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CONFORMATION OF ACETYLAMINOFLUORENE AND AMINOFLUORENE MODIFIED GUANOSINE AND GUANOSINE DERIVATIVES

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Summary

Acetylaminofluorene and aminofluorene modified Guo, GMP, d(GpA) and d(ApG) have been studied by circular dichroism and ^1H nuclear magnetic resonance. Aminofluorene modified Guo is preferentially in the anti conformation and acetylaminofluorene modified Guo in the syn conformation. It is proposed that the anti conformation of aminofluorene modified Guo is stabilized by an intra molecular hydrogen bond between the NH group of aminofluorene residue and the 5'-OH group of the sugar. The results on the modified dinucleoside monophosphates are analyzed according to this hypothesis.

Introduction

In the in vivo reaction between the carcinogen N-hydroxy-N-acetyl-aminofluorene and DNA, substitutions occur on the C(8) of guanine residues and at least two different adducts are formed. One is well identified as N(deoxyguanosin-8-yl)-acetyl-aminofluorene and the other one is probably N-(deoxyguanosin-8-yl)-aminofluorene (general review 1). The covalent binding of these two bulky fluorene residues to in vitro modified synthetic and natural DNAs leads to different conformational changes (2-5). For example in low salt concentration, the conformation of AAF-modified poly(dG.dC). poly(dG.dC) is the Z-form (or a Z-like form) and that of AF-modified poly (dG.dC).poly(dG.dC) is the B-form (5).

Studies of AAF-modified oligoribonucleotides have been already performed and have led to the proposal of the base displacement model

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Abbreviations

We will write Guo-AAF (or -AF), GMP-AAF, d(GpA)-AAF and d(ApG)-AAF for acetylaminofluorene (or aminofluorene) modified Guo, GMP, d(GpA) and d(ApG) respectively.

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(3). In this model, the modified guanosine has the syn conformation and there is a stacking interaction between AAF-residues and a base adjacent to the modified guanine. This model is in agreement with the insertion-denaturation model deduced from the study of AAF-modified DNA (2).

We have undertaken a study of AF-modified oligodeoxyribonucleotides. In this paper, we report circular dichroism and ^{1}H nuclear magnetic results on AF (and AAF)-modified guanosine, guanosine 5'-monophosphate and deoxydinucleoside monophosphates showing important differences in the conformations of AF and AAF derivatives.

Material and Methods

Guanosine (Guo), guanosine 5'-monophosphate (GMP), 2'-deoxyguanylyl(3'-5')-2'-deoxyguanosine (d(GpA)) and 2'-deoxyadenylyl (3'-5')-2'-deoxyguanosine (d(ApG)) were purchased from Sigma or P. L. Biochemicals. The AAF-modified compounds were prepared and purified as already described (6). The AF-modified compounds were obtained by alkali treatment of the corresponding AAF-modified compounds (7). They were purified on a L-H 20 Sephadex column.

^{1}H nuclear magnetic resonance spectra were recorded with a Bruker WH 90 spectrometer operating in the FT mode. Assignements were based on homonuclear decoupling experiments and comparison with literature data. Circular dichroism spectra were recorded with a Roussel-Jouan dichrograph 3.

Results

Circular dichroism The CD spectra of the modified compounds are presented in figure 1.

The CD spectra of Guo-AF and GMP-AF (figure 1) are similar in shape but differ in intensity. Larger changes are observed in the comparison between the spectra of Guo-AAF and GMP-AAF but one spectrum is not an inversion of the other.

The CD spectra of d(ApG)-AF and d(GpA)-AF are similar in shape (a first negative band centered at 310 nm and a second positive band centered at 265 nm) but differ in intensity (the signal of d(ApG)-AF being larger than that of d(GpA)-AF). Large variations are observed as a function of temperature even above 300 nm where almost all the light absorption is due to AF-residues. At 58°C, $\Delta\varepsilon_{310nm}$ d(ApG)-AF is about two times larger than $\Delta\varepsilon_{310nm}$ d(GpA)-AF at 5°C.

