Conformation of Aromatic-Substituted Dinucleoside Monophosphates: An Extension of the Base-Displacement Theory of Carcinogenesis†

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ABSTRACT: The conformations of 12 dinucleoside monophosphates containing N^4-phenylcytidine (C^{Ph}) or N^4(β-naphthyl)cytidine ($C^{\beta N}$) residues have been studied, using circular dichroic spectroscopy. The following compounds had aqueous spectra resembling their parent compounds, which lacked the modifying aromatic substituent: $C^{Ph}pU$, $C^{\beta N}pU$, UpC^{Ph}, $UpC^{\beta N}$, $C^{Ph}pG$, $C^{\beta N}pG$, GpC^{Ph}, and $C^{Ph}pA$. The spectra of $GpC^{\beta N}$ and $ApC^{\beta N}$ were almost the reverse of the unmodified compounds, while $C^{\beta N}pA$ and ApC^{Ph} were intermediary in character. When the spectra were run in methanol, all major differences between the modified and unmodified compounds disappeared. This result suggested that the differences observed in aqueous solution were the result of stacking interactions between the aromatic ring and a neighboring purine. When the aromatic ring was naphthalene, the modified cytidine occupied the 3′-terminal position, and, when the purine was adenine, the effect was enhanced. These conclusions were supported by a consideration of chemical shifts in the ^{1}H NMR spectra of $ApC^{\beta N}$, and $GpC^{\beta N}$, as compared to those of the unmodified compounds and $C^{\beta N}pG$. A study of molecular models of $ApC^{\beta N}$ and $GpC^{\beta N}$ revealed a unique conformation in which the purine rotates to a syn position, in order to allow a close stacking interaction with the naphthalene ring. No such conformation is available for $C^{\beta N}pA$ and $C^{\beta N}pG$, and the best partial stacking interaction occurs in a conformation with the purine in the anti conformation. The base-displacement theory of carcinogenesis (Levine, A. F., Fink, L. M., Weinstein, I. B., and Grunberger, D. (1974), *Cancer Res. 34,* 319) describes the conformational changes resulting from the attachment of a bulky aromatic residue at the 8 position of guanine in RNA or DNA, and attributes biological importance to the event. The changes that occur upon substitution of the amino group of cytosine differ in detail from the above, but would be expected to produce similar biological results. Base-displacement effects need not be limited, therefore, to a particular substitution position in a nucleic acid.

The base-displacement theory of carcinogenesis (Levine et al., 1974; Weinstein and Grunberger, 1974) provides an elegant description of the way in which conformational changes resulting from the modification of a base in DNA may ultimately lead to mutations and the initiation of carcinogenesis (Ames et al., 1972). Modification of guanine in a nucleic acid with *N*-acetoxy-2-acetylaminofluorene results in the substitution of a bulky aromatic group in the 8 position. To relieve steric strain, the modified guanine rotates to a syn conformation, allowing the fluorene system to stack with adjacent bases in the polynucleotide chain. In DNA, these changes cause localized denaturation, which may result in frameshift and other mutations.

The details of the theory depend, however, on the geometry of the binding of *N*-acetoxy-2-acetylaminofluorene at the 8 position of guanine, and need not apply to other carcinogens. Even in the case of this carcinogen, there is some doubt about the identity of the significant lesion. An uncharacterized adenine reaction may be responsible for A-T to G-C mutations produced by AAAF[1] (Levine et al., 1974). Reaction of AAAF with guanine also takes place at the amino group, and this product, rather than the 8-substituted product, is the one which persists in DNA in vivo (Kriek, 1972). Another carcinogen, 7-bromomethylbenz[a]anthracene, has been reported to react at the amino group of guanine, as well as those of adenine and cytosine, in DNA (Dipple et al., 1971). Furthermore, the amino group of guanine has also been shown to be a site of reaction of the biologically important tetrahydrodiol epoxide metabolite of benzo[a]pyrene (Jeffrey et al., 1976).

Some years ago we proposed that a naphthyl residue bound to the cytosine amino group of DNA could displace the guanine opposite to it and insert into the stacked bases of DNA (Shapiro and Klein, 1967). We called this effect "covalent intercalation". The development of the bisulfite-catalyzed transamination reaction (Shapiro and Weisgras, 1970) has provided a means by which oligonucleotides and polynucleotides modified at the amino group of cytosines can be prepared conveniently. In this paper, we present certain dichroic and nuclear magnetic resonance spectra of dinucleoside monophosphates modified with phenyl and β-naphthyl residues. We will then compare the conformational changes deduced from these spectra with those observed in dinucleoside monophosphates modified by AAAF (Nelson et al., 1971).

Experimental Procedure

Materials. β-Naphthylamine (technical grade, Eastman) was recrystallized three times from methanol-water (mp 110–111 °C). Aniline (Baker) was distilled over zinc. Nucleosides and dinucleoside monophosphates were purchased from Worthington Biochemical Corp. Deuterated solvents for ^{1}H NMR spectroscopy were obtained from Stohler Isotope Chemicals, Inc.

