Polyamine-induced hydrolysis of apurinic sites in DNA and nucleosomes

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ABSTRACT

The ability of different polyamines to catalyze hydrolysis of phosphodiester linkages in apurinic and apyrimidinic (AP) sites has been investigated in supercoiled, relaxed and denatured DNA, and also in core and chromatosome particles. The rate constants for the hydrolysis in the DNAs have been determined. In general the order of effectiveness of the polyamines were: spermine > spermidine > putrescine > cadaverine. A 9 fold difference in rate constants was found between spermine and cadaverine. No difference in the rate of hydrolysis was seen between AP-sites in supercoiled and relaxed DNAs, whereas the rate for the single-stranded DNA and DNA in core and chromatosome particles was only half of that in the double-stranded DNA. All AP-sites in both free DNA and DNA-histone particles were hydrolyzed in the presence of polyamines. For all polyamines, with the exception of spermine, increasing concentration of both Mg\(^{++}\) and salts such as KCl both led to a large decrease in the rate of polyamine-induced hydrolysis of AP-sites. The rate of hydrolysis increased markedly with increasing pH in the pH range pH 6 - pH 11.

INTRODUCTION

Apurinic and apyrimidinic sites in DNA (AP-sites) are formed by spontaneous hydrolysis of the base-sugar linkages in DNA, by severe UV- and γ-irradiation and by the action of certain DNA repair enzymes, the DNA glycosylases. AP-sites can also be generated \textit{in vitro} by exposing the DNA to acidic conditions. The mechanism of repair of AP-sites has been investigated in a number of laboratories. It appears that repair takes place via the excision repair pathway (1,2). The phosphodiester linkage in the AP-site must first be broken and the sugar-phosphate residue removed by nucleases before gap-filling by DNA polymerase and ligase joining can take place. Hydrolysis of the phosphodiester linkages in AP-
sites can be accomplished by means of specific AP-endonucleases present in the cell (3). The hydrolysis of such linkages can, however, also be achieved by a non-enzymatic mechanism in the presence of certain basic amino acids, peptides, proteins and amines (4,5).

The polyamines spermine, spermidine, putrescine and cadaverine constitute a special class of biological important amines widely distributed in nature and found mostly associated with the nucleic acids and other negatively charged macromolecules. Only limited information is available with regard to their role in phosphodiester bond hydrolysis during DNA repair (6). The fact that polyamines and other amines as well are capable of hydrolysing phosphodiester linkages in AP-sites was first described by Chargaff and co-workers (7,8). Lindahl and Andersson (4) have shown that putrescine catalyze hydrolysis of phosphodiester linkages in AP-sites in double-stranded DNA.

In the present report we have examined in more detail the properties of the various polyamines with regard to hydrolysis of phosphodiester linkages in AP-sites in different DNAs and also in core and chromatosome particles.

MATERIALS AND METHODS

Chemicals. Methyl methane sulphonate (MMS) was obtained from Merck. Dimethyl sulphate (DMS) was from Koch-Light laboratories, and [methyl-\(^3\)H]-thymidine (52 Ci/m mole) and \(\gamma^{-32}\)P-ATP (3000 Ci/m mole) from The Radiochemical Centre, Amersham.

The nitrocellulose membrane filters (Metrical GN-6, diameter 25 mm) were from Gelman. E.coli exonuclease III was obtained from New England Biolabs. Micrococcal nuclease was purchased from Boehringer Mannheim. Spermine tetrahydrochloride, spermidine trihydrochloride, putrescine dihydrochloride and cadaverine dihydrochloride were products of Sigma Chemical Company.

Enzymes. An endonuclease specific for AP-sites in DNA was isolated from mouse plasmacytoma cells (MPC 11) according to Nes (9). The enzyme recovered from the phosphocellulose column was used. The AP-endonuclease had no detectable
T<sub>4</sub>-polynucleotide kinase was purified as previously described (10) and was a gift from J.R. Lillehaug.

