



HAL
open science

Synthesis, thermal stability and reactivity towards 9-aminoellipticine of double-stranded oligonucleotides containing a true abasic site

J.J. Vasseur, Jean-Jacques Vasseur, Bernard Rayner, Jean-Louis Imbach, Jean-Rémi Bertrand, Jean-Jacques Vasseurl, Bernard Rayner, Jean-Louis Imbachl, Jacques Paoletti, Claude Paoletti, et al.

► To cite this version:

J.J. Vasseur, Jean-Jacques Vasseur, Bernard Rayner, Jean-Louis Imbach, Jean-Rémi Bertrand, et al.. Synthesis, thermal stability and reactivity towards 9-aminoellipticine of double-stranded oligonucleotides containing a true abasic site. *Nucleic Acids Research*, 1989, 17 (24), pp.10307-10319. <10.1093/nar/17.24.10307>. <hal-03008450>

HAL Id: hal-03008450

<https://hal.science/hal-03008450v1>

Submitted on 16 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Synthesis, thermal stability and reactivity towards 9-aminoellipticine of double-stranded oligonucleotides containing a true abasic site

Jean-Rémi Bertrand, Jean-Jacques Vasseur¹, Bernard Rayner¹, Jean-Louis Imbach¹, Jacques Paoletti, Claude Paoletti and Claude Malvy

Laboratoire de Biochimie-Enzymologie, UA 147 CNRS, UA 140 INSERM, Institut Gustave-Roussy, 94805 Villejuif Cédex and ¹Laboratoire de Chimie-Bioorganique, UA 488 CNRS, Université des Sciences et Techniques du Languedoc, place Eugène-Bataillon, 34600 Montpellier Cédex, France

Received August 29, 1989; Accepted November 6, 1989

ABSTRACT

A 13 mers abasic oligonucleotide was synthesized. It was therefore possible to compare thermal stability and reactivity of duplex oligonucleotides either with an apurinic/aprimidinic site or without any lesion. An important decrease in the melting temperature appeared for duplexes with an abasic site. The chemical reaction of these modified oligonucleotides with the intercalating agent 9-aminoellipticine was studied by gel electrophoresis and by fluorescence. The formation of a Schiff base between 9-aminoellipticine and abasic sites was rapid and complete with duplexes at 11°C. Schiff base related fluorescence and β -elimination cleavage were more important with the apyrimidinic sites than with the apurinic ones. When compared to previous results obtained with the model d(TprpT) some unexpected behaviours appeared with longer and duplex oligonucleotides. For instance only partial β -elimination cleavage was observed. It is likely that stacking parameters in the double helix play a great role in the studied reaction.

INTRODUCTION

Cleavage of the N-glycosidic bond of nucleosides in DNA generates (apurinic/aprimidinic) sites (1). Such biological important lesions consist of a 2'-deoxyribose residue linked to neighboring nucleosides through 3'- and 5'-phosphodiester bonds.

Introduction of such an abasic site into synthetic oligonucleotides, in order to have a model assay system for enzymatic, chemical or structural studies, was a challenging problem for the chemists as these lesions are subject to easy basic hydrolysis (2) including amino reagents treatment (3).

For this reason, various synthetic models were obtained including cyclic tetrahydrofuran substituted (4,5,6) or not (4,7,8) in pseudoanomeric position and acyclic moieties such as propanediol (8,9) and deoxyribitol (8). These analogues are stable enough to be introduced in oligodeoxynucleotides by conventional chemical methods.

However none of those models was satisfactory to study the reactivity of true abasic sites (ie deoxyribose) owing to the fact that the ring-opened aldehyde form A of deoxyribose is responsible of the reactivity of abasic sites (2), even if the cyclic deoxyribofuranose form B is predominant (8,10) (Figure 1).

We reported for the first time the chemical synthesis of a simple abasic oligonucleotide model d(TprpT) (where r is a deoxyribose moiety) containing a real abasic site (10) and we studied its reactivity towards the action of some amino derivatives (11,12). However this model oligonucleotide was too short to modelise the reactivity of an abasic site in double-stranded DNA.

This prompted us to prepare longer abasic oligonucleotides and to study their reactivity in double-stranded duplexes with the intercalating agent 9-aminoellipticine (9-AE) which

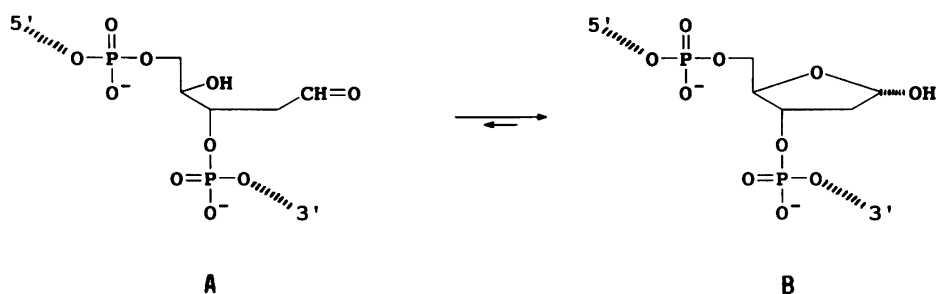


Figure 1: Tautomeric equilibrium between the different forms of deoxyribose in an abasic site.

is one of the most potent compounds known to induce apurinic DNA breakage (13,14,15) and is a specific inhibitor of DNA repair (23). According to the sequence of the complementary synthetic oligonucleotide, we had also the possibility of studying the action of 9-AE on apurinic and apyrimidinic sites. We had indeed published that, in opposition with AP-endonucleases, 9-AE cleaves apurinic, but not apyrimidinic, sites in pBR322 circular supercoiled DNA (16). However the precise localisation of apyrimidinic sites, generated by the action of uracil DNA glycosylase on missincorporated uracil, was not known. A kinetic study on a defined substrate became possible with oligonucleotides. These oligonucleotides were also able to help in the understanding of the mechanism of reaction between 9-AE and apurinic sites. Similarities in the fluorescence spectrum of intermediate compounds had been indeed observed in the reaction of 9-AE with d(TprpT) and with apurinic DNA (15). However velocity of the reaction was very different. Analysis of the different steps of the pathway with apurinic DNA were difficult because of the difficulty of detecting intermediates. This became possible, thanks to gel electrophoresis, with synthetic apurinic oligonucleotides.

