# Interstrand DNA crosslinks due to AP (apurinic/apyrimidinic) sites

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Storage of a solution of DNA containing apurinic sites, even at 4°C leads to the appearance of interstrand crosslinks. Possible biological consequences of these crosslinks, when they appear in cell DNA, are briefly discussed. Formation of interstrand crosslinks in DNA containing tritium-labelled thymine and kept in an aqueous solution might be due, at least partly, to the loss of bases by the autoirradiated DNA.

*AP site* *Interstrand DNA crosslink* *Ionizing radiation*

## 1. INTRODUCTION

Authors in [1] observed that keeping DNA at acid pH just below the melting temperature of the double-helix resulted in the appearance of interstrand crosslinks. Acid pH causes depurination and the authors suggested that the aldehyde function at carbon 1′ of the base-free deoxyribose residue could react with a chemical group on the opposite strand creating an interstrand crosslink. Authors in [2] published a similar observation: heating DNA alkylated with methyl methanesulfonate, at 50°C and neutral pH, led to the formation of crosslinks resistant to formamide denaturation. The number of crosslinks increased with the duration of heating and was proportional to the number of apurinic sites; they calculated that there was about 1 interstrand crosslink for 140 apurinic sites.

Here, [$^3$H]DNA from T7 phage was depurinated at pH 4.0 and 37°C for a short time; the depurinated [$^3$H]DNA, which contained about 6 apurinic sites per molecule (25 200 kDa), was then kept at 4°C for several months. Already immediately after depurination, we observed a few molecules with interstrand crosslinks resistant to 95% formamide at 37°C; their number increased with time of storage at 4°C and, after 3 months, nearly all T7 DNA molecules were crosslinked. These crosslinks were labile at alkaline pH which, moreover, produced nicks near the apurinic sites.

Freshly prepared [$^3$H]DNA from T7 phage is completely and irreversibly denatured in 95% formamide at 37°C. However, after a long storage of the untreated [$^3$H]DNA solution at 4°C, part of the molecules were found to be crosslinked; the ratio was however, far less than when apurinic sites were produced by an acid treatment. These interstrand crosslinks in untreated [$^3$H]DNA could be due to spontaneous depurination and/or to the action of the ionizing radiations.

## 2. MATERIALS AND METHODS

### *2.1. Preparation of [$^3$H]DNA from T7 phages*

DNA from T7 phages, labelled with tritium in the thymine methyl group, was prepared as in [3]; its specific radioactivity was 329000 dpm/$\mu$g. It was kept at 4°C in 0.15 M NaCl, 0.015 M EDTA (pH 7.0), 0.1% benzyl alcohol, at 340 $\mu$g/ml.

### *2.2. Preparation of depurinated [$^3$H]DNA*

To 1 volume of T7 [$^3$H]DNA solution was added 2 vol. of 0.5 M acetate buffer, final pH 4.0. After a 50-min incubation at 37°C, the solution was



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brought back to pH 7.0, and dialyzed against 5 mM NaCl, 1.5 mM Na citrate, 0.1 mM EDTA, pH 7.0. Immediately after depurination, the [$^3$H]DNA contained an average of 0.84 breaks and 2.80 intact apurinic sites (i.e., apurinic sites not associated with breaks) per DNA strand (12600 kDa). The solution of depurinated [$^3$H]DNA (60 $\mu$g/ml) was kept at 4°C.

