CIRCULAR DICHROISM STUDY OF 2'-5' DINUCLEOSIDE MONOPHOSPHATES MODIFIED WITH N-2-ACETYLAMINOFLUORENE

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The conformational properties of four 2'-5' dinucleoside monophosphates modified with N-2-acetylaminofluorene have been studied by circular dichroism spectroscopy. Covalent binding of this chemical carcinogen at the C_8 position of guanosine in the 2'-5' dinucleoside monophosphates induces striking changes in their circular dichroic spectra depending on their base sequence and composition. The changes in CD spectra, redshift of the extrema and change of their polarity, not observed in the spectra of corresponding 3'-5' derivatives modified with N-2-acetylaminofluorene are correlated with the difference in the configuration of 2'-5' and 3'-5' dinucleoside monophosphates and discussed in respect to the intramolecular stacking interactions.

INTRODUCTION

Oligonucleotides have been used in many applications as model compounds for studies on nucleic acid interactions. We have previously studied the effect of binding of N-2-acetylaminofluorene to some 3'-5' dinucleoside monophosphates in an effort to explain the mechanism by which the covalent attachment of this chemical carcinogen at the C_8 position of guanosine residues might modify the structure of nucleic acids and thereby their biological activity (1,2). It was shown that the covalent binding of AcNH-Fln to C_8 of guanosine residues resulted in major changes in conformation properties of the modified oligonucleotides. These changes included rotation of the guanine base around the glycosidic linkage $N_9 - C_1'$ and intramolecular stacking of the fluorene residue with the adjacent base (3,4). The modified guanine residue is then shifted out from its normal coplanar

Abbreviations: G*, guanosine modified with N-2-acetylaminofluorene; AcNH-Fln, N-2-acetylaminofluorene; AcN(OAc)-Fln, N-acetoxy-2-acetylaminofluorene.

relation with adjacent bases. This structure was designated as the "base displacement model" (5,6). Similar conformation changes were proposed by Michelson et al. (7) and Fuchs and Daune (8) for DNA modified with AcNH-Fln. The remarkable difference in conformational behavior of dinucleoside monophosphates with naturally occurring 3'-5' isomers (9,10), led us to the suggestion that similar differences in conformation might be observed between the corresponding modified dinucleosides.

In the present study, we have explored the conformation changes induced in four 2'-5' dinucleoside monophosphates after modification with AcNH-Fln by circular dichroism.

MATERIALS AND METHODS

AcN(OAc)-Fln was synthesized with small modifications of the published procedure (11) as reported elsewhere (3). 2'-5' dinucleoside monophosphates were prepared as described by Lapidot and Barzilay (12).

Modification and isolation of 2'-5' dinucleoside monophosphates. 40 μmoles of 2'-5' dinucleoside monophosphates were dissolved in 1.5 ml of 0.01 M Tris-HCl, pH 7.2 and reacted with 107 μmoles of Ac (OAc)-Fln dissolved in 2 ml ethanol at 37° for 3.5 hours in the dark. The reaction mixture was then extracted 5 times with 3.5 ml ether to remove the unreacted drug. The separated underphase was applied to a 2 cm x 25 cm Sephadex LH-20 (Pharmacia Fine Chemicals Inc.) chromatography column for isolation of the modified 2'-5' dimers. The unmodified material was eluted with 0.01 M ammonium bicarbonate, whereas the modified product was eluted with 0.01 M ammonium bicarbonate in 50% ethanol.

The concentration of dinucleoside monophosphates in solutions for spectroscopic measurements were in the range of 1-3 x 10^{-4} M and were determined by the measurement of optical density at 260 nm (3). This data was not corrected for hyperchromicity (13).

Circular Dichroism. CD spectra were recorded on a Cary 61 spectropolarimeter with 0.01 and 0.1 cm selected Suprasil cells at 27°C. Experimental data are expressed as mean residue ellipticity $[\theta]_A$, deg cm^2 d mol^{-1} and not corrected for solvent refractive index. 0.1 M Tris pH 7.2 was used as solvent unless stated otherwise.

Ultraviolet spectra. UV absorption spectra were recorded on a Cary Model 14 spectrophotometer. 0.1 M Tris pH 7.2 was applied as solvent.