MATERIALS AND METHODS

1) Oligonucleotide synthesis

Synthesis of the oligonucleotide d(CTCTCCrTTCCTC) 1 was achieved by depurination upon acidic treatment (17) of the parent oligonucleotide d(CTCTCCATTCTC) (Figure 2). The abasic oligonucleotide was then purified as already described by Vasseur and al (10). Treatment of the crude oligomer with an excess of methoxyamine gave a stable adduct 2 (3,18), which was easily purified by HPLC chromatography. The structure of this adduct is a O-methylaldoxime as it has been previously described for the model compound d(TprpT) (10). Acid catalysed displacement reaction was performed upon treatment of the methoxyamine-adduct with acetaldehyde and yielded pure abasic oligonucleotide.

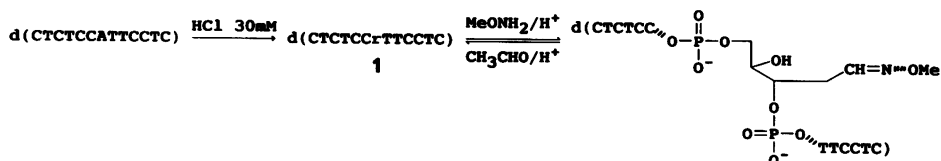


Figure 2: Chemical synthesis of d(CTCTCCrTTCCTC)

2) Thermal stability

Optical measurements were performed on a UVIKON 810 spectrophotometer (KONTRON), interfaced with an IBM PC compatible microcomputer. The temperature control was through a HUBER PD4 temperature programmer connected to a refrigerated water bath (HUBER Ministat). Cuvettes were 1 cm pathlength quartz cells and nitrogen was continuously circulated through the cuvette compartment. Prior to the experiments, the sequences to be studied were mixed together in 10 mM cacodylate, PH 7.0, 1M NaCl, and allowed to incubate at 80°C for a length of time sufficient to allow the optical density of the mixture to be perfectly stable (about 1 hour). Digitized absorbance values and temperatures were stored by the computer for subsequent plotting and analysis. The computer collected absorbance and temperature every 20 sec while the temperature variation was 0.5°C/min. This resulted in more than 450 points for an annealing curve ranging from 80°C to 0°C.

The results were fitted with a non linear least squares program based upon the Marquart algorithm (19, 20) and using the method described by Turner et al (21). The oligomers being non self-complementary, the changes in enthalpy, δH° , and entropy, δS° , for the reaction are related to α , the fraction of strands in the double-stranded state through: $\delta H^\circ - T\delta S^\circ = -RT\text{Log}[2\alpha/(1-\alpha)^2Ct]$ were Ct is the total strand concentration (each strand at a concentration Ct/2). The program fitted the annealing curves with temperature dependant absorptions for both the helix and the sum of the separated single strands assuming that these absorptions are linear functions of temperature and with apparent enthalpy and entropy for the transition. In order to allow an easier comparison between the annealing profiles corresponding to different oligomers, the normalized absorbance was plotted as a function of temperature. Absorbances were normalized to the absorbance at 70°C.

3) Reactivity with 9-AE

a) *Hybrid formation*: Single-stranded complementary oligonucleotides (2 μM each) were pooled in 1 ml of 10 mM cacodylate, 1M NaCl, pH 7. They were then heated at 80°C for 10 minutes and slowly cooled for 3 hours until 10°C. Oligonucleotide concentrations prior to hybridization were obtained through 260 nm absorbance at 80°C in order to minimize stacking.

b) *[5'-³²P] labelling of oligonucleotides*: The abasic oligonucleotide was phosphorylated with 1 μl of T4 polynucleotide kinase (Biorad) and 5 μl of [γ -³²P] ATP (3000 Ci/mmole, Amersham) in 20 mM Tris HCl, 10 mM MgCl₂, 100 mM KCl, 0.1 mM DTT, pH 7.5 (34 μl final volume). Incubation was performed for 1 hour at 37°C. Three extractions with chloroform; isoamyl alcohol (24/1, v/v) and three diethylether extractions were performed to remove proteins. Double strand formation was then allowed to occur as upper described.

c) *Strand break measurement*: Either single or double strand abasic oligonucleotides (2 μM each) were incubated at 11°C with 10 μM 9-AE in 10 mM cacodylate, 1 M NaCl, pH 7. Aliquots (5 μl) were withdrawn at various times and treated with 3 μl of 10 M sodium borohydride at room temperature. The sample was then neutralised with 23 μl of 1 M HCl. They were then ethanol precipitated overnight at -20°C using 2 μl of *E. coli* tRNA solution (1 mg/ml, Boehringer). After a 20 minutes centrifugation at 12000 rpm the supernatant was removed and the pellet dissolved in water in order to get 2000 cpm per μl . The experiment was ended by a denaturing gel electrophoresis in 20% acrylamide, 7 M urea followed by autoradiography. In order to get quantified values the film was scanned with a densitometer (Vernon, France). The size of the fragments was estimated, according to Maniatis (22) by comparison with the migrations of xylene cyanol (which corresponds