Spectroscopic Methods. Ultraviolet studies were performed on a Cary 15 spectrophotometer. All ^{1}H NMR studies were performed on a Varian XL 100 spectrometer. The β-naphthyl-modified dinucleoside monophosphates were purified for

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[1] Abbreviations used are: AAAF, *N*-acetoxy-2-acetylaminofluorene; G^{AAF}, 8-(*N*-2-fluorenylacetamido)guanosine; C^{Ph} or C^{ϕ}, N^4-phenylcytidine; $C^{\beta N}$, N^4-(β-naphthyl)cytidine; ϕCN, sugar-base torsion angle (Donahue and Trueblood, 1960).

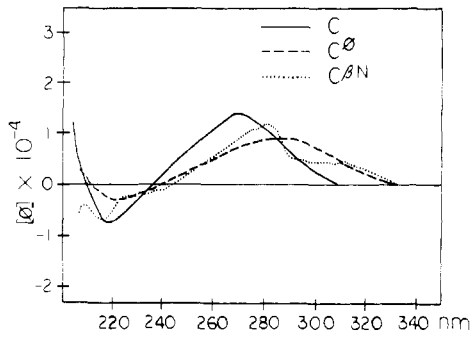

FIGURE 1: Circular dichroic spectra of cytidine (C), N^4-phenylcytidine (C^{ϕ}), and N^4-(β-naphthyl)cytidine ($C^{\beta N}$) in aqueous buffer.

^{1}H NMR spectroscopy by chromatography on a 1 × 20 cm Sephadex LH-20 column in 0.06 M ammonium bicarbonate (the second of the two peaks was collected), followed by desalting on a Sephadex G-25 column in water. They were then dried under vacuum for 24 h at 50 °C over phosphorus pentoxide and dissolved in 0.02 M deuterated potassium phosphate buffer, pD 7.4, at a concentration of 0.001 M. The ^{1}H NMR spectra represent the accumulation of about 1500 pulse responses on a Fourier transform. The chemical shifts were referenced to an internal sodium 4,4-dimethyl-4-silapentane-5-sulfonate peak. Circular dichroic spectra were recorded on a Cary 60 spectrophotometer equipped with a Cary, Model 6001, circular dichroic accessory, using a circular cell with 1-cm path length. All solutions were filtered through glass wool before the spectra were taken. Concentrations of samples in 0.001 M potassium phosphate buffer, pH 7.15, varied according to their extinction coefficients at the λ_{max} and were adjusted so that the absorption at λ_{max} was in the range of 0.6–1.2 optical density units. That value corresponded to about 1×10^{-4} M for unmodified dinucleoside monophosphates. Ellipticities (θ) and extinction coefficients are expressed on a molar basis for both nucleosides and dinucleoside monophosphates. If the values for the latter are desired per mole of base residue, the constants given here should be divided by two. The unit for the molar ellipticity is (deg/mol^{-1} cm^{-1})100. Molar concentrations of the samples were calculated from ultraviolet spectra using the known extinction coefficients of the unmodified dinucleoside monophosphates ϵ_{261}(ApC) = 21.0 × 10^3; ϵ_{261}(CpA) = 21.0 × 10^3; ϵ_{255}(GpC) = 18.2 × 10^3; ϵ_{251}(CpG) = 19.2 × 10^3; ϵ_{265}(CpU) = 16.8 × 10^3; ϵ_{265}(UpC) = 16.8 × 10^3 (Warshaw and Tinoco, 1965), unmodified nucleosides, N^4-phenylcytidine (ϵ_{292} = 18.5 × 10^3) (Shapiro and Klein, 196?), and N^4-(β-naphthyl)cytidine (ϵ_{281} = 29.1 × 10^3) (Shapiro and Klein, 1967). Extinction coefficients of the modified dinucleoside monophosphates were estimated from those of the respective monomer constituents with appropriate consideration of hypochromic effects (Cantor and Tinoco, 1965), (ϵ_{262}(ApC^{Ph}) = 23 × 10^3; ϵ_{262}(C^{Ph}pA) = 22 × 10^3; ϵ_{257}(ApC$^{\beta N}$) = 39 × 10^3; ϵ_{257}(C$^{\beta N}$pA) = 41 × 10^3; ϵ_{253}(GpC^{Ph}) = 20 × 10^3; ϵ_{253}(C^{Ph}pG) = 20 × 10^3; ϵ_{249}(GpC$^{\beta N}$) = 35 × 10^3; ϵ_{249}(C$^{\beta N}$pG) = 35 × 10^3; ϵ_{269}((UpC^{Ph}) = 19 × 10^3; ϵ_{268}(C^{Ph}pU) = 19 × 10^3; ϵ_{259}(UpC$^{\beta N}$) = 36 × 10^3; ϵ_{260}(C$^{\beta N}$pU) = 35 × 10^3. In a typical calculation, ϵ_{257}(ApC$^{\beta N}$) = ϵ_{257}(ApC) − ϵ_{257}(C) + ϵ_{257}(C$^{\beta N}$). The assumption has been made in these calculations that the interaction between the chromophores that leads to hypochromicity is the same for both unmodified and modified dinucleoside monophosphates. This need not be correct. However, hypochromicity effects at the dimer level rarely exceed 10%, therefore, the error in the concentration and spectral coefficients would not exceed that value. Temperature-dependent circular dichroic spectra were recorded using a water-jacketed cell thermostatically controlled by a Lauda, Model K2, circulator. All spectra were run at low speed. In regions of low signal-to-noise ratio, the pen response time was increased to maximum. Spectra of the corresponding modified and unmodified compounds were always taken together using the same baseline.

