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Conformational changes induced in DNA by the *in vitro* reaction with the mutagenic amine: 3-N,N-acetoxyacetylamino-4,6-dimethyldipyrido (1,2-a : 3',2'-d) imidazole

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ABSTRACT

The conformation of synthetic or natural DNAs modified in vitro by covalent binding of N-AcO-A-Glu-P-3 was investigated by fluorescence and circular dichroïsm. In all cases, substitution occurs mainly on the C_8 of guanine residues. In modified poly(dG-dC).poly(dG-dC) or poly(dA-dC).poly(dG-dT) in B conformation, A-Glu-P-3 residues interact strongly with the bases whereas in Z conformation these residues are largely exposed to the solvent and interact weakly with the bases. A-Glu-P-3 and N-acetyl-2-aminofluorene (AAF) residues are equally efficient to induce the B-Z transition of poly(dG-dC).poly(dG-dC) and of poly(dA-dC).poly(dG-dT). Modifications of poly(dG).poly(dC) and calf thymus DNA indicate strong interactions between A-Glu-P-3 and the bases.

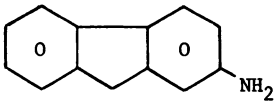
INTRODUCTION

Recent studies have shown that mutagenic compounds are formed in the charred parts of cooked food such as meat, fish, bakery and cereals (1-7) and in pyrolysis of amino acids and proteins (7-13). Two new highly mutagenic heterocyclic amines have been identified in pyrolysate of L-glutamic acid : 2-amino-6-methyldipyrido (1,2-a:3',2'-d) imidazole and 2-aminodipyrido (1,2-a:3', 2'-d) imidazole, respectively named Glu-P-1 and Glu-P-2 (10).

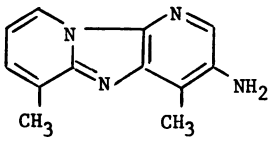
We have recently reported that a related compound : 3-amino-4,6 dimethyldipyrido (1,2-a:3',2'-d) imidazole, named Glu-P-3 is even more mutagenic than Glu-P-1 and Glu-P-2 (14). In vitro Glu-P-3 does not bind to DNA but a supposed metabolite N-acetoxy-N-acetyl-Glu-P-3 binds to DNA and deoxyguanosine.

It is known that all the carcinogens bind covalently to DNA and it is generally assumed that this covalent binding is important in cancer process (15). In this paper, we report some results on the conformational changes induced by the covalent binding of A-Glu-P-3 residues to natural and synthetic polynucleotides. There are already numerous studies on the conformation of nucleic acids modified by carcinogens. Nevertheless, it seemed to us of interest to better characterize the binding of this new heterocyclic amine to DNA and to compare with the well-known carcinogen acetyl-2-aminofluorene. Glu-P-3 and

2-aminofluorene show significant structural similarities ; formulas of the two compounds are:



2-aminofluorene



Glu-P-3

Moreover, Glu-P-3 is fluorescent in normal conditions. This offers a new possibility to locate the Glu-P-3 residues with respect to the bases residues. In parallel, a study of the modified DNAs has been performed by circular dichroïsm.

MATERIALS AND METHODS

Chemicals

3-N-acetoxy-N-acetylamino-4,6 dimethyldipyrido (1,2-a:3',2'-d) imidazole (N-AcO-A-Glu-P-3) and 3-N(2'deoxyguanosin-8-yl-5'monophosphate), N-acetylamino-4,6 dimethyl dipyrido(1,2-a:3',2'-d) imidazole (dGMP-A-Glu-P-3) were prepared as already described (14). Poly(dG-dC).poly(dG-dC), poly(dA-dC).poly (dG-dT) and poly(dG).poly(dC) were bought from P.L. Biochemicals and calf thymus DNA (Type I) from Sigma. Potassium iodide (KI) is obtained from Merck.

