. Thus the primary interest of this study is to learn more about the properties of minor bases which may be related to the structure of RNA. Dinucleoside phosphates have been used as models for single stranded RNA. If an understanding of how minor bases affect the structure of these simple compounds can be obtained, then a basis exists for predicting how a minor base might influence the structure of the region of RNA in which it occurs.

## MATERIALS AND METHODS

### Chemicals

IpU, IpI, poly I, and poly (G,I) (1:1) were purchased from Miles Laboratories and were used without further purification except as noted.* Monomers used were from P-L Biochemicals. IpA and IpI were prepared in this laboratory as described below. ApI was given to us by Dr. O. Uhlenbeck. ApA was purchased from Cal-biochem and from Zellstoffabrik Waldhof. Nitrosyl chloride gas was purchased from Matheson. Reagent-grade Trizma Base from Sigma was used. Dimethylformamide was reagent grade from Matheson, Coleman and Bell. All other chemicals used were reagent grade from either Baker and Adamson or Mallinckrodt.

### Deamination of ApA

The dimers IpA and IpI were obtained by deamination of ApA using nitrosyl chloride. The procedure of Sigel and Brintzinger[5] in slightly modified form was used. An ethylene glycol constant temperature bath with a circulating pump was used to maintain the reaction vessel at −8°C. The reaction is carried out in *N*,*N*-dimethylformamide (previously dried by refluxing at 153°C for 0.5 hr and stored under dry $N_2$). Dimethylformamide (0.1 ml) is added to 3–5 mg ApA and the slurry cooled to −8°C. Gaseous NOCl is added and will liquify at this temperature.[6] After adding NOCl, gas evolution is noticed, and the slurry begins to clear. For a partial reaction care must be taken to avoid an excess of NOCl. This can easily be checked by noting the color of the reaction mixture, because NOCl is a dark red-brown color. Anything darker than a light yellow indicates that too

* Abbreviations used: A, U, G, C, I signify the ribonucleosides adenosine, uridine, guanosine, cytidine, and inosine, respectively.

much NOCl has been added. A dark brown color in the reaction mixture should never be tolerated; it indicates that extensive degradation to monomers will occur. Excess NOCl can be removed by flushing the reaction vessel with dry $N_2$. After 15 min, more NOCl is added. The second addition of NOCl is omitted for a partial reaction. The reaction mixture is left at $-8°C$ for approximately 1 hr for a partial reaction, or up to 3 hr for a fairly complete reaction. After this time the reaction mixture is slowly warmed to room temperature. While it is warming, most of the unreacted NOCl can be removed by flushing with $N_2$. The mixture is then recooled to $-8°C$, 0.1 ml water added, and the mixture neutralized with solid $KHCO_3$. Sufficient $KHCO_3$ is added so that a few crystals remain on the bottom of the vessel. This will usually insure a pH between 7 and 8.

Deaminations of A and pA were used for controls following the same procedure.

After neutralization, the reaction mixture is lyophilized and redissolved in a small volume of water. It is then spotted on washed Whatman 3MM paper for descending chromatography and the paper equilibrated overnight. The solvent used is 99% *n*-butyl alcohol saturated with water plus 1% concentrated $NH_4OH$. The chromatogram is developed for about 5 days at 22°C. This is an important step in the separation, because compounds which contain inosine move very little in this system, while other compounds and salts are washed away. Here we may add that $KHCO_3$ appears to be the best means of neutralization; other salts do not wash away from the origin as well. Ammonium formate (pH 6.8), pH 7 phosphate buffer, and NaOH have been tried with unsatisfactory results. The origin of the chromatogram is then eluted and the material respotted on washed Whatman 3 MM paper for electrophoresis. This was carried out at pH 3.6 in 0.025*M* ammonium formate. The paper was equilibrated for at least 1 hr, the material spotted, and the electrophoresis run at 2900 V for 2.5 hr at 22°C. Four spots will usually result (this depends on reaction conditions) in the area towards the positive pole. The first, third and fourth have been identified as IpA, IpI, and Ip (and/or pI), respectively. There was never enough of the second spot for conclusive identification, though it may be ApI.

If necessary the material from electrophoresis was subjected to column chromatography on AG 1 × 2 resin to remove background contamination from the paper.

