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Properties of damage-dependent DNA incision by nucleotide excision repair in human cell-free extracts

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ABSTRACT

Nucleotide excision repair (NER) is the primary mechanism for the removal of many lesions from DNA. This repair process can be broadly divided in two stages: first, incision at damaged sites and second, synthesis of new DNA to replace the oligonucleotide removed by excision. In order to dissect the repair mechanism, we have recently devised a method to analyze the incision reaction *in vitro* in the absence of repair synthesis (1). Damage-specific incisions take place in a repair reaction in which mammalian cell-free extracts are mixed with undamaged and damaged plasmids. Most of the incision events are accompanied by excision. Using this assay, we investigated here various parameters that specifically affect the level of damage-dependent incision activity by cell-free extracts *in vitro*. We have defined optimal conditions for the reaction and determined the kinetics of the incision with cell-free extracts from human cells. We present direct evidence that the incision step of NER is ATP-dependent. In addition, we observe that Mn^{2+} but no other divalent cation can substitute for Mg^{2+} in the incision reaction.

INTRODUCTION

The control of genetic stability is dependent upon accurate DNA replication and efficient DNA repair processes. Among the DNA repair pathways, nucleotide excision repair (NER) plays a major role (2, 3). This repair process, which recognizes and removes a wide variety of DNA lesions, is present from bacteria to human cells. The current view of the NER mechanism stems largely from a model derived from extensive genetic and biochemical studies of *Escherichia coli* (2, 4–7). The repair process consists of five steps: (i) damage recognition, (ii) asymmetric incision of the lesion-containing DNA strand on both sides of the lesion, (iii) excision of the damaged oligonucleotide, (iv) synthesis of new DNA using the complementary strand as template and (v) ligation.

NER activity has been reproduced *in vitro* with purified repair proteins from *E.coli* (6). Nevertheless, additional cellular factors are necessary to account for the reaction *in vivo*. For example, in *E.coli*, yeast and mammalian cells, lesions are removed from the transcribed strand of active genes faster than from the non-transcribed strand or the genome overall (8–10) and evidence has accumulated for the dual use of some proteins in both DNA repair and transcription (11–14).

Several lines of research have been undertaken in order to understand the individual steps of NER in mammalian cells at the molecular level. They take advantage of the availability of several repair mutants in yeast (15–17) and rodents (18) and of repair-deficient human syndromes such as xeroderma pigmentosum (XP) (19). The study of mammalian NER has been approached by the cloning of repair genes and the purification of repair proteins (3) and, in parallel, by biochemical analysis of the molecular repair mechanism. Permeabilized cell systems were used initially (20) and, more recently, a cell-free system has been developed that can carry out NER on damaged plasmid DNA using extracts from mammalian cells (21, 22) or from yeast (23). In this repair assay, specific repair reactions are detected by radiolabeled repair patches on plasmid DNA. A repair synthesis assay has also been adapted recently to a more chromatin-like DNA (24, 25). Nevertheless, nucleotide excision on plasmid DNA sufficiently resembles genomic repair, since defective repair has been observed with extracts from repair-deficient xeroderma pigmentosum cells belonging to complementation groups A–G (26–28).

Though all the proteins involved in the repair complex are not yet characterized, studies using cell extracts *in vitro* has proved to be very informative. The incision step appears to be slow and rate-limiting, while gap-filling and ligation probably proceed very rapidly (29, 30); moreover, the repair complex might not be processive (31). It has been reported that the fragments excised by human cell-free extracts ranged in length from 27–32 nucleotides (30) according to *in vivo* estimates (32) and had 5′-P and 3′-OH termini corresponding to enzymatic hydrolysis of mainly the 22–24th and the 5th phosphodiester bonds, 5′ and 3′ to the lesion respectively (33). Human single-stranded DNA binding protein (HSSB) is involved in carrying out or stabilizing incisions in damaged DNA *in vitro* (34, 35) and the proliferating cell nuclear antigen (PCNA)-dependent DNA pol δ/ε may be required *in vitro* for the repair synthesis step (29, 36, 37). In addition, this system has been used *in vitro* for purification of

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a protein factor complementing the defect of XP-A cell extracts (38), for the identification of the repair defect in XP-G cell extracts (39) and for the characterization of potential repair complexes (40, 41).