The reaction between calf thymus DNA and N-AcO-A-Glu-P-3 was performed as already described (14). Denaturated samples were obtained by heating nDNA-A-Glu-P-3 at 100°C during 15 mn and then by rapid cooling in ice. The reaction between synthetic polynucleotides and N-AcO-A-Glu-P-3 was performed according to the following procedure : polynucleotide (100µg) was dissolved in 500 µl of 1 mM phosphate buffer pH 7.3 and 5 µl of a dimethylformamide solution of N-AcO-A-Glu-P-3 was added (the ratio of carcinogen to phosphate was 1). After incubation at 37°C for 2 hours, the solution was treated twice with phenol and then exhaustively dialyzed against 1 mM phosphate buffer, 0.1 mM EDTA pH 7.3. The percentage of modified bases was deduced from the ultraviolet absorption spectra (14). We will write DNA-A-Glu-P-3 (0.10) for a modified sample having 10 % modified bases. Enzymatic hydrolysis was performed as described in ref. 14 and radioimmunoassays have been already described (35).

Instrumentation

Ultraviolet spectra were recorded on a Cary 210 spectrophotometer, circular dichroïsm spectra on a Roussel-Jouan Auto-dichrograph Mark IV. Fluo-

rescence measurements were performed with a Farrand Mark I spectrofluorimeter. The circular dichroïsm results are given in ΔA and not in $\Delta\varepsilon$ M^{-1} cm^{-1} because Glu-P-3 and nucleotide residues can participate to the CD signal.

RESULTS

Nature of the adducts

Several synthetic polynucleotides : poly(dG-dC).poly(dG-dC) ; poly (dG).poly(dC), poly(dA-dC).poly(dG-dT) and calf thymus DNA have been reacted with N-AcO-A-Glu-P-3. After removal of the unbound metabolite, the samples were digested by DNAse I and P_1 nuclease as previously described (37) and then analyzed by HPLC. In these conditions, only one adduct resulting from the substitution on the C_8 of deoxyguanosine was detected. (14)

Fluorescence

Glu-P-3 and some of its derivatives are highly fluorescent. In figure 1 are drawn the absorption and the fluorescence spectra of dGMP-A-Glu-P-3 and of nDNA-A-Glu-P-3 (0.05) in low salt conditions. Above 310 nm the absorption spectra are similar in shape.

The fluorescence spectra have also a similar shape with a maximum at 415 nm, but the fluorescence intensities I_F are different. A-Glu-P-3 residues are about thirty times more fluorescent when bound to DNA than to dGMP. The fluorescence intensity is almost the same for A-Glu-P-3 residues bound to poly (dG-dC).poly(dG-dC), poly(dA-dC).poly(dG-dT), poly(dG).poly(dC) or nDNA. On the other hand, I_F of dGMP-A-Glu-P-3 is very sensitive to the nature of the medium. I_F is much larger in ethanol (almost 10 times) than in water (not shown).

The fluorescence intensity of dGMP-A-Glu-P-3, nDNA-A-Glu-P-3 and poly

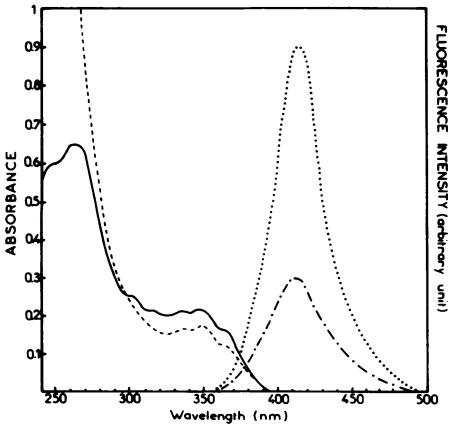


Figure 1 - left : absorption spectra of nDNA-A-Glu-P-3 (0.05) (---) and dGMP-A-Glu-P-3 (—) A-Glu-P-3 concentration : $3x10^{-5}$ M ; right : fluorescence spectra of dGMP-A-Glu-P-3 (—·—) (sensitivity : 0.1) and nDNA-A-Glu-P-3 (0.05) (.....) (sensitivity : 1) A-Glu-P-3 concentration : $3x10^{-7}$ M, λ_{exc} 350 nm. Solvent 2 mM citrate buffer pH 7.3, 10 mM NaCl, 0.1 mM EDTA.

