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Protection of DNA by salts against thermodegradation at temperatures typical for hyperthermophiles

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Abstract The effect of physiological concentrations of KCl and $MgCl_2$ on the chemical stability of double-stranded and single-stranded DNA has been studied at temperatures typical for hyperthermophiles. These two salts protect both double and single-stranded DNA against heat-induced cleavage by inhibiting depurination. High KCl concentrations also protect DNA cleavage at apurinic sites, while high $MgCl_2$ concentrations stimulate this cleavage. It has been previously proposed that salt protects double-stranded DNA against depurination by stabilizing the double helix. However, the inhibition of the depurination of single-stranded DNA by KCl and $MgCl_2$ indicates that this effect is more probably due to a direct interaction of salts with purine nucleotides. These results suggest that the number and nature of heat-induced DNA lesions which have to be repaired might be quite different from one hyperthermophile to another, depending on their intracellular salt concentration. High salt concentrations might be also useful to protect DNA in long polymerase chain reaction (PCR) experiments and for long-term preservation.

Key words Hyperthermophiles · DNA thermodegradation · Depurination · PCR · DNA repair · Histone

Introduction

DNA molecules are exposed to very high temperatures (80°–110°C) for long periods in hyperthermophiles and for short periods in biotechnological procedures involving hyperthermophilic enzymes. These temperature stresses can potentially induce various chemical modifications, the main one being depurination followed by cleavage of the nearby phosphodiester bond (for a review, see Lindahl 1993). Heat-induced DNA damage should be repaired in hyperthermophiles (Peak et al. 1995), but it is not clear how efficient their repair systems have to be. On the other hand, DNA damage induced by high temperature in vitro cannot be repaired and might lead to various types of error cascade. In addition, similar damage can accumulate, even at much lower temperatures, in DNA stored for very long periods (fossil DNA). These considerations have recently rejuvenated the interest in studies focusing on DNA thermodegradation and on the general problem of long-term DNA stability.

Most studies on DNA thermodegradation in vitro have been initially performed using linear double-stranded DNA at temperatures below the melting temperature (T_m) (usually 70°–80°C) (Greer and Zamenhof 1962; Lindhal and Nyberg 1972; Lindhal and Andersson 1972). We have recently initiated similar investigations at temperatures more typical for hyperthermophiles, i.e., from 90° to 110°C, using supercoiled plasmids (Forterre et al. 1992; Marguet and Forterre 1994). Plasmids are much more resistant to thermodenaturation than linear DNA, since the topological links between the two strands cannot be eliminated as long as they are covalently closed (Vinograd et al. 1968). We have shown that a bacterial plasmid is indeed resistant to denaturation, at least up to 107°C, but is rapidly cleaved at such temperatures (Marguet and Forterre 1994). Although hyperthermophiles possess an enzyme, reverse gyrase, which produces positively supercoiled DNA (for reviews, see Duguet 1995; Forterre et al. 1996), positively supercoiled plasmids are no more resistant to depurination and cleavage than negatively supercoiled ones (Marguet and Forterre 1994). In the course of this work, we noticed that DNA thermodegradation was strongly reduced in the presence of physiological concentrations of either monovalent (50–500 mM KCl, NaCl) or divalent salts (1–25 mM $MgCl_2$) (Forterre et al. 1992; Marguet and Forterre 1994). Kozyavkin et al. (1995) also reported minimal DNA cleavage at 110°C in the presence of 1.5 M K-glutamate. These effects could be related to previous observations that the depurination rate was enhanced in buffers of low ionic strength (Greer and Zamenhof 1962; Lindhal and

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Nyberg 1972; Lindhal and Andersson 1972). Lindhal and Andersson (1972) suggested that this effect was due to destabilization of the double helix at low salt because single-stranded DNA is depurinated about four times more rapidly than double-stranded DNA (Lindhal and Nyberg 1972).

The protective effect of KCl and $MgCl_2$ against DNA cleavage could be relevant for hyperthermophilic life, since some hyperthermophiles exhibit very high intracellular salt concentrations (Scholz et al. 1992). It may be also significant for DNA manipulations in vitro in the presence of magnesium. Here, we show that KCl and $MgCl_2$ protect both double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA) against heat-induced cleavage, by inhibiting depurination. This shows that salt protection of double-stranded DNA against heat-induced cleavage is due not to stabilization of the double helix, but to direct protection of the purine N-glycosidic bond at high temperature.

Materials and methods

Chemicals and DNA preparations

Indubiose A 37 NA was from BioSepra (Villeveuve la Garenne, France). All the other chemicals were from Prolabo (France). Plasmid pTZ18 was isolated from *Escherichia coli* JM109 growing at 37°C, as described by Sambrook et al. (1989). M13 DNA was isolated from bacteriophage M13mp19 as described by Sambrook et al. (1989).