Hydrogen-Bonding Studies. All ^{1}H NMR spectra were taken at −9 °C using a mixture of deuterated (99.5%) dimethyl sulfoxide and deuterated (99.5%) dimethylformamide (7.3; v/v) as a solvent. The chemical shifts were referenced to an internal tetramethylsilane peak. The concentration of each nucleoside was 0.2 M.

Preparation and Characterization of Modified Nucleosides and Dinucleoside Monophosphates. N^4-Phenylcytidine and N^4-(β-naphthyl)cytidine were prepared according to the procedure of Shapiro and Klein (1967). β-Naphthyl- and phenyl-modified dinucleoside monophosphates were prepared and purified as described elsewhere (Brown and Shapiro, 1977). Their composition was established by enzymatic hydrolysis to monomers, followed by a comparison of the resulting monomers with reference compounds using thin-layer chromatography and ultraviolet spectroscopy.

Results

The ultraviolet spectra of cytidine, N^4-phenylcytidine and N^4-(β-naphthyl)cytidine (Shapiro and Klein, 1967) differ in that the β-naphthyl and phenyl groups introduce a characteristic absorption at 310 and at 293 nm, respectively. The circular dichroic spectra of cytidine and its derivatives are shown in Figure 1. The positive band in the 300–330-nm region of the spectrum of N^4-(β-naphthyl)cytidine and the Cotton effect at 290 nm in the spectrum of N^4-phenylcytidine are attributed to the optical activity introduced into the aromatic rings by attachment to cytidine. It is apparent that the only significant changes induced in the circular dichroic spectra of cytidine by the aromatic residues are in the region where cytidine absorbs little or not at all. This fact and the fact that the Cotton effects of cytidine, N^4-phenylcytidine, and N^4-(β-naphthyl)cytidine correspond to their ultraviolet maxima suggest that no significant conformational changes occur in the cytidine moiety as a result of the covalent attachment of either a phenyl or a β-naphthyl group (Warshaw and Tinoco, 1966). This result was expected, as a study of molecular models showed no steric interaction between the phenyl or naphthyl rings and the ribose residue in these compounds.

In Figures 2 and 3, the circular dichroic spectra of UpC, CpU, and their modified derivatives are given. The spectra are similar in shape in the region above 230 nm. In that region, the Cotton effects of UpC and CpU closely correspond, in both positions and signs, to the Cotton effects of their respective modified analogues. The only differences are in the intensities of some of the corresponding bands.

The spectra of CpG, C^{Ph}pG, and C$^{\beta N}$pG are shown in Figure 4. Both modified dinucleoside monophosphates have more complex spectra than CpG, and one can observe a number of overlapping bands. The maximum at 281 nm, characteristic of CpG is shifted to 295 nm in C^{Ph}pG and the spectrum of C^{Ph}pG has a positive band at 252 nm not present in that of CpG. The Cotton effects present in the spectrum of C$^{\beta N}$pG correspond to those of CpG in both wavelength and sign. The optical activity in the 300–348-nm region of the spectra of both modified dinucleoside monophosphates are probably due to the aromatic residues. The general similarity of the magni-

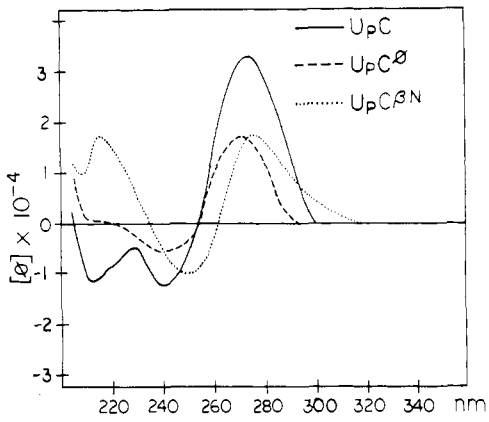

FIGURE 2: Circular dichroic spectra of UpC and its modified derivatives. Ellipticity is given per mole of dimer.

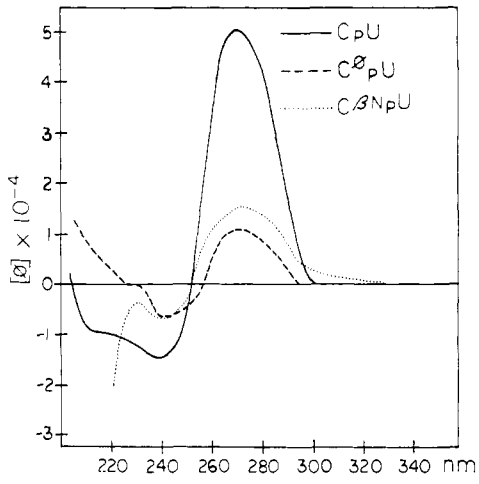

FIGURE 3: Circular dichroic spectra of CpU and its modified derivatives. Ellipticity is given per mole of dimer.