When only IpI was needed the reaction mixture was separated directly on AG 1 × 2 resin at pH 3.6 with a NaCl gradient. IpI is eluted first and is quite pure.

## Experimental Procedures

All spectra were taken with a Cary 15 recording spectrophotometer or a Cary 60 recording spectropolarimeter with a 6001 CD attachment. The data were reduced by an on-line PDP 8/S computer (Digital Equipment

Corp.) by using a program written by Drs. M. S. Itzkowitz and B. L. Tomlinson.[7] This program smooths both high- and low-frequency noise and records the data in both punched tape and printed format. Spectral integrals were computed by a subroutine written into the main program by C. Formoso. This subroutine uses a Simpson's Rule numerical integration, allowing the operator to choose the range of integration. No attempt at curve resolution is made.

Hypochromism was then calculated from the oscillator strengths of monomers and dimers as follows:

$$\%h = [1 - (f_D/f_M)]100$$

where $f_D$ is the oscillator strength of the dimer and $f_M$ is the oscillator strength of the monomers. Hypochromicity is calculated for a given wavelength $\lambda$:

$$\%h = \{1 - [\epsilon_M(\lambda)/\epsilon_D(\lambda)]\}100$$

where $\epsilon_D(\lambda)$ is the extinction coefficient of the dimer at the desired wavelength and $\epsilon_M(\lambda)$ is the extinction of the monomers at the same wavelength.

Temperature control was obtained by use of a cell block designed by Dr. D. M. Gray. This block features Dynatech Frigistor heating-cooling modules and a circulating water heat sink. The Frigistors are controlled from a Hallikainen Thermotrol 1053A.

Unless otherwise stated, the sample concentrations used were such that an optical density near 1 was obtained at $\lambda_{max}$ with a 1-cm light path. Though the intensities of some of the CD spectra reported here are low, all spectra have been repeated several times with independent samples and the results have been consistent, except where noted.

## RESULTS AND DISCUSSION

### IpI

We have observed the CD spectrum of IpI under various conditions of temperature, ionic strength, and dimer concentration. It is well known that the structure of poly I is dependent on ionic strength; it is single-stranded at low ionic strengths and is thought to become triple-stranded as salt concentration approaches $1M$.[8-10] IpI in essentially zero ionic strength solution (less than $10^{-8}M$ $Cl^-$) was obtained by gel filtration on a Bio Gel P-2 column. A solution of IpI in $1M$ NaCl was prepared by lyophilizing a portion of the zero-salt sample and redissolving it in $1M$ NaCl solution. Neither of these samples was buffered, but this does not cause any difficulty because the pH of distilled water is always on the acid side of neutrality within a range where there is no protonation of I. The results were checked with samples buffered at pH 7 and no differences were found. Figure 1 shows the CD spectra of the zero-salt sample at various temperatures, and also the CD spectrum of the monomer pI for comparison. There was no change in the CD spectrum of the monomer with salt concentration in the

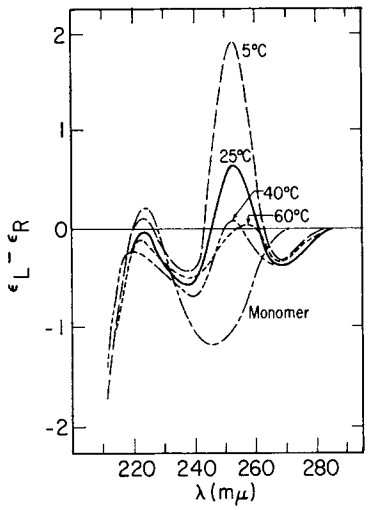


Fig. 1. CD of IpI in zero salt solution at (– –) 5°C, (——) 25°C, (– – – –) 40°C, and (– –) 60°C; and (– – –) the CD of pI.