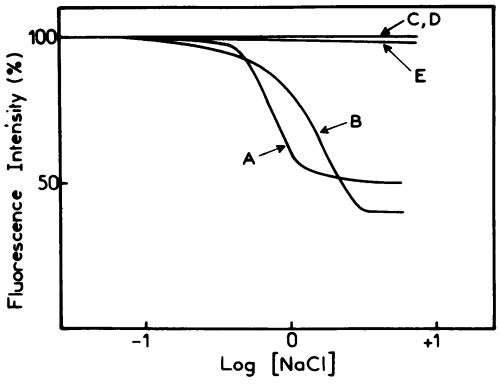


Figure 2 : Fluorescence intensity percentage at 415 nm as a function of NaCl concentration : (A) poly(dA-dC).poly(dG-dT)-A-Glu-P-3 (0.12) ; (B) poly(dG-dC)-A-Glu-P-3 (0.05) ; (C) poly(dG).poly(dC)-A-Glu-P-3 (0.065) ; (D) nDNA-A-Glu-P-3 (0.05) ; (E) dGMP-A-Glu-P-3. Solvent : 1 mM phosphate buffer pH 7.3, 0.1 mM EDTA, λ_{exc} 350 nm, temperature 25°C A-Glu-P-3 concentration : 3×10^{-7} M.

(dG).poly(dC)-A-Glu-P-3 is independant of salt concentration up to 4 M NaCl. It depends strongly upon salt concentration when A-Glu-P-3 residues are bound to poly(dG-dC).poly(dG-dC) or poly(dA-dC).poly(dG-dT). In both cases, there is a transition occuring in a cooperative manner, the midpoints of which are respectively 1,4 M and 0.8 M for poly(dG-dC)-A-Glu-P-3 (0.05) and for poly(dA-dC).poly(dG-dT)-A-Glu-P-3 (0.12) (figure 2).

These results strongly suggest that the location of A-Glu-P-3 residues depends upon salt concentration in some polynucleotides but not in all of them.

The location of A-Glu-P-3 residues can be better determined by a study of the accessibility of these residues to ions which are known to quench fluorescence.

If one assumes proportionnality between fluorescence intensity and quantum yield of fluorescence, the quenching of a fluorophor is expressed by the Stern-Volmer law :

$$F_o/F = 1 + K_Q c$$

where F_o and F are the fluorescence intensities in absence of in presence of quencher, c the concentration of quencher and K_Q the Stern-Volmer constant.

Our purpose is to characterize A-Glu-P-3 residues bound to natural DNA in which the accessibility of the fluorophor might depend upon the base composition. The analysis of the quenching of an heterogenous system can be done using a modified form of the Stern-Volmer law as originally proposed by

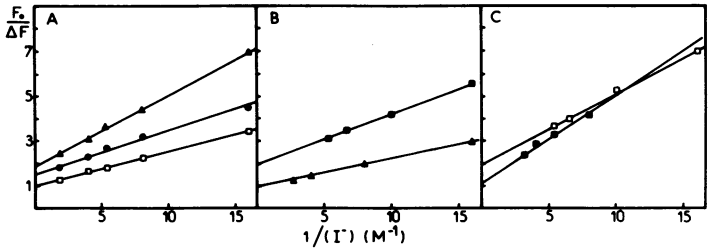


Figure 3 : Modified Stern-Volmer plot of the quenching of A-Glu-P-3 fluorescence by iodide : A. nDNA-A-Glu-P-3 (0.11) (▲), dDNA-A-Glu-P-3 (0.11) (●), dGMP-A-Glu-P-3 (□) in (NaCl + KI) 0.5 M
B. poly(dG-dC)-A-Glu-P-3 (0.05) in (NaCl + KI) 0.2 M (■), 2.5 M (▲)
C. poly(dA-dC).poly(dG-dT)-A-Glu-P-3 (0.12) in (NaCl + KI) 0.2 M (□), 2 M (■) Solvent : 1 mM phosphate buffer pH 7.3, 0.1 mM EDTA, λ_{exc} 350 nm, λ_{em} 415 nm. Temperature 25°C, A-Glu-P-3 concentration : 3×10^{-7} M.

Lehrer (16) : $F_o/\Delta F = 1/Fa + 1/Fa\ K_Q c$ where $\Delta F = F_o - F$ with the same signification as above for F_o and F and where $Fa = \sum_{i=o}^{m} Fi$ summed over the m accessible fluorophors is the fractional maximum accessible fluorescence (Fi being the fractional fluorescence of the fluorophor i).

The experiments were performed in presence of IK, I^- being known to be an effective fluorescence quencher.