DNA temperature treatment for agarose gel analysis

DNA thermodegradation at 95°C was analyzed by incubating 500ng pTZ18 or 1μg M13mp19 in 20μl 25mM hydroxyethylpiperazine ethanesulfonic acid (Hepes), pH 7.5 covered with H_2O-saturated paraffin oil to prevent evaporation. The pH and temperature of the incubation mixtures were controlled with temperature and pH probes. For DNA depurination, 500ng pTZ18 or 1μg M13mp19 was incubated in 10μl 50mM Na_2HCO_3, pH 5.5, at 75°C and mixed with water and salt to give a final volume of 20μl. To study the thermodegradation of depurinated DNA, 10μg pTZ18 or 20μg M13mp19 was incubated in 200μl sodium acetate pH 5.5 at 75°C. Depurinated DNA was precipitated by addition of 20μl 3M sodium acetate and 600μl ethanol, and resuspended in 200μl 50mM Hepes buffer, pH 7.5. Depurinated DNA (10μl) was mixed with water and salt to give a final volume of 20μl, and incubated at 95°C.

Agarose gel analyses

For neutral gel electrophoresis, 20-μl DNA samples were run at room temperature in a 0.7% agarose gel in 45mM Tris-borate, 1mM ethylene diamine tetraacetic acid (EDTA), pH 8 (TBE buffer) for 16h at $2 V.cm^{-1}$. For alkaline gel electrophoresis, gels were prepared in 50mM NaCl, 4mM EDTA and soaked for at least 1h in running buffer containing 30mM NaOH and 2mM EDTA. DNA samples were incubated for 15min in 0.5M NaOH (see Marguet and Forterre 1994) and run for 16h at $1 V.cm^{-1}$ in a 0.7% agarose gel (with recirculation of the buffer). The gels were washed with water and stained with ethidium bromide. Polaroid photographs were taken under UV transillumination at 254nm. Densitometric analysis was performed with a Sony (OSI-Fisher, EPan court, France) CCD video-camera. The images were analyzed by UVP image store and Scan analysis software (Biosoft, Cambridge, England).

Results

KCl and $MgCl_2$ protect double-stranded DNA against heat-induced cleavage

The effect of various concentrations of KCl and $MgCl_2$ on plasmid heat-induced cleavage is illustrated in Fig. 1. Plasmids were incubated for one hour at 95°C in Hepes buffer

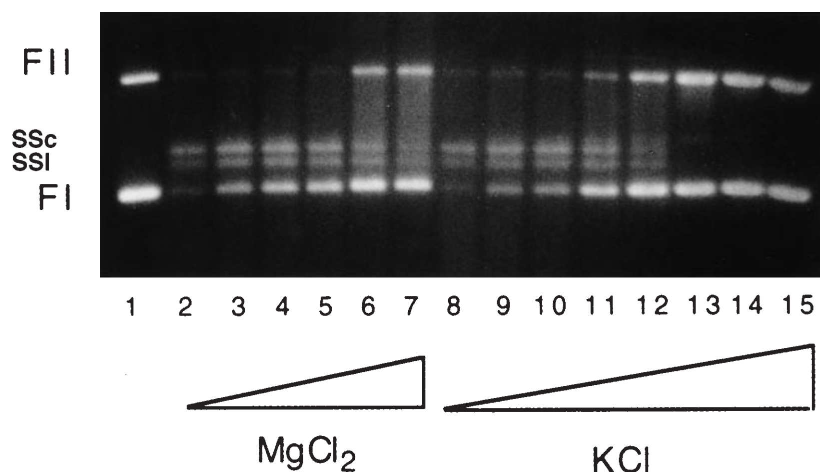

Fig. 1. Effect of KCl and $MgCl_2$ on heat-induced cleavage of supercoiled DNA. Plasmid pTZ18 was incubated for one hour at 95°C in 25mM Hepes buffer pH 7.5. After incubation, the DNA samples were run on a neutral agarose gel. *Lane 1*, nonheated DNA control; *lane 2*, DNA heated without salt; *lanes 3–7*, DNA heated in the presence of 0.25, 0.5, 1, 5, and 10mM $MgCl_2$, respectively; *lanes 8–15*, DNA heated in the presence of 1, 5, 10, 50, 250, 500mM, and 1 and 2M KCl, respectively. *FI*, supercoiled plasmid; *FII*, open circular form; *SSc*, single-stranded circular form; *SSl*, single-stranded linear form

and cleavage was monitored by running heat-treated DNA on agarose gels. In the absence of salt, the band corresponding to supercoiled DNA has nearly disappeared after the heat-treatment (lane 2). In addition, nicked molecules (form II), either originally present in the plasmid preparation, or produce by nicking of the supercoiled plasmid, have been denatured, leading to the appearance of circular and linear single-stranded DNA (SSc and SSl, respectively). The addition of salt inhibited DNA cleavage, as indicated by the presence of supercoiled plasmids in lanes corresponding to DNA heat-treated in the presence of $MgCl_2$ or KCl. Protection was already detectable at low salt concentrations (0.5 mM $MgCl_2$ and 5 mM KCl, lanes 3 and 9, respectively) and increased with salt concentrations up to 10 mM $MgCl_2$ and 1 M KCl (lanes 7 and 14, respectively). At high salt concentrations, one also observes the reappearance of form II DNA, because of the renaturation of circular and linear single-stranded DNA. Experiments in which cleavage was monitored by spectrophotometry at 107°C (as described in Marguet and Forterre 1994) indicated that protection by KCl continued to increase up to 2 M (data not shown).

