cause or be caused by significant changes in the protein conformation.

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Circular Dichroism Study of the Effects of Magnesium Perchlorate and Temperature on the Solution Conformation of Uridine 5′-Monophosphate, Uridine 3′-Monophosphate, Uridine, and Uridylyl-(3′→5′)-uridine†

Carl Formoso

ABSTRACT: The circular dichroism (CD) spectra of uridine 5′-monophosphate, uridine 3′-monophosphate, uridine, and uridylyl-(3′→5′)-uridine were obtained under a variety of solution conditions generated by adjusting magnesium perchlorate concentration and temperature. The CD results for the monomers are discussed in comparison to recent nuclear magnetic resonance (nmr) results. Both the CD and nmr of these compounds are sensitive to temperature and salt concentration, indicating that conformational changes are induced by these solution conditions. Evidence is presented which indicates that the CD of the monomers is primarily affected by the position of the base moiety relative to the furanose ring. The CD results clearly show that the effects of increasing temperature are quite different from the effects of increasing magnesium perchlorate concentration. Discrepancies with the nmr results are discussed. From an examination of the uridylyl-(3′→5′)-uridine CD spectra, with proper accounting for monomer conformational processes, it is concluded that the conformation of the dinucleoside phosphate achieved by high magnesium perchlorate concentration is similar to the conformation achieved by high temperature. Based on the conformation-perturbing effectiveness of magnesium perchlorate as a function of temperature, it is suggested that magnesium perchlorate acts both by direct binding of the Mg^{2+} ion and by disruption of solvent structure.

Recent nuclear magnetic resonance studies (Prestegard and Chan, 1969; Schleich *et al.*, 1972) have indicated that conformational changes occur in uracil nucleosides and nucleotides upon addition of certain salts to solution, and with temperature. These conformational changes are thought to involve both the position of the base relative to the sugar

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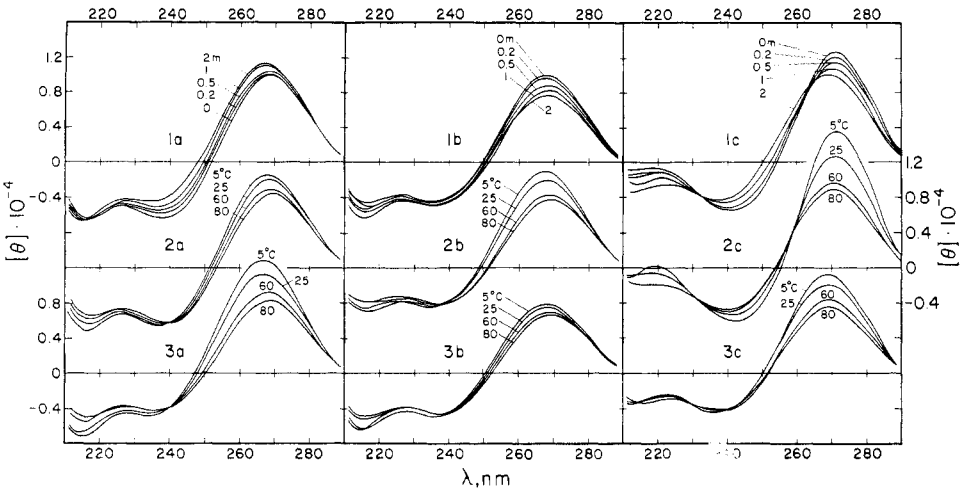

FIGURE 1: The CD of (a) 5′-UMP, (b) 3′-UMP, and (c) UpU at 25° and the indicated concentrations of $Mg(ClO_4)_2$.

FIGURE 2: The CD of (a) 5′-UMP, (b) 3′-UMP, and (c) UpU in zero $Mg(ClO_4)_2$ solution at the indicated temperatures. The scale for the ordinate is on the right.