In figure 3 are shown the variations of $F_o/\Delta F$ as a function of the inverse of IK concentration. The value of $F_o/\Delta F$ at infinite IK concentration is equal to 1/Fa, Fa being the fractional maximum accessible fluorescence. In the case of dGMP-A-Glu-P-3, Fa = 1 and thus the fluorophor is completely accessible to the quencher. It is less accessible in denaturated DNA and still less in native DNA (fig. 3A).

On the other hand, the influence of the percentage of base modified in native calf thymus DNA has been examined and we found that Fa values remain the same whatever is the level of modification in the range 0.01-0.11 (not shown).

Poly(dG-dC)-A-Glu-P-3, poly(dA-dC).poly(dG-dT)-A-Glu-P-3 (figure 3, B and C) and poly(dG).poly(dC)-A-Glu-P-3 (not shown) in low salt behave as nDNA; in all the cases, Fa = 1/2. In high salt conditions, Fa is still equal to 1/2 for modified nDNA and poly(dG).poly(dC) but is equal to 1 and 0.9 for modified poly(dG-dC).poly(dG-dC) and poly(dA-dC).poly(dG-dT) respectively.

The values of Fa and of the Stern-Volmer constant K_Q are reported in the table 1.

Circular dichroïsm

It is well known that poly(dG-dC).poly(dG-dC) has the B conformation

Table I : Quenching of A-Glu-P-3 fluorescence by iodide at 25°C

Substrate	salt concentration (M)	Fa	$K_Q(M^{-1})$
nDNA-A-Glu-P-3 (0.01)	0.5	0.52	3.8
nDNA-A Glu-P-3 (0.05)	0.5	0.52	6.3
nDNA-A-Glu-P-3 (0.11)	0.5	0.52	6.1
dDNA-A-Glu-P-3 (0.11)	0.5	0.67	6.6
dGMP-A-Glu-P-3	0.5	1	6.1
poly(dG-dC)-A-Glu-P-3 (0.05)	0.2	0.5	9.1
poly(dG-dC)-A-Glu-P-3 (0.05)	2.5	1	8
poly(dA-dC).poly(dG-dT)-A-Glu-P-3 (0.12)	0.2	0.5	4.6
poly(dA-dC).poly(dG-dT)-A-Glu-P-3 (0.12)	2	0.9	4.2
poly(dG).poly(dC)-A Glu-P-3 (0.065)	0.2	0.5	10

in 0.1 M NaCl and the Z conformation in 4 M NaCl (17, 18, 19). The cooperative transition of poly(dG-dC)-A-Glu-P-3 (0.05) detected by fluorescence suggested that such a B-Z transition also occured with this modified polynucleotide as the salt concentration was increased. This has been confirmed by circular dichroïsm.

In figure 4A are shown the CD spectra of poly(dG-dC).poly(dG-dC) in

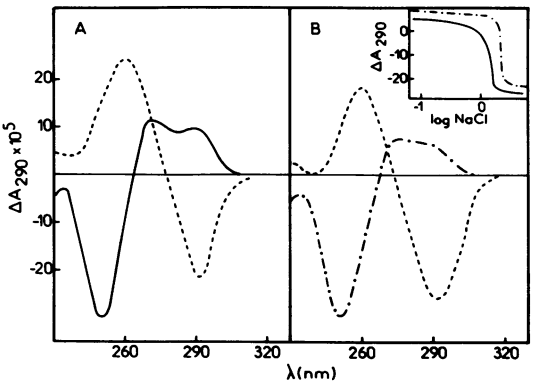


Figure 4 : Circular dichroïsm spectra : (A) poly(dG-dC).poly(dG-dC) in 1 mM phosphate buffer pH 7.3, 0.1 mM EDTA (—), in 3 M NaCl, 0.1 mM EDTA (---) ; (B) poly(dG-dC)-A-Glu-P-3 (0.05), in 1 mM phosphate buffer pH 7.3, 0.1 mM EDTA (-·-), in 1.8 M NaCl, 0.1 mM EDTA (---). Inset : $\Delta A_{290} \times 10^5$ as a function of NaCl concentration of poly(dG-dC).poly(dG-dC) (-·-), poly(dG-dC)-A-Glu-P-3 (0.05) (—). The absorbance at 260 nm was 0.4 ; temperature 25°C.