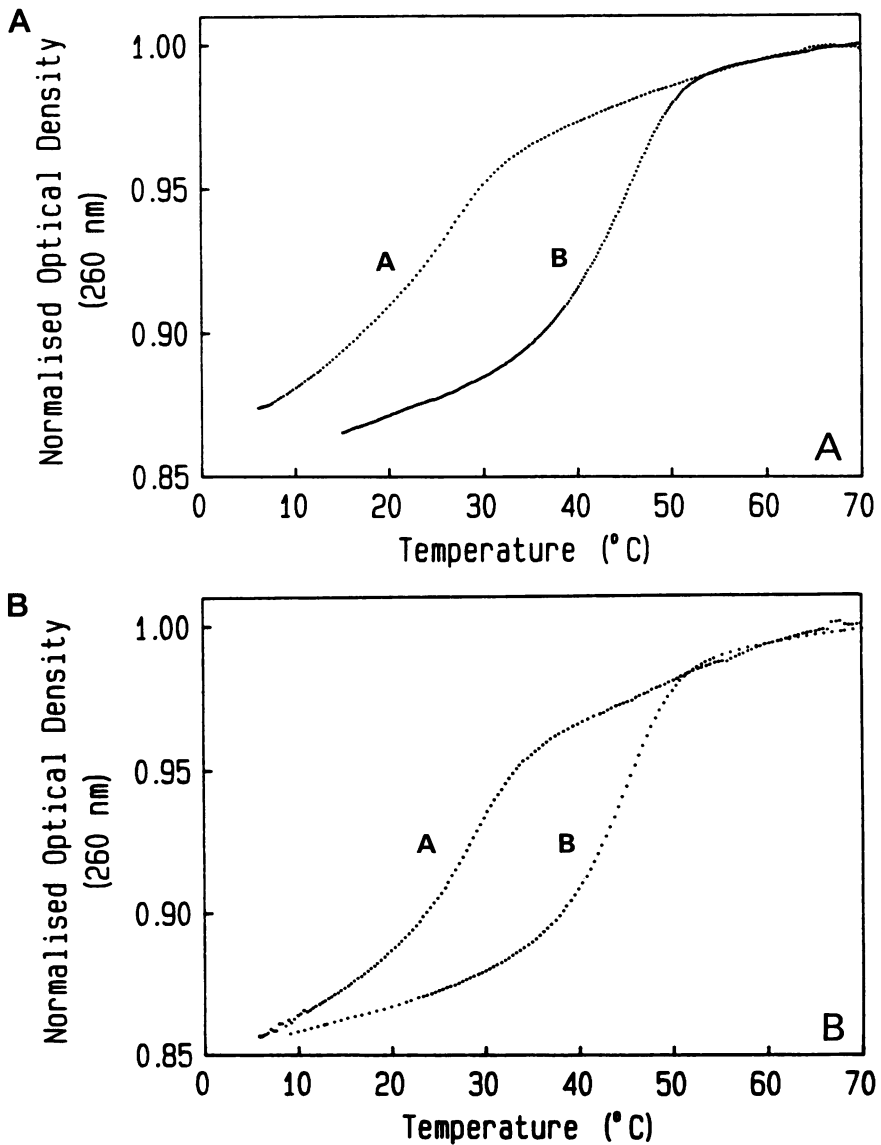


Figure 3: Variation of absorbance as a function of temperature for apurinic and apyrimidinic duplex oligonucleotides
 Panel A: Apurinic duplex oligo (A), control duplex oligo (B); Panel B: Apyrimidinic duplex oligo (A), control duplex oligo (B).

to 28 bases) and of bromophenol blue (which corresponds to 8 bases).

d) Fluorescence measurements: 2 μM of either single- or double- stranded oligonucleotides were mixed with 10 μM 9-AE in 10 mM cacodylate, 1 M NaCl, pH 7. Time dependant fluorescence was detected in a 1 ml quartz cuvette thermostated to 11°C using a SFM

Table 1: Comparison of melting temperatures obtained with intact duplexes, and apurinic/apyrimidinic duplexes.

	Apurinic site	Apyrimidinic site
Control oligo	d-5'CTCTCC A TTCCTC3' d-3'GAGAGG T AAGGAG5'	d-5'CTCTCC T TTCCTC3' d-3'GAGAGG A AAGGAG5'
T _m	45.8°C	45°C
Abasic oligo	d-5'CTCTCC r TTCCTC3' d-3'GAGAGG T AAGGAG5'	d-5'CTCTCC r TTCCTC3' d-3'GAGAGG A AAGGAG5'
T _m	25°C	28°C

23/B spectrofluorimeter (KONTRON). Experiments were performed at 322 nm for excitation and 547 nm for emission according to earlier published data (14,15).

RESULTS

1) Thermal stability

The study of thermal denaturation of double-stranded oligonucleotides with or without an abasic site shows that introduction of one abasic site decreases the melting temperature of the duplex of about 19°C (Figures 2 and 3, and table 1). However this decrease is higher for an apurinic site (20.8°C) than for an apyrimidinic site (17°C). The removal of a purine base might trigger a greater destabilisation of the double helix than the removal of a pyrimidine base because of the role of stacking forces.

