Differential reactivity of 9-NH_2-ellipticine on apurinic and apyrimidinic sites in circular DNA

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Endonucleases for apurinic sites as well as chemical compounds reacting with aldehydes do not generally differentiate between apurinic and apyrimidinic sites. We have studied the effect of the apurinic site reagent, 9-NH_2-ellipticine, on apyrimidinic sites enzymatically generated on PBR322 DNA and compared it to its' action on apurinic PM2 and PBR322 DNAs. In conditions where this compound induces breakage of apurinic sites, it does not display any action on apyrimidinic sites.

Apyrimidinic site *Apurinic site* *DNA structure* *Ellipticine*

1. INTRODUCTION

The N-glycosidic bond between bases and deoxyribose in DNA can be either spontaneously broken by base damage or radiation or enzymatically hydrolysed by repair enzymes such as glycosidases. The sites generated are named apurinic or apyrimidinic according to the excised base and are mutagenic and toxic in bacteria [1,2] and in mammalian cells [3]. They might constitute an intermediate in chemical mutagenesis [4]. The DNA intercalating agent, 9-NH_2-ellipticine, is already known to induce breakage of DNA at apurinic sites at a low dose [5]. To measure its action on apyrimidinic sites induced by *E. coli* uracil-DNA glycosidase we have chosen a PBR322* DNA from a mutant *E. coli* strain, containing some uracil which have been substituted for thymines [6], as a model substrate. For comparison with breakage at heat induced apurinic sites we have used PM2 DNA as already described in the literature [7,14]. However a similar effect is observed with apurinic PBR322* DNA. We confirm that the tripeptide Lys-Trp-Lys and *E. coli* exonuclease III induce breakage of DNA at apyrimidinic sites whereas 9-NH_2-ellipticine has no effect.

Abbreviation: PBR322* DNA, PBR322* DNA with uracil substituted thymines

2. MATERIALS AND METHODS

2.1. *Chemicals*

9-NH_2-ellipticine was synthesized by Dr Viel (Chatenay-Malabry) according to a published method [8]. Adenine, guanine, cytosine, thymine and uracil were from Sigma. [6-^{3}H]Uridine (24.3 Ci/mmol) was from NEN (Boston).

2.2. *Nucleic acids*

PBR322 plasmidic DNA was from Boehringer (Grenoble). The first steps in the preparation of uracil containing PBR322* DNA were performed according to a published protocol [6] starting with the following strain: *E. coli* KL 16 (thi$^-$, icl A) [6] deficient in uracil-DNA glycosidase (ung mutation) and in deoxyuridine triphosphatase (dut mutation). However, a few steps of additional purification were necessary: DNA was treated for 2 h at 37°C with pancreatic RNase at 50 μg/ml in order to obtain an efficient removal of any possible RNA contamination. RNase was eliminated through a treatment with proteinase K 1 mg/ml,

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for 12 h at 37°C, followed by 2 phenol extractions.

PM2 DNA was purchased from Boehringer (Mannheim) and purified by alcohol precipitation.

2.3. *Heat depurination of DNA*

Circular DNAs were depurinated by heating at 70°C for 7 min (PM2) or 15 min (PBR322) at pH 5 in 25 mM sodium acetate, acetic acid.

2.4. *Quantification of uracil incorporated into PBR322* DNA*

Uracil containing PBR322* DNA as well as control PBR322 DNA were hydrolysed by 1 M perchloric acid at 100°C for 1 h [9]. Analysis of bases was then performed in high-performance liquid chromatography on a C18 microbondapak reverse-phase column (Waters, Millipore) with the following buffer: 2% methanol, 98% of 50 mM $NH_4H_2PO_4$, pH 4. The different peaks were identified through comparison with pure bases injected with the hydrolysate. Fractions were collected every 30 s and the radioactivity measured by scintillation counting.

2.5. *PBR322* DNA treatment by uracil-DNA glycosidase*

Uracil-DNA glycosidase from *E. coli* was purified by J.P. Leblanc according to the method of Lindahl [10]. 1.8 μg of DNA was mixed with 2 μl of purified enzyme in the following buffer: 7 mM Hepes NaOH, 37.5 mM NaCl, 2 mM EDTA, 5 mM dithiothreitol, pH 6.8. Total volume was 30 μl. The reaction was stopped after a given reaction time by addition of 0.3 mM uracil. DNA was then alcohol precipitated and resuspended either in 1 mM sodium cacodylate, 0.1 mM EDTA, pH 7, for treatment with 9-NH_2-ellipticine or the tripeptide Lys-Trp-Lys (Serva, Heidelberg) and with 0.15 M NaCl, 0.015 M sodium citrate, 0.005 M $CaCl_2$ for treatment with exonuclease III (BRL Bethesda).

The activity of uracil-DNA glycosydase was controlled through the excision of [^{14}C]uracil on PBS1 DNA [11].