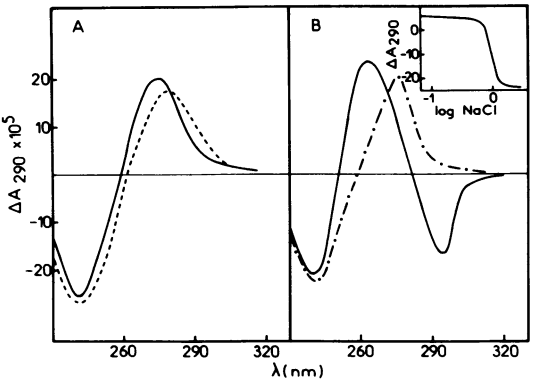


Figure 5 : Circular dichroïsm spectra : (A) poly(dA-dC).poly(dG-dT) in 1 mM phosphate buffer pH 7.3, 0.1 mM EDTA (—), in 1.9 M NaCl, 0.1 mM EDTA (---) ; (B) poly(dA-dC).poly(dG-dT)-A-Glu-P-3 (0.12) in 1 mM phosphate buffer pH 7.3, 0.1 mM EDTA (-·-), in 1.1 M NaCl, 0.1 mM EDTA (——). Inset : ΔA_{290} x 10^5 as a function of NaCl concentration of poly(dA-dC).(dG-dT)-A-Glu-P-3 (0.12). The absorbance at 260 nm was 0.4 ; temperature 25°C.

low and high salt conditions. They are in good agreement with those already reported (19).

The CD spectrum of poly(dG-dC)-A-Glu-P-3 in low salt presents a positive band with a maximum at 275 nm and then a negative band centered at 250 nm (fig. 4B). In 1.8 M NaCl, the spectrum looks like the spectrum of poly(dG-dC).poly(dG-dC) in Z conformation. The variation of ΔA_{290} as a function of salt concentration shows that the transition occurs according to a cooperative process and the midpoint of the transition is at 1.4 M (inset of figure 4B). The midpoint of the transition depends upon the amount of bound A-Glu-P-3. As judget by CD, poly(dG-dC)-A-Glu-P-3 (0.20) is in the Z conformation in 0.1 M NaCl (not shown).

In figure 5 are shown the spectra of poly(dA-dC).poly(dG-dT) and of poly(dA-dC).poly(dG-dT)-A-Glu-P-3 (0.12) in low and high salt conditions. The CD spectra of the unmodified polynucleotide are almost the same, with a first positive band centered at 275 nm and then a negative band centered at 240 nm (fig. 5A). In low salt conditions, the CD spectrum of the modified polynucleotide looks like that of the unmodified polynucleotide.

On the other hand, in 1.1 M NaCl, the CD spectrum presents a first negative band centered at 295 nm, then a positive band centered at 263 nm and then a negative band (fig. 5B). This spectrum presents large similarities with that of poly(dG-dC)-A-Glu-P-3 in 1.8 M NaCl (fig. 4B).

In the inset of fig. 5B the variation of ΔA_{290} as a function of salt

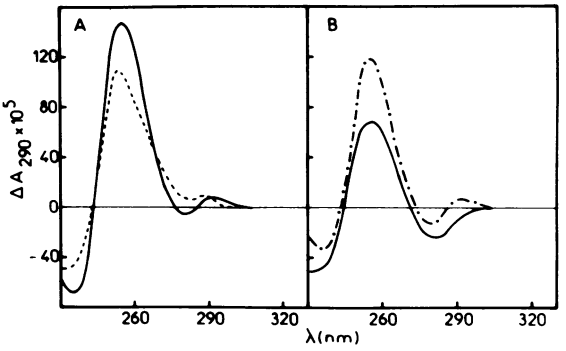


Figure 6 : Circular dichroïsm spectra : (A) poly(dG).poly(dC) in 1 mM phosphate buffer pH = 7.3, 0.1 mM EDTA (——), in 1.1 M NaCl, 0.1 mM EDTA (---) ; (B) poly(dG).poly(dC)-A-Glu-P-3 (0.065) in 1 mM phosphate buffer pH = 7.3, 0.1 mM EDTA (-·-), in 2 M NaCl, 0.1 mM EDTA (——). The absorbance at 260 nm was 0.4 : temperature 25°C.

concentration shows that the midpoint of the cooperative transition occurs at 0.8 M.