FIGURE 3: The CD of (a) 5′-UMP, (b) 3′-UMP, and (c) UpU in 2 *m* $Mg(ClO_4)_2$ solution at the indicated temperatures.

moiety, and changes within the sugar. While there has been extensive work with circular dichroism (CD) (or optical rotatory dispersion) of the nucleic acid monomer units (Ikehara *et al.*, 1972; Miles *et al.*, 1971; Rogers and Ulbricht, 1970; Yang *et al.*, 1966), no CD work has appeared which monitors the conformational changes seen by nuclear magnetic resonance (nmr). It is useful to do this, since the two techniques are sensitive to different aspects of molecular structure, and a better understanding of that molecular structure can thus be obtained.

The CD spectra are observed over a range of 5–80° and 0–2 *m* $Mg(ClO_4)_2$. This salt was chosen since it generally had the largest effect in the nmr results (Prestegard and Chan, 1969). Part of this work is an effort to correlate changes caused by solution conditions in CD spectra and nmr spectra for 5′-UMP, 3′-UMP, and U.[1] The dinucleoside phosphate containing these two monomer units, UpU, is also examined in an attempt to determine how important monomer conformational changes are in oligomers, and the extent to which oligomer structure depends on solution conditions. UpU is a good choice for this study since it shows little stacking interaction, and the CD of the monomers contribute a large part of the UpU CD.

Materials and Methods

U and 5′-UMP were from Calbiochem. UpU, 3′-UMP, iU, and iUMA were from Sigma. All chemicals were reagent grade.

Ultraviolet absorption spectra were taken on a Cary 14 spectrophotometer and CD spectra were taken on a Cary 61 spectropolarimeter. The Cary 61 was operated at a scan speed of 1 Å/sec, with a slit program which maintained a spectral bandwidth of 1.5 nm. The CD data were processed by an on-line Varian 620/i computer. The data were digitized every 1 nm and then smoothed by a 13-point function (Savitzky and Golay, 1964). CD data are reported as molar ellipticity per base residue. In the 260-nm region the reproducibility of the data is $[\theta] = \pm 300$ (deg cm²)/dmole. No correction was made for the refractive index of the solvent. Difference spectra were obtained using the digitized, smoothed data. The temperature was controlled electronically to ±0.5° in a manner similar to that described by Allen *et al.* (1972). When CD spectra were taken at elevated temperatures the ultraviolet (uv) absorbance before and after the CD measurement was identical within 1%.

All solutions were buffered by 0.01 M Na^+, pH 7 phosphate buffer. A concentrated stock of each sample was prepared in buffer prior to beginning a series of experiments. From this, 50-μl portions were taken and stored at −20°. When the material was needed for a CD spectrum, it was thawed and 3.0 ml of the appropriate solution added. The optical density at 260 nm was between 0.8 and 1.05 for all CD work. Neither the dilution due to the 50 μl of sample, nor the concentration of buffer is considered in the reported salt concentrations.

Results

Eleven CD spectra were taken for each compound (except the isopropylidene derivatives) under varying conditions of temperature and $Mg(ClO_4)_2$ concentration. The spectra for 5′-UMP, 3′-UMP, and UpU are shown in Figures 1, 2, and 3. The figures are arranged so that looking down a column the same compound is shown; looking across a row, the same type of solution conditions are shown. The results for U are similar to those for 5′-UMP except that $Mg(ClO_4)_2$ has quite a small effect on the CD spectra of U. Increasing $Mg(ClO_4)_2$ produces quite different effects on the CD of 5′-UMP, 3′-UMP, and UpU as seen in Figure 1. The presence of 2 *m* $Mg(ClO_4)_2$ magnifies the temperature dependence of the CD for both 5′-UMP and U, but diminishes the temperature dependence for 3′-UMP and UpU (Figures 2 and 3). At 25° in zero salt there are small differences between the CD of 5′-UMP and 3′-UMP, primarily at low wavelength. The CD of U is almost identical to that of 3′-UMP under these conditions. A similar difference in low-wavelength CD has been seen with A and 5′-AMP (Ikehara *et al.*, 1972). The CD of UpU is closer to the monomer CD at higher concentrations of $Mg(ClO_4)_2$. At 80° in zero salt the CD of UpU approaches

[1] Abbreviations used are: 5′-UMP uridine 5′-monophosphate; 3′-UMP, uridine 3′-monophosphate; U, uridine; UpU, uridylyl-(3′→5′)-uridine; iU, 2′,3′-isopropylidenuridine; iUMA, 2′,3′-isopropylidenuridine 5′-monoacetate.