2) Reactivity with 9-AE

a) Detection and identification of oligonucleotides in gel electrophoresis: The reactivity of 9-AE with the abasic oligonucleotide was studied by electrophoresis on a denaturing gel (Figure 4). In line 1 the oligonucleotide was pretreated by sodium borohydride, in order to reduce the aldehydic function of abasic sites and to prevent β -elimination cleavage, before mixing with 9-AE. Only one compound is obtained which corresponds to a 13 mers oligonucleotide (1'). In absence of reduction by sodium borohydride one more compound is obtained (line 2, band 4). According to published results (15) this compound should correspond to a 6 mer oligonucleotide obtained by β -elimination cleavage (4). A treatment of the abasic oligonucleotide was performed by sodium hydroxide (followed by sodium borohydride, line 3) which is known to cleave apurinic sites by β - and δ -elimination (17). Two more compounds are obtained, which is consistent with published results (25). As a consequence we suggest that one compound which migrates more slowly (band 4) corresponds to a 6 mer oligonucleotide linked to α - β -unsaturated deoxyribose (4'), the other one (band 5) corresponding to the δ -elimination cleavage compound which is a 6 mers (5). Sodium cyanoborohydride is less powerful as a reducing agent than sodium borohydride. It reduces the Schiff base formed between 9-AE and the apurinic site, however

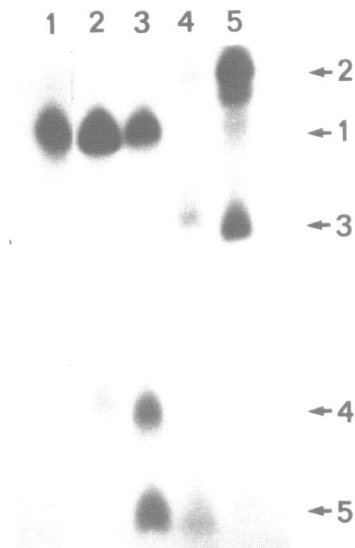


Figure 4: Denaturing gel electrophoresis of [5' -³²P] abasic single stranded oligonucleotide: line 1: control + sodium borohydride; line 2: control without sodium borohydride; line 3: control + sodium hydroxyde then sodium borohydride; line 4: control + sodium hydroxyde, then 9-AE + cyanoborohydride; line 5: control + 9-AE + cyanoborohydride. Incubation were performed at 25°C for one hour as indicated in methods.

it does not reduce its aldehydic function. When the abasic oligonucleotide was incubated at the same time with 9-AE and sodium cyanoborohydride (line 5) we observed a great diminution of the original compound (band 1) and the appearance of two new compounds, one with a slower migration (band 2), which is in fact likely composed of 2 bands, and one with a faster migration (band 3). According to published results (15) band 2 should be the adduct between 9-AE and the oligonucleotide (2'), obtained through reduction of the Schiff base, and we suggest band 3 to be a similar adduct on the β -elimination cleaved oligonucleotide (3'). In order to check this hypothesis about band 3 a new experiment was performed: the abasic oligonucleotide was first treated by sodium hydroxyde, in order to creat 4 and then, after neutralization, mixed with 9-AE and sodium cyanoborohydride before electrophoresis (line 4). Band 3 becomes the major one and only traces of 2 are detected likely due to the incomplete cleavage by sodium hydroxyde (line 5). Band 3 could therefore in part, however with a low yield, derive from 4. This therefore supports the hypothesis on the nature of 3': a reduced Schiff base on the β -elimination cleaved oligonucleotide.

2) *Kinetic studies of the reaction between 9-AE and abasic oligonucleotides*

a) *Single stranded abasic oligonucleotide:* 5' labeled abasic oligonucleotide (2 μ M) was mixed with 9-AE (10 μ M). Sodium borohydride reduced aliquots, withdrawn at various times, were studied by denaturing gel electrophoresis. Results are displayed on Figure