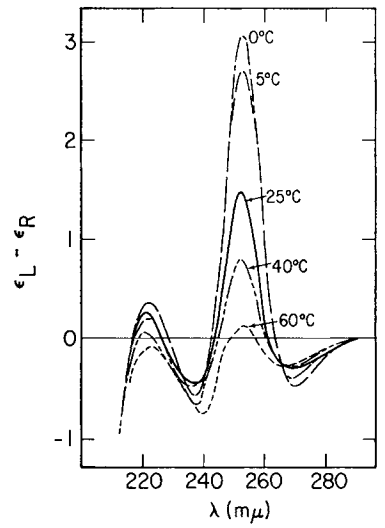


Fig. 2. CD of IpI in 1*M* NaCl solution at (– – –) 0°C, (– –) 5°C, (——) 25°C, (– – – –) 40°C, and (– –) 60°C.

range 0–1*M* NaCl, with temperature between 0°C and 60°C, or with monomer concentration of 1–20 $OD_{248}$/ml. Also the CD spectrum of I is identical to that of pI. The CD spectra at various temperatures of the 1*M* NaCl sample of IpI are shown in Figure 2. It can be seen that the CD of IpI is not like the CD of pI under any of the conditions studied, and, as has been reported for other dimers,[11–13] the CD of IpI approaches that of the monomer as the temperature is raised. Thus, we may conclude that the base-

base interaction is considerable at room temperature (25°C) and below, while at 60°C the interaction is diminished, but not insignificant. Using the standard two-state analysis,[12] we determine $\Delta H^\circ$ of unstacking to be about 14 kcal/mole, which is considerably higher than $\Delta H^\circ$ values reported for the usual dimers.[11] The extrapolations necessary to analyze the data in this manner, however make the $\Delta H^\circ$ value somewhat uncertain.

Observe also that ionic strength does affect the CD spectrum of IpI, indicating a structural change. This fact has also been reported for IpIp by Inoue and Satoh.[14] The CD in high salt solution is increased in magnitude, but is of approximately the same shape. The increase in magnitude is about the same as would occur if the temperature of the low salt solution were lowered by about 20°C. The changes in magnitude can also be appreciated by looking at the rotational strengths reported in Table I. To learn more about this structural change we have observed the CD and/or ORD spectra of IpI over a 200-fold concentration range in both zero salt and 1$M$ salt solutions. For this purpose cylindrical quartz cells of path lengths 0.05 cm, 1 cm, and 10 cm were employed. Since the Cary 60 CD attachment will only hold up to a 3-cm path length cell, it was necessary to revert to ORD for measurements using the cell of 10 cm path length. However, there was no change in the ORD for either the high salt or the low salt case in going from the 1 cm cell to the 10 cm cell (diluting from $8 \times 10^{-5}M$ to $8 \times 10^{-6}M$ in base residue). Obviously there would likewise be no change ir the CD.

In low salt solution, using the 0.05 cm cell, there was no change in CD up to 39.0 $OD_{248}$/ml ($3.25 \times 10^{-3}M$). In high salt solution we believe that there is no change in CD when the 0.05 cm cell is used, but we did observe one exception. Previous to this, the IpI prepared by deamination was similar in all ways to that purchased commercially. But the 1$M$ NaCl sample prepared from the commercial IpI (concentration $1.77 \times 10^{-3}M$) showed an altered CD spectrum, while three samples prepared with our IpI, with concentrations $1.48 \times 10^{-3}M$ (in 1$M$ NaCl), $1.84 \times 10^{-3}M$ (1$M$ NaCl), and $2.70 \times 10^{-3}M$ (1.7$M$ NaCl), showed no change in CD. Thinking that this might be due to a trace amount of metal ion in the commercial

TABLE I
Rotational Strengths Calculated from CD Spectra of IpI

| | $R_{230} \times 10^{38}$, esu-cgs[a] | | $R \times 10^{38}$, esu-cgs[b] | |
|---|---|---|---|---|
| $T$, °C | Zero salt | 1$M$ MaCl | Zero salt | 1$M$ NaCl |
| 0° | — | 1.28 | — | 1.63 |
| 5° | 0.60 | 1.30 | 1.00 | 1.48 |
| 25° | 0.46 | 0.45 | 0.36 | 0.72 |
| 40° | −0.75 | −0.68 | −0.12 | 0.21 |
| 60° | −0.88 | −1.42 | −0.14 | −0.17 |

[a] Integration from 350 mμ to 230 mμ.