Finally in fig. 6 are shown the spectra of modified poly(dG).poly (dC)-A-Glu-P-3 in low and high salt conditions. There are some variations in the spectra as the salt concentration is increased, but they are smaller than those observed with the alternated polymers.

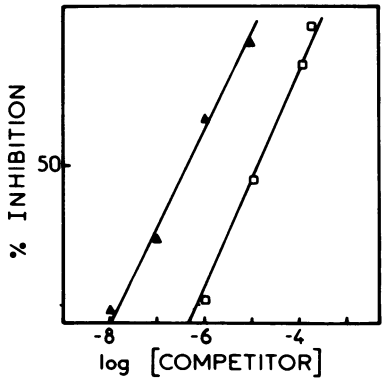


Figure 7 : Inhibition of tracer-antibody binding by poly(dA-dC).poly(dG-dT)-A-Glu-P-3 (0.12) in competitive RIA. Tracer [^{3}H] poly(dG-br^{5}dC).poly(dG-br^{5}dC), c = 3 x 10^{-7} M. Antiserum dilution 1/1000. Inhibitors (▲) poly(dG-br^{5}dC).poly (dG-br^{5}dC), (□) poly(dA-dC).poly(dG-dT)-A-Glu-P-3 (0.12). Solvent 1.1 M NaCl, 1 mM phosphate buffer pH 6.5, 0.1 mM EDTA. Temperature 4°C.

Radioimmunoassays

The affinity of antibodies against Z-DNA towards the modified poly (dA-dC).poly(dG-dT) was studied by radioimmunoassays.

The tracer was [^{3}H] poly(dG-br^{5}dC).poly(dG-br^{5}dC). As shown in fig. 7 in 1.1 M NaCl, the poly(dA-dC).poly(dG-dT)-A-Glu-P-3(0.12) interacts with the antibodies to Z-DNA. It is recognized to a less extent than poly(dG-br^{5}dC).poly (dG-br^{5}dC) ; about 50 times more poly(dA-dC).poly(dG-dT)-A-Glu-P-3 (0.12) than poly(dG-br^{5}dC).poly(dG-br^{5}dC) are necessary to get 50 % inhibition.

DISCUSSION

We have studied by fluorescence and circular dichroism the conformational changes induced by the binding of 3-N-acetylamino-4,6 dimethyldipyrido (1,2-a:3',2'-d) imidazole (A-Glu-P-3) residues to the C_8 of guanine residues in natural and synthetic nucleic acids (Glu-P-3 is related to the mutagenic compounds Glu-P-1 and Glu-P-2 formed in the pyrolysis of glutamic acid).

The fluorescence emission spectrum of dGMP-A-Glu-P-3 presents a maximum at 415 nm. The fluorescence intensity depends upon the nature of the medium, being much larger in ethanol than in water. In low salt conditions, the fluorescence intensity of several modified polynucleotides poly(dG-dC).poly (dG-dC), poly(dA-dC).poly(dG-dT), poly(dG).poly(dC) and DNA is about the same and larger than that of dGMP-A-Glu-P-3 in water. This suggests that A-Glu-P-3 residue bound to DNA and synthetic polynucleotides is not totally in contact with water but rather interact with the base residues which provide an hydrophobic environment.

The study of poly(dG-dC)-A-Glu-P-3 gives strong support to this hypothesis. Poly(dG-dC).poly(dG-dC) has the B conformation in low salt concentration and the Z conformation in high salt concentration (17,18). The CD spectra of poly(dG-dC).poly(dG-dC) and poly(dG-dC)-A-Glu-P-3 in 1 mM phosphate on one hand and in 3 M NaCl on the other hand are similar. As judged by circular dichroïsm, poly(dG-dC)-A-Glu-P-3 adopts the Z conformation in high ionic strength. The B-Z transition of the modified polymer is cooperative and occurs at lower salt concentration than that of the unmodified polymer. The binding of A-Glu-P-3 residues to poly(dG-dC).poly(dG-dC) stabilizes the Z conformation (a poly(dG-dC)-A-Glu-P-3 (0.20) is in the Z conformation in 0.1 M NaCl, not shown).