RESULTS AND DISCUSSION

Isolation of modified 2' 5' dinucleoside monophosphates. The modified 2'-5' dinucleoside monophosphates were separated from unmodified materials by Sephadex LH-20 column chromatography as described in Materials and Methods. Figure 1 demonstrates the separation of modified A2'-5'G* (fractions 30-40) from the unmodified dimer (fractions 12-22). The presence of AcNH-Fln in the second peak (A2'-5'G*) is indicated by the absorption at 305 nm which

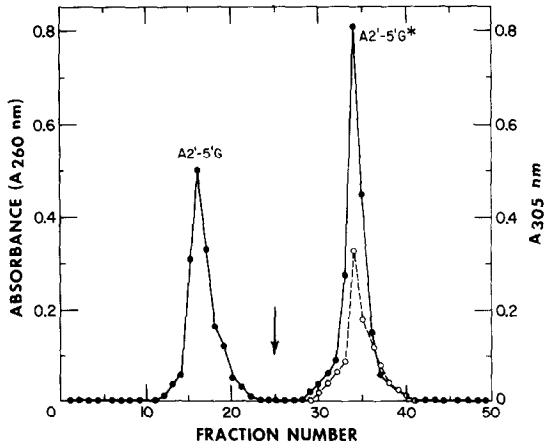


Figure 1. Sephadex LH-20 column chromatogram (1.5 x 20 cm) of products after reaction of A2'-5'G with AcN(OAc)-Fln. Elution with 0.01 M ammonium bicarbonate was at a flow rate of 40 ml/hr at arrow indicated, elution with ethanol-0.02 M ammonium bicarbonate (1:1, V/V) was at a flow rate of 20 ml/hr. Approximately 4.0 ml fractions were collected and ultraviolet absorption of 0.1 ml of each fraction diluted to 1 ml was measured at 260 nm (—●—●—), and 305 nm (—o—o—).

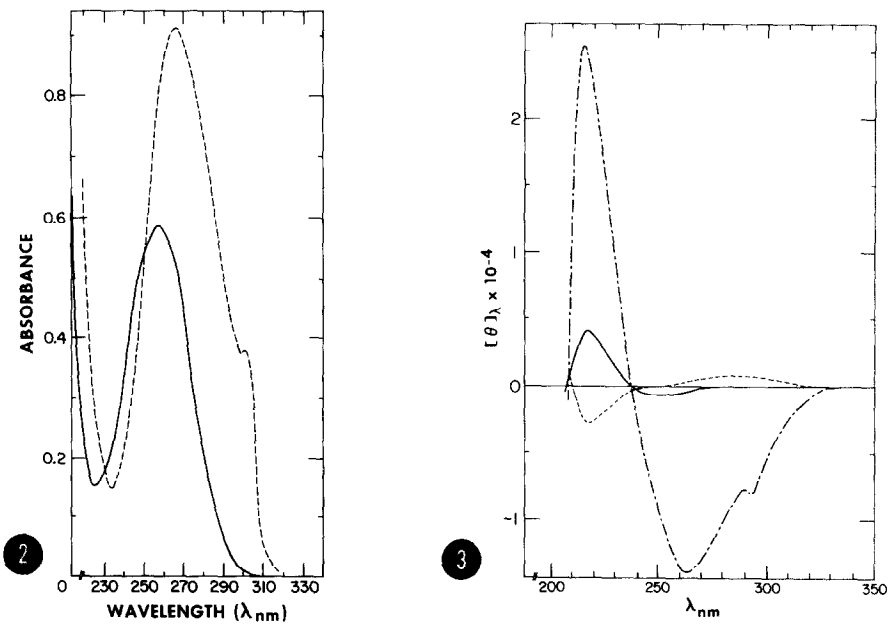
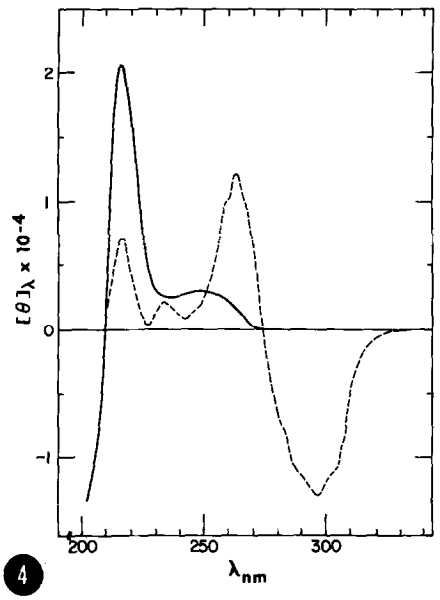
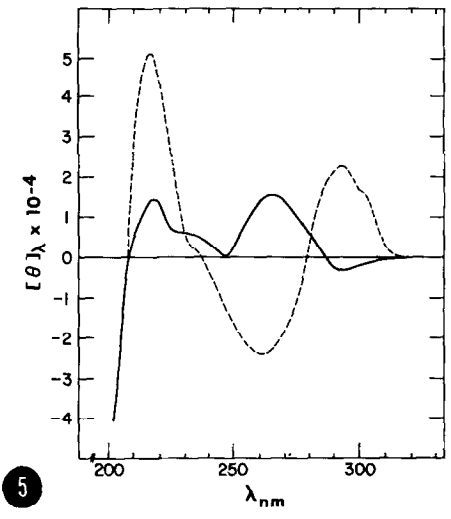
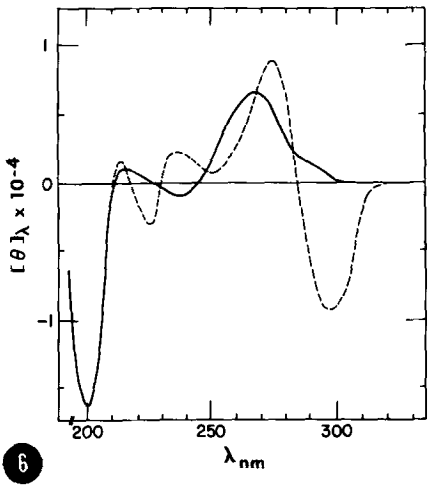