[b] Integration over the region of the first positive band; 264 mμ to 244 mμ.

sample, we added $MgCl_2$ to the remaining portion of our most concentrated high salt solution. With $10^{-2}M$ $Mg^{++}$, $1.4M$ NaCl, and a base residue concentration of $2.32 \times 10^{-3}M$, there was no change in CD. We are investigating this further, but the bulk of the present evidence indicates that there is no CD change.

We, therefore, must conclude that there is no aggregation of IpI in $1M$ NaCl at ordinary optical concentrations. The structural change caused by the increase in ionic strength must thus be an internal one, i.e., a change in stacking geometry. We have reproduced the ORD data of Inoue and Satoh in Figure 3 for comparison with our data. It can be seen that the terminal phosphate on IpIp causes a change in ORD. This effect can not be due to steric factors, since the ORD of IpIp in $1M$ KCl becomes very like the ORD

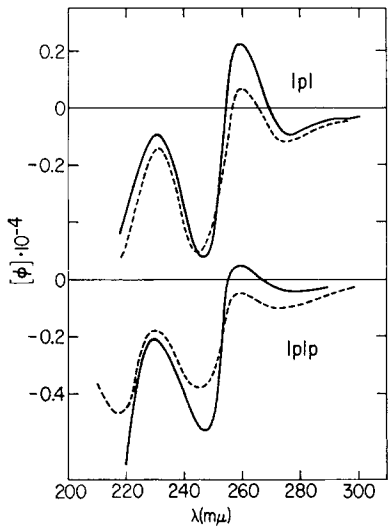


Fig. 3. ORD of IpI and IpIp: upper curves, IpI in 1 $M$ NaCl (——) and zero salt (- -); (lower curves) IpIp in $1M$ KCl (——) and $0.1M$ KCl (- -) (from Inoue and Satoh).

of IpI in low salt. This is probably because of electrostatic shielding by the $K^+$ ion. Thus the stacking geometry is also quite sensitive to the coulombic influence of the terminal phosphate group.

The extinction coefficient and oscillator strength of IpI were determined by first taking the ultraviolet spectrum of a standard solution, then hydrolyzing with NaOH, reneutralizing with $HClO_4$ and taking the ultraviolet spectrum of the resultant monomers. This was done in triplicate. After correcting for dilution, the concentration of IpI in the original solution was determined and the extinction coefficient calculated. The oscillator strength was calculated from the spectrum as described above. The extinction coefficient of IpI in $1M$ NaCl solution was determined by preparing solutions of equal IpI concentration in zero and $1M$ NaCl, respectively. The results are given in Table II.

TABLE II
Values Determined from Ultraviolet Absorption Spectra

| Compound | Experimental conditions | $\lambda_{max}$, mμ | $\epsilon_{max} \times 10^{-3}$ | $f_{230}$[a] | % $H$ | % $h$[b] |
|---|---|---|---|---|---|---|
| pI | pH 7.3, 25°C | 248 | 12.2 | 0.261 | | |
| IpI | Zero salt, 25°C | 248 | 12.0 | 0.254 | 2.7 ± 0.2 | 1.5 ± 0.2 |
| IpI | 1*M* NaCl, 25°C | 248 | 11.9 | 0.250 | 4.2 ± 0.2 | 2.5 ± 0.2 |
| pA + pI | pH 7.3, 25°C | 252 | 12.3 | 0.288 | | |
| IpA | pH 7.3, 25°C | 251 | 11.0 | 0.261 | 9.4 ± 0.9 | 10.6 ± 0.9 |
| ApI | pH 7.3, 25°C | 252 | 11.6 | 0.281 | 2.5 ± 1.6 | 6.7 ± 2.9 |
| pU + pI | pH 7.3, 25°C | 251 | 9.7 | 0.246 | | |
| IpU | pH 7.3, 25°C | 251 | 9.6 | 0.243 | 1.2 ± 0.4 | 0.8 ± 0.5 |

[a] Integration of ultraviolet spectrum was from 360 mμ to 230 mμ.
[b] Calculated at $\lambda_{max}$.