The B form-Z form transition of poly(dG-dC)-A-Glu-P-3 (0.05) can be followed by fluorescence. The fluorescence intensity I_F is larger in 0.1 M than in 3 M NaCl. This is not due to a direct interaction between NaCl and A-Glu-P-3 residues since in the same salt range, I_F of dGMP-A-Glu-P-3 is almost

constant. I_F of poly(dG-dC)-A-Glu-P-3 (0.05) decreases sharply near 1 M (midpoint of the transition 1.4 M).

The value of midpoint of the transition is in good agreement with that determined by CD experiments. Thus the interactions between A-Glu-P-3 residues and the nucleotide residues are smaller in the Z conformation than in the B conformation. This is confirmed by the determination of the fractional maximum accessible fluorescence. Experiments are performed in presence of I^-. I^- quenches the fluorescence when it is in close contact with the fluorophor. The results are analyzed with a modified form of the Stern-Volmer law (16). The linear plot of $F_o/\Delta F$ versus $1/(I^-)$ (figure 3) suggests that each fluorophor is subject to a similar degree of fluorescence quenching. On the other hand the degree of quenching depends upon the conformation of the polynucleotide. About 50 % of A-Glu-P-3 fluorescence is accessible for quenching by iodide when A-Glu-P-3 residues are bound to poly(dG-dC).poly(dG-dC) in the B conformation and 100 % when bound to poly(dG-dC).poly(dG-dC) in the Z conformation (or to dGMP). It can be noted that the values of the quenching constants are almost independent of salt concentration.

Thus our conclusion from CD and fluorescence experiments is that A-Glu-P-3 residues are largely exposed to the solvent and do not interact (or interact weakly) with the base residues when bound to poly(dG-dC).poly(dG-dC) in the Z conformation. On the other hand, these residues interact strongly with the base residues and are only partially exposed to the solvent when bound to poly(dG-dC).poly(dG-dC) in the B conformation.

CD and fluorescence experiments were also performed with modified poly(dA-dC).poly(dG-dT) and poly(dG).poly(dC).

The behaviour of poly(dA-dC).poly(dG-dT)-A-Glu-P-3 resembles that of poly(dG-dC)-A-Glu-P-3. In low ionic strength the CD spectrum presents a first positive band centered at 275 nm and then a negative band centered at 240 nm. About 50 % of A-Glu-P-3 residues fluorescence is accessible for quenching by iodide. As the ionic strength is increased, a cooperative transition occurs. The midpoint of the transition determined by CD and fluorescence are in good agreement. In high ionic strength, about 90 % of A-Glu-P-3 residues fluorescence is accessible for quenching by iodide. The CD spectrum presents a first negative band centered at 295 nm and then a positive band centered at 263 nm. This part of the spectrum looks like the one of poly(dG-dC).poly(dG-dC) in the Z conformation and thus suggests that the modified polymer has the Z conformation. This assumption is confirmed by the radioimmunoassays data which clearly indicate that poly(dA-dC).poly(dG-dT)-A-Glu-P-3 (0.12) is recognized by the an-

tibodies to Z-DNA. Thus, the A-Glu-P-3 residues bound to poly(dG-dC).poly(dG-dC) and to poly(dA-dC).poly(dG-dT) behaves similarly when the polymers have on one hand the B conformation and on the other hand the Z conformation.

In 4.5 M NaCl, the B-Z transition of linear poly(dA-dC).poly(dG-dT) does not occur while poly(dG-dC).poly(dG-dC) has the Z conformation. A-Glu-P-3 residues stabilize the Z form of poly(dG-dC).poly(dG-dC) and poly(dA-dC).poly (dG-dT). It is interesting to compare the effects of A-Glu-P-3 and AAF residues. Both residues bound to poly(dG-dC).poly(dG-dC) behave similarly (this work and references 20-22, 31). On the other hand, A-Glu-P-3 seems to be more efficient than AAF residues to stabilize poly(dA-dC).poly(dG-dT) in the Z conformation (23).

As far as concerning the modified poly(dG).poly(dC), the fluorescence intensity remains constant in the range 1 mM-2 M NaCl and about 50 % of A-Glu-P-3 residues fluorescence is accessible for quenching by iodide as for the previously investigated polymers in low ionic strength. These results indicate again that A-Glu-P-3 residues interact with the bases residues. CD spectra which are not drastically changed for modified poly(dG).poly(dC) with respect to unmodified sample, even on increasing the ionic strength suggest that modification by A-Glu-P-3 does not induce the B-Z transition of poly(dG).poly(dC).