Figure 2. Ultraviolet absorption spectra of A2'-5'G (——) and A2'-5'G* (---). UV absorption spectra of the materials after separation on Sephadex LH-20 column were measured in 0.1 Tris pH 7.2.

Figure 3. Circular dichroism spectra of guanosine monophosphate (——), N-2-acetylaminofluorene (---) and guanosine monophosphate modified with N-2-acetylaminofluorene (—.—.—.—). For details on CD spectra see Materials and Methods.







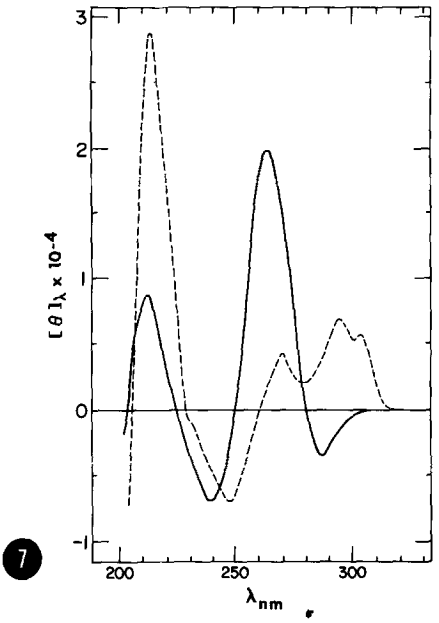


Figure 4. Circular dichroism spectra of A2'-5'G (—) and A2'-5'G* (---).

Figure 5. Circular dichroism spectra of G2'-5'A (—) and G*2'-5'A (---).

Figure 6. Circular dichroism spectra of U2'-5'G (—) and U2'-5'G* (---).

Figure 7. Circular dichroism spectra of G2'-5'U (—) and G*2'-5'U (---).

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is characteristic for AcNH-F1 modified guanine residues. Similar results were obtained from the separation of G2'-5'A, U2'-5'G and G2'-5'U from the

corresponding modified dinucleoside monophosphates. Figure 2 compares the absorption spectra of A2'-5'G from the first peak of Figure 1 with that of the modified A2'-5'G* in the second peak. The modified product shows a redshift in absorption maximum from 257 nm to 265 nm indicating stacking interaction. Shoulder at about 305 nm originates from AcNH-Fln residue.