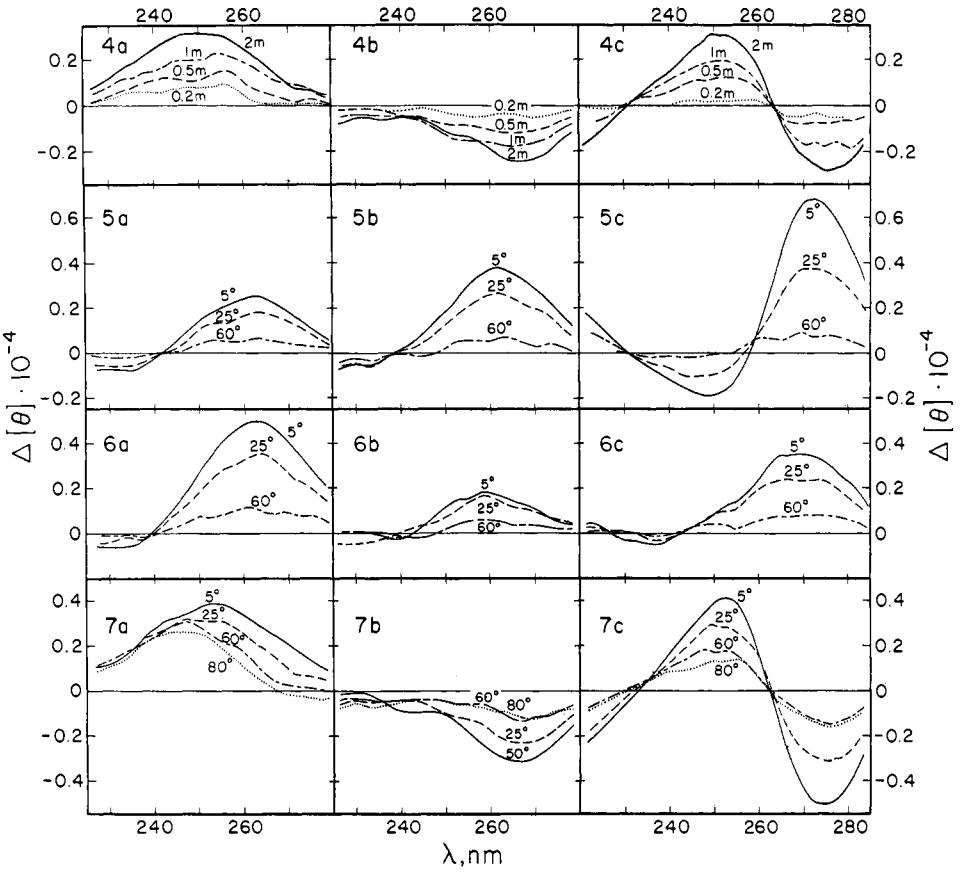

FIGURE 4: The salt-induced CD difference spectra of (a) 5′-UMP, (b) 3′-UMP, and (c) UpU at 25°. The CD spectrum in zero $Mg(ClO_4)_2$ was subtracted from the CD spectrum at the indicated salt concentration in each case.

FIGURE 5: The temperature-induced CD difference spectra of (a) 5′-UMP, (b) 3′-UMP, and (c) UpU in zero $Mg(ClO_4)_2$ solution. The CD spectrum at 80° was subtracted from the CD spectrum at the indicated temperature in each case.

FIGURE 6: The temperature-induced CD difference spectra of (a) 5′-UMP, (b) 3′-UMP, and (c) UpU in 2 *m* $Mg(ClO_4)_2$ solution. The CD spectrum at 80° was subtracted from the CD spectrum at the indicated temperature in each case.