Preparation of DNAs. ØX174 RFI and RFII DNA were labelled with[methyl-<sup>3</sup>H]-thymidine and isolated according to a method of Nes and Nissen-Meyer (11). The RFI DNA contained maximum 5 % RFII DNA, and the specific activity was (0.6-1) x 10<sup>5</sup> cpm/µg. RFII DNA had a specific activity of 0.4 x 10<sup>5</sup> cpm/µg. The ØX174 [<sup>3</sup>H]-DNAs were stored in 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 10 % ethanol at 4 °C. Relaxed covalently closed circular DNA was prepared from ØX174 RFI DNA by use of an extract from L-cells containing topoisomerase I (12).

Denaturation of ØX174 RFII was achieved by incubation of the DNA at 100 °C for 5 min. After heating the solution was quickly frozen in ethanol-dry ice followed by slow thawing on ice.

Preparation of [<sup>3</sup>H]-labelled and 5'-[<sup>32</sup>P]-labelled core particles and chromatosomes. [<sup>3</sup>H]-labelled core particles were prepared from L-cells by growing the cells in the presence of 1 µM [methyl-<sup>3</sup>H]-thymidine (50 µCi/µmole) for 2-3 generations (13), and the further purification were essentially as previously described (14), except that the reaction mixture was made 0.1 M KCl after the micrococcal nuclease treatment. The core particles were further purified using sucrose gradient centrifugation (5-28.8 % sucrose in 1 mM EDTA, 36.000 rpm for 18 hrs in a Beckman L8-70 centrifuge, SW 41Ti rotor). The pellet from the 0.1 M KCl centrifugation, containing chromatosomes and compact dimers, was resuspended in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA and 0.1 mM PMSF. Insoluble material was removed by centrifugation. The chromatosomes and compact dimers were purified by sucrose density gradient centrifugation as described above. The purified DNA from core particles and chromatosomes had a specific activity of 800-2600 cpm/µg DNA. In some experiments the 5'-OH ends of unlabelled core particles and chromatosomes were labelled with [<sup>32</sup>P] using T4 polynucleotide kinase.
Modification of DNA. Depurination/depyrimidination of supercoiled and relaxed øX174 RFII [³H]-DNA was carried out by heating the DNA at pH 5.0 at 70 °C for 10-15 minutes (11). This treatment resulted in generation of 0.8 to 1.3 AP-site per DNA molecule. Native and denatured øX174 RFII [³H]-DNA were depurinated by methylation with MMS and heating as previously described (15).

Depurination of core particles and chromatosomes. Core particles (A₂₆₀ = 2.5) in 100 mM sodium cacodylate, (pH 7.2) were methylated with 100 mM DMS for 20 min at 22 °C (16). The reaction was stopped by chilling on ice followed by addition of 2-mercaptoethanol to a final concentration of 50 mM and excess DMS was immediately removed by rapid gel filtration. Partial depurination was achieved by incubating the particles for 30 minutes at 58 ± 2 °C (17).

The chromatosomes were treated in a similar manner but only 20 mM sodium cacodylate (pH 7.2) and 50 mM DMS was used in order to prevent aggregation. The pH did not drop below 6.5 by this treatment of core particles and chromatosomes. The core particles and chromatosomes were used immediately after the depurination reaction.

Detection of AP-sites in DNA. Hydrolysis of AP-sites in DNA were detected either by a simple nitrocellulose filter assay (A) (11) or by precipitation with perchloric acid (B) (9) or trichloroacetic acid (C) (18).

Assay A. The constant ionic strength buffer of Miller and Golder (19) was used. Unless otherwise stated, the standard reaction mixture in the nitrocellulose filter assay contained: 2 mM Na⁺-veronal HCl, and 18 mM NaCl (μ=0.02), 2 mM EDTA, 40-70 ng øX174 RFI [³H]-DNA and 1 mM polyamine. The total volume was 0.1 ml. The mixture was incubated at 37 °C for 20 minutes, and the further treatment was as previously described (11). The activity was calculated as number of induced breaks per DNA molecule, assuming a Poisson distribution of AP-sites in DNA.