KCl and $MgCl_2$ protect double-stranded DNA against depurination

Heat-induced cleavage of DNA occurs in two steps, depurination and subsequent cleavage of the phosphodiester bond by β-elimination at an apurinic site (Fig. 2) (for a detailed analysis of this reaction, see Suzuki et al. 1994). To study the effect of salt on depurination, we searched for conditions that would allow us to uncouple depurination and cleavage. Plasmids were incubated for various times at 75°C in an acidic buffer (25 mM potassium acetate pH 5.5), i.e., in conditions that would a priori favor depurination but reduce subsequent cleavage [Fig. 3a,b (DS panels)]. Degradation was monitored by running DNA in neutral gels (Fig. 3a) while depurination was monitored by running the same samples in alkaline gels after prior treatment with an alkaline solution (Fig. 3b). This procedure induces the cleavage of all phosphodiester bonds nearby apurinic sites (Marguet and Forterre 1994). Three bands were visible in an alkaline gel (Fig. 3b): a band of random coiled plasmids (RC) and bands of circular (SSc) and linear (SSl) single-stranded DNA. Random coiled plasmids were those which had been denatured by the alkaline treatment but were still topologically closed, whereas single-stranded plasmid forms were produced by denaturation of the open circular plasmids (FII in Fig. 3a). Comparison of the kinetics of cleavage in neutral (Fig. 3a) and alkaline gels (Fig. 3b) shows that the disappearance of covalently closed circular plasmids occurred faster in alkaline gels than in neutral gels. This indicated that depurination occurred more rapidly than subsequent cleavage under these experimental conditions, as expected from the fact that cleavage occurred preferentially at apurinic sites.

We looked at the effect of KCl and $MgCl_2$ on plasmidic DNA incubated for 10 min at 75°C in acidic buffer. Few intact plasmid molecules remained visible in alkaline gels after this heat-treatment (see Fig. 3b and Fig. 4 lane 2). Addition of low magnesium concentrations (0.25–1 mM) strongly inhibited depurination, as indicated by the increasing amount of DNA visible in the alkaline gel (Fig. 4 lanes 3–5). DNA migration was disturbed above 1 mM $MgCl_2$ (Fig. 4 lanes 6, 7). The strong band appearing in lanes 3 and 4 at the level of single-stranded linear DNA (SSl) corresponded to intact supercoiled plasmids which had not been denatured by the alkaline treatment in the presence of $MgCl_2$ (see Marguet and Forterre 1994, Fig. 3b). This was confirmed by the disappearance in lanes 3 and 4 (Fig. 4) of the random coiled form (RC), which was still visible after incubation in the absence of salt (lane 2).

The addition of KCl also inhibited depurination. The protective effect was first observed at 25 mM KCl (Fig. 4

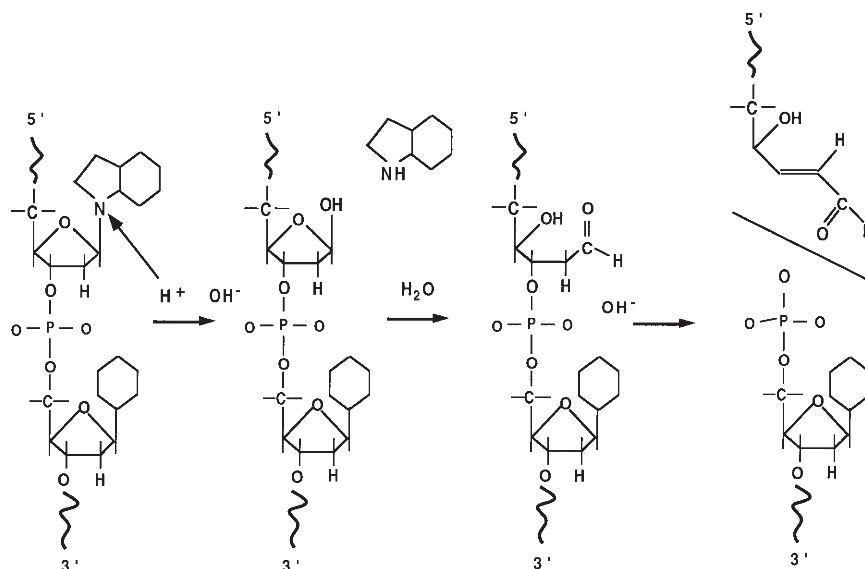

Fig. 2. Mechanism of depurination and subsequent cleavage at the apurinic site, adapted from Suzuki et al. (1994)