The CD spectra of d(GpA)-AAF and d(ApG)-AAF present large differences in shape and in intensities. In the region where mainly

Table I

^3J(Hz)	H(1')-H(2')	H(2')-H(3')	H(3')-H(4')
Guo-AF	7.9	6	2.2
Guo-AAF	6.7	5.1	# 2.2
GMP-AF	6	6.5	4.5
GMP-AAF	6.2	6.2	unresolved

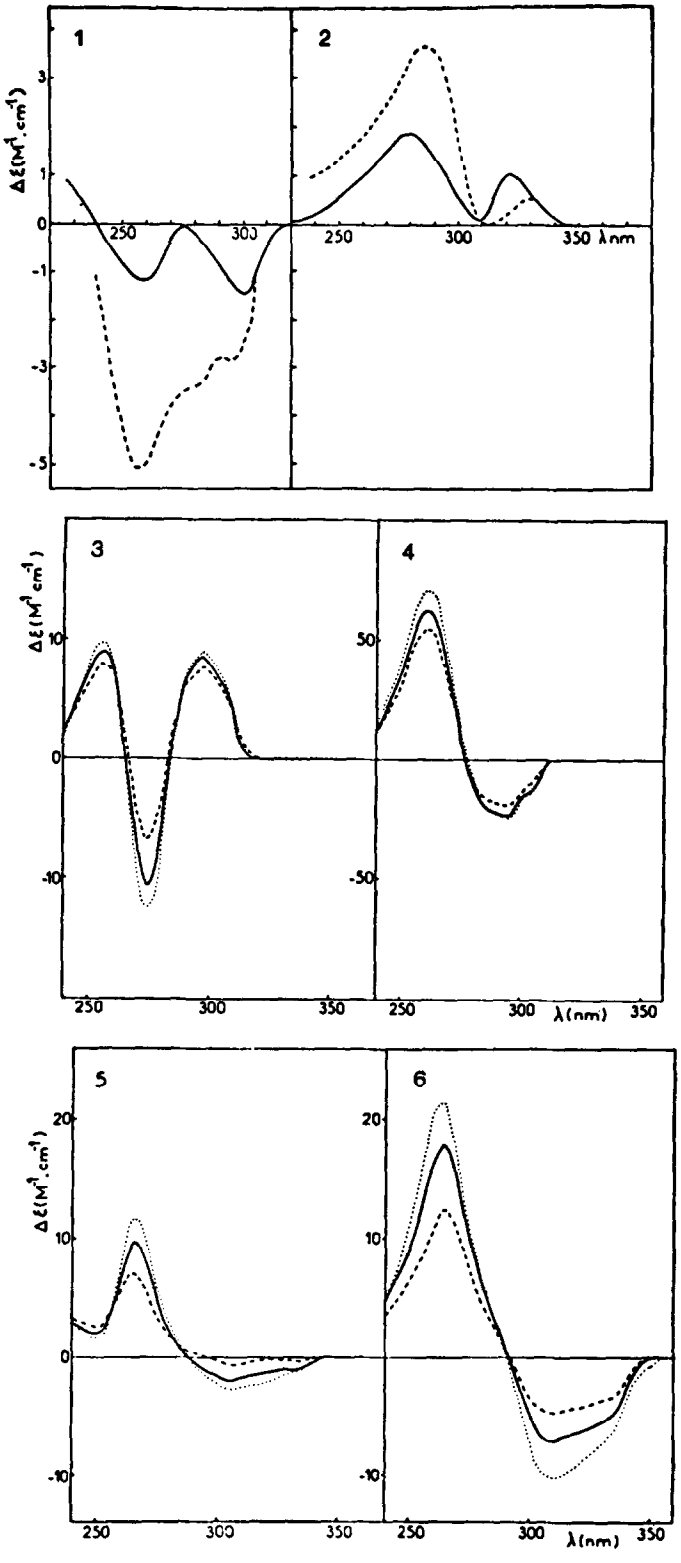
The coupling constants are accurate to ± 0.2 Hz. Temperature 23°C. Possible second order effects were corrected by simulation of the spectra (Brucker ITRCAL programm).

the fluorene absorbs, the first band of d(ApG)-AAF spectrum is negative while the first band of d(GpA)-AAF is positive. In both cases, there is a positive band centered about at 260 nm but $\Delta\varepsilon_{max}$ d(ApG)-AAF is about seven times larger than $\Delta\varepsilon_{max}$ d(GpA)-AAF . There are some variations of the intensities as a function of temperature but there are smaller than those observed on the AF derivatives.