Assay B was carried out under the same conditions as A, except that the substrates were depurinated single- or double-stranded øX174 RFII [³H]-DNA, [³H-Tdr]-core particles.
or \([^3\text{H}]-\text{Tdr}\)-chromatosomes. The concentrations of polyamines and DNAs were as indicated in the legends to figures. The reactions were terminated by addition of 25 \(\mu\)l calf thymus DNA (2 mg/ml) and further treatment were as described in reference 9.

Assay C. This method was used for the detection of nicks in end-labelled DNA of core particles and chromatosomes. The DNA was precipitated with cold trichloroacetic acid (18).

RESULTS

Influence of different polyamines. The time course of hydrolysis of depurinated \(\varnothing X174\) RFI DNA by 4 different polyamines is shown in Figure 1. Spermine was the most efficient polyamine followed by spermidine, putrescine and cadaverine. The plateau values obtained for spermine and spermidine correspond to the maximum number of nicks obtained by alkali treatment of the DNA as well as with AP-endo-nuclease. Similar experiments were carried out using relaxed covalently closed \(\varnothing X174\) RFI, the nicked RFII form and heat denatured RFII. With the exception of the denatured form similar rates were found for these DNAs using the same polyamine. The rate constants have been determined for the different polyamines and DNAs at 2 different concentrations of polyamines and the results are presented in Table I. A 9 fold difference in rate constants was found between the most

![Figure 1. Time course of polyamine-induced hydrolysis of AP-sites in \(\varnothing X174\) RFI \([^3\text{H}]-\text{DNA}\). The standard reaction mixture, assay A, was employed and the concentration of polyamines was 1 mM. Temperature 37 \(\text{\degree C}\).](image)
Table I

Rate constants for the polyamine-induced hydrolysis of AP-sites in various DNAs

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>DNA</th>
<th>Polyamine conc.</th>
<th>$k_{obs} \times 10^4$ (sec$^{-1}$)</th>
<th>$k_{corr} \times 10^4$ (sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ØX174 RFI</td>
<td>Spermine</td>
<td>1</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>Spermidine</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>Putrescine</td>
<td>1</td>
<td>5.9</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>Cadaverine</td>
<td>1</td>
<td>3.7</td>
</tr>
<tr>
<td>5</td>
<td>Relaxed ØX174 RFI</td>
<td>Spermine</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>Spermidine</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>ØX174 RFII</td>
<td>Spermine</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>Spermidine</td>
<td>1</td>
<td>4.3</td>
</tr>
<tr>
<td>9</td>
<td>&quot;</td>
<td></td>
<td>10</td>
<td>7.2</td>
</tr>
<tr>
<td>10</td>
<td>Single stranded ØX174</td>
<td>Spermine</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>11</td>
<td>&quot;</td>
<td></td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>12</td>
<td>&quot;</td>
<td>Spermidine</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>13</td>
<td>&quot;</td>
<td></td>
<td>10</td>
<td>4.2</td>
</tr>
<tr>
<td>14</td>
<td>[3H]-Core particles</td>
<td>Spermine</td>
<td>1</td>
<td>8.6</td>
</tr>
<tr>
<td>15</td>
<td>&quot;</td>
<td></td>
<td>10</td>
<td>11</td>
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<tr>
<td>16</td>
<td>&quot;</td>
<td>Spermidine</td>
<td>10</td>
<td>7.5</td>
</tr>
<tr>
<td>17</td>
<td>[3H]-Deprot.core particl.</td>
<td>Spermine</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>18</td>
<td>&quot;</td>
<td></td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>19</td>
<td>[3H]-Core particles</td>
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</tr>
<tr>
<td>20</td>
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<td>21</td>
<td>5'-[32P]-Core particles</td>
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<tr>
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<td>5'-[32P]-Chromatosomes</td>
<td></td>
<td></td>
<td>0.9</td>
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</table>