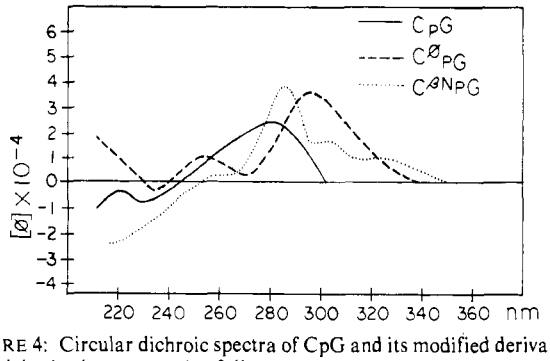

FIGURE 4: Circular dichroic spectra of CpG and its modified derivatives. Ellipticity is given per mole of dimer.

tudes, signs, and positions of the Cotton effects of CpG to those of C^{Ph}pG and C$^{\beta N}$pG is an indication that no significant conformational changes are introduced into CpG by the attachment of a phenyl or β-naphthyl group.

The circular dichroic spectra of CpA, C^{Ph}pA, and C$^{\beta N}$pA are compared in Figure 5. Specific attachment of a phenyl group to the cytidine residue in CpA produces a red shift of the Cotton effects at 235 and 273 nm to 258 and 290 nm, respectively. Similar effects were observed in the spectrum of CpG. The differences between the circular dichroic spectra of CpA and C$^{\beta N}$pA are much greater than those between CpG and C$^{\beta N}$pG. An obvious feature of the spectrum of C$^{\beta N}$pA is a

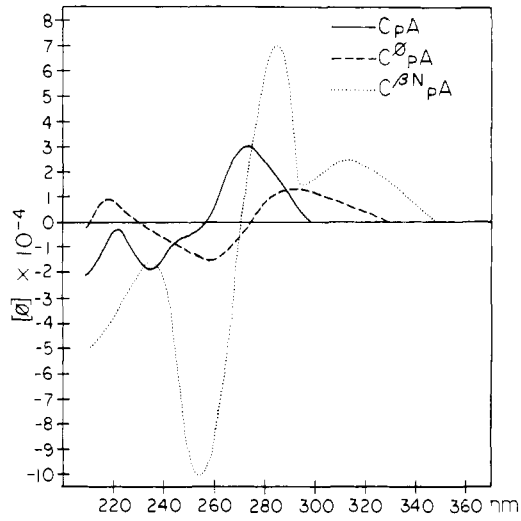

FIGURE 5: Circular dichroic spectra of CpA and its modified derivatives. Ellipticity is given per mole of dimer.

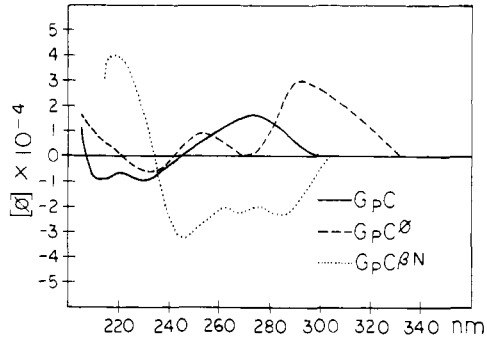

FIGURE 6: Circular dichroic spectra of GpC and its modified derivatives. Ellipticity is given per mole of dimer.

strong negative extremum at 255 nm with a magnitude about five times greater than the corresponding negative maxima of CpA and C^{Ph}pA. It is likely that the attachment of the β-naphthyl group affected the conformation of C$^{\beta N}$pA to a greater extent than that of C$^{\beta N}$pG.

Figure 6 illustrates the circular dichroic spectra of GpC, GpC^{Ph}, and GpC$^{\beta N}$. There is a striking difference in the spectra of GpC and GpC$^{\beta N}$, in both the magnitudes and the signs of their respective Cotton effects. The most significant properties of GpC$^{\beta N}$ include the strong positive Cotton effect at 217 nm, the complex pattern of negative bands at 247, 272, and 286 nm, and the lack of dichroic bands above 305 nm characteristic of the β-naphthyl chromophore. It is possible that the lack of the circular dichroic activity at higher wavelengths is due to overlapping bands of comparable magnitudes and opposite signs, canceling each other. Comparison of the spectra of GpC and GpC$^{\beta N}$ shows that they are almost mirror images of each other, with that of GpC$^{\beta N}$ being almost three times greater in magnitude and more complex. The dramatic differences between the circular dichroic spectral properties of GpC$^{\beta N}$ and those of GpC strongly indicate that major conformational changes follow the specific attachment of β-naphthyl to cytidine in GpC. It is significant that the attachment of the phenyl group does not considerably affect the spectral properties of GpC. The effect of phenyl on the spectrum of GpC (Figure 6) is comparable to that of CpG (Figure 4), which indicates the lack of sequence dependence. The effect of a β-naphthyl group, on the other hand, is clearly sequence