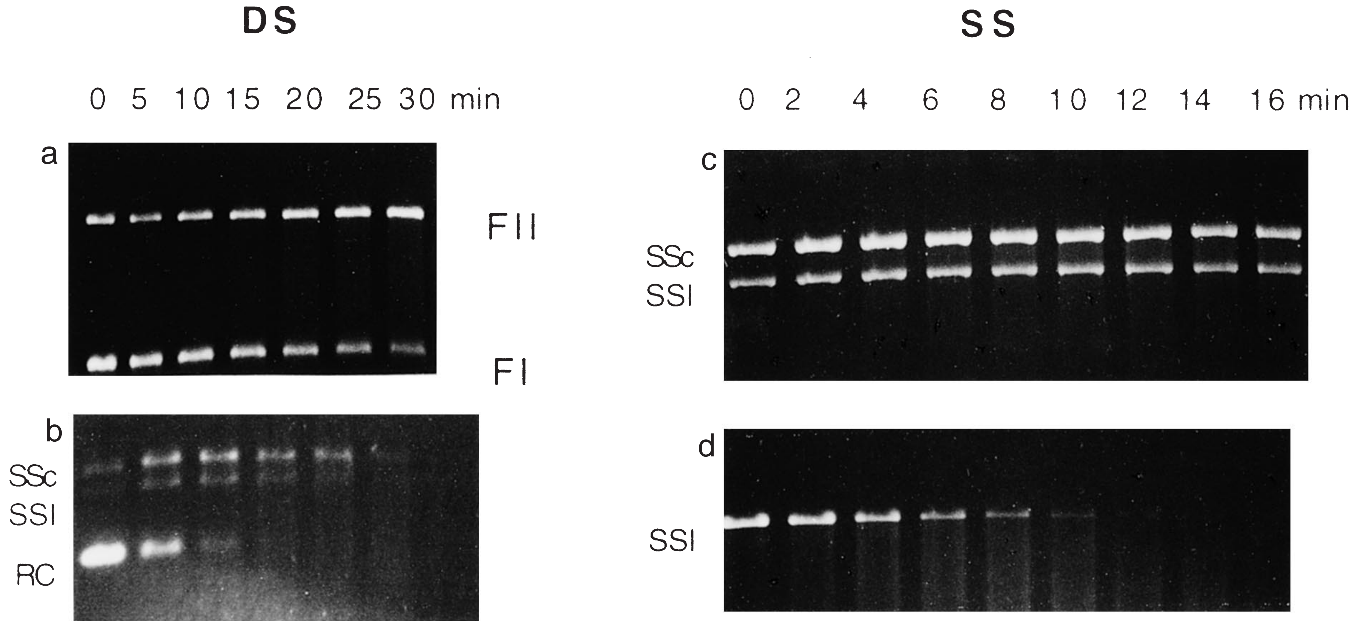

Fig. 3. Kinetics of pTZ18 and M13 heat-induced cleavage and depurination at 75°C. **a,b** Double stranded (DS): plasmid pTZ18 was incubated for various times at 75°C in an acidic buffer (25 mM potassium acetate pH 5.5). Cleavage was monitored by running DNA on a neutral gel (**a**) while depurination was monitored by running the same samples on an alkaline gel (**b**). **c,d** Single stranded (SS). M13 DNA was incubated in the same conditions and analyzed in a neutral gel (**c**) and an alkaline gel (**d**). *FI*, supercoiled plasmid; *FII*, open circular form; *SSc*, single-stranded circular form; *SSl*, single-stranded linear form

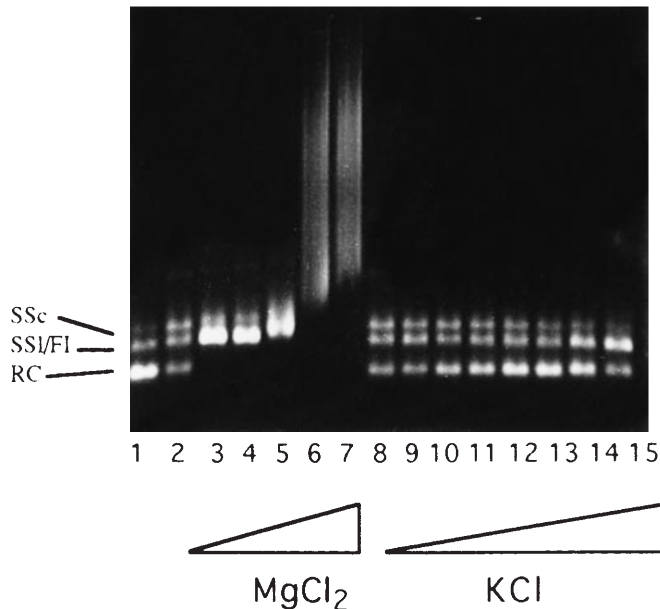

Fig. 4. Effect of KCl and $MgCl_2$ on depurination. Plasmid pTZ18 was incubated for 10 min at 75°C in acidic buffer (25 mM potassium acetate pH 5.5). Samples were run in alkaline gel. *Lane 1*, nonheated pTZ18; *lane 2*, DNA heated without salt; *lanes 3–7*, DNA heated in the presence of 0.25, 0.5, 1, 5, and 10 mM $MgCl_2$, respectively; *lanes 8–15*, DNA heated in the presence of 5, 10, 25, 50, 100, 250, and 500 mM and 1 M KCl, respectively. SSc, single-stranded circular form; *FI*, supercoiled plasmid; *SSl*, single-stranded linear form; *RC*, random coiled form

lane 10) and increased with KCl concentration up to 1 M, an effect similar to that observed on cleavage. As in the case of $MgCl_2$, supercoiled plasmids are present in samples which had been treated in the presence of high KCl concentrations (lanes 14, 15), corresponding to supercoiled molecules which were not denatured by the alkaline treatment at high ionic strength.