In order to understand the molecular mechanism of human NER, it is necessary to characterize each step of the reaction independently. However, when NER is performed under the usual *in vitro* conditions, all the steps of the repair pathway are carried out, from the initial DNA strand incision up to the ligation that retores strand continuity (42). Therefore the properties of the incision reaction cannot be investigated.

In order to focus the analysis on the incision step of NER, we have reported recently a rapid and simple method to quantify repair incisions in plasmid DNA in the presence of whole cell extracts (1). As expected for NER, this method detected damage-dependent incision of plasmids treated with UV, cisplatin and 8-methoxypsoralen. Under these conditions, the yield of incision activity measured was dependent upon the extent of plasmid modification (1). Incision was abolished with extracts from xeroderma pigmentosum excision repair-deficient cell lines, but activity could be restored by mixing XP extracts from different complementation groups. Using this method, we report here some biochemical properties of damage-specific incision by NER in human cell-free extracts.

MATERIAL AND METHODS

Cell lines and extracts

Human lymphoblastoid cell line AHH1 (healthy donor) was from Dr W.Thilly via Dr E.Moustacchi (Institut Curie, Paris, France). The HeLa S3 cell line was obtained from the stock of the European Molecular Biology Laboratories (Heidelberg, Germany). All the cells were cultured in suspension in 1 l spinner bottles at 37°C. Culture medium was RPMI 1640 medium (Gibco BRL) supplemented with glutamine (2 mM), 7% or 10% fetal calf serum (Gibco BRL) for HeLa or AHH1 cell lines respectively, penicillin (2×10^5 U/l) and streptomycin (50 mg/l). Cells were regularly tested and found to be free of contamination by *Mycoplasma* (Mycoplasma Detector Kit; Boehringer).

Cells were collected at a density of about 6×10^5/ml. Whole cell extracts were performed according to the method of Manley (43) with minor modifications as previously described (21). After preparation, extracts were immediately frozen and stored at −80°C.

Preparation of plasmids

The 2959 bp plasmid pBluescript KS$^+$ (Stratagene) and the related 3738 bp pHM14 plasmid (a gift from Dr R.D.Wood, ICRF, UK) were prepared by the alkaline lysis method from *E.coli* JM109 (relevant genotype: *recA1*, *endA1*, *gyrA96*, *hsdR17*). Both plasmids were carefully purified by one cesium chloride and two neutral sucrose gradient centrifugations as previously described (44). DNAase I-nicked plasmid was prepared by treatment of pBluescript KS$^+$ plasmid with 1 ng/ml pancreatic DNAase I (Gibco BRL) at 25°C for 10 min.

In vitro repair reactions

Repair synthesis assay. Except as otherwise indicated, standard 50 μl reaction mixtures contained 300 ng each of damaged pBluescript KS$^+$ and untreated pHM14 closed circular plasmids, 74 kBq of [α-^{32}P]dATP (110 TBq/mmol; Amersham), human cell extract protein (typically 100 μg) and 60 mM KCl in reaction buffer containing 45 mM HEPES−KOH (pH 7.8), 7.4 mM $MgCl_2$, 0.9 mM dithiothreitol, 0.4 mM EDTA, 2 mM ATP, 20 μM each dGTP, dCTP and dTTP, 4 μM dATP, 40 mM phosphocreatine, 2.5 μg of creatine phosphokinase (Type I; Sigma), 3.4% glycerol and 18 μg of bovine serum albumin as described (21). Plasmid DNA was purified from reaction mixtures as described (21), linearized with *Hin*dIII and electrophoresed overnight on a 1% agarose gel containing 0.5 μg/ml ethidium bromide. When necessary, open circular and closed circular forms of plasmid DNA were recovered according to the same purification protocol, except that the *Hin*dIII linearization step was omitted.