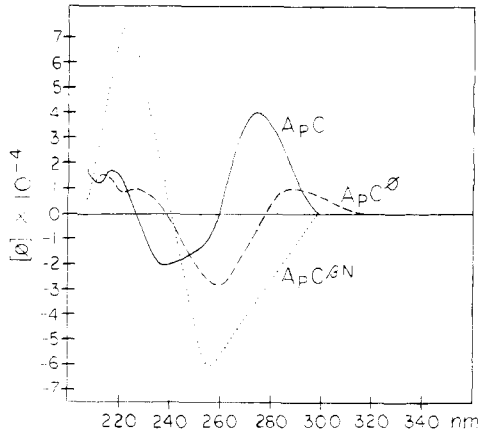

FIGURE 7: Circular dichroic spectra of ApC and its modified derivatives. Ellipticity is given per mole of dimer.

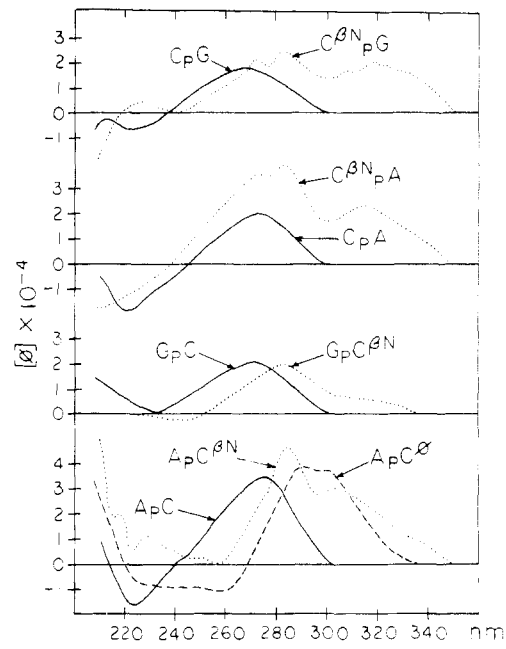

FIGURE 8: Circular dichroic spectra in methanol of dinucleoside monophosphates modified on cytosine by phenyl (ϕ) or β-naphthyl (βN) groups, and of the unmodified analogues. Ellipticity is given per mole of dimer.

dependent and much greater in GpC than in GpC.

The circular dichroic spectra of ApC, ApC^{Ph}, and ApC$^{\beta N}$ are presented in Figure 7. It can be seen that, as in C^{Ph}pA, the specfic attachment of the phenyl group to cytidine caused a red shift of the two long-wavelength Cotton effects (at 259 and 288 nm) as compared to those of ApC (239 and 273 nm, respectively). There are no significant differences between ApC and ApC^{Ph} in the 210–225-nm region. Comparison of the spectra of ApC and ApC$^{\beta N}$ reveals the spectacular differences in the positions, signs, and magnitudes of the Cotton effects. The Cotton effects of ApC$^{\beta N}$ have opposite signs to those of ApC in the 225–240-nm and the 260–300-nm regions. There are no dichroic bands above 300 nm. The magnitudes of the Cotton effects of ApC$^{\beta N}$ are about 2.5 times greater than the corresponding Cotton effects of ApC. The dramatic circular dichroic spectroscopic differences between ApC and ApC$^{\beta N}$ indicate that the attachment of β-naphthyl is associated with significant conformational changes.

It is noteworthy that, although the circular dichroic spectra of ApC$^{\beta sN}$ and GpC$^{\beta N}$ are very different from their unmodified and phenyl-modified analogues and from their isomers C$^{\beta N}$pA and C$^{\beta N}$pG, they resemble each other closely. The conformational change is likely to be very similar in these two compounds. The sequence and the presence of the naphthyl ring are important factors, while the identity of the purine is less important. A more general examination of Figures 1 through 7 reveals that changes in the spectra become greater as the identity of the neighboring base changes from U to G to A, as naphthyl replaces phenyl, and when the modified cytosine is the 3′-terminal residue.

A possible explanation for our data was that stacking interactions between the β-naphthyl group and an adjacent purine led to conformational changes reflected in the circular dichroic spectra. In order to examine this possibility further, the circular dichroic spectra of several modified dinucleoside monophosphates were studied as a function of temperature and solvent.

The values ($[\theta] \times 10^4$) observed at different wavelengths and temperatures for ApC$^{\beta N}$ were, at 259 nm, −6.0 at 25 °C and −3.8 at 80 °C; at 224 nm, 7.8 at 25 °C and 5.0 at 80 °C. For GpC$^{\beta N}$, the values were, at 286 nm, −2.4 at 25 °C and −0.6 at 80 °C; at 247 nm, −3.2 at 25 °C and −1.0 at 80 °C; at 217 nm, 4.0 at 25 °C, and 1.4 at 80 °C. The intensity of the spectra of GpC$^{\beta N}$ and ApC$^{\beta N}$ decreased with increasing temperature, but the shape and position of the bands remained the same. This decrease of circular dichroic intensity is characteristic of stacking interactions (Warshaw and Tinoco, 1965; Davis and Tinoco, 1968).