FIGURE 7: The difference in CD in zero salt and 2 *m* $Mg(ClO_4)_2$ solution (2–0 *m*) as a function of temperature for (a) 5′-UMP, (b) 3′-UMP, and (c) UpU.

that of the monomers, the largest deviation from the monomers CD being in the 220-nm region (Figure 2). In 2 *m* salt at 80° the largest deviation from the monomers CD is again in the 220-nm region (Figure 3).

To obtain a clearer view of the spectral changes caused by $Mg(ClO_4)_2$ and temperature, CD difference spectra were determined, and are shown in Figures 4, 5, 6, and 7. Representative CD difference spectra are shown in Figure 8 for U, and in Figure 9 for iU and iUMA. For 5′-UMP and 3′-UMP the CD difference spectra induced by $Mg(ClO_4)_2$ are not only of different sign, but also the maxima occur at different wavelengths (Figure 4a,b). The curves for 5′-UMP have an isosbestic point at 216 nm (not shown), but there is no apparent isosbestic point in the 3′-UMP curves. The CD difference spectra induced by $Mg(ClO_4)_2$ for UpU (Figure 4c) show a negative peak at 275 nm and a positive peak near 250 nm, with isosbestic points at 263 and 232 nm.

In zero $Mg(ClO_4)_2$ the effects of temperature on the CD of 5′-UMP (Figure 5a) 3′-UMP (Figure 5b) and U (Figure 8) are very similar, with the CD of 5′-UMP showing a slightly smaller temperature dependence. In 2 *m* $Mg(ClO_4)_2$ the temperature dependence of the CD of both 5′-UMP (Figure 6a) and U (Figure 8) is increased, but that of 3′-UMP (Figure 6b)

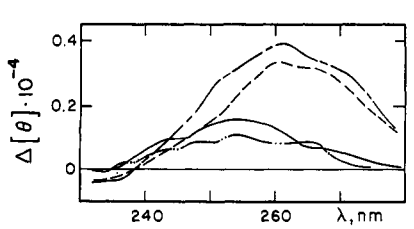

FIGURE 8: CD difference spectra for U: (——) at 25° and (— · —) at 80° the CD in zero salt subtracted from the CD in 2 *m* $Mg(ClO_4)_2$; (- - - -) in zero salt and (— — —) in 2 *m* $Mg(ClO_4)_2$ the CD at 80° subtracted from the CD at 5°.

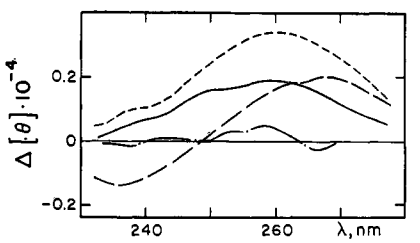

FIGURE 9: CD difference spectra for iU and iUMA: in zero salt (——) for iU and (- - - -) for iUMA the CD at 80° subtracted from the CD at 5°; at 25°C (— —) for iUMA and (— · —) for iU the CD in zero salt subtracted from the CD in 2 *m* $Mg(ClO_4)_2$.

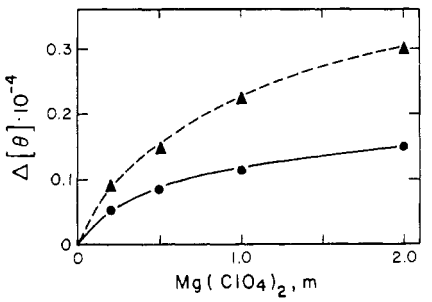

FIGURE 10: The magnitude of the CD difference spectrum at 255 nm for U (●) and 5′-UMP (▲) as a function of $Mg(ClO_4)_2$ concentration at 25°.

is decreased. 5′-UMP shows the largest temperature dependence in 2 *m* salt.

As expected, the temperature dependence of the CD of UpU (Figure 5c) in zero $Mg(ClO_4)_2$ is quite different from that of the monomers. There are isosbestic points at 259 and 231 nm in the CD difference spectra for UpU, and at 242 nm for 5′-UMP, 238 nm for 3′-UMP, and 240 nm for U.