The hypochromism of 2.7% and the hypochromicity are small but consistent with the conclusion that there is a stacking interaction in IpI. While the hypochromicity is only 1.5% at 248 mμ($\lambda_{max}$) a maximum difference in extinction of 550 is reached at 259 mμ which gives a hypochromicity of 7%. This closely agrees with the hypochromicity of 6% reported by Inoue and Satoh for IpIp at 260 mμ. The hypochromism of 4.2% in 1*M* NaCl solution would imply that stacking is increased by increasing ionic strength.

These results are quite interesting since it is generally held that salt concentration has little effect on the optical properties of oligonucleotides. Recent studies[15] have demonstrated conformational changes in mononucleotides with salt concentration, but we have found no structural change in I or pI that is detectable by CD. We do not expect that the dependence of IpI structure on salt concentration could be solely due to changes in monomer conformation. It seems more likely that the balance of forces giving rise to the particular dimer geometry is upset as ionic environment changes. If the magnitude of the positive ORD maximum at 260 mμ is used as a criterion for stacking, an ordering can be seen: IpI (1*M* salt) > IpI (low salt) ~ IpIp (1*M* salt) > IpI (low salt). It might be that other dimers would show a similar ordering, but with changes of smaller magnitude. The unusual nature of our results with IpI is not that there is a change in stacking with salt concentration, but that the change is so large. It is, of course, unknown why IpI and IpIp respond to changes in salt concentration, but it appears to not be inherent in inosine. Other inosine dimers studied in this work (discussed below) did not show a corresponding effect.

## Poly I

Although ORD[10] and CD[16] spectra of poly I have previously been reported, we have taken the CD spectra (see Figs. 4 and 5) for direct comparison with our IpI data. The polymer concentrations were determined by us-

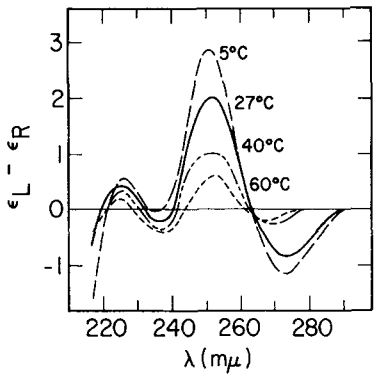


Fig. 4. CD of poly I in low salt solution (commercial sample, as received, dissolved in glass-distilled water) at (– –) 5°C, (——) 27°C, (– – – –) 40°C, and (- -) 60°C.

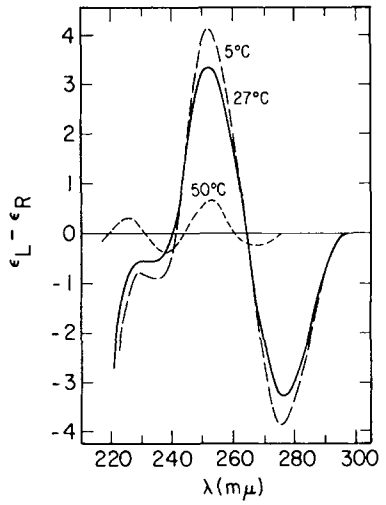


Fig. 5. CD of poly I in 0.85*M* NaCl solution at (– –) 5°C, (——) 27°C, and (- -) 50°C.

ing an extinction coefficient of 10,200 for poly I at 248 mμ in 0.1*M* NaCl solution,[10] and assuming it was identical for our low salt sample (commercial sample, as received, dissolved in $H_2O$). The samples were prepared in such a way that the polymer concentration would be identical in both the low salt and high salt samples. This gave an extinction coefficient at 248 mμ for multistranded poly I in 0.85*M* NaCl of 6500, which is comparable to that obtained for other sources.[9,17]

Our CD spectra for poly I do not agree exactly with the previously reported CD.[16] While the general shape is the same, our curves show a larger negative $\epsilon_L - \epsilon_R$ at 275 mμ. Both the low-salt (Fig. 4) and 0.85*M* NaCl (Figure 5) data show this discrepancy with the previous results.[16] The previous workers' low-salt sample was more concentrated in both polymer (1

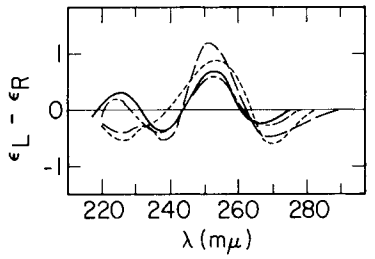


Fig. 6. Comparison of experimental and calculated CD for poly I at high temperature (50–60°C): (——) high salt, measured; (– –) high salt, calculated; (– – –) low salt, measured; (- -) low salt, calculated.