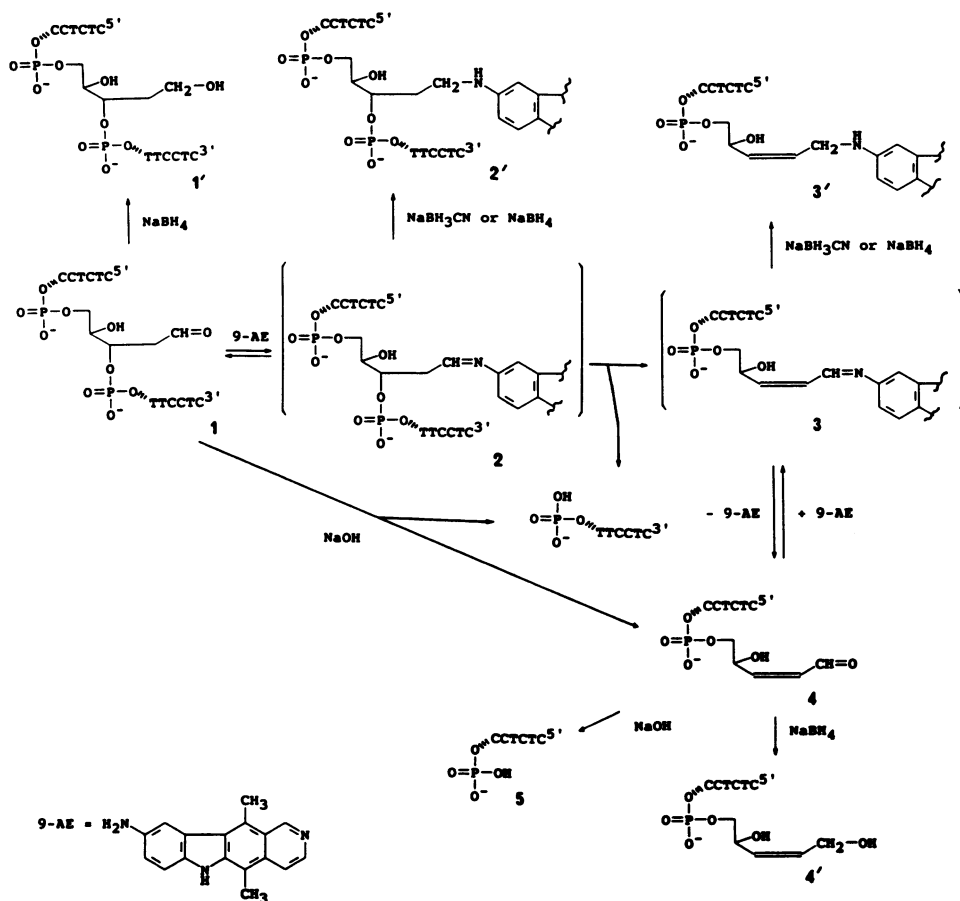


Figure 5: Supposed structure for the compounds corresponding to the pathways of reaction between 9-AE and the abasic oligonucleotide. The different products corresponding to detected bands in denaturing gel electrophoresis are labelled with a ' indicating a treatment with borohydride or cyanoborohydride.

6. The parent oligonucleotide, *1*, undergoes a 20% decrease in 10 minutes before levelling. This is nearly concomitant with the grow up of compound *4'*, which appears to be the main cleavage product (12% of total radioactivity). One observes the formation in less than 2 minutes of compound *2'* which is the reduced Schiff base before cleavage. It is likely that *2'* does not accumulate because it gives rise to *4'* and *3'* (issuing from *4* and *3* by reduction) which are minor compounds in this reaction.

b) Double-stranded apurinic oligonucleotide: As can be observed in Figure 7 the reaction is now very different. The parent oligonucleotide *1* disappears in less than 2 minutes. Almost simultaneously the reduced Schiff base *2'* appears and the conversion of *1* into *2* is nearly complete. *2'* is at maximum between 2 and 4 minutes after the onset of the reaction and decreases to level off at a value around 65% after 20 minutes. The main degradation product is *3'* which reaches 20% of the total radioactivity after 30 minutes and is then stable. A minor degradation product is *4'* which reaches a maximum of 10% of the total radioactivity

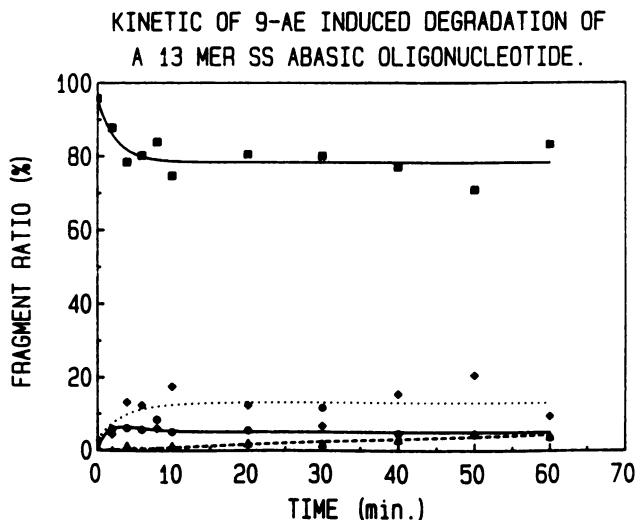


Figure 6: Kinetic variation according to time of the abasic single stranded oligonucleotide and its degradation products when mixed with 9-AE at 11°C, pH 7.

- (—■—) 13 mers abasic oligo reduced (compound 1')
- (—●—) Reduced adduct between 9-AE and 13 mers abasic oligo (compound 2')
- (—▲—) Reduced adduct between 9-AE and broken abasic oligo (compound 3')
- (··◆··) Reduced abasic oligo after breakage (compound 4')

after 20 minutes before decreasing. Under these conditions 30–35% of the Schiff bases between 9-AE and apurinic sites undergo a β -elimination cleavage.

c) *double-stranded apyrimidinic oligonucleotide*: The rapid decrease of the parent oligonucleotide 1 as well as the rapid build up of the Schiff base 2' is similar to what happens with the apurinic oligonucleotide (Figure 8). However after 4 minutes the pattern

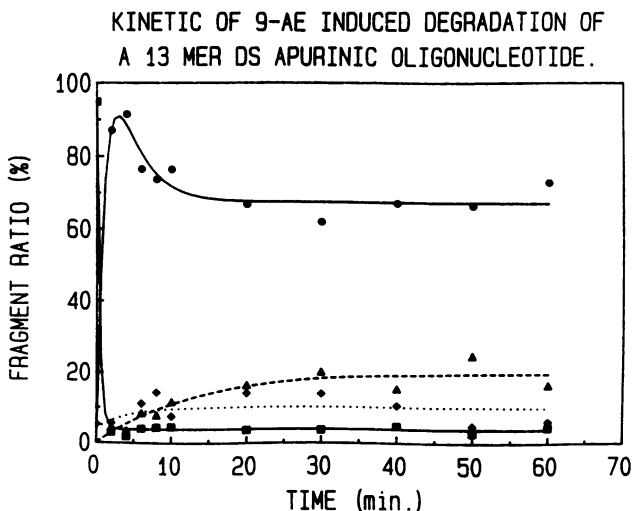


Figure 7: Idem than 6 but with the apurinic duplex oligonucleotide.