Nuclear magnetic resonance Due to their low solubility in water, Guo-AF and Guo-AAF were studied in methanol. Striking differences appear in the resonances of the ribose protons. Relatively to Guo-AF, H(1') of Guo-AAF is upfield shifted (- 0,21 ppm), H(5'), (5'') (- 0,02 ppm) whereas H(2') is downfield shifted (+ 0,35 ppm)(conventionally, a negative $\Delta\delta$ corresponds to a upfield shift). The coupling constants are given in table I.

The modified 5'-ribonucleotides are soluble in water and a typical NMR spectrum is shown in figure 2. Relatively to GMP-AF, H(1') of GMP-AAF is slightly downfield shifted (0,07 ppm), H(4') and H(5'), (5'') are slightly upfield shifted (- 0,07 ppm) and H(2') remains downfield shifted (0,33 ppm). The coupling constants of ribose protons are given in table I. For GMP-AF, a small increase of ^3J H(1')-H(2') as a function of temperature was observed (0.4 Hz/20°C).

Figure 1 : Circular dichroism. (1) (----) Guo-AAF (taken from ref. 7); (——) GMP-AAF, (2) (----) Guo-AF (ref. 7), (——) GMP-AF, room temperature, neutral pH. (3) d(GpA)-AAF, (4) d(ApG)-AAF, (5) d(GpA)-AF, (6) d(ApG)-AF. Solvent 0.2 M NaCl, 5 mM Tris-HCl pH 7.5, 0.1 mM EDTA. In 3-6, temperatures are 5°C (···), 25°C (——), 58°C (---).


1
2
3
4
5
6
Δε(M⁻¹.cm⁻¹)
250
300
350
λnm
λ(nm)

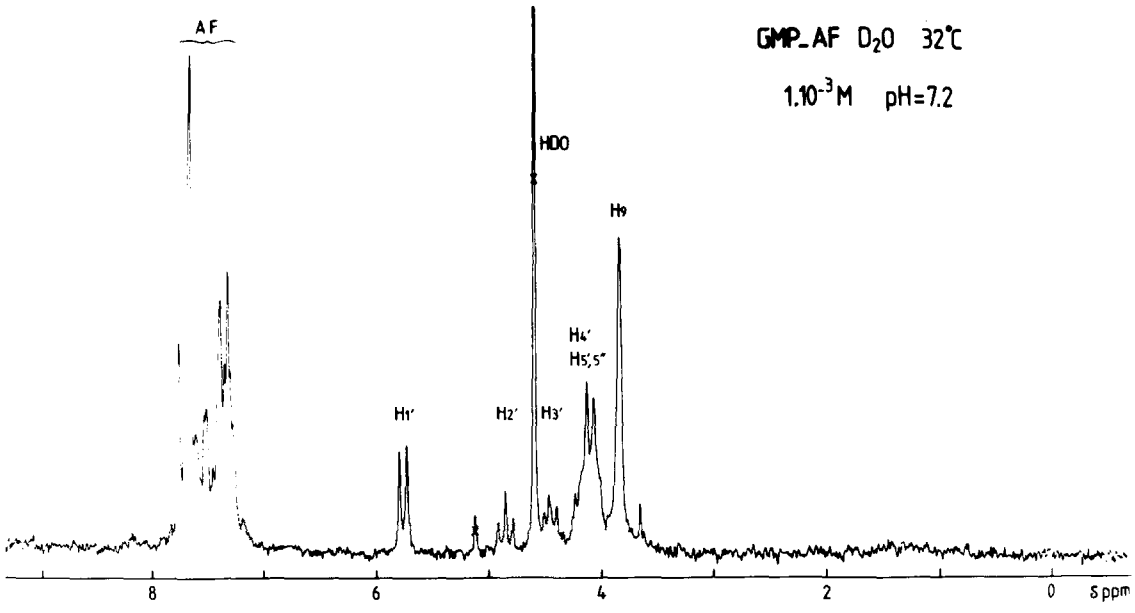


Figure 2 : ^{1}H 90 MHz NMR spectrum of GMP-AF in D_2O. The chemical shifts are measured relatively to external DSS.