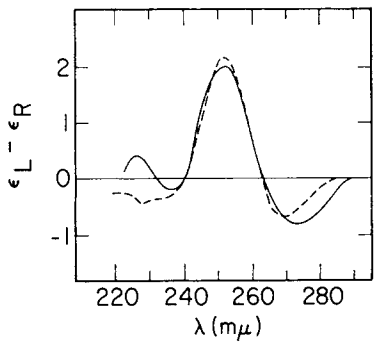


Fig. 7. Comparison of experimental and calculated CD for poly I at 27°C in low salt: (——) measured; (- -) calculated.

and 2 mm light path) and salt (0.1$M$ NaCl, 0.01$M$ Tris, $10^{-4}M$ EDTA), and the CD taken at a lower temperature (0°C) than ours, but they clearly showed that their low-salt sample was single-stranded.[16] Thus it is very likely that poly I is also single-stranded at our low-salt conditions. The previous work in high-salt solution was at 0.5$M$ NaCl,[16] so the possibility exists that different forms in high salt solution have been observed. Though the sources of the CD discrepancies are not at all clear, and the matter should be further studied, it is not our purpose to do so here. In the following discussion, our CD results with poly I are used.

The CD data for poly I can be compared with that for monomer and dimer by the following equation:[18]

$$\Delta\epsilon_{\text{polyI}} = 2\Delta\epsilon_{\text{IpI}} - \Delta\epsilon_{\text{pI}}$$

If the secondary structure of dimer and polymer are identical, and if only nearest-neighbor interactions contribute to the CD, then this equation will be a good approximation. In Figures 6, 7, and 8 we show the results of this calculation compared to the experimental CD of single-stranded poly I. For poly I at high temperature (50–60°C), the calculated CD is not too bad for either the high-salt or low-salt case. While the agreement between

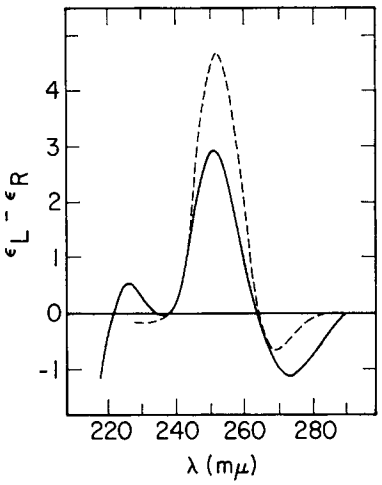


Fig. 8. Comparison of experimental and calculated CD for poly I at 5°C in low salt: (——) measured; (- -) calculated,

experiment and calculation is good for low salt at 27°C, it is poor for low salt at 5°C. Therefore, it is likely that the temperature dependence of the structure of the polymer is different from that of the dimer. As we have stated above, I–I stacking interactions are sensitive to electrostatic environment. Certainly the electrostatic environment of the polymer is different from that which the dimer experiences. This may be sufficient to cause a difference in stacking geometry and temperature dependence. Perhaps it is only fortuitous that the secondary structures of dimer and polymer are approximately the same at room temperature. Both IpI and single-stranded poly I show rather sharp melting profiles. This would mean that slight differences in stability could lead to considerable differences in structure.

## Poly (G, I)

The CD of poly (G, I) (1:1) was observed in low salt (commercial sample, as received, dissolved in $H_2O$) and 1*M* NaCl solution. The polymer concentration was estimated by assuming an extinction coefficient of $10^4$ at 251 mμ ($\lambda_{max}$). Since we were interested mainly in determining if multiple strands formed, this was sufficiently accurate for our purpose. The CD (Fig. 9) is independent of salt concentration indicating that the strandedness of poly (G, I) is also independent of this factor in the range studied. The CD is unlike that of poly I, and there is more of a similarity to that of poly G.[19] Thus G–G interactions probably dominate the various contributions to the poly (G, I) CD spectrum.