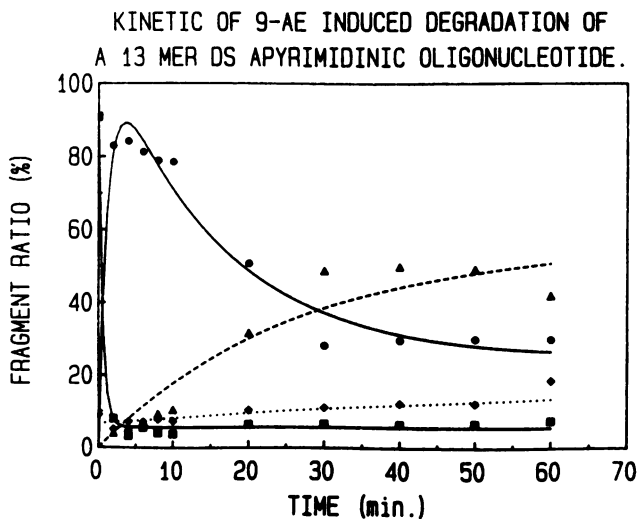


Figure 8: Idem than 6 but with the apyrimidinic duplex oligonucleotide.

becomes different. 2' decreases until 40 minutes to reach 29% of the total radioactivity and at the same time 3' increases to reach a maximum which exceeds 50% of the total radioactivity after 40 minutes. A minor degradation product is 4' which grows until 10% of the total radioactivity after 10 minutes. Nearly 60–70% of the apyrimidinic oligonucleotide is cleaved after 40 minutes of reaction which is twice the effect observed in the same conditions with the apurinic oligonucleotide.

3) Fluorescence studies

We had shown that the interaction of 9-AE with AP sites can be followed by fluorescence in a continuous way (14,15). This presents the interest of not having to use sodium borohydride to stop the reaction. Fluorescence was therefore monitored at the known DNA maxima: excitation 322 nm; emission 547 nm (14).

a) *Single-stranded abasic oligonucleotide*: Under these conditions of fluorescence, 9-AE alone or 9-AE in the presence of the non abasic oligonucleotide displays a basal fluorescence which is not time dependant (Figure 9A). This is also true for the abasic oligonucleotide itself. However when the abasic oligonucleotide and 9-AE are mixed a characteristic kinetic of fluorescence is displayed (Figure 9A). This fluorescence follows an exponential like curve during the 25 first minutes and is then followed by a linear increase.

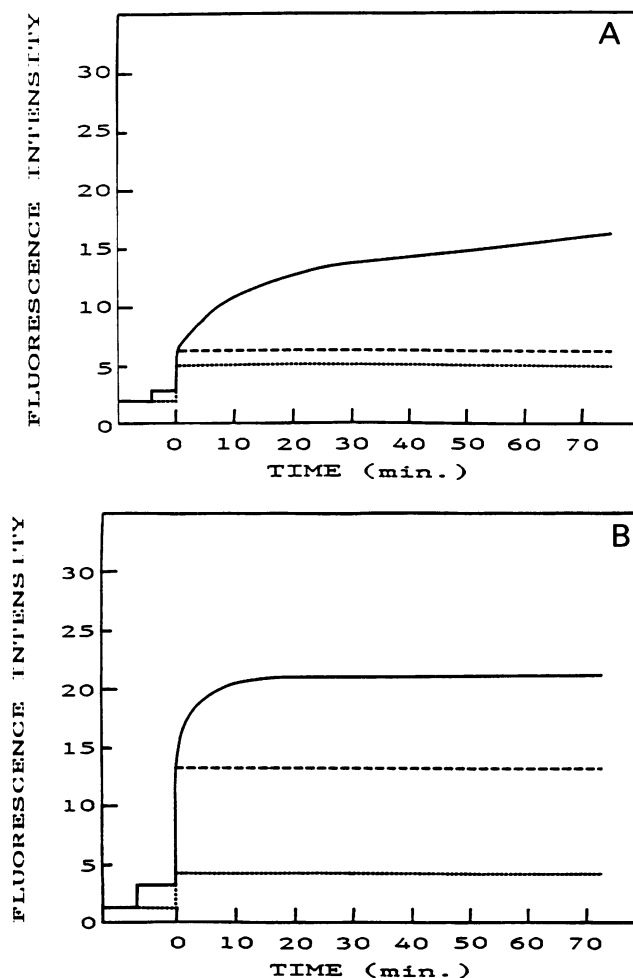
b) *Double stranded-apurinic oligonucleotide*: It can be observed on Figure 9B that the initial increase of fluorescence when 9-AE is mixed with the non apurinic double stranded oligonucleotide is higher than for the single stranded one. This confirms the intercalation of 9-AE in the double stranded oligonucleotide. In the presence of the apurinic oligonucleotide the instantaneous fluorescence increase is identical but is then followed by an exponential build up of fluorescence which reaches a stable plateau after 10 minutes.

c) *Double stranded-apyrimidinic oligonucleotide*: The instantaneous fluorescence variations display the same characteristics than those upper described for the apurinic oligonucleotide. However the time dependant build up of fluorescence in the presence of 9-AE reaches a maximum after 15 minutes which is much higher than observed either with apurinic

double stranded or with abasic single stranded oligonucleotides (Figure 9C). Fluorescence then decreases to reach a stable value after 70 minutes.