The temperature dependence of the CD of UpU in 2 *m* $Mg(ClO_4)_2$ (Figure 6c) is more like that of the monomers (Figure 6a,b), but significant differences remain. The peak for 3′-UMP (262 nm) and the peak for 5′-UMP (263 nm) are different from the peak for UpU (268 nm). The UpU CD difference spectra (Figure 6c) show isosbestics at 243, 226, and 215 nm (not shown). The isosbestic for either 5′-UMP or U is at 238 nm, while 3′-UMP does not show an isosbestic point.

Figure 7 shows the effect of temperature on the CD differences induced by 2 *m* $Mg(ClO_4)_2$. The effect is slightly larger for 5′-UMP (Figure 7a) than for 3′-UMP (Figure 7b), and both are larger than that for U (Figure 8). The changes for 5′-UMP and 3′-UMP are mainly in the region above 245 nm. There are no apparent isosbestic points in the difference spectra for either 5′-UMP or 3′-UMP. The corresponding CD difference spectra for UpU (Figure 7c) show isosbestics at 263 and 235 nm. For all compounds there is very little effect on the salt-induced CD difference spectra between 60 and 80° (Figure 7), and a substantial effect of 2 *m* $Mg(ClO_4)_2$ remains at 80°.

The group of CD difference spectra for the monomers studied show peaks or shoulders in three wavelength regions: 245, 255, and 270 nm. The presence of three peak locations in the CD difference spectra may be an indication that there are three electronic transitions which make important contributions to the CD in this wavelength region. Usually only two π–π^* transitions are considered to be in this region (Clark and Tinoco, 1965); the third transition may be an n–π^* transition.

Discussion

In the monomers the conformational changes which primarily concern us are the rotations around the glycosidic bond, and the puckerings of the furanose ring. The torsion angle, ϕ_{CN}, defined by Donohue and Trueblood (1960) characterizes the rotation of the base relative to the furanose ring. A $\phi_{CN} = -30°$ corresponds to the "anti" conformation usually found in crystals (Wilson and Rahman, 1971). The furanose ring configuration is usually defined by the puckering at the 2′ or 3′ positions. In endo structures the atom referred to is displaced to the same side of the ring as $C_{5'}$, and in exo structures it is displaced opposite $C_{5'}$.

The nmr results indicate that both glycosidic rotation and sugar puckering change with salt and temperature (Prestegard and Chan, 1969; Schleich *et al.*, 1972). While CD will be sensitive to these changes in molecular conformation, it could also be sensitive to changes in electronic structure (for example, solvent shifts) induced by $Mg(ClO_4)_2$. While no uv absorption differences caused by $Mg(ClO_4)_2$ were observed outside the level of experimental error (1–2% due to volumetric work), this does not rigorously exclude changes in electronic structure since the observed CD differences are rather small. However, since nmr is less sensitive to overall electronic structure, and it is thus quite likely that conformational changes do occur, the CD differences are completely attributed to differences in molecular conformation. This is also supported by the fact that the CD of U or iU is changed very little by the addition of $Mg(ClO_4)_2$.

In the dinucleoside phosphate, UpU, the conformational possibilities inherent in the phosphodiester linkage are added to the monomer conformational processes. It is fairly certain that temperature induces conformational changes in dinucleoside phosphates (Davis and Tinoco, 1968; Glaubiger *et al.*, 1968; Smith *et al.*, 1969; Powell *et al.*, 1972), and the CD differences induced by $Mg(ClO_4)_2$ will be shown to result from similar conformational changes.

Monomer Units. The CD results show that for the monomers studied the effects of increasing $Mg(ClO_4)_2$ concentration is always different from the effects of increasing temperature. The effects of salt and temperature are in different directions for 5′-UMP and U. While the salt and temperature dependence for 3′-UMP are in the same direction, the shapes of the CD difference spectra are not similar (Figures 4b and 5b), and the difference spectra for 3′-UMP induced by temperature show an isosbestic point while those induced by salt do not. From the nmr results either increasing salt or increasing temperature causes an upfield shift of the H_6 resonance for 3′-UMP and U (Schleich *et al.*, 1972). For 3′-UMP and U increasing temperature generally caused an increase in the furanose proton coupling constants while increasing $NaClO_4$ concentration caused an increase for some of the coupling constants for 3′-UMP and a general decrease for U. Thus agreement between the nmr results and the CD results is not apparent at this point.