Assuming a random sequence, one can estimate the I–G interaction by using the nearest-neighbor formulation for circular dichroism:[20]

$$\Delta\epsilon_{IpG} + \Delta\epsilon_{GpI} = 2\Delta\epsilon_{poly\ GI} - (\Delta\epsilon_{IpI} + \Delta\epsilon_{GpG}) + \Delta\epsilon_{pI} + \Delta\epsilon_{pG}$$

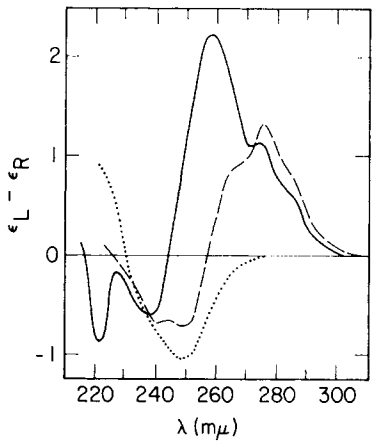


Fig. 9. CD of poly(G,I) (1:1) in low salt (commercial sample, as received, dissolved in glass-distilled water) or 1*M* NaCl and calculated CD for IpG + GpI (per mole of base residue): (——) poly(G,I); (– –) (IpG + GpI); (· · ·) monomers.

The result of this calculation is also shown in Figure 9 (CD data for GpG from Warshaw and Cantor[21]). Since the calculated spectrum is significantly different from the monomers' spectra, we conclude that at least one and perhaps both of the dimers, IpG and GpI, stack. This would not be surprising, since purines usually do show a tendancy to stack. The calculation was also made by using the CD data for poly I and poly G in place of those for IpI and GpG. While the CD calculated in this manner differed considerably from that presented above, the conclusion concerning stacking in IpG and GpI is not altered. The differences between the two calculated spectra may be due to aggregation in poly G or to other unknown factors. Certainly, both calculations are subject to doubt because poly (G, I) may not be sufficiently random in sequence; and also it may not be completely single-stranded.

## IpA

The CD spectrum of IpA at three temperatures is shown in Figure 10. The CD spectrum of the monomers is also shown for comparison. It was found that ionic strength did not affect the ORD between 0 and 1*M* NaCl. There was also no change in ORD in diluting the sample from $10^{-4}M$ (1-cm cell) to $10^{-5}M$ (10-cm cell).

The extinction coefficient of IpA was determined by a triplicate hydrolysis with venom phosphodiesterase (Worthington). The dimer concentration was determined from the ultraviolet absorption and known extinction coefficients[22,23] of the monomers. The results are given in Table II.

The base–base interaction in IpA is quite extensive as may be concluded from the CD data, hypochromism of 9.4% and the hypochromicity of 10.6% (Table I). There is no change in structure with changes in salt concentration.

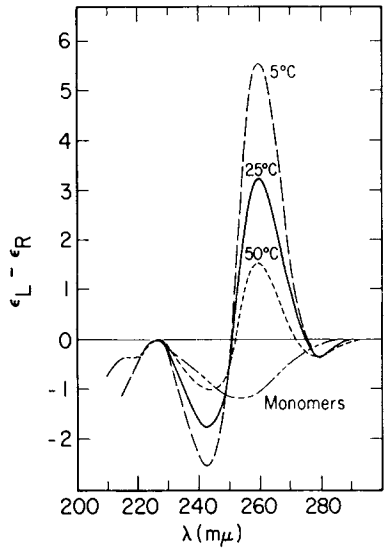


Fig. 10. CD of IpA at pH 7.3 0.1*M* NaCl: (——) 5°C, (– –) 25°C, and (- -) 50°C; (— — — —) CD of monomers.