Incision assay. Standard 50 μl reaction mixtures contained 300 ng each of damaged pBluescript KS$^+$ and untreated pHM14 closed circular plasmids, extract protein (typically 100 μg), the reaction buffer as above except that deoxyribonucleotides were omitted and 4.5 μM aphidicolin was included as 1 μl of a suitably diluted solution in Me_2SO. Reaction was carried out at 30°C for 2 h. After termination of the reaction by the addition of EDTA to 25 mM, the mixture was treated with 200 μg/ml proteinase K (37°C, 30 min) in the presence of 0.5% SDS. Plasmid DNA was purified by phenol−chloroform extraction under gentle mixing conditions and ethanol precipitated. DNA was then incubated for 10 min in a 10 μl reaction mixture containing 90 mM HEPES−KOH, (pH 6.6), 10 mM $MgCl_2$, 74 kBq of [α-^{32}P]dATP (110 TBq/mmol; Amersham), 2 mM dithiothreitol, 20 μM each dGTP, dCTP and dTTP, 2 μM dATP and 1 U of *E.coli* DNA polymerase I large fragment (Gibco BRL). Reaction was terminated by addition of EDTA to 50 mM and unlabeled dATP to 1 mM. The mixture was treated with 50 μg/ml of bovine pancreatic ribonuclease A (37°C, 10 min). DNA was purified by phenol−chloroform extraction, ethanol precipitated,

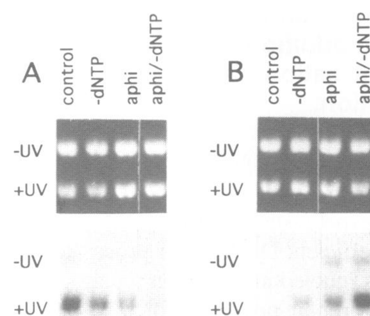

Figure 1. Effect of aphidicolin and dNTP on DNA repair synthesis (A) and DNA incision (B) by extracts from AHH1 cell line. (**A**) 300 ng each of UV-irradiated pBluescript KS$^+$ and untreated pHM14 control plasmids were incubated for 3 h at 30°C with 100 μg protein of repair-proficient AHH1 cell extract under conditions as indicated. Plasmids were then purified, linearized with *Hin*dIII and electrophoresed. Control, standard repair synthesis reaction; −dNTP, deoxyribonucleotides omitted (4 μM unlabelled dATP were present in order to maintain the isotopic dilution); aphi, as in control but in the presence 9 μM aphidicolin. Upper panel: photograph of the ethidium bromide-stained agarose gel. Lower panel: autoradiograph of the dried gel. (**B**) 300 ng each of UV-irradiated pBluescript KS$^+$ and untreated pHM14 control plasmids were incubated as in Fig. 1A but without [α-^{32}P]dATP. After purification by standard procedures, plasmids were labeled in the presence of Klenow polymerase (1 U, 10 min, 20°C). Plasmids were then purified, linearized with *Hin*dIII and electrophoresed. Upper panel: photograph of the ethidium bromide-stained agarose gel. Lower panel: autoradiograph of the dried gel.

then linearized with *Hin*dIII and electrophoresed as described above.

Quantification of repair. Data were quantified by autoradiography, scintillation counting of excised DNA bands and densitometry of the photographic negative of the gel to normalize for plasmid DNA recovery in each reaction sample (Scanning Laser Densitometer; Biocom, France).

Treatment of plasmids with mung bean nuclease

UV-treated or untreated pBluescript KS^+ plasmid purified from the incision reactions with cell extracts or plasmid pretreated with pancreatic DNAase I (~ 0.5 nicks/molecule) were incubated with mung bean nuclease. The assay was achieved in a 10 μl reaction mixture containing 300 ng plasmid DNA, 10 mM sodium acetate (pH 5.0), 50 mM NaCl, 0.1 mM zinc acetate, 5% glycerol (v/v) and the units of mung bean nuclease as indicated. Incubation was for 30 min at 30°C. The reaction was stopped by addition of EDTA to 50 mM, and DNA was purified as described above.