Prestegard and Chan (1969) showed that for 5′-UMP the H_6 resonance is shifted upfield by $Mg(ClO_4)_2$, $NaClO_4$, and several other salts. The shift is much larger and more abrupt than for 3′-UMP or U (which were about the same). Since the presence of the charged phosphate group has a large effect on H_6 resonance, they interpreted the large upfield shift for 5′-UMP at low salt concentrations as due to diamagnetic ion binding to the phosphate. The CD results for 5′-UMP do not show a sharp change at low $Mg(ClO_4)_2$ concentration (Figure 10) indicating that there is no sharp change in molecular conformation.

Both of the nmr reports interpreted the upfield shift of H_6 resonance as a change in ϕ_{CN} to more negative values. Prestegard and Chan (1969) observed only the $H_{1'}$–$H_{2'}$ coupling constants and concluded that the furanose conformation was in equilibrium between 2′ endo and 3′ endo, and that $Mg(ClO_4)_2$ causes a change toward 3′ endo. Schleich *et al.* (1972) conclude that the furanose ring conformation is a rapid blend of several conformers, but that the effects of temperature and $NaClO_4$ are not always in the same direction. Though they do not specify which conformers are involved,

it is quite likely that 3′ endo and 2′ endo are the predominant species.

Recent calculations by Teng *et al.* (1972) are presently the best basis for interpretation of the CD results in terms of molecular geometry. Their results indicate that the rotational strength (CD intensity) of uridine becomes more positive as ϕ_{CN} deviates from the anti position toward more positive values. This is in qualitative agreement with the results of Miles *et al.* (1969). Calculations for 2′-dU indicate that a more positive rotational strength is also consistent with a change in furanose conformation toward 2′ endo (Teng *et al.*, 1972). While this indicates a discrepancy with the nmr results, the quantitative aspects of such calculations must be taken with reservation. In addition, CD cannot observe glycosidic rotation and furanose puckering independently, so that it is difficult to know the relative contribution to the CD of these two conformational possibilities. There is evidence that glycosidic angle is coupled to furanose conformation (Wilson and Rahman, 1971), and the presence of isosbestic points in several of the CD spectra reported here indicates that the system consists of only two optical states, and that changes in glycosidic angle and ribose puckering do not occur independently.

The isopropylidene derivatives were studied to examine this point. Figure 9 shows that though the furanose ring is much more rigid in these derivatives, the effects of temperature and $Mg(ClO_4)_2$ on the CD spectra are only slightly smaller in magnitude than for the parent compounds. $Mg(ClO_4)_2$ (2 *m*) had practically no effect on the CD spectrum of iU, but the CD of iU is anomalously small. The positive lobe for iU has $[\theta]_{max} = 5700$ while for all the other monomers studied $[\theta]_{max} = 9000$–10,000. It should also be recalled that $Mg(ClO_4)_2$ had a very small effect on the CD of U. With this one exception, the general conclusion from the study of the isopropylidene derivatives is that the conformational processes giving rise to the CD differences are still occurring, and that therefore the change in ϕ_{CN} is making the dominant contribution for the monomers studied in this work. A similar argument has been used by Rhodes and Schimmel (1971) to eliminate furanose puckering as the relaxation process they observe for adenosine by ultrasonic absorption.

If this is adopted as a working hypothesis the apparent discrepancy with the nmr results is enlarged, since the nmr workers indicate that ϕ_{CN} is always changed in the same direction by the conditions studied here; whereas the CD results indicate that the changes in ϕ_{CN} caused by temperature and $Mg(ClO_4)_2$ are not the same.