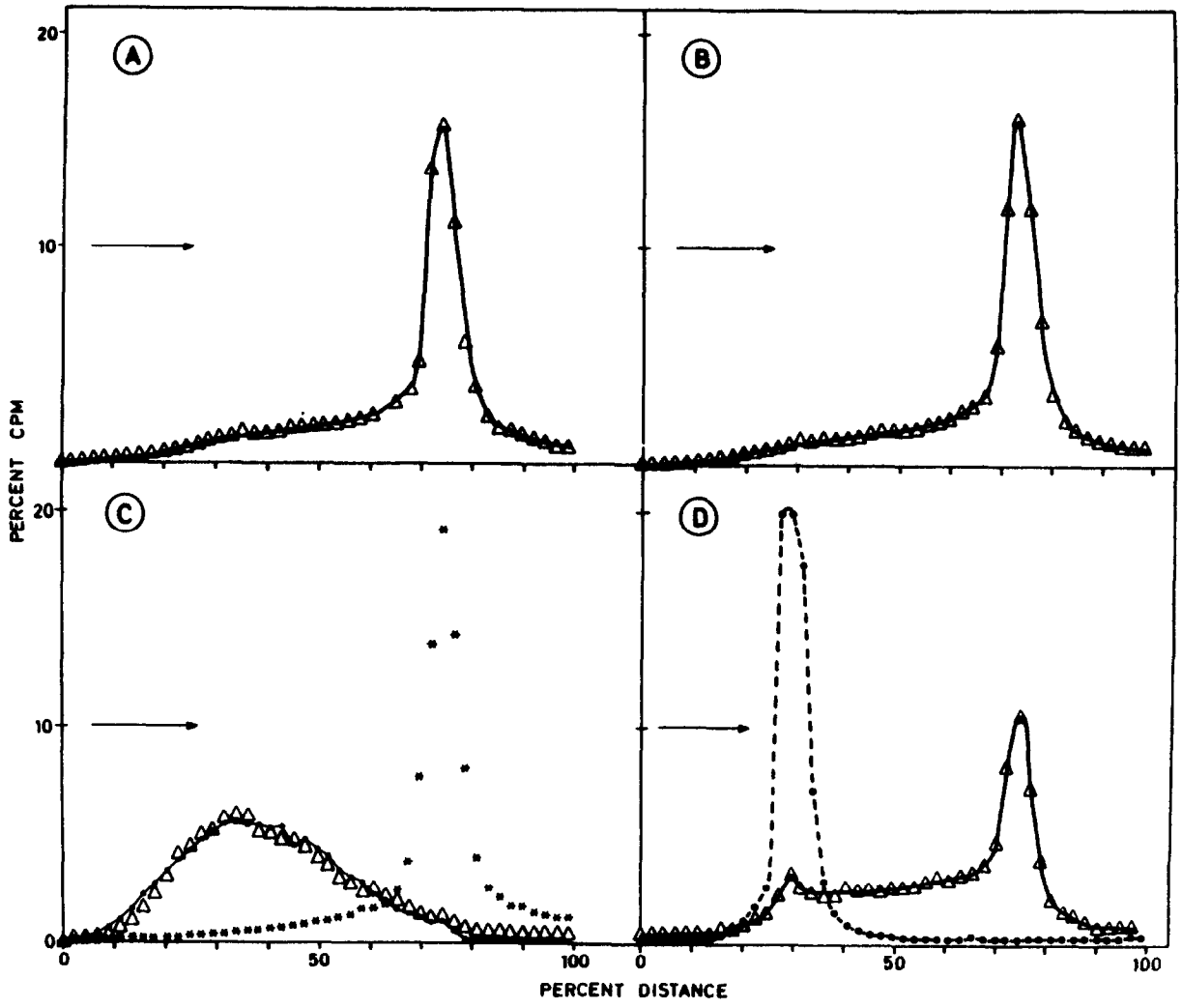


Fig.1. Sedimentation profiles of freshly prepared T7 [$^3$H]DNA, untreated (A,B) or depurinated (C,D). The [$^3$H]DNA was denatured with NaOH (A,C) or formamide (B,D); after neutralization or elimination of the formamide, the sample was placed on a linear 5–20% sucrose neutral gradient and centrifuged as in [4]. The tubes were emptied from the top (arrow indicates the direction of centrifugation); the radioactivity of the 45 collected fractions was measured and plotted as a % of total radioactivity (% cpm) *vs* the sedimented distance ($\Delta$; heavy line). The profile of intact DNA strands is the reference; breaks are introduced randomly to find the theoretical curve which has the best fit with the experimental data [4]. For the sake of clarity, the reference profile (★) and the best theoretical curve (●---●) are given only in (C) where the heavy line joining the experimental data has been omitted. The average number of breaks/T7 DNA strand for the untreated [$^3$H]DNA is 0.24 after NaOH denaturation (A) as well as after formamide denaturation (B); for the depurinated [$^3$H]DNA, it is 3.64 after NaOH denaturation (C) and about 0.84 after formamide denaturation (D). There is a larger error on this latter result because of the small renaturation peak; otherwise the error is less than $\pm$0.1. The sedimentation profile of native T7 [$^3$H]DNA (●; broken heavy line) is given in (D).

*2.3. Denaturation with formamide or with NaOH, centrifugation in neutral sucrose gradients and determination of the average numbers of breaks and intact AP sites per T7 DNA strand*

The method was as in [4]. The sedimentation profiles were analyzed with a computer. The breaks found after formamide denaturation preexisted in the native DNA. The breaks found after NaOH denaturation were the preexisting breaks and those produced near the intact AP sites that were present in the native DNA. Intact AP sites were counted by difference.

