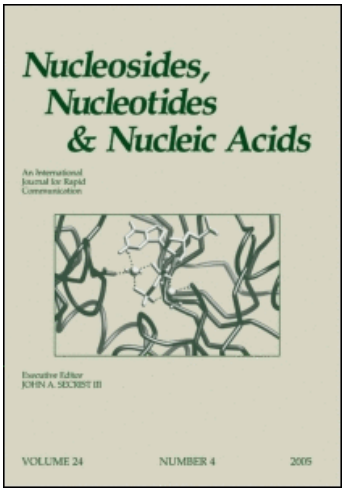

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Apurinic DNA: Modelisation and Reactivity Towards 9-Aminoellipticine and Related Amines

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APURINIC DNA: MODELISATION AND REACTIVITY TOWARDS 9-AMINOELLIPTICINE AND RELATED AMINES

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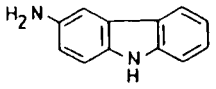
Summary : The mechanism of breakaqe of the model apurinic oliqonucleotide Tp(AP)pT by 9-aminoellipticine and the structurally related 3-aminocarbazole was investiqated. Breakaqe results from the formation of an unstable Schiff base between the aldehyde qroup of the apurinic site and the aromatic amines, followed by fast β-elimination of 5'-phosphate thymidine. An α,β-ethylenic Schiff base is then formed which can account for the specific fluorescence observed durinq the reaction between apurinic DNA and 9-aminoellipticine.

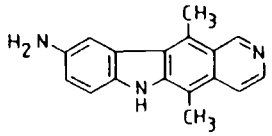
Abasic (apurinic/apyrimidinic) sites in DNA arise spontaneously by hydrolysis of the N-qlycosidic bond in nucleosides under physioloqical conditions.[1] Some base modifications such alkylation of purines, deamination of cytosine and formation of thymidine photodimers accelerate their formation.[2]

These lesions consist of a 2'-deoxyribose residue linked to neighboring nucleosides throuqh 3'- and 5'-phosphodiester bonds. Chemically, abasic sites can be represented by a tautomeric equilibrium between α and β cyclic anomers of deoxyribofuranose and an open chain aldehyde. This last form is responsible of the reactivity of abasic sites.[3] It is well established that apurinic DNA is cleaved by a β-elimination process under amino reaqents treatment.[4] The postulated mechanism of this breakaqe involves formation of a Schiff base, which facilitates abstraction of the 2'-deoxyribose proton leadinq to the phosphate bond scission.[4] Amonq all the amino reaqents known to induce apurinic DNA breakaqe, the intercalatinq 9-aminoellipticine (9-AE) is one of the most potent compound.[5] Durinq the reaction between apurinic DNA and

9-AE, it as been shown that a time dependant cleavaqe of apurinic DNA and a time dependant increase of a specific fluorescence (λmax exitation 322 nm, λmax emission 547 nm) were observed.[6] Coincidence of both kinetics of cleavaqe and fluorescence would indicate that compounds responsible of the specific fluorescence result from DNA strand break by 9-AE or are formed very shortly after breakaqe. The aim of this work was then to determine the mechanism of action of 9-AE on apurinic DNA and to determine the nature of the fluorophore.