While the conformational equilibria of the furanose ring were considered in the nmr work, the range of allowed glycosidic angle was not. Recent investigation of the nuclear Overhauser effect for iU has shown that a wide range of glycosidic angle is necessary to fit the experimental results (Schirmer *et al.*, 1972). While these results were obtained in Me_2SO solution, it could be expected that the conclusion would also hold for H_2O solutions. Also, only the effect of $O_{1'}$ was considered in interpreting the H_6 chemical shifts as arising from more negative values of ϕ_{CN}. Another interesting possibility is to consider the rotation of the 5′-CH_2OH group. In the gauche-gauche, anti, conformation (defined by Schleich *et al.*, 1972) $O_{5'}$ can make a very close approach to H_6. One can expect that this would have a deshielding effect on H_6. The H_6 resonance in uridine is in fact 0.34 ppm downfield from the uracil resonance (Prestegard and Chan, 1969). This specific deshielding has previously been suggested by Smith *et al.* (1969). Schleich *et al.* (1972) found that the gauche-gauche

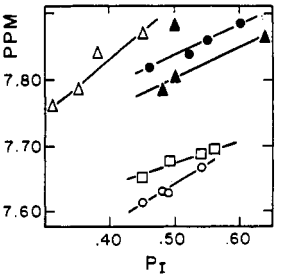

FIGURE 11: The chemical shift of the H_6 resonance as a function of the population of the gauche-gauche rotamer (P_I) for U (●), β-ψ(○), dU (△), 3′-UMP (▲), 3′-β-ψMP (□). The data are taken from Schleich *et al.* (1972).

conformation becomes less populated with increasing $NaClO_4$ or temperature, which could thus partially withdraw the deshielding effect of $O_{5'}$. This would lead to an apparent shielding, and an upfield shift. Except for 5′-UMP the shifts induced by salt and temperature are considerably smaller than 0.34 ppm. Each of the five compounds studied by Schleich *et al.* (1972) shows a fairly good correlation between the population of the gauche-gauche rotamer and the H_6 chemical shift. Their data are plotted in Figure 11.

If the H_6 resonance is sensitive to the rotation of the 5′-CH_2OH group as suggested, the discrepancies between the CD results and the nmr results are greatly relieved; but this would also mean that the nmr results make no clear statement on the changes in ϕ_{CN}. The interesting possibility of observing ^{13}C–^{1}H coupling constants recently suggested by Lemieux *et al.* (1972) could be quite useful in this context. Their preliminary results indicate that the glycosidic angle does not change very much with temperature, but this is not necessarily inconsistent with the CD results which cannot easily be converted into a quantitative value for change in ϕ_{CN}. It would be expected that increasing temperature would primarily allow larger fluctuations of ϕ_{CN} to occur, which would not necessarily lead to a change in the average value of ϕ_{CN}. Increased fluctuations of ϕ_{CN} is consistent with a decreased CD intensity as observed. On the other hand it is unlikely that increasing $Mg(ClO_4)_2$ merely causes fluctuations in ϕ_{CN}; it probably also leads to a new average value of ϕ_{CN}. The fact that $Mg(ClO_4)_2$ can cause both increased and decreased CD intensity indicates this, as does the residual effect of $Mg(ClO_4)_2$ at high temperatures.

Dinucleoside. From Figures 1c, 2c, and 3c it is seen that the effect of high $Mg(ClO_4)_2$ concentration or high temperature of UpU is primarily to cause unstacking. But the unstacking processes do not appear similar as seen in Figures 4c and 5c. In order to properly compare these dinucleoside CD spectra the monomer conformational changes must be accounted for. This is done by the following equation: CD[UpU, 2 *m*, 25°] = CD[UpU, 0, 80°] − 0.5CD[3′-UMP, 0, 80°] − 0.5CD[5′-UMP, 0, 80°] + 0.5CD[3′-UMP, 2 *m*, 25°] + 0.5CD[5′-UMP, 2 *m*, 25°], where CD[*x*,*y*,*z*] is the CD spectrum of compound *x* at salt concentration *y* and temperature *z*. This equation holds remarkably well; the largest and only possibly significant difference between the measured and the calculated CD spectra is 800 (deg cm²)/dmole at 215 nm. For any other choice of monomer units (*i.e.*, U and 3′-UMP or U and 5′-UMP), however, the calculation does not work. This probably indicates that the 3′ and 5′ isomers in this case more closely approximate the conditions of the monomers in the dinucleoside. If this is generally true, it suggests that the appropriate