## 3. EXPERIMENTS AND RESULTS

Freshly prepared T7 [$^3$H]DNA was denatured with NaOH or formamide and analyzed on neutral sucrose gradients. Fig. 1A,B shows a peak of intact DNA strands, which has sedimented a distance equal to 74% of the gradient height, followed by

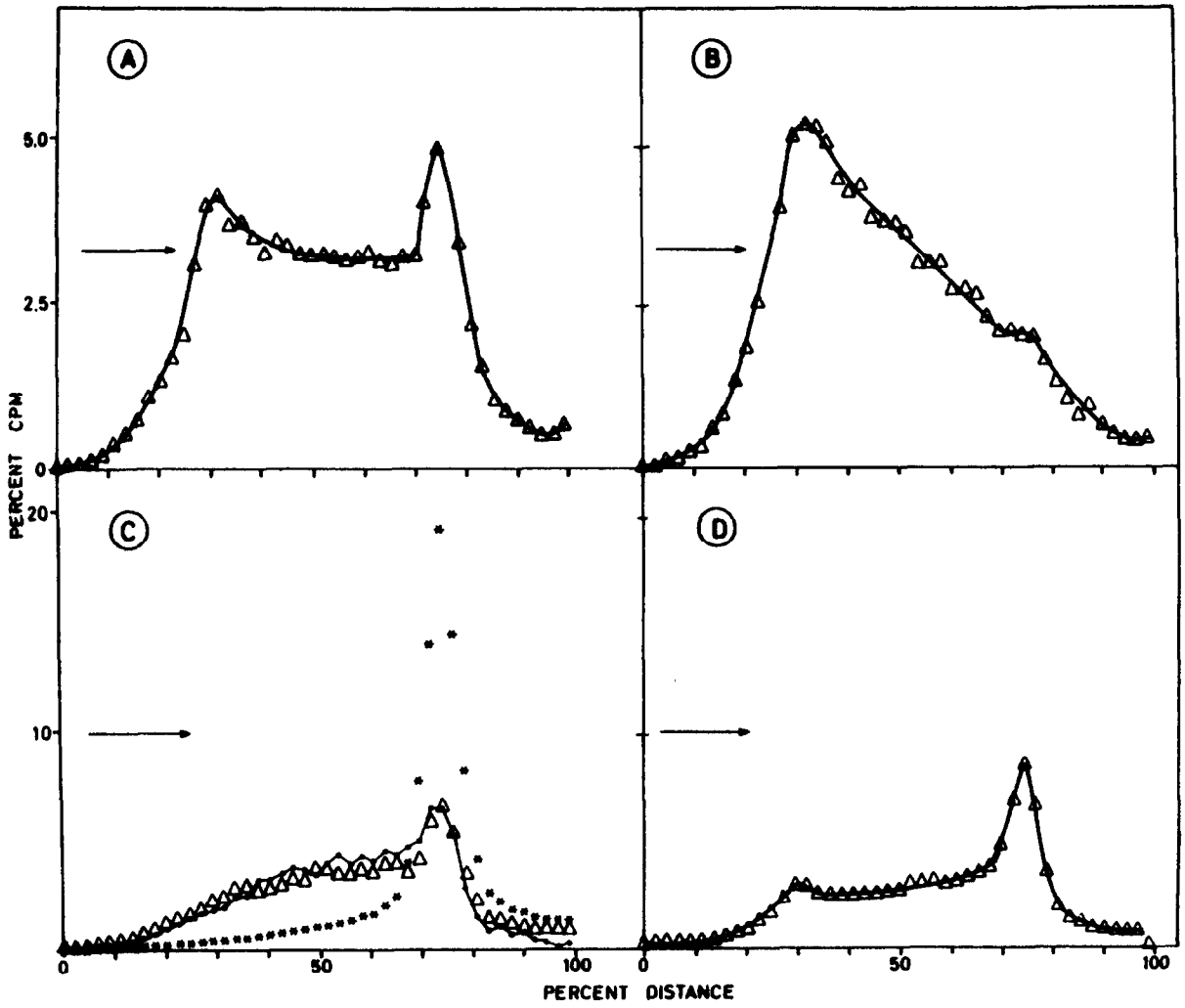


Fig.2. Sedimentation profiles of T7 [$^3$H]DNA, depurinated (A,B) or not (C,D), after storage at 4°C. The depurinated [$^3$H]DNA presented in fig.1D was kept at 4°C for 55 days (A) or 85 days (B); it was then denatured with formamide and analyzed on neutral sucrose gradients. The untreated [$^3$H]DNA presented in fig. 1A,B was kept at 4°C for 120 days; it was then denatured with NaOH (C) or formamide (D) and sedimented on neutral sucrose gradients. The average number of breaks after NaOH denaturation is 1.24/T7 DNA strand (C). Symbols are defined in the legend of fig. 1.

a tail of broken strands. Computer analysis indicated an average of 0.24 breaks/strand in both cases, indicating that this DNA did not contain intact AP sites. The tail of broken strands after formamide denaturation had a normal profile (fig. 1B).