The rate constants were all determined at 37°C and the polyamine concentrations were as indicated. Assay method A was used for experiments no. 1-6, assay B for 7-20 and assay C was used in experiments no. 21 and 22. The amount of DNA per assay employed were: ØX174 RFI supercoiled and relaxed 40-70 ng, ØX174 RFII native and denatured 0.6 μg [3H]-labelled core particles and chromatosomes 1.4-5 μg and 1.5 μg [32P]-labelled core particles and chromatosomes. The principles of assay B and C are based on acid solubility of DNA fragments and therefore correction factors have to be used in order to make possible a comparison with assay A. The estimated factors are based on the assumptions that AP-sites in DNA are Poisson distributed and the acid soluble fragments of DNA are ≥20 nucleotides in length (R. Male and K. Kleppe, unpublished).
efficient polyamine, spermine, and the least efficient, cadaverine, for the hydrolysis of supercoiled DNA. The rate of hydrolysis of AP-sites in single-stranded DNA by spermine and spermidine was only approximately half of that for the double-stranded form. The same rate constants were found when the DNA concentration was doubled, clearly suggesting a first order reaction. The rate was proportional to the concentration of polyamines only at low levels of polyamines as shown in Figure 2 for spermidine. A hyperbolic saturation curve was obtained, indicative of the catalytic nature of the reaction. At high concentrations of polyamines, particularly spermine and spermidine, the DNA used will form aggregates (20). However, the rates of the polyamine-induced hydrolysis in the aggregates were found to be similar to those of the free DNA.

**Effect of pH.** The influence of pH on the polyamine-induced hydrolysis of AP-sites was investigated. In these experiments a constant ionic strength buffer was used (19). A marked stimulation was seen with increasing pH for spermidine and putrescine, Figure 3. The rate of hydrolysis was almost 10 fold higher at pH 10 than at neutral pH. Similar results were obtained with spermine and cadaverine. Due to the short incubation time used there was no significant hydrolysis in the absence of polyamines.

**Influence of salt and divalent cations.** Both polyamines and mono- and divalent cations will stabilize the DNA in a double-stranded form. Hence, the different cations will compete with the polyamines for the binding to the negatively charged phosphate groups on the DNA, and thus the concentration of cations could influence the polyamine-induced hydrolysis of AP-sites. In the presence of increasing concentrations of KCl the polyamine-induced hydrolysis decreased markedly for spermidine, putrescine and cadaverine, Figure 4. At a concentration of 0.3 M KCl virtually no hydrolysis was seen in the presence of the latter polyamines. The spermine induced hydrolysis was not affected by salt to the same extent and at 0.3 M KCl; only 20 % reduction in rate was seen which could be due to the tighter binding of spermine to DNA.
Figure 2. Influence of the spermidine concentration on the observed rate constant of hydrolysis. Assay method B was employed, using 0.6 μg/assay of ØX174 RFII [3H-Tdr]-labelled DNA; temperature 37 °C.

Figure 3. Effect of pH on nicking activity of polyamines at AP-sites in ØX174 RF1 [3H]-DNA. Standard incubation mixtures were employed, assay A, and the buffers had a constant ionic strength of μ = 0.02. The following buffers were used: pH 6.0-7.5 Na₃HPO₄/NaH₂PO₄; pH 8.0-9.0 Na-veronal/HCl; 9.5-11 glycine/NaOH. The polyamine concentration was 1 mM and the temperature 37 °C. The incubation period with spermidine and spermine was 5 min and with putrescine and cadaverine 20 min. -○-○- spermidine; -■-■- putrescine; -△-△- control, no polyamines, 20 min incubation time at 37 °C.

Similar results were obtained with NaCl and Na₂HPO₄.

The presence of increasing concentration of Mg²⁺ resulted in a similar decrease in rate of hydrolysis as with KCl, Figure 5. Thus at 20 mM Mg²⁺ only approximately 20 % of the original rate was seen for spermidine, putrescine and cadaverine versus 60 % for spermine.