Effect of KCl and $MgCl_2$ on the cleavage of previously depurinated double-stranded DNA

To check the effect of KCl and $MgCl_2$ on the cleavage reaction at apurinic sites, we first prepared depurinated DNA by incubating plasmid pTZ18 for 10 min at 75°C in acidic buffer. Depurinated DNA was then precipitated, resuspended in Hepes buffer, and incubated for 30 min at 95°C. Depurinated DNA was strongly degraded by this treatment, as indicated by the reduction of the supercoiled band (about 85% was cleaved) and the appearance of two bands of single-stranded DNA (Fig. 5 lane 3). In the same conditions, about 35% of the DNA which had not been depurinated was cleaved (not shown). We observed some protection by salt of depurinated DNA against cleavage, as indicated by the presence of some supercoiled plasmids in samples which had been heat-treated in the presence of salt. However, this protection was lower than in the case of DNA which had not been depurinated. In fact, we only observed good protection with 500 mM KCl (lane 11).

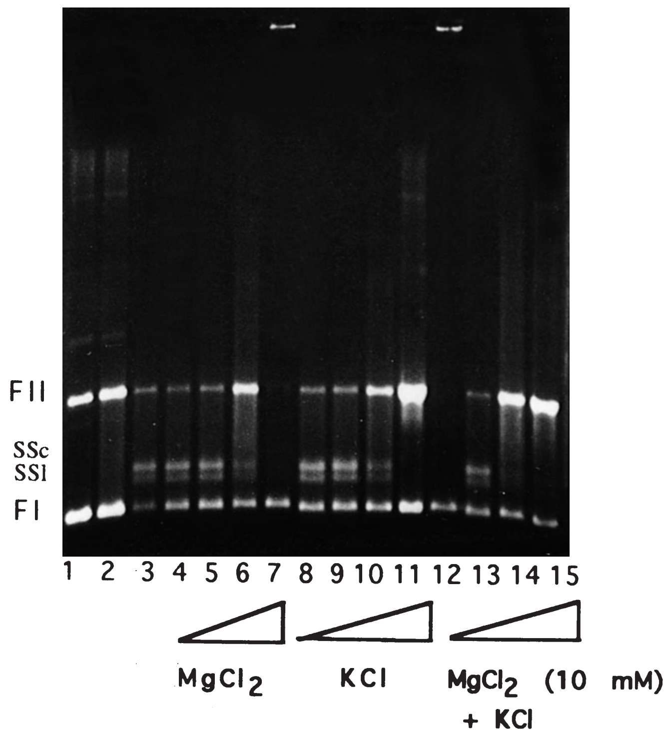

Fig. 5. Effect of KCl and $MgCl_2$ on the cleavage of previously depurinated DNA. Plasmid pTZ18 was first depurinated by incubation in acidic buffer (25 mM potassium acetate pH 5.5), then incubated for 30 min at 95°C in Hepes buffer. The samples were run on a neutral gel. *Lane 1*, nonheated DNA control; *lane 2*, nonheated, depurinated DNA; *lane 3*, depurinated DNA heated without salt; *lanes 4–7*, depurinated DNA heated in the presence of 0.5, 1, 5, and 10 mM $MgCl_2$, respectively; *lanes 8–11*, depurinated DNA heated in the presence of 10, 25, 100, and 500 mM KCl, respectively; *lanes 12–15*, heated, depurinated DNA in the presence of 10 mM $MgCl_2$ and 10, 25, 100, and 500 mM KCl, respectively. *FI*, supercoiled plasmid; *FII*, open circular form; *SSc*, single-stranded circular form; *SSl*, single-stranded linear form

With KCl and $MgCl_2$ combined, 10 mM $MgCl_2$ reduced the extent of protection by 50 mM KCl (compare lanes 11 and 15).

At high concentrations of both salts, the two forms of single-stranded DNA (SSc and SSl) were renatured to produce open circular double-stranded forms (FII). These topologically open forms specifically remained in the well in the presence of 10 mM $MgCl_2$ (lanes 7, 12). This aggregation occurred only at high temperature (not shown). Interestingly, even low KCl concentrations prevented this phenomenon (lane 13).