T7 [$^3$H]DNA was depurinated as described in section 2 and analyzed 48 h later. One part was denatured with NaOH and another part with formamide before sedimentation on neutral sucrose gradients. Fig. 1C indicates that, after alkaline denaturation, the sedimentation profile was normal; it corresponds to a T7 DNA with 3.64 breaks/strand. The sedimentation profile after formamide denaturation (fig.1D) showed an anomaly: a small peak was present at 30% distance from the top. Native [$^3$H]DNA from T7 phage sedimented exactly at the same position (fig.1D). One can conclude that some depurinated T7 [$^3$H]DNA molecules have renatured. We have shown that this renaturation was not due to the presence of contaminating proteins since an additional phenol extraction did not change the results (not shown).

The depurinated [$^3$H]DNA from T7 phage was also analyzed after 55 (fig.2A), and 85 (fig.2B) days of storage at 4°C. Formamide denaturation was followed by sedimentation on neutral sucrose gradients as usual. One can see that the peak of renatured molecules was more and more prominent and that, after 85 days, there were practically no free intact strands left.

The non-depurinated [$^3$H]DNA was also studied after 120 days of storage at 4°C. One aliquot was denatured with formamide and another with NaOH. The sedimentation profile after alkaline denaturation (fig.2C) was normal; it corresponds to a T7 DNA with 1.24 breaks/strand. The sedimentation profile after formamide denaturation (fig.2D) showed, at 30% from the top of the gradient, a small peak of renatured DNA molecules.

## 4. DISCUSSION

Authors in [1,2] have shown that depurination leads to interstrand crosslinking of DNA: the aldehyde group of the apurinic site is likely to react with an amino group of the opposite strand to form a Schiff base that resists formamide denaturation but not an alkaline treatment. Authors in [1] thought that these crosslinks could be formed only near the melting temperature of the double-helix; our results show that such a high temperature is not necessary and that there is enough freedom in the depurinated molecule at 4°C for the reaction to occur. In our experiment, we observed about 1 crosslink for 6 apurinic sites after 85 days of storage at 4°C, and it is possible that even higher frequencies might be reached. Crosslinking at AP sites when the temperature is low appears, however, to be a slow process and one wonders whether it has biological consequences. Several questions can be raised: can an AP site involved in an interstrand crosslink be recognized by an AP endodeoxyribonuclease? Can it be repaired in the cell? Is it an insuperable block to the progression of the replication fork when the cell DNA replicates?

[$^3$H]DNA from T7 phage, which has not been intentionally depurinated, also gets crosslinked on storage at 4°C; the magnitude of the phenomenon is, however, much smaller than with T7 [$^3$H]DNA containing 6 apurinic sites/molecule, so that the role of AP sites in the crosslinking of depurinated DNA cannot be disputed.

But what is the cause of the formation of interstrand crosslinks in untreated [$^3$H]DNA during storage at 4°C? During the 120 days, there was an average of 2.38 tritium disintegrations per T7 DNA molecule and ionizing radiations are known to produce crosslinks although the chemical nature of these crosslinks is still poorly understood.

Could AP sites be responsible for the crosslinks observed in stored untreated [$^3$H]DNA? We have seen that, as with the crosslinks due to apurinic sites, they do not resist an alkaline treatment (fig.2C). On the other hand, one calculates from data in [15] that, during 120 days at 4°C, spontaneous depurination must have led to the formation of about 0.1 AP site/T7 DNA molecule, but it is likely that base modifications by the ionizing radiations have significantly increased the loss of bases by the [$^3$H]DNA. The experimental data show the appearance, during the 120 days of storage, of $(1.24-0.24)\times 2 = 2.00$ breaks and AP sites/T7 DNA molecule. The possibility that some interstrand crosslinks forming in untreated [$^3$H]DNA on storage at 4°C are due to AP sites is thus worth investigating.

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