RESULTS AND DISCUSSION

Damage-specific DNA incisions in the absence of repair synthesis

Recently, we have devised an original method to measure the capacity of cell extracts to incise damaged DNA (1). The principle of this assay is summarized in Figure 1. In order to obtain damage-dependent incised intermediates from the repair reaction *in vitro*, we blocked the repair synthesis polymerization. Since DNA pol δ/ϵ may be required for the repair synthesis step (29, 36, 37), we used aphidicolin, an inhibitor of eukaryotic DNA polymerases α, δ and ϵ in order to inhibit the DNA resynthesis stage of the NER process. When aphidicolin was added to reactions mixtures without dNTP, repair synthesis in damaged DNA was inhibited up to 95% (Fig. 1A, compare lane control and lane aphi/−dNTP). Under these conditions, incisions accumulated mainly in the damaged plasmid (1; see also Figs 2 and 4). These could be subsequently radiolabeled by using the resulting incisions in purified plasmids as primers for DNA synthesis by the large fragment of *E.coli* DNA polymerase I (Klenow polymerase) and in the presence of [α-^{32}P]dATP (Fig. 1B). The conditions of incubation with Klenow polymerase permitted a linear increase of [α-^{32}P]dAMP incorporation with the number of 3′-OH ends/plasmid molecule (1).

The first steps in the repair process consist of a double incision followed by displacement of the damaged oligonucleotide. We asked at which stage the process stopped when DNA resynthesis was inhibited under our assay conditions. If excision was impaired, the incised plasmids recovered would contain mostly nicks; in contrast, they would contain gaps if excision proceeded normally. However, both steps could conceivably produce 3′-OH ends recognized as primer by Klenow polymerase. In order to discriminate between these possibilities, we employed mung bean nuclease, which degrades single-stranded DNA opposite gaps but not nicks in plasmid DNA (45). UV-treated and untreated plasmids incubated with HeLa extracts under conditions of damage-dependent incision were subsequently treated with various amounts of mung bean nuclease (Fig. 2). In the presence of cell extracts, conversion of superhelical plasmid molecules to relaxed covalently closed circular DNA occured, as well as preferential nicking of the UV-treated plasmids. A DNAase I-treated plasmid containing an equivalent average number of nicks (~ 0.5 nicks/molecule) was incubated in parallel with mung bean nuclease as a control for cleavage activity on nicked DNA. The treatment with mung bean nuclease clearly generated a band corresponding to linear plasmid DNA exclusively from the UV-treated plasmids pre-incised by HeLa extracts (Fig. 2). This specific cleavage strongly argued for the production of gaps by

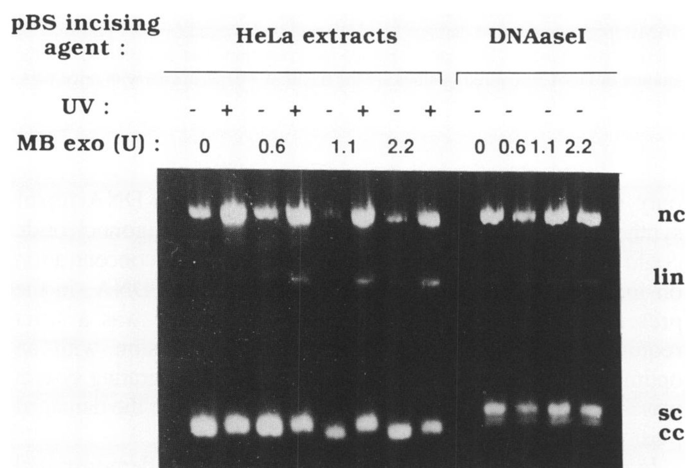

Figure 2. Plasmid DNA cleavage with mung bean nuclease. 300 ng of UV-treated (UV: +) or untreated (UV: −) pBluescript KS^+ (pBS) plasmids were incubated for 2 h at 20°C with 250 μg of repair-proficient HeLa cell extract in the absence of dNTP and with 4.5 μM aphidicolin. After purification by standard procedures, plasmids were incubated in the presence of the indicated number of mung bean nuclease units (referred to as MB exo) for 30 min at 30°C. 300 ng DNAase I-nicked pBS (~ 0.5 nicks/molecule) were incubated in parallel. After purification, the plasmids were electrophoresed on a 1% agarose gel containing 0.5 μg/ml ethidium bromide. The picture shows the photograph of the agarose gel. nc, nicked circular form; lin, linear form; sc, supercoiled form; cc, closed circular form.