Temperature dependence. The polyamine-induced hydrolysis showed a normal temperature dependence, in the temperature range from 0-50 °C. When the data were plotted in an Arrhenius type plot a straight line was obtained (results not shown). The activation energy for chain breakage in AP-sites in supercoiled DNA was estimated to be 19.6 Kcal/mole. This value is similar to that reported for certain basic peptides (5).
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Figure 4. Effect of KCl on polyamine induced hydrolysis of AP-sites in ØX174 RFI [3H]-DNA. Different concentrations of KCl were included in the standard reaction mixture, assay A. The concentration of polyamines used was 1 mM and the temperature 37 °C. The activities were monitored by the nitrocellulose filter assay and are expressed as percent of breaks induced at standard conditions. -o-o- spermine, -•-•- spermidine, -•-•- putrescine, -D-D- cadaverine.

Figure 5. Hydrolysis of AP-sites in ØX174 RFI [3H]-DNA by different polyamines in the presence of increasing amounts of MgCl₂, using stand assay system, assay A, and the temperature 37 °C. The activity was detected by the nitrocellulose filter technique. A 1 mM concentration of the following polyamines were used: -o-o- spermine, -•-•- spermidine, -•-•- putrescine -D-D- cadaverine.

Hydrolysis of AP-sites in nucleosomes. In eukaryotic cells the DNA and histones are organized into nucleosome particles. These can be isolated either as core particles, consisting of a 146 base pair DNA plus 2 molecules each of histone H2A, H2B, H3 and H4, or as chromatosomes which contains a DNA of 168 base pairs and histone H1 in addition to the 8 core histone molecules. It is of importance to clarify whether polyamines also will induce nicking of AP-sites in these particles and the rate at which this occur. Partially depurinated core particles and chromatosomes were prepared as described in Material and Methods. The depurinated particles had the same sedimentation properties as the unmodified complexes. All polyamines tested induced hydrolysis
of phosphodiester linkages in AP-sites in the histone-DNA particles. The time course of hydrolysis of [\(^3\)H]-Tdr -labelled core particles with spermine is shown in Figure 6. Similar rates were found for the chromatosomes (results not shown). The corresponding rate constants determined under varying conditions are listed in Table I. The rate of hydrolysis of AP-sites in the two DNA-histone particles proceeded at a rate approximately half of that found in the free DNA. Also there was less difference in rate of hydrolysis between the various polyamines than for free DNA. All AP-sites on the DNA-histone particles appeared to be hydrolyzed in the presence of polyamines. In the absence of polyamines the AP-sites in the DNA-histone particles were found to be considerably more unstable than in the corresponding free deproteinized DNA. Such spontaneous hydrolysis is most likely due to histone-induced nicking of the DNA in the particles. The rate of spontaneous hydrolysis of 5'-[\(^32\)P]-labelled chromatosomes was two times higher than for the corresponding [\(^3\)H -Tdr]-uniformly labelled particle, suggesting that AP-sites at the ends are easier attacked than in

Figure 6. Time course of the polyamine-induced and spontaneous hydrolysis of AP-sites in [\(^3\)H-Tdr]-labelled core particles. The depurinated core particles were prepared as described in Materials and Methods. The standard reaction mixture, assay B, was used with 2 µg DNA and the concentration of spermine was 1 mM. Temperature 37 °C. 100 % activity corresponds to the fraction of acid soluble counts obtained for the alkali-treated DNA (38 %). Core particles plus spermine -o-o-; control core particles minus spermine -o-o-; deproteinized DNA from core particles plus spermine -e-e-, and minus polyamine -e-e-. 
the internal sites. The fact that the rates of spontaneous hydrolysis of 5'\-[^{32}P]\-labelled and \([^{3}H]\)-uniformly labelled core particles were the same may indicate that histone H1 is responsible for the enhanced nicking of the 5'-end in chromatosomes.

The influence of pH on hydrolysis of AP-sites in DNA-histone particles was also investigated. Contrary to the polyamine-induced nicking the spontaneous hydrolysis of AP-sites on DNA-histone particles was not influenced to any large extent by variation of pH.