The results on the modified deoxyribodinucleoside phosphates in aqueous solution can be summarized as follows.

Relatively to d(GpA), the protons of d(GpA)-AF are shifted : H(8) (- 0.06 ppm), H(2) (- 0.1 ppm), H(1') (- 0.04 ppm) for adenosine and H(1') (0.23 ppm), H(2'), (2'') (0.05 ppm) for guanosine. An increase of temperature from 23°C to 40°C produces downfield shifts of almost all resonances but mainly H(2) (0.22 ppm) for adenine, H(1') (0.18 ppm), H(2'), (2'') (0.2 ppm) for guanosine.

More drastic changes are produced by the binding of AAF residues. Relatively to d(GpA), important upfield shifts of adenosine protons are observed, i.e., H(8) (- 0.36 ppm), H(2) (- 0.48 ppm), H(1') (-0.25 ppm) and H(2'), (2'') (- 1 ppm). H(1') of guanosine is upfield shifted (- 0.16 ppm) and shows a poorly resolved structure at room temperature. No changes in the chemical shifts of H(8) and H(2) of adenine and of the aromatic protons of fluorene are observed as a function of temperature (in the range 20-60°C). On the other hand, H(1') and H(2'), (2'') of guanosine are downfield shifted and their resolutions are greatly increased. The resolution of fluorene residues is increased as the concentration is decreased from 1 mg/mL to 0.3 mg/mL.

The binding of AF-residues to guanine residue in d(ApG) induces important upfield shifts of H(8) (- 0.3 ppm), H(2) (- 0.4 ppm) and H(1') (-0.26 ppm), H(2'), (2'') (-0.4 ppm) of adenosine. These shifts are smaller than those measured for the same protons in (ApG)-AAF(8).

Discussion and Conclusion

It is generally assumed that a bulky substituent on the C(8) of purine nucleoside favours the syn conformation. However, the existence of an anti conformation stabilized by specific interactions between the C(8) substituent and the 5'-OH of modified adenosines was recently shown (9, 10). In NMR studies, the existence of syn and anti conformations is usually correlated to the conformation of ribose ring and to the shielding of ribose protons (mainly the H(2'), 11, 12).

In our study of modified ribonucleosides, the $^3J_{1'2'}+{}^3J_{3'4'}$ sums: 10, 1 Hz (Guo-AF) and 8, 9 Hz (Guo-AAF) as well as the $^3J_{2'3'}$ values, 6 Hz (Guo-AF) and 5.1 Hz (Guo-AAF) clearly indicate differences in the ribose ring conformations. A fitting with standard relationships correlating coupling constants and ribose conformations (13, 14) is not quite satisfactory, probably because of large differences in the pseudo rotation angles (P, τ). Qualitatively, one can assume a higher population of the N(C(3') endo - C(2') exo) conformer for Guo-AAF as compared to Guo-AF. Such a trend associated with the large deshielding of H(2') proton suggests a predominant syn conformation of Guo-AAF as compared to Guo-AF. The presence of a 5'-phosphate group modifies the conformational behaviour of the ribose ring. No important differences between AF and AAF derivatives are found but the $^3J_{2'3'}$ values are higher than in unmodified 5'-ribonucleotides. According to the $^3J_{1'2'}/{}^3J_{3'4'}$ ratio, the population of N and S conformers are not drastically different in GMP-AF. On the other hand, the large deshielding of H(2') in GMP-AAF relatively to GMP-AF again suggests a predominance of the syn conformation for the AAF derivatives. For GMP-AF, the H(2') shows only a small downfield shift (0.08 ppm) as compared to GMP. This strongly suggests an anti conformation for the AF derivative. We propose that this anti conformation is due to interactions between the NH group of AF residue and the 5'OH or the 5' phosphate group. These interactions which depend upon the rotation about the C(8)-NH-C(2) bonds, modulate differently the ri-

bose ring of nucleoside and of nucleotide but do not mainly influence the H(2') chemical shift. It has to be noted that the CD spectra of Guo-AF and GMP-AF are similar in shape which suggests a similar conformation for these two derivatives.