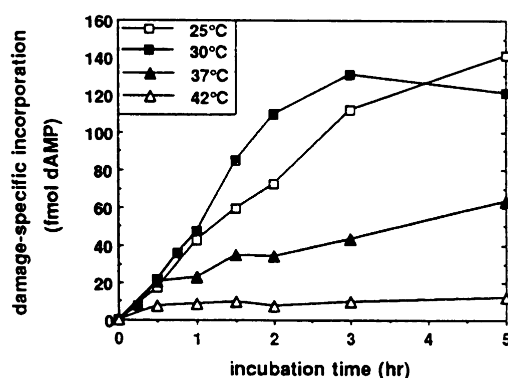

Figure 3. Kinetics of damage-dependent DNA nicking activity by HeLa extracts at various temperatures. 300 ng each of UV-treated pBluescript KS^+ and untreated pHM14 (pHM) control plasmids were incubated for different time intervals at various temperatures with 150 μg HeLa protein extracts. Incubation was carried out in the absence of added dNTP and with 4.5 μM aphidicolin. After purification by standard procedures, plasmids were labeled in the presence of Klenow polymerase (1 U, 10 min, 20°C). Plasmids were then purified, linearized with *Hin*dIII and electrophoresed. The figure shows fmol dAMP incorporated into damaged plasmids by Klenow polymerase. For each sample, incorporation was normalized for the amount of DNA recovered and the amount of background incorporation in the pHM14 control plasmid was subtracted from incorporation in the pBluescript KS^+ plasmid.

the excision repair pathway in the absence of DNA resynthesis. Since the linear form co-migrated with full-length plasmid, this indicated that the gaps might extend over very few nucleotides. An accurate quantification of the nick/gap ratio was not undertaken, since the efficiency of mung bean nuclease on short gaps was unknown.

In addition, we have measured the ligation efficiency of T4 DNA ligase on UV-damaged plasmid substrates which have been pre-incised by cell extracts by following the inhibition of Klenow polymerase activity. T4 ligase rejoined <20% of the primer sites of Klenow polymerase on the damaged plasmids pre-incised by cell extracts under conditions where it ligated ~90% of the DNAase I nicks on a control plasmid (data not shown).

Taken together, these results indicate that although the resynthesis step of the nucleotide excision repair process is prevented, the excision step goes to completion. Consequently, most of the damage-specifically incised plasmids recovered under our conditions contain gaps, corresponding to a normal release of the damaged oligonucleotide.

Kinetics of damage-dependent incision and effect of temperature

The kinetics of damage-dependent incision at various temperatures of incubation are shown in Fig. 3. At 30°C, kinetics were linear for 2 h and then reached a plateau. At 25°C, the kinetics appeared slower than at 30°C and were linear up to 3 h. However, the reactions performed at 25 or 30°C yielded an identical level of incisions after 3–5 h. In contrast, higher temperatures (37 or 42°C) greatly reduced the extent of lesion-specific incision.

The kinetics of incision were identical with the time-course obtained when repair synthesis was assessed (21, 22). This result is consistent with the hypothesis that the rate-limiting steps are the first (damage recognition, DNA incision) rather that the late stages (polymerization, ligation) of the NER reaction. Likewise, in the case of xeroderma pigmentosum, UV-dependent repair synthesis can be restored by pre-incision of the damaged DNA by pyrimidine dimer-specific glycosylases in systems of microinjected or transfected repair-deficient cell lines (46, 47) and supplemented cell extracts (21, 26). The time-course of incision has been previously analyzed under conditions that inhibit repair synthesis (absence of PCNA; 29) and a plateau was obtained after 1 h; in that case, the early completion of the reaction might reflect the lack of turnover of the 'excision nuclease' complex which could partly depend on PCNA (30). In contrast, the mechanism of DNA synthesis inhibition by aphidicolin does not prevent the assembly of the DNA resynthesis complex, including PCNA (48), and, consequently, the release of the incision enzymes from the nicked site probably occurs normally under our conditions. Accordingly, we believe that most of the incised intermediates generated in damaged DNA in our incision reaction contained gaps (Fig. 2).