Circular dichroism spectroscopy. Figure 3 shows the CD spectra of AcNH-Fln, Gp and Gp modified by AcNH-Fln (Gp*). There is practically no signal from AcNH-Fln and from Gp between 340-240 nm. The weak extrema at 215 nm observed with both AcNH-Fln and Gp are comparable in size but opposite in sign. (AcNH-Fln spectrum have been obtained in ethanol because of its low solubility in water.) The CD spectra of G*p is a typical example of how the addition of another interacting chromophore modifies the optical properties of either chromophore. Changes in the intrinsic molecular asymmetry of modified Gp after covalent attachment of AcNH-Fln due to the stacking interactions between G and fluorene ring of the drug result in extrema comparable in magnitude, position and complexity with the CD spectra of some dinucleoside monophosphates.

The CD spectra of four 2'-5' dinucleoside monophosphates (Figs. 4-7) show a remarkable difference frome those of the isomers with 3'-5' phosphodiester linkage (9). The spectra of G2'-5'A (Fig. 5) and G2'-5'U (Fig. 7) are more complex than those of A2'-5'G (Fig. 4) and U2'-5'G (Fig. 6). All spectra show an additional extremum at about 290 nm found neither with Gp nor AcNH-Fln but distinct for Gp* and characteristic for 2'-5' dinucleoside monophosphates modified with AcNH-Fln. A distinct redshift of extrema characteristic for stacking interactions (mainly at 260 nm) was found in CD spectra of all modified 2'-5' dinucleoside monophosphates (Figs. 4,6,7) with the exception of G*2'-5'A (Fig. 5). However, the correlation of the redshift of AcNH-Fln modified dinucleoside monophosphates with the base sequence and the base composition is not straight-forward, e.g. 2'-5' dinucleoside monophosphates with uracil exhibit redshift of about 8 nm

regardless if G* is in 2' or 5' position. Derivative with adenine shows a redshift of 15 nm, i.e. almost doubled when G* is in the 5' position.

The CD spectra of modified 2'-5' dinucleoside monophosphates in Figs. 4-7 are too complex for a detailed comparison but the changes of $[\theta]_\lambda$ observed in the main bands in the wave length regions ca. 215, 265, and 295 nm after AcNH-Fln binding are significant. There are again certain similarities between G*2'-5'A (Fig. 5) and G*2'-5'U (Fig. 7) and also between A2'-5'G* (Fig. 4) and U2'-5'G* (Fig. 6). The CD spectra of G*2'-5'A and G*2'-5'U have a pronounced increase of the positive extremum at approximately 215 nm while the derivatives A2'-5'G* with reversed sequence of bases show remarkable decrease of this extremum in comparison with the unmodified dimers. The extremum at approx. 260 nm decreased in the case of G*2'-5'U and changed to a negative value in G*2'-5'A, while increase of $[\theta]_{260}$ has been observed in A2'-5'G* and U2'-5'G*. Similarly at 290 nm the mean residue ellipticity increased in G*2'-5'U from negative values to positive ones while in the case of A2'-5'G* and U2'-5'G*, with no signal at this wave length in the unmodified 2'-5' dimers, dominant negative extrema were observed. These changes in extrema can be correlated with the base sequence and to some extent with the base composition as it has been shown with the unmodified 3'-5' and 2'-5' dinucleoside monophosphates (14-19). The largest conformation changes (as indicated by the magnitude of the extrema at 215, 260 and about 290 nm) have been observed in dinucleoside monophosphates with both bases being purines and with modified guanine in the 2' end. The maximum effect was therefore, measured with G*2'-5'A (Fig. 5) and minimum effect with U2'-5'G* (Fig. 6).

A similar trend has been reported earlier by Nelson *et al.* (3) on AcNH-Fln modified dinucleoside monophosphates but no change of polarity which we found for the studied 2'-5' dinucleoside monophosphates when G* was in the 5' position. The changes in polarity of the extrema

can be related to the differences in the spatial arrangement of hydroxyl and phosphate groups in 2'-5' and 3'-5' dinucleoside monophosphates which affect the phosphate-ribose backbone and the interaction with AcNH-Fln. However, the explanation of this effect in 2'-5' dinucleoside monophosphates is still not clear because of the controversies about the role and hydrogen bonding of the 2'-hydroxyl group of the sugar and about the steric influences of purine and/or pyrimidine bases in these derivatives (16,17,21,22).

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