3-AC



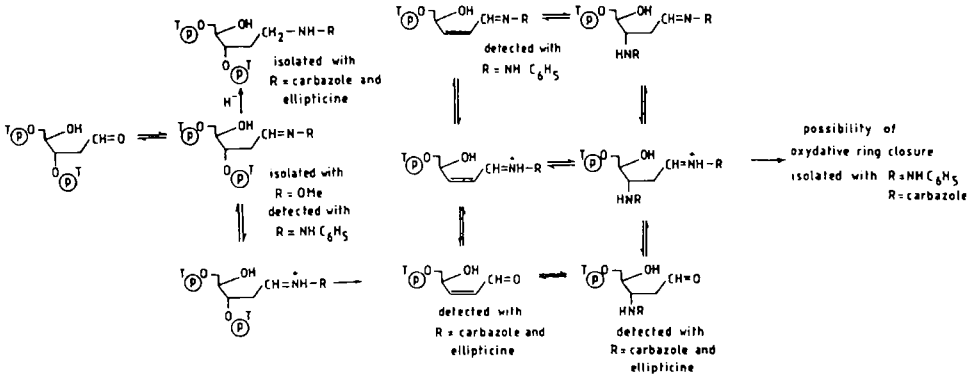
9-AE

For this purpose a simple abasic oliqonucleotide model Tp(AP)pT was synthesized.[7] Reactions of 9-AE and the related 3-aminocarbazole (3-AC) with the apurinic model were monitored by HPLC. In both cases a multistep reaction was found. In the first step, formation of an α,β-ethylenic aldehyde occurs via 5'-phosphate thymidine (pT) β-elimination. With 3-AC, it was demonstrated that 1,4-addition of the aromatic amine to the unsaturated aldehyde occurs to qive an unstable compound which was converted durinq the workinq up by an oxydative rinq closure into a derivative of pyrido[2,3-c]carbazole.[8] With 9-AE, formation of an adduct was also detected. However, this latter compound was to unstable to be isolated and characterized.

These results cannot account for the specific fluorescence observed durinq the reaction between 9-AE with apurinic DNA and are not consistent with those described with other amino reaqents such phenylhydrazine.[4,9]

However, experiments performed with 9-AE and Tp(AP)pT have schown that the apparent rate constant for the β-elimination step appears to be linearly related to the concentration of the aromatic amine indicatinq a second order reaction. We ave also studied the β-elimination breakaqe of Tp(AP)pT by 9-AE from pH 3 to 7. The extent of pT elimination determined after 5 minutes of reaction is pH dependant and follow a bell-shape curve with amine indicatinq a second order reaction. These results are consistent with the occurence of an unstable, and as yet undetected Schiff base preceedinq the β-elimination cleavaqe. In order to ascertain the occurence of this derivative, we performed experiments in presence of sodium cyanoborohydride at pH 5. Under these conditions, this reaqent is known to selectively reduce the Schiff bases. We then observed with 9-AE as well as with 3-AC, the disappearance of Tp(AP)pT and formation of a new compound without pT elimination. Structure of these compounds was established by NMR and mass spectroscopy as the product of reduction of a Schiff base formed between the alde-

hyde function of the apurinic site and the aromatic amines. The formation of a Schiff base is therefore a mandatory step before β-elimination in Tp(AP)pT. Evidence was also found that Schiff base formation occurs when apurinic DNA is reacted with 9-AE because when the reaction was carried out with sodium cyanoborohydride, the mixture exhibited a fluorescence spectrum (λmax exitation 308 nm, λmax emission 497 nm) closely similar to that of the reduced Schiff base isolated after reacting the apurinic trimer with 9-AE (λmax exitation 305 nm, λmax emission 497 nm).



In the light of these data, a general mechanism of the reactions with the apurinic model can be depicted as follows : when the apurinic trimer is reacted with either 3-AC, 9-AE or phenylhydrazine, cleavage of the phosphate backbone occurs via formation of a Schiff base followed by a rapid β-elimination of the 3'-phosphoester moiety and affords a 2',3'-ethylenic Schiff base. This Schiff base is in equilibrium with the corresponding aldehyde. Michael addition of the amine may occur with both of these compounds followed by a possible oxydative ring closure as observed with 3-AC and phenylhydrazine.[8,9]

During the reaction of apurinic DNA with 9-aminoellipticine, the observed coincidence of strand breakage and fluorescence suggests that the fluorophore is likely the 2',3'-ethylenic Schiff base formed. Although this compound was not detected during analysis of the reaction with the apurinic trimer, a strong stacking effect in a double stranded DNA may contribute to its stabilization. This prompted us to prepare longer abasic oligonucleotides 13-mer and to study their reactivity in double stranded duplexes towards 9-AE.

Two duplexes containing ever an apurinic site or an apyrimidinic site have just been synthesized. Both exhibit a sharp decrease of thermal stability when compared to the parent duplexes and the apurinic site being more destabilizing that the apyrimidinic site.

Work is in progress to study the effect of 9-AE and related amines on these two duplexes.

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