The behaviour of nDNA-A-Glu-P-3 resembles that of modified synthetic polynucleotides in the B conformation. About 50 % of A-Glu-P-3 residues fluorescence is accessible for quenching by iodide. This value is independent of the level of modification (in the range 0.01-0.11). There are no changes in the fluorescence intensity in the range 1 mM-2 M NaCl. These results suggest that A-Glu-P-3 residues interact strongly with the base residues and that the accessibility to the quencher is not sensitive to the base composition. The A-Glu-P-3 residues are more exposed in denaturated DNA. About 63 % of the fluorescence is accessible for quenching by iodide.

Up to this point, we have to compare the conformational changes induced by the binding of A-Glu-P-3 and AAF residues to nucleic acids in B conformation. In vitro, AAF is mainly bound to the C_8 of guanine residue (24). The solution studies show that AAF modified DNA of random sequences adopts a conformation named insertion-denaturation (25a-27) or base-displacement model (28-30). In this conformation, the carcinogen is stacked with the adjacent base and the guanine is syn and outside of the double helix. When the carcinogen is bound to poly(dG-dC).poly(dG-dC) or to poly(dA-dC).poly(dG-dT) it stabilizes the Z conformation (20-23). The guanine are syn, paired with cytosine and it is assumed that the AAF residues are outside the double helix (26-31). Mini-

mized semi-empirical potential energy calculations for AAF adducts with dCpdG have yielded molecular views of the adduct conformation (31,32).

The experiments here reported on modified nucleic acids in low ionic strength and those in reference (14) relative to the thermal stability of nDNA-A-Glu-P-3 and the hydrolysis by S_1 nuclease are in agreement with the insertion-denaturation or base-displacement models. The fluorescence quenching by iodide indicates that the A-Glu-P-3 residues are not completely inside the double helix but partially exposed to the solvent. This can explain, in the case of AAF, the recognition of DNA-AAF by the anti Guo-AAF antibodies (33,34).

On the other hand, A-Glu-P-3 residues stabilize the Z conformation of poly(dG-dC).poly(dG-dC) and poly(dA-dC).poly(dG-dT). The fluorescence quenching by iodide demonstrates that A-Glu-P-3 residues are outside of the double helix.

A recent paper in the case of AAF modified DNA underlines the possible role of Z-DNA in mutational hotspots (36). Because of the similar conformational changes induced by AAF and A-Glu-P-3 residues, we are interested in establishing the DNA binding spectrum of A-Glu-P-3. Work is in progress to determine such a spectrum.

Abbreviations

Glu-P-3 : 3-amino-4,6 dimethyldipyrido(1,2-a:3'2'-d) imidazole.
N-AcO-A-Glu-P-3 : 3-N,N-acetoxyacetylamino-4,6-dimethyldipyrido(1,2-a:3'2'-d) imidazole.
A-Glu-P-3 : 3-N-acetylamino-4,6-dimethyldipyrido (1,2-a:3'2'-d) imidazole.
dGMP-A-Glu-P-3 : N-(deoxyguanosin-8-yl-5'monophosphate)-3-N-acetylamino-4,6-dimethyldipyrido (1,2-a:3',2'-d)imidazole.
nDNA : native DNA.
dDNA : denatured DNA.
DNA-A-Glu-P-3 : DNA that has reacted with N-AcO-A-Glu-P-3.
poly(dG-dC)-A-Glu-P-3 : poly(dG-dC).poly(dG-dC) that has reacted with N-AcO-A-Glu-P-3.
poly(dA-dC).poly(dG-dT)-A-Glu-P-3 : poly(dA-dC).poly(dG-dT) that has reacted with N-AcO-A-Glu-P-3.
poly(dG).poly(dC)-A-Glu-P-3 : poly(dG).poly(dC) that has reacted with N-AcO-A-Glu-P-3.
AAF : N-acetyl-2-aminofluorene.
Guo-AAF : N-(guanosin-8-yl)-2-N-acetylaminofluorene.
DNA-AAF : DNA that has reacted with N-acetoxy-N-acetyl-2-aminofluorene.

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