The circular dichroic spectra in methanol of the following unmodified compounds: C$^{\beta N}$pG, C$^{\beta N}$pA, GpC$^{\beta N}$, ApC^{Ph}, and ApC$^{\beta N}$ and those of their unmodified analogues are given in Figure 8. It can be seen that the solvent effect on the spectra of the unmodified compounds is small. The spectra of the modified compounds in methanol in all cases resemble the corresponding unmodified ones, with a shift of the positive maxima to longer wavelengths. In those cases where the aqueous spectrum of the modified compound differed greatly from that of the unmodified one, the solvent effect is very large. This result again indicates that hydrophobic forces are involved in the conformational changes reflected by the circular dichroic spectra.

To obtain further evidence of base stacking in modified dinucleoside monophosphates, the 100 MHz proton magnetic resonance spectra of the aromatic protons were measured. Stacking interactions between a β-naphthyl group and a purine base require that their aromatic rings be parallel and above each other. Thus, strong shielding of the aromatic protons occurs, which can be detected by ^{1}H NMR spectroscopy. Table I contains the values of nucleosides and dinucleoside phosphates in 0.02 M deuterated potassium phosphate buffer, pD 7.4. The spectral assignments of the protons of nucleosides and dinucleoside monophosphates have been made previously by Ts'o et al. (1969). The 8 protons of purine bases were identified by D_2O exchange (2 h at 90 °C). It was not possible to assign the 2 proton of the adenine base in ApC$^{\beta N}$ due to its overlap with the aromatic protons of the β-naphthyl group. Its approximate position was, however, determined by comparison of the spectra of ApC and ApC$^{\beta N}$. The 6 protons of the modified cytidine derivatives were not assigned for the same reason. No attempt was made to resolve a complex peak due to the β-naphthyl protons. Instead, the areas of their chemical shifts in the spectra of C$^{\beta N}$ and β-naphthyl-modified dinucleoside monophosphates were compared.

The following facts emerge from comparison of the chemical

TABLE I: Chemical Shifts of Aromatic Protons (δ) in Nucleosides and Dinucleoside Monophosphates.

Compound	H-2 (A)	H-8 (A or G)	H-6 (C)	H-5 (C)	β-Naphthyl Protons
C			7.69	5.91	
$C^{\beta N}$			7.95	6.20	8.17; 8.04–7.52
GpC		8.03	7.83	5.73	
$GpC^{\beta N}$		7.91		6.20	8.06–7.32
CpG		8.02	7.72	5.91	
$C^{\beta N}pG$		8.00		5.92	8.18; 8.02–7.48
ApC	8.23	8.40	7.76	5.75	
$ApC^{\beta N}$	<7.94[a]	8.17		6.16	7.94–7.30

[a] There were no peaks below δ 7.94 other than that of the 8 proton at δ 8.17.

TABLE II: Chemical Shifts of the Protons of Guanosine, Cytidine, and N^4-(β-Naphthyl)cytidine in $(CD_3)_2SO$-$DCON(CD_3)_2$, 7:3.

	δ Values from Tetramethylsilane Internal Standard					
	H-8 (G)	H-1 (G)	NH_2 (G)	H-5 (C)	H-6 (C)	NH_2 (C)
C				5.80	7.94	7.26
G	8.08	10.92	6.71			
C + G	8.10	12.52	7.50	5.85	8.08	7.78
$C^{\beta N}$				6.22	8.26	10.35
$C^{\beta N}$ + G	8.10	11.42	6.99	6.23	8.27	10.35

shifts of the modified and unmodified dinucleoside monophosphates: (1) In $ApC^{\beta N}$, the 2 proton of adenine is shifted by at least 0.29 ppm upfield and the 8 proton of adenine by 0.23 ppm upfield, as compared with the corresponding protons in ApC. (2) The chemical shift of the 8 proton of guanine in $GpC^{\beta N}$ is at 0.12 ppm higher field than the 8 proton of guanine in GpC. (3) The area where the aromatic protons of the β-naphthyl group appear is shifted upfield by at least 0.23 ppm in $ApC^{\beta N}$ and by at least 0.11 ppm in $GpC^{\beta N}$ as compared to $C^{\beta N}$. (4) There are no significant changes in the chemical shifts of the 8 protons of the guanine bases and of the aromatic protons of the β-naphthyl group in $C^{\beta N}pG$, as compared to those of CpG and $C^{\beta N}$, respectively.