KCl and $MgCl_2$ protect single-stranded DNA against heat-induced cleavage

To determine whether KCl and $MgCl_2$ also protect single-stranded DNA (ssDNA) against heat-induced cleavage, we incubated M13 DNA for one hour at 95°C in Hepes buffer, pH 7.5, in the presence of various $MgCl_2$ and KCl concentrations. As seen in Fig. 6a, M13 DNA migrated as two

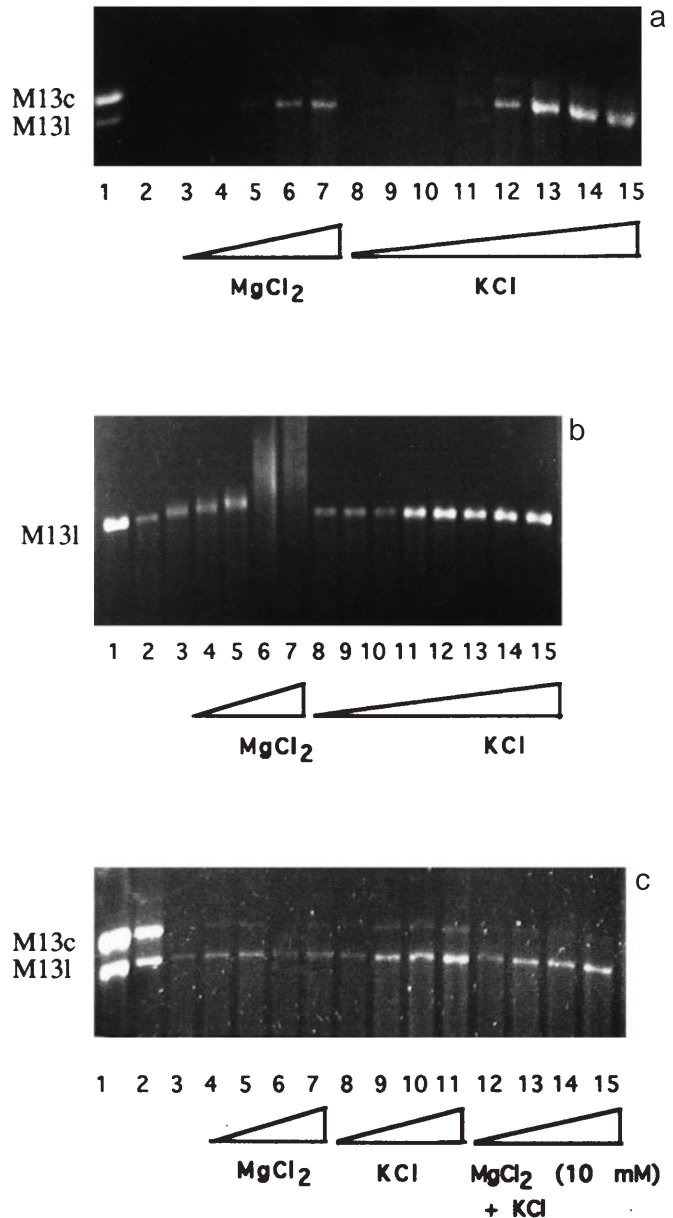

Fig. 6. Effect of KCl and $MgCl_2$ on heat-induced cleavage (**a**) and depurination (**b**) of single-stranded DNA, and on the cleavage of previously depurinated single-stranded DNA (**c**). **a** M13 DNA was incubated for 1 h at 95°C in Hepes buffer. Samples were run on a neutral gel; *lane 1*, nonheated DNA; *lane 2*, DNA heated without salt; *lanes 3–7*, DNA heated in the presence of 0.25, 0.5, 1, 5, and 10 mM $MgCl_2$, respectively; *lanes 8–15*, DNA heated in the presence of 1, 5, 10, 50, 250, 500 mM, and 1 and 2 M KCl, respectively. **b** M13 DNA was incubated for 10 min at 75°C in acidic buffer (25 mM potassium acetate pH 5.5). The samples were run on an alkaline gel. *Lane 1*, nonheated DNA; *lane 2*, DNA heated without salt; *lanes 3–7*, DNA heated in the presence of 0.25, 0.5, 1, 5, and 10 mM $MgCl_2$, respectively; *lanes 8–15*, DNA heated in the presence of 1, 5, 10, 50, 100, 250, and 500 mM and 1 M KCl, respectively. **c** depurinated M13 DNA was incubated for 30 min at 95°C in Hepes buffer. DNA samples were run in neutral gel. *Lane 1*, nonheated DNA (not depurinated); *lane 2*, nonheated, depurinated DNA; *lane 3*, heated, depurinated DNA without salt; *lanes 4–7*, depurinated DNA heated in the presence of 0.5, 1, 5, and 10 mM $MgCl_2$, respectively; *lanes 8–11*, depurinated DNA heated in the presence of 10, 25, 100, and 500 mM KCl, respectively; *lanes 12–15*, depurinated DNA heated with 10 mM $MgCl_2$ and 10, 25, 100, and 500 mM KCl, respectively. *M13c*, circular M13 DNA; *M13l*, linear M13 DNA

bands in neutral agarose gels, the upper one corresponding to circular molecules (M13c) and the lower one to linearized molecules (M131). M13 DNA was completely cleaved by the heat-treatment (lane 2). Both $MgCl_2$ and KCl protected M13 DNA against thermodegradation at 95°C, as indicated by the persistence of linearized M13 DNA and some intact circular molecules in samples heat-treated in the presence of salt. Protection was visible for concentrations of at least 1 mM $MgCl_2$ (lane 5) or 50 mM KCl (lane 11), which are again in the physiological concentration range.