DISCUSSION

The result of the present work confirm and extend earlier studies with regard to the polyamine induced hydrolysis of phosphodiester linkages in AP-sites. Of the four polyamines tested a 9 fold difference in rate between the most and least active, namely spermine and cadaverine, was found. The rate constant obtained for putrescine in the present study agrees well with that reported by Lindahl and Andersson (4) when the latter is corrected for the temperature, Mg\(^{++}\) and pH effects. The most likely explanation for the varying rates obtained for the different polyamines is that the polyamines have different binding affinities for DNA. The order of binding to DNA and the ability to aggregate DNA correspond well with the ability of the polyamines to induce hydrolysis of AP-site linkages (20). No differences were found in the rate of hydrolysis of AP-sites in supercoiled and relaxed DNAs. This observation is contrary to that described for certain basic peptides (5). However, in these cases intercalation may play a role in binding of the peptide and the possibility for this may be different for the two DNAs. Polyamines do not normally intercalate in the DNA structure and hence no difference in rates would be expected. In the case of the nucleosome complexes it is clear that the binding of the DNA to the basic histone complex obviously will decrease the possiblity of interaction of the polyamines with the DNA. The finding that AP-sites in single-stranded DNA are hydrolyzed at only half the rate of that in double-
stranded DNA may be a reflection of the way polyamines are known to bind to DNA.

Polyamines increase the stability of double-stranded DNA by binding to phosphate groups. X-ray crystallographic data indicate that polyamines bind across the narrow groove of the DNA (21). In the case of spermine each of the four amine groups interact with one phosphate group, two groups on each strand of the double helix. The - (CH\(_2\))\(_4\) - moiety, located between the two secondary amino groups, bridges the gap of narrow groove of the double helix and the primary amino groups then bind to adjacent phosphates along the two single polynucleotide chains. A similar model has been postulated for spermidine. Mono- and divalent metal ions compete directly with the polyamines for binding to the DNA. The effect of these ions on the polyamine-induced hydrolysis of AP-sites can therefore be explained in terms of displacement of the polyamines from the DNA.

The mechanism of chain cleavage by amines, basic proteins and peptides is a complex reaction. It is possible that the reaction takes place via \(\beta\)-elimination or cyclic phosphate formation. Results obtained by Pierre and Laval (5) for the tripeptide Lys-Trp-Lys suggest that \(\beta\)-elimination is the favoured, since the termini generated had 3'-OH and 5'phosphate groups. Furthermore, a role of the free aldehyde groups of the sugar is implicated since reduction of the DNA with \(\text{NaBH}_4\) virtually abolished the nicking activity of the polyamines. The marked increase in reaction rate with increasing pH for the free DNA obtained in the present work also are in agreement with a \(g\)-type elimination reaction since this is also known to be enhanced by OH\(^-\) ions (4). In the case of the spontaneous hydrolysis of AP-sites on DNA-histone particles other factors such steric hindrance obviously also are of importance.

The present work suggest that polyamines may play a role in repair of DNA damages \textit{in vivo} by hydrolyzing phosphodiester linkages at AP-sites both in prokaryotic and eukaryotic cells. The finding that AP-sites on core and chromatosomes particles are hydrolysed at considerable rates clearly
suggests that in eukaryotic chromatin the polyamine action are not limited only to the linker region. Experiments are currently being carried out using polyamine-deficient strains of *E.coli* (22) with the aim of assessing the role of polyamines in DNA repair in prokaryotic cells (7).

ACKNOWLEDGEMENT

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ABBREVIATIONS

MMS; methyl methane sulphonate. DMS, dimethyl sulphate.
AP is the collective term of apurinic and apyrimidinic. RFI, the supercoiled replicative form I of øX174 DNA. RFII, the nicked form of RFI DNA. PMSF, phenylmethyl sulfonyl fluoride.

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REFERENCES

8. Tamm, C., Shapiro, H.S. and Chargaff, E. (1952) J. Biol. Chem. 199, 313-327