In order to proceed with the construction of molecular models of the β-naphthyl-modified dinucleoside monophosphates, it was necessary to consider the conformation of N^4-(β-naphthyl)cytidine. In constructing molecular models of this compound, it was not found possible to have the amino group coplanar with both the pyrimidine and naphthalene rings. Steric hindrance between the ortho protons on naphthalene, on the one hand, and the 5 proton and lone pair of electrons at the 3 position of the pyrimidine rings, on the other, prevented coplanarity. In order to obtain information about the actual conformation, the hydrogen bonding of guanine with cytidine and N^4-(β-naphthyl)cytidine was studied by ^1H NMR spectroscopy (Katz and Penman, 1966; Shoup et al., 1966; Patel and Tonelli, 1974) in a 7 to 3 mixture of deuterated dimethyl sulfoxide and deuterated dimethylformamide at −9 °C. A similar study has been used to obtain information on the conformation of N^4-methylcytidine (Engel and von Hippel, 1974). The results are given in Table II.

Although the N-1 and amino protons of guanosine and the amino protons of cytidine in the cytidine and guanosine pair are shifted downfield by 1.60, 0.69, and 0.52 ppm, respectively, the downfield shifts of the corresponding protons in the N^4-(β-naphthyl)cytidine + guanosine pair are much smaller (0.50 for H-1(G), 0.28 for NH_2(G), and none for NH_2(C)). At the same time, only negligible changes in the δ values of the aromatic protons in both pairs are observed. These results suggest that two of the normal three hydrogen bonds have been weakened and the third one has been completely eliminated by the covalent binding of the naphthyl group to cytidine. These results are not conclusive, but are consistent with the situation where the amino group of N^4-(β-naphthyl)cytidine is coplanar with the naphthylamine ring and not available for hydrogen bonding. The carbonyl group and the lone pair of electrons at the 3 position of the pyrimidine ring would remain available for weakened hydrogen bonding with guanosine.

Discussion

A comparison of the circular dichroism of phenyl- and naphthyl-modified dinucleoside monophosphates in water (Figures 1–7) reveals that the most dramatic changes, almost complete reversal of the spectra, occurred with $ApC^{\beta N}$ and $GpC^{\beta N}$. In both of these cases, the modifying group was naphthyl rather than phenyl, the modified cytosine was the 3′-terminal base, and the adjacent base was a purine. Lesser changes were observed in the case of $C^{\beta N}pA$ and ApC^{Ph}, indicating the greater effect when adenine, rather than guanine, is the neighboring base. The spectra of UpC^{Ph}, $UpC^{\beta N}$, $C^{Ph}pU$, $C^{\beta N}pU$, $C^{Ph}pG$, $C^{\beta N}pG$, GpC^{Ph}, and $C^{Ph}pA$ resembled those of the unmodified compounds. All major differences between the modified and unmodified compounds were abolished when the spectra were run in methanol (Figure 8). The above results suggest that the spectral changes observed were the results of conformational shifts, which involved stacking between the naphthalene ring and an adjacent purine residue. The stacking ability of the bases increases in the order of uracil ≪ guanine < adenine (Warshaw and Tinoco, 1965, 1966), which is consistent with our results. The greater effect of the naphthyl ring, as compared to phenyl, is consistent with the fact that the degree of stacking increases with greater aromaticity (Ts'o et al., 1969). This interpretation is also consistent with the temperature study. However, the decrease in the spectrum observed with temperature could be due to an increase in the freedom

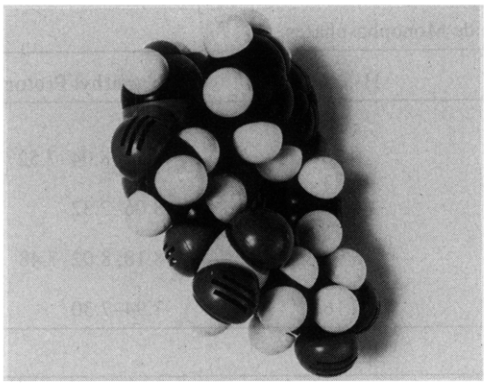

FIGURE 9: Space-filling CPK models of a proposed conformation for $ApC^{\beta N}$.

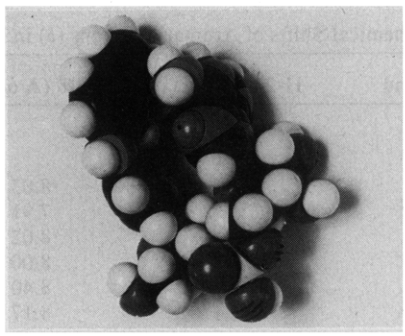

FIGURE 10: Space-filling CPK models of a proposed conformation for $C^{\beta N}pA$.

of other types of rotational movement, rather than a decrease in stacking (Emerson et al., 1966).

The data from ^1H NMR spectra (Table I) also support our hypothesis. Downfield shifts of the adenine, guanine, and naphthalene ring protons in $ApC^{\beta N}$ and $GpC^{\beta N}$ were observed, as was an upfield shift of the cytosine 5 proton. These results were expected, if the purine ring was stacking with naphthalene, rather than cytosine. In $C^{\beta N}pG$, where no conformational change was indicated by the circular dichroic spectrum, no such shifts in the ^1H NMR spectrum were observed.