DISCUSSION

We first found that the introduction of an abasic site in a double stranded oligonucleotide induces an important decrease in the melting temperature (around 20°C). This means that the removal of the base nearly acts as a strand break as far as the energy of the double helix is concerned. The apurinic strand would therefore behave as 2 independant strands located in 3' and in 5' of the abasic site. As expected it is slightly more difficult to dissociate the apyrimidinic oligonucleotide than the apurinic one. This might indeed result from the greater stacking effect of the purine opposite to the apyrimidinic site when compared to the one of the pyrimidine opposite to the apurinic site. This difference in the nature of the base opposite to the abasic site is obviously the only difference in the structure of apurinic



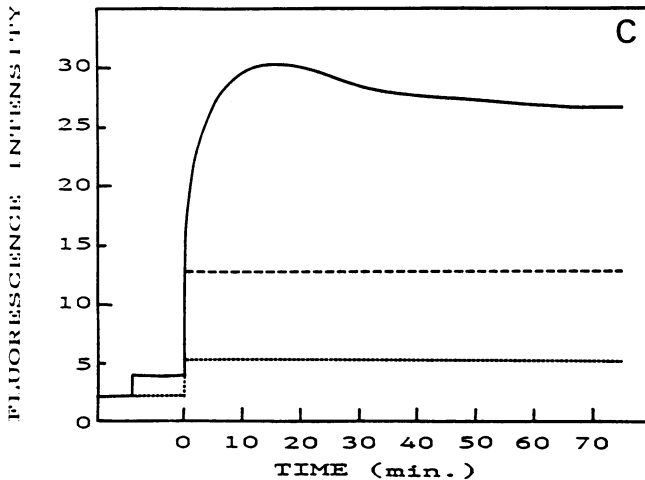


Figure 9: Time dependent variation of fluorescence of abasic oligonucleotides mixed with 9-AE at 4°C, pH 7 (excitation: 322 nm; emission: 547 nm).
 A) Single stranded abasic oligonucleotide
 B) Duplex apurinic oligonucleotide
 C) Duplex apyrimidinic oligonucleotide
 (—) Abasic SS or DS oligo (2 μ M)
 (---) Control SS or DS oligo (2 μ M)
 (.....) 9-AE alone (10 μ M)

and apyrimidinic sites. We already proposed (16) that this difference accounts for the absence of apyrimidinic sites cleavage by 9-AE in supercoiled circular pBR322 when apurinic sites are cleaved in the same conditions of incubation. However we unexpectedly found that the apyrimidinic duplex oligonucleotide is more extensively cleaved, by 9-AE, than the apurinic one. This indicates that there is probably no direct relation between the structures of an apyrimidinic site in a 13 mers oligonucleotide and in supercoiled pBR322. This could be checked by inserting the oligonucleotide in pBR322. One could expect at this time that 9-AE does not break any more the apyrimidinic site in the oligonucleotide. We notice two other important elements in the observed breakage kinetics: time scales required to get the maximum cleavage, level of this maximum. When compared to time scales required to get cleavage either with d(TprpT) or with apurinic DNA, the results obtained here, mostly with double stranded oligonucleotides, indicate that they are nevertheless good models for reaction with apurinic DNA. It is more difficult to compare the absolute values of cleavage because although quantification is easy with the apurinic oligonucleotide, it becomes more difficult with apurinic linear DNA (15). It is however striking that in spite of an excess concentration of 9-AE, (95% of 9-AE is not bound to the oligonucleotide by intercalation), we did not observe a complete cleavage of abasic sites. This is not an artefact caused by the method because it can be observed on figures 6,7,8 that there is an obvious conservation of the oligonucleotide concentration. New compounds indeed appear as long as degradation occurs. In order to clarify the understanding of the reaction the different supposed structures of intermediate oligonucleotides are given in Figure 5. The reaction begins by the formation of a Schiff base which appears to generate few cleavage either when using an abasic single stranded oligonucleotide or with an apurinic duplex

oligonucleotide. This result is rather unexpected and could be explained by the occurrence of different conformations of the Schiff base, with different behaviours for β -elimination induced strand cleavage. This hypothesis is also supported by the observation that band 2 is in fact likely composed of 2 products (Fig 4). The intensity of these 2 bands for apurinic and apyrimidinic duplexes is consistent with the occurrence of one compound prone to cleavage whereas the other is resistant (result not shown). The rapid formation of a Schiff base is a stimulating result for the use of 9-AE as an inhibitor of base excision repair in cancer chemotherapy (23,24) either with inducers of apurinic sites (alkylating agents) or with inducers of apyrimidinic sites (ionising radiations or radiomimetic drugs). The kinetic of breakage studies also show the formation of strand breaks and of cleaved structures forming Schiff bases with 9-AE. All these molecules could play a role in inhibition of repair.

We already observed (13 and unpublished results) that 9-AE induces cleavage on depurinated purified *E. coli* DNA but that total extent of cleavage is inferior to the number of apurinic sites. This was attributed to a heterogeneity in the apurinic sites. However the same phenomenon is observed with homogeneous apurinic oligonucleotides and other causes have therefore to be considered.