monomer unit to use in comparison with the properties of longer oligomers may be the 3′,5′-diphosphate. If it is accepted that the proper monomer units in the present case are 3′-UMP and 5′-UMP, then it follows that the unstacked structure of UpU is the same whether achieved by high temperature or by high salt. Using any other choice of monomer units would lead to the conclusion that unstacking is not a unique process and/or that the monomer conformational processes are altered in the dinucleoside phosphate.

Conformational Perturbation by $Mg(ClO_4)_2$. Neutral salts may perturb biological molecules by either specific ion binding to the molecule or specific effects on solvent structure which then induce changes in molecular conformation (von Hippel and Schleich, 1969). Based on the liquid structure temperature concept of Bernal and Fowler (1933), Schleich *et al.* (1972) suggested that simple solvent structure effects are not operating in their system since the effects of $NaClO_4$ and temperature are not always in the same direction. If this argument is to be followed, a stronger case is made by the CD results since they show that for the monomers the effects of salt and temperature are never the same. For UpU, however, the effects of salt and temperature are the same, if correction is made for the monomer conformational changes. Thus the action of $Mg(ClO_4)_2$ on oligonucleotide structure may be accountable only on the basis of solvent disruption.

For the compounds studied here 2 *m* $Mg(ClO_4)_2$ was a less effective perturbant of conformation at higher temperatures, but a plateau is apparently reached at 60°, and little change occurs from 60 to 80°. Thus it may be possible to separate the action of $Mg(ClO_4)_2$ into a temperature-dependent phase and a temperature-independent phase. This would suggest that at least two processes are occurring, but it is difficult to know at this stage which would be associated with ion binding and which with solvent disruption. An indication comes from considering the UpU spectra (Figure 7c) where the high-temperature residual of the effect of $Mg(ClO_4)_2$ is almost completely due to the monomers (one-half the sum of the 5′-UMP and 3′-UMP curves). Thus if UpU conformation is solely sensitive to solvent structure as suggested above, the temperature-dependent phase may perhaps be associated with disruption of solvent structure by $Mg(ClO_4)_2$. The charged phosphate group must be involved in both phases, however, since 5′-UMP and 3′-UMP show both a larger temperature-dependent phase and a larger residual CD difference at high temperature than does U. This does, however, implicate Mg^{2+} in the binding; as the results of Prestegard and Chan (1969) also do. An interesting side line on this study is that for the sum of the monomers (5′-UMP and 3′-UMP) the CD difference spectrum induced by 2 *m* $Mg(ClO_4)_2$ is independent of temperature.

Conclusions

The study of circular dichroism spectra shows that $Mg(ClO_4)_2$ and temperature have different effects on the solution conformation of the uracil nucleosides and nucleotides studied in this work. Increasing temperature is thought to result in increased fluctuation in the position of the base relative to the sugar, with little change in the average position; while increasing $Mg(ClO_4)_2$ is thought to result in a change in the average glycosidic angle. Concomitant changes in furanose conformation undoubtedly occur, but the studies with isopropylidene derivatives indicate that CD is primarily sensitive to changes in the glycosidic angle. The change induced by $Mg(ClO_4)_2$ may be towards more positive values of ϕ_{CN} for 5′-UMP and U, and toward more negative values of ϕ_{CN} for 3′-UMP.

It is likely that 5′-UMP and 3′-UMP are the best monomer units for comparison with the properties of UpU. When correction is made for the monomer conformational processes, it appears that either high temperature or high $Mg(ClO_4)_2$ concentration will lead to the same unstacked structure for UpU.

Both "direct" and "indirect" action of $Mg(ClO_4)_2$ are expected to be important for the monomer units, but solvent structure disruption alone may explain the pertubation of UpU conformation by $Mg(ClO_4)_2$.

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