Our results show a striking parallel to the studies of dinucleoside monophosphates in which guanine has been modified at the 8 position by attachment of an acetylaminofluorene resisdue (Nelson et al., 1971; Levine et al., 1974). In that series, the circular dichroic effects and the ^1H NMR shifts resembled those we have observed. A stacking interaction between the fluorene ring and the neighboring adenine (but not uracil) was proposed. The effect was greater when the modified guanine occupied the 3′-terminal position.

Space-filling CPK atomic models of $ApC^{\beta N}$ and $C^{\beta N}pA$ were examined in order to find a conformation in which a close parallel approach of the adenine and naphthalene rings was attained. To simplify this search, a right-handed turn of the (3′ → 5′) screw axis was maintained (Ts'o et al., 1969), and unfavorable steric interactions were avoided. As the naphthylamine group was located directly opposite the glycosyl linkage on the cytosine ring, rotation of the latter did not affect the spatial position of the former. The cytosine ring was therefore held in the normal anti conformation (Emerson et al., 1966; Berthod and Pullman, 1971).

A single uniquely favorable conformation of $ApC^{\beta N}$ was encountered, which is illustrated in Figure 9. The most significant feature of this model is the syn conformation of the adenine ring, with the ϕ_{CN} in the range between +120° and +150°. The amino group is coplanar with the naphthalene ring, rather than the pyrimidine ring, as suggested by our hydrogen-bonding studies (see Results). The rings of naphthalene and adenine residues are parallel and closely overlapping. Rotation of the purine ring to the anti range significantly diminished the overlap necessary for stacking.

In the most favorable conformation of $C^{\beta N}pA$, both the adenosine and cytidine residues are held in the anti conformation, and the naphthalene ring is coplanar with the amino group of cytosine. As in the case of $ApC^{\beta N}$, the adenine and naphthalene rings in $C^{\beta N}pA$ are parallel but only partially overlapping, with a less favorable stacking interaction (Figure 10).

Models of $GpC^{\beta N}$ and $C^{\beta N}pG$ were also constructed, and their properties were very similar to those of $ApC^{\beta N}$ and $C^{\beta N}pA$, respectively.

Our model for the conformational shift in $ApC^{\beta N}$ and $GpC^{\beta N}$ has both similarities and differences to the base-displacement model of the AAF series (Nelson et al., 1971; Levine et al., 1974). In both models, a purine rotates and the modifying aromatic residue stacks with an adjacent base. However, in the G^{AAF}-containing dinucleoside monophosphates the modified nucleoside changes its conformation, whereas in $ApC^{\beta N}$ and $GpC^{\beta N}$ it is the adjacent purine base that rotates around its glycosidic linkage. Furthermore, the change in the conformation of G^{AAF} arises from the severe steric hindrance between the fluorene and the ribose ring when G^{AAF} is in the anti form. In N^4-(β-naphthyl)cytidine, the β-naphthyl group does not interact with the ribose ring and is unaffected by the torsional angle around the glycosyl bond. The driving force for the conformational shift in $GpC^{\beta N}$ and $ApC^{\beta N}$ must be the favorable stacking interaction between the naphthalene ring and the adjacent purine ring. Purines in dinucleoside monophosphates appear to have a modest preference for the anti conformation (Ts'o et al., 1969). This preference can be overcome when the stacking group is β-naphthyl, but not when it is phenyl. This is a particularly striking feature because of the great importance that polycyclic (but not monocyclic) aromatic compounds have had as carcinogens.

Although the details of the conformational shift in our case are different than in the base-displacement model, as applied to AAAF, one would expect the same effects. A purine held in the syn conformation cannot form normal Watson–Crick hydrogen bonds, and should misfunction in a mRNA (Grunberger and Weinstein, 1971; Grunberger et al., 1974) and in DNA (Levine et al., 1974; Millette and Fink, 1975). We would predict that appropriate triplets modified with β-naphthylamine should fail to stimulate binding of aminoacyl-tRNAs to ribosomes (Grunberger and Weinstein, 1971; Grunberger et al., 1974). DNA modified by β-naphthylamine should show regions of localized denaturation (Levine et al., 1974) and reduced activity as a template for transcription (Millette and Fink, 1975). We hope to test these predictions in the future.

Our results demonstrate that base displacement or covalent intercalation effects can result from substitutions on the amino group of cytosine, as well as the 8 position of guanine. It is plausible that they may operate at other attachment sites in the nucleic acids as well. It has not yet been demonstrated, of course, that any specific chemical lesion is the one responsible for mutagenesis (or carcinogenesis) by aromatic carcinogens. The uncertainties with respect to *N*-acetoxy-2-acetylaminofluorene have been discussed in the introduction. The situation with respect to β-naphthylamine is even less clear. An activation route similar to that of 2-acetylaminofluorene (Miller,

1970) has been suggested (Radomski et al., 1971; Ong and de Serres, 1972), but not yet demonstrated. An alternative metabolic pathway has been proposed (Belman et al., 1968). If the base-displacement theory of carcinogenesis is correct, however, then the significant alterations induced in nucleic acids by aromatic carcinogens will be those which produce conformational effects resembling the ones described here and in the original statement of the theory. Further confirmation or refutation of the theory must await the structure determination of a number of these important reaction products.

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