The formation of a Schiff base between 9-AE and the apurinic site was shown to trigger a specific fluorescence (15). This fluorescence allowed us to follow in a continuous way the reaction. However this fluorescence is likely to result from the addition of the fluorescence of several intermediate compounds in the reaction. With single-stranded abasic oligonucleotides we observed an increase of fluorescence whereas cleavage, in the same conditions, is at a plateau. This means that the reaction proceeds at the cleavage site as was already demonstrated for 3-NH₂-carbazole which presents important similarities with 9-AE (11). With the double stranded apurinic and apyrimidinic oligonucleotides the build up of fluorescence is much faster. This can be related to the quick formation of the Schiff base with 9-AE. The greater increase in fluorescence observed with the apyrimidinic site may be due to the greater stacking in the apyrimidinic oligonucleotide if one supposes that 9-AE linked by a Schiff base to the oligonucleotide is intercalated. This stacking could also be responsible of the levelling of the reaction after 10 to 30 minutes whereas there is a further evolution with d(TrpT) (15) or with the abasic single stranded oligonucleotide.

In conclusion we synthesised original abasic oligonucleotides which allowed us to show that the removal of a base introduces an important structural modification in a duplex oligonucleotide. It also made possible a quantitative and kinetic measurement of the reaction between 9-AE and apurinic sites in linear single and double stranded DNA. This model showed that some apparently minor changes such as the shift from apurinic to apyrimidinic structure, without known incidence on AP endonucleases, can trigger important behaviour differences of a compound such as 9-AE. Besides known capacity of 9-AE to potentiate alkylating agents which induce apurinic sites (24), our results, which show the formation of a Schiff base with apyrimidinic sites, suggest experiments to potentiate anticancer agents such as radiations or radiomimetic drugs which are known to induce apyrimidinic sites in DNA.

ACKNOWLEDGEMENTS

We thank Dr E. Lescot for synthesis of 9-AE. This work was supported by grants from Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique and Association de la Recherche sur le Cancer.

REFERENCES:

1. Friedberg, E.C. (1985) in 'DNA repair', Freeman, W.H., pp 1-77 New York.
2. Kotchetkov, N.K., and Budovsky, E.I. (1972) in 'Organic Chemistry of Nucleic Acids', part B, pp 477-532, Plenum Press, New York.
3. Coombs, M.M., and Livingston, D.C. (1969) *Biochim. Biophys. Acta.* *174*, 161-173.
4. Millican, T.A., Mock, G.A., Cahancy, M.A., Patel, T.P., Eaton, M.A.W., Gunning, J., Cutbush, S.D., Neidle, S., Mann, J. (1984) *Nucleic. Acid. Res.*, *12*, 7435-7453.
5. Pochet, S., Huynh-Dinh, T., Neumann, J.M., Tran-Dinh, S., Adam, S., Taboury, E., Taillandier, E., and Igoen, J. (1986) *Nucleic. Acid. Res.*, *14*, 1107-1126.
6. Raap, J., Dreef, C.E., van der Marcel, G.A., and van Boom, J.H. (1987) *J. Biomol. Struct. Dyn.*, *5*, 219-247.
7. Eritja, R., Walker, P.A., Randall, S.K., Goodman, M.F., Kaplan, B.E. (1987) *Nucleosides Nucleotides*, *6*, 803-814.
8. Takeshita, M., Chang, C.N., Johnson, F., Will, S. and Grollman, A.P., (1987) *J. Biol. Chem.*, *262*, 10171-10179.
9. Seela, F. and Kaiser, K. (1987) *Nucleic. Acid. Res.*, *15*, 3113-3129.
10. Vasseur, J.J., Rayner, B., and Imbach, J.L., (1986) *Biochem. Biophys. Res. Commun.*, *134*, 1204-1208.
11. Vasseur, J.J., Rayner, B., Imbach, J.L., Verna, S., Mc Closkey, J.A., Lee, M., Chang, D.K. and Lown, J.L. (1987) *J. Org. Chem.*, *52*, 4994-4998.
12. Vasseur, J.J., Rayner, B., Imbach, J.L. (1988) *J. Het. Chem.*, *25*, 389-392.
13. Malvy, C., Prevost, P., Gansser, C., Viel, C., and Paoletti, C. (1986) *Chem. Biol. Interact.* *57*, 41-53.
14. Bertrand, J.R., Malvy, C. and Paoletti, C. (1987) *Biochem. Biophys. Res. Commun.* *143*, 768-774.
15. Bertrand, J.R., Vasseur, J.J., Gouyette, A., Rayner, B., Imbach, J.L., Paoletti, C., and Malvy, C. (1989) *J. Biol. Chem.* in press
16. Malvy, C. and Bertrand, J.R. (1986) *FEBS letters.* *208*, 155-157.
17. Bailly, V. and Verly, G. (1987) *Biochem. J.* *242*, 565-572.
18. Liuzzi, M. and Talpaert-Borle, J. (1985) *J. Biol. Chem.* *260*, 5252-5258.
19. Marquardt, D.W. (1963) *J. Soc. Ind. Appl. Math.* *11*, 431-441.
20. Bevington, P. R. (1969) *Data Reduction and Error Analysis for the Physical Sciences*, Chapter 11, Mc Graw-Hill, New York
21. Petersheim, M. and Turner, D. H. (1983) *Biochemistry* *22*, 256-263.
22. Maniatis, T., Fritseh, E.F. and Sambrook, J. (1982) *Molecular Cloning : A Laboratory Manual*, pp 185, Cold Spring Harbor Laboratory Press, Cold Sping Harbor.
23. Lefrançois, M., Bertrand, J-R. and Malvy, C. (1989) Submitted for publication to *Mutation Research*.
24. Malvy, C., Safroui, H., Bloch, E. and Bertrand, J.R. (1988) *Anti-Cancer Drug Design.* *2*, 361-370
25. Bailly, V. and Verly W.G. (1988) *Biochem. J.* *253*, 553-559.

This article, submitted on disc, has been automatically converted into this typeset format by the publisher.