## ApI

The CD of ApI is given in Figure 11. There was only a very slight increase in both positive and negative CD bands when the salt concentration

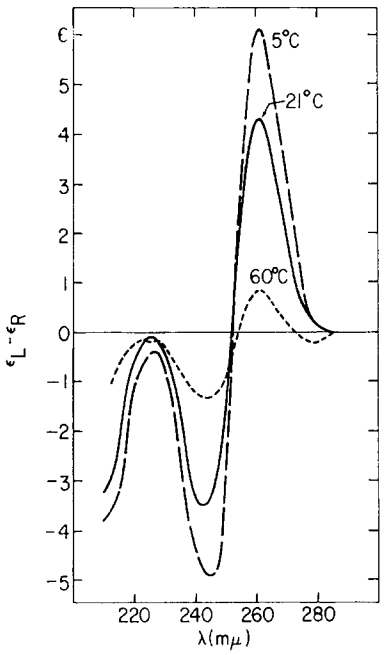


Fig. 11. CD of ApI at pH 7.3, 0.1*M* NaCl: (– –) 5°C; (——) 21°C; (- -) 60°C.

was raised to 1*M* NaCl. The extinction coefficient was determined by enzymatic hydrolysis as with IpA, and these results are listed in Table II.

The CD results demonstrate that there is stacking in ApI, and this is supported by the hypochromism of 2.5% and the hypochromicity of 6.7%. There is only a small change in CD with ionic strength. Thus the sensitivity of inosine to this factor may be restricted to self interaction.

## IpU

The CD spectrum of IpU is reported at three temperatures, along with the monomers' CD, in Figure 12. There was only a very small increase in CD in 1*M* NaCl solution. The extinction coefficient was determined by the same method used with IpA and ApI and the results are given in Table II.

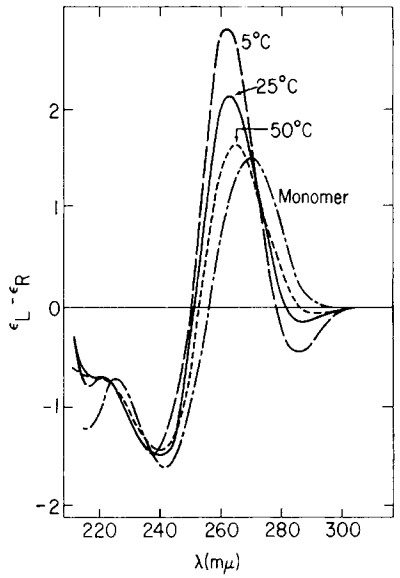


Fig. 12. CD of IpU at pH 7.3, 0.1*M* NaCl: (– –) 5°C, (——) 25°C, and (- -) 50°C; (– - –) CD of monomers.

The CD spectrum of IpU is not very different from the sum of the monomers CD at room temperature. But the difference that does exist indicates some degree of interaction. The hypochromism of 1.2% also indicates that there is some interaction. This is consistent with previous results,[24,25] with dimers containing U; they usually exhibit less stacking at room temperature than do other dimers.

## CONCLUSIONS

We can conclude from our studies that IpI, IpA, and ApI stack considerably at room temperature, but that IpU does not. The stacking does not appear unusual unless I endows stability to a structure not allowed by other

bases. This cannot be determined from CD studies without a great deal of calculation. Of course, the hydrogen bonding properties of I are unlike those of normal bases, but to a good approximation I base pairs like G. What does seem important, and unique, is the sensitivity to ionic environment of the conformation of oligomers of I. While this may be influential in the single to triple strand conversion of poly I, there is no clear interpretation in relation to tRNA structure since the sequence —IpI— does not occur in any known tRNA. Perhaps in the geometrically specific environment of the ribosome complex or the synthetase complex this sensitivity of I may play an important role. Inosine seems to appear only in the anticodon loop in the "wobble" position.[26]

Looking at specific sequences, —UpIpG— occurs in yeast alanine and yeast serine[2] tRNA's, while —UpIpA— occurs in yeast valine[2] and *T. utilis* isoleucine[27] tRNA's. Thus of the nearest neighbor interactions involved we can only make a definite statement on IpA. However, the calculated spectrum of IpG + GpI suggests that there is stacking of IpG. We suppose that there will be little stacking in UpI since there is little in IpU. But in the results of Warshaw and Tinoco[25] UpG shows evidence of stacking while GpU shows little. Thus UpI may be able to stack, while IpU does not. We therefore conclude that I does not disrupt stacking in the anticodon loop, but allows the loop to maintain a normally rigid structure with the possibility of subtle structural variations caused by the sensitivity of I to the ionic environment.

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