The poor resolution of NMR spectra of modified dinucleoside monophosphates prevents an analysis of the deoxyribose ring conformations. The more stricking result is the broadening of H(1') (Guo) in d(GpA)-AAF at room temperature. This suggests a conformational equilibrium involving the ribose ring and the base position around the glycosidic bond, frequency exchange of which rapidly increases as a function of temperature. The upfield shifts of H(8) and H(2) protons of adenine can be considered as a proof of adenine stacking with another conjugated ring. We propose that the fluorene ring is involved, the resonances of which being broadened and upfield shifted (specially H(9), ref. 8). Qualitatively, one can assume more stacking in d(GpA)-AAF than in d(GpA)-AF and in d(ApG) than in d(GpA) sequences. The CD results are in agreement with this assumption. The absolute intensity of the first band mainly due to the light absorption by fluorene residue is the smallest for d(GpA)-AF and the largest for d(ApG)-AAF. Different effects as a function of temperature were observed on AF and AAF-derivatives. The intensities of the first band in the spectra of d(GpA)-AAF and of d(ApG)-AAF are almost insensitive to the variation of temperature while they largely decrease in d(GpA)-AF and in d(ApG)-AF as the temperature is increased from 5°C to 60°C.

In conclusion, it is tempting to correlate the ability of Guo-AF and GMP-AF to remain in the anti conformation and the weak AF-adenine stacking in d(GpA)-AF. On the other hand, the anti conformation of guanosine seems more difficult to maintain in d(ApG)-AF because of the steric hindrance of the adenosine sugar, as found on space-filling CPK models. In d(ApG)-AAF, the NH group is acetylated (as compared to d(ApG)-AF). The acetylation restricts the rotational freedom, increases the steric hindrance and the hydrogen bond is no longer possible. This favours the syn conformation or guanosine residues. It results that the adenine residues interact strongly with the fluorene residues, which is in agreement with the results on AAF-modified ribodinucleoside phosphates (3, 8).

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References

1. Kriek, E. and Westra, J.G. (1979) in "Chemical Carcinogens and DNA" (Grouer, P.L., ed.) vol. 2, 2-28, CRC Press
2. Daune, M.P. and Fuchs, R.P.P. (1977) in "Réparation du DNA, Mutagénèse, Cancérogénèse Chimique" (CNRS, ed.) 83-97
3. Grumberger, D. and Weinstein, I.B. (1979) in "Chemical Carcinogens and DNA" (Grouer, P.L., ed.) vol. 2, 59-93, CRC Press
4. Spodheim-Maurizot, M., Saint-Ruf, G. and Leng, M. (1979) Nucleic Acids Res. 6, 1683-1694
5. Sage, E. and Leng, M. (1980) Proc. Nat. Acad. Sci., USA, in press
6. Guigues, M. and Leng, M. (1979) Nucleic Acids Res. 6, 733-744
7. Spodheim-Maurizot, M., Dreux, M., Saint-Ruf, G. and Leng, M. (1979) Nucleic Acids Res. 7, 2347-2356
8. Nelson, J.H., Grumberger, D., Cantor, R.C. and Weinstein, I.B. (1971) J. Mol. Biol. 62, 331-346
9. Evans, F.E. and Kaplan, N.O. (1976) J. Biol. Chem. 251, 6791-6797
10. Neidle, S., Sanderson, M.R., Subbiah, A., Chattopadhyaya, J.B., Kuroda, R. and Reese, C.B. (1979) Biochim. Biophys. Acta 565, 379-386
11. Sarma, R.H., Lee, C.H., Evans, F.E., Yathindra, N. and Sundaralingam, M. (1974) J. Am. Chem. Soc. 96, 7337-7348
12. Schweizer, M.P. and Robins, R.K. (1973) in "Conformation of Biological Molecules and Polymers" (Bergman, E.E. and Pullman, B. eds) vol. 5, 329-343, Israel Academy of Sciences and Humanities, Jerusalem
13. Altona, C. and Sundaralingam, M. (1973) J. Am. Chem. Soc. 95, 2333-2344
14. Davies, D.B. and Danyluk, S.S. (1974) Biochemistry 13, 4417-4434.