KCl and $MgCl_2$ protect single-stranded DNA against depurination

To study the depurination of ssDNA, we first incubated M13 DNA for various times at 75°C in our depurination buffer. Samples were then treated with alkali and run in parallel on a neutral (Fig. 3c) and on an alkaline gel (Fig. 3d). As with dsDNA, depurination occurred much more rapidly than cleavage. We selected an incubation time of 10 min to check the effect of salt on depurination, since M13 DNA was still intact in the neutral gel after 10 min of depurination but was extensively cleaved in the alkaline gel. Figure 6b shows the result of a depurination experiment performed in the presence of various KCl and $MgCl_2$ concentrations in the depurination buffer, and analyzed in an alkaline gel. We observed protection with 1 mM $MgCl_2$ (lane 5) and concentrations of KCl of at least 50 mM (lanes 11–15). Protection increased with increasing KCl concentrations while the presence of either 5 or 10 mM $MgCl_2$ induced perturbation in the migration of DNA (formation of a smear) in the alkaline gel (lanes 6, 7).

As seen in Fig. 6b, nonheated M13 DNA migrated as a linear DNA in alkaline gels [see also Fig. 3b (SS)]. This indicates that all molecules in our M13 DNA preparation already contained at least one apurinic site before the depurination treatment.

Effect of KCl and $MgCl_2$ on the cleavage of previously depurinated single-stranded DNA

To check the effect of KCl and $MgCl_2$ on the cleavage reaction at apurinic sites in ssDNA, M13 DNA was first depurinated by incubation for 10 min at 75°C in acidic buffer, then precipitated, resuspended in Hepes buffer pH 7.5, and finally incubated for 30 min at 95°C in the presence of different salt concentrations (Fig. 6c). In the absence of salt, depurinated M13 was extensively cleaved (lane 3). As with dsDNA, cleavage of previously depurinated DNA was slightly reduced in the presence of KCl, the protection increasing with KCl concentrations from 25 to 500 mM (lanes 9–11). We did not observe clear-cut protection with $MgCl_2$. In fact, as with dsDNA, the addition of 10 mM $MgCl_2$ reduced the extent of protection by 500 mM KCl (compare lanes 11 and 15).

Discussion

It is well known that monovalent and divalent salts protect DNA against melting at high temperature (thermodenaturation) by screening the negative charges of the phosphate groups. Here, we showed that physiological concentrations of KCl and $MgCl_2$ also protect DNA against heat-induced DNA cleavage and depurination of both ds and ssDNA (thermodegradation). In the case of dsDNA, our results confirm and extend previous work, which has shown that the rate of depurination was reduced in the presence of 100 mM NaCl or sodium phosphate (Greer and Zamenhof 1962; Lindahl and Nyberg 1972), and that 10 mM $MgCl_2$ slightly reduced depurination (30%) (Lindahl and Nyberg 1972). In the case of ssDNA, this is a new result which has important implications for the mechanism of salt protection against cleavage. In particular, Lindahl and Nyberg (1972) suggested that $MgCl_2$ protected DNA against depurination by stabilizing the double helical structure, because ssDNA is depurinated more rapidly than dsDNA. However, the protection of ssDNA by salt against depurination suggests instead that salt directly interacts with the purine base to prevent depurination and consequently heat-induced cleavage. This fits well with our previous observations that (1) the double-helix is stable in the conditions of our test since we did not detect ssDNA upon incubation of supercoiled DNA at 95°C by densitometric analyses, and (2) that negatively supercoiled DNA (with a greater unwinding potential) is not degraded more rapidly than positively supercoiled DNA (Marguet and Forterre 1994). A possible explanation for the effect of salt is that K^+ and Mg^{2+} compete with protons for binding to the nitrogen atom of the purine imidazole ring (see Fig. 2).

We observed some protection by KCl against cleavage of previously depurinated ds and ssDNA. However, the interpretation of this result is complicated by the fact that KCl should in any case protect against new depurination that takes place during incubation of previously depurinated DNA at high temperature. Since KCl protection against cleavage of depurinated ssDNA is clearly less efficient than protection against depurination itself (compare Fig. 6b and c), we conclude that KCl indeed prevents heat-induced cleavage of depurinated DNA by inhibiting further depurination, and has probably no effect on heat-induced cleavage at apurinic sites. In the case of $MgCl_2$, we also observed some protection against cleavage of depurinated DNA, but the effect was lower than with KCl, especially in the case of ssDNA. Moreover, 10 mM $MgCl_2$ enhances cleavage at apurinic sites of both ss and dsDNA in the presence of KCl. This last observation agrees with a previous report that 10 mM $mgCl_2$ doubles the rate of cleavage at apurinic sites (Lindahl and Andersson 1972). In the case of $MgCl_2$, it is thus clear that this salt can only prevent heat-induced cleavage by inhibiting depurination, since once depurination has occurred, it stimulates the cleavage. The opposite effects of $MgCl_2$ on depurination and subsequent cleavage are not unexpected, considering the different mechanism of the two reactions (Fig. 2).