2.6. *Quantification of breakage on DNA*

The breaks were displayed thanks to the relaxation of DNA form 1 caused by one single strand break. DNA form 2 and form 1 were separated by gel electrophoresis and the number of breaks quantified according to a published method [12].

2.7. *Background breakage on control PBR322* DNA*

The amount of ^{3}H cpm in the fraction corresponding to uracil in HPLC after hydrolysis of DNA (cf. section 2.4) as well as absorbance measurements indicate 40 uracil residues per molecule of PBR322* DNA (4% of thymines are substituted). The background effects of 9-NH_2-ellipticine and exonuclease III on this DNA are unexpected (for conditions see section 3). They induce 0.3 breaks per DNA molecule after 2 h at 37°C whereas no breakage is detected in control PBR322.

2.8. *Action of purified* E. coli *uracil-DNA glycosidase on PBR322**

The activity of *E. coli* uracil-DNA glycosidase on PBR322*, measured by alcohol soluble radioactivity is low. This has already been observed by Martin and Sicard [6] according to the number of alkali sensitive sites created. In order to correct for unspecific effects caused by adsorption of [^{3}H]uracil on the tube surface, control experiments were performed with an inhibitor of uracil-DNA glycosidase, uracil, at a concentration of 0.3 mM (KI = 0.1 mM [10]). A relative 2.5 times increase in alcohol soluble radioactivity was then measured after 30 min of contact with active uracil-DNA glycosidase (10 uracil residues per molecule of PBR322 excised).

3. RESULTS AND DISCUSSION

We have compared the action of 9-NH_2-ellipticine, Lys-Trp-Lys and *E. coli* exonuclease III on apurinic and apyrimidinic sites. Lys-Trp-Lys (1 mM, 2 h at 37°C) and *E. coli* exonuclease III (3 units/μg of DNA, 2 h at 37°C) cause the same amount of breaks as 9-NH_2-ellipticine (1 μM, 2 h at 37°C) on PM2 DNAs with different numbers of apurinic sites caused by heating (fig.1). In the same conditions 9-NH_2-ellipticine induces the same level of breakage as exonuclease III on apurinic PBR322 DNA (cf. fig.1) and no breaks in control PBR322 DNA. Experiments were then performed with 2 different preparations of apyrimidinic PBR322*. The effect of 9-NH_2-ellipticine was compared to exonuclease III on the first preparation, and to Lys-Trp-Lys on the second one. Exonuclease III,

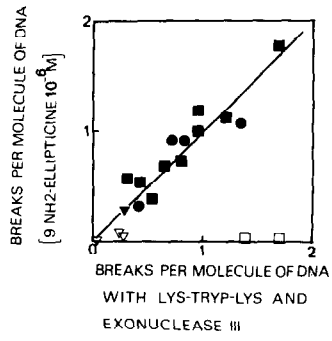

Fig.1. Relationship between breakage by 9-NH_2-ellipticine on one hand, Lys-Trp-Lys and *E. coli* exonuclease III on the other, on DNAs with various amounts of apurinic sites and apyrimidinic sites. (■) Lys-Trp-Lys on apurinic PM2 DNA; (▼) exonuclease III on apurinic PM2 DNA; (●) exonuclease III on apurinic PBR322 DNA; (▽) exonuclease III on apyrimidinic PBR322* DNA; (□) Lys-Trp-Lys on apyrimidinic PBR322* DNA. All treatments are performed for 2 h at 37°C. For each substrate, breakage obtained with 9-NH_2-ellipticine (10^{-6} M) is plotted against breakage obtained with Lys-Trp-Lys (10^{-3} M) or exonuclease III (3 units per μg of DNA). For all values background breakage is subtracted.

with Ca^{2+} instead of Mg^{2+} [13] and Lys-Trp-Lys induce a number of breaks related to depyrimidination time whereas breakage caused by 9-NH_2-ellipticine occurs at a background level (fig.1).

No specificity is known for chemicals breaking DNA at apurinic sites. The tripeptide Lys-Trp-Lys has thus been shown to break DNA at apurinic sites [14,15] and then at apyrimidinic sites [16]. Likewise endonucleases for apurinic sites are reported not to be specific, except *E. coli* endonuclease VII which is specific for apyrimidinic sites [17].

It may be suggested that heat induced apurinic sites are in partially single-stranded DNA [18]. However 2 results [5] indicate that 9-NH_2-ellipticine induces breakage of apurinic sites in double-stranded DNA: (i) acido-soluble fragments are generated in double-stranded apurinic *E. coli* DNA, (ii) ethidium bromide relaxed apurinic circular PM2 DNA is nicked by 9-NH_2-ellipticine. By another method, a CPK model of a double-stranded oligonucleotide clearly indicates that an apurinic site is perfectly adapted for a partial insertion of 9-NH_2-ellipticine via the small groove whereas it is impossible for an apyrimidinic site. This interaction is not possible in double-stranded DNA because of steric hindrance and therefore no breakage occurs.

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