Our results have implications for the behavior of DNA at high temperature both in vitro and in vivo. For example, high concentrations of monovalent or divalent salts should reduce template degradation in PCR experiments. This could be especially important in the case of experiments involving long exposure of DNA at very high temperatures and/or using hyperthermophilic archaeal DNA polymerases. A balance should be considered between salt concentrations optimal to allow denaturation of the template after the polymerization step and those optimal for protection against DNA cleavage. It would also be important to consider these salt effects in dealing with ancient DNA. In particular, one should expect better preservation of DNA included in salt minerals, and one should consider the salt problem in handling heavily depurinated DNA: for example, by avoiding incubation with high magnesium concentrations.

In the course of our experiments, we also observed that the presence of 5 or 10 mM $MgCl_2$ in DNA samples exposed at high temperatures can strongly disturb the migration of some DNA forms, either in neutral or alkaline gels. In particular, ssDNA aggregates instead of renaturing in the presence of 10 mM $MgCl_2$ (Fig. 5). One should be aware of such phenomena when studying DNA-modifying enzymes isolated from hyperthermophiles, especially when using techniques relying on the agarose gel methodology.

Our results indicate that DNA in hyperthermophiles should be strongly protected against depurination by physiological concentrations of salt, especially in those hyperthermophiles with very high intracellular potassium concentrations. Some hyperthermophilic archaea indeed contain high levels of K^+ with various compatible solutes as counter-ions, such as cyclic 2,3-diphosphoglycerate (cDPG), or di-myo-inositol-1,1′-phosphate (DIP) and 2-*O*-mannopyranosyl-glycerate (Hensel and König 1988; Scholz et al. 1992; Ciulla et al. 1994). In a number of methanogens, it has been shown that as the intracellular concentration of cDPG increases from 300 to 1.2 M, there is an increase in the maximal growth temperature of the organism, correlating with corresponding K^+ concentrations of 700 mM, 1 M, and 2.3 M (Hensel and König 1988). These K^+ concentrations afford very efficient protection against DNA depurination in our in vitro experiments. DIP also accumulates in response to temperature in *Methanoccoccus jannashi* (Ciulla et al. 1994) and *Pyrococcus furiosus* (Martins and Santos 1995). It would be interesting to test whether DNA protection is similar with Cl^-, or with other specific compatible solutes found in hyperthermophiles as counter-ions.

Some hyperthermophiles have a low intracellular salt concentration and should thus be more susceptible to DNA thermodegradation. For example, the intracellular concentration of K^+ ions in *Thermoproteus tenax* is less than 100 mM (Hensel et al. 1987). The number and nature of heat-induced DNA lesions which have to be repaired might thus be quite different from one hyperthermophile to another, depending on their intracellular salt concentration. The role of the intracellular pH should be also very important in thermoacidophiles, which can grow at up to 85°C, since low pH increases dramatically the rate of depurination (Lindhal and Nyberg 1972), and an intracellular pH of 5.6 has been determined for *Sulfolobus acidocaldarius* (Lübben and Schäfer 1989).

In the course of our experiments, we also realized that depurinated DNA is quite resistant to subsequent cleavage, even at temperatures typical for hyperthermophiles. For example, only about 50% of depurinated DNA was cleaved after incubation for 15 min at 95% (not shown), which is in agreement with the rate of cleavage at apurinic sites reported by Lindhal and Andersson (1972). This suggests that repair enzymes, such as *AP* endonucleases, have probably enough time to prevent uncontrolled DNA cleavage at apurinic sites. However, special mechanisms could be required to prevent immediate denaturation at high temperature of the double-helix of nicked or gapped DNA produced during repair. This task could be fulfilled, at least partly, by nonspecific DNA-binding proteins. Indeed, it has been shown that archaeal histones or histone-like proteins increase the T_m of DNA (for a review see Grayling et al. 1996). It is usually assumed that these proteins can help the overall stabilization of DNA in hyperthermophiles. However, this must be viewed in the light of evidence that shows that topologically closed DNA is resistant to denaturation at temperatures typical for hyperthermophiles (Marguet and Forterre 1994). The major histones or histone-like proteins could be very useful in preventing denaturation of DNA regions which are no longer topologically closed following DNA cleavage. It would be interesting to test whether archaeal DNA-binding proteins can directly prevent DNA depurination and/or cleavage. It has been shown, for example, that the DNA-binding protein MCl from the mesophilic archaeon *Methanosarcina* sp. CHTI55 can protect DNA against fast neutrons and gamma ray irradiation (Isabelle et al. 1993). We have shown that the archaeal histone HMf does not protect DNA against thermodegradation (unpublished results), but other archaeal DNA-binding proteins could be involved in such a task. This should be an interesting area of research for the future in hyperthermophiles.

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