The inhibition of incision activity at temperatures higher than 30°C was not related to plasmid degradation, since the yield of plasmid recovery did not change significatively between 20 and 42°C. Higher temperature might be incompatible with assembling of the multiprotein complexes that are required for NER (3).

Requirement for ATP

As observed in *E.coli* (6), the eukaryotic NER process is ATP-dependent. However, this result has been previously observed only with mammalian cell-free extracts in which DNA repair synthesis (21, 22) or removal of the damaged oligonucleotide could occur (33). Fig. 4 shows the effect of ATP concentration on incision in damaged and undamaged plasmid DNA, in the presence of an ATP-regenerating system. There was a strict requirement for ATP for damage-specific incision with an optimum around 2 mM. In addition, the ATP-regenerating system was also indispensable to detect specific incisions in the damaged plasmid (data not shown).

In permeabilized human fibroblasts it has been demonstrated that at least one ATP-requiring step occured before or during incision (49, 50); in these systems, however, processes like nucleosomal rearrangements or topological changes in DNA might also require ATP, rather than the incision reaction *per se*. In contrast, NER in cell-free extracts takes place on naked DNA rather than chromatin (51) and on plasmid circles relaxed by ATP-independent topoisomerase I (52, 53). Therefore, our result is direct evidence that the incision step of eukaryotic NER is an ATP-dependent process.

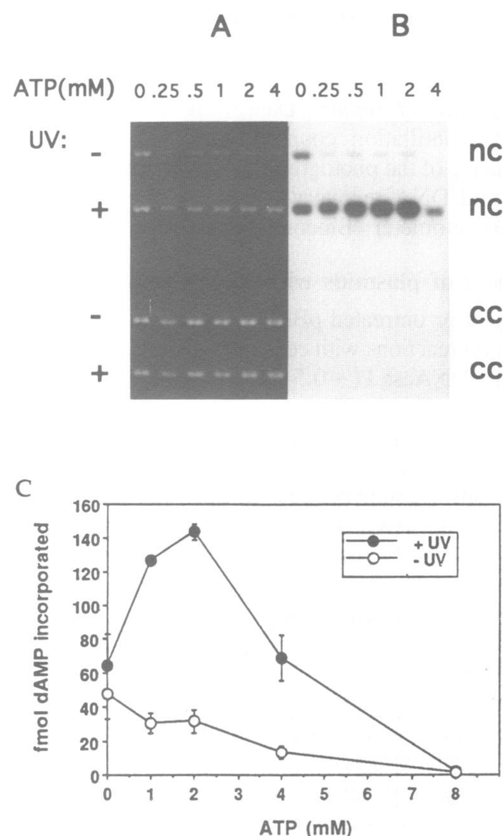

Figure 4. Effect of ATP concentration on damage-dependent DNA nicking activity by HeLa extracts. 300 ng each of UV-treated pBluescript KS$^+$ and untreated pHM14 (pHM) control plasmids were incubated for 2 h at 30°C with 100 μg HeLa protein extract. After purification by standard procedures, plasmids were labeled in the presence of Klenow polymerase (1 U, 10 min, 20°C). Plasmids were then purified and electrophoresed without linearization. nc, nicked circular form; cc, closed circular form. (**A**) Photograph of the ethidium bromide-stained agarose gel. (**B**) Autoradiograph of the dried gel. (**C**) The figure shows fmol dAMP incorporated into damaged (+UV) and undamaged (−UV) plasmids by Klenow polymerase. For each sample, incorporation was normalized for the amount of DNA recovered. Values are the mean of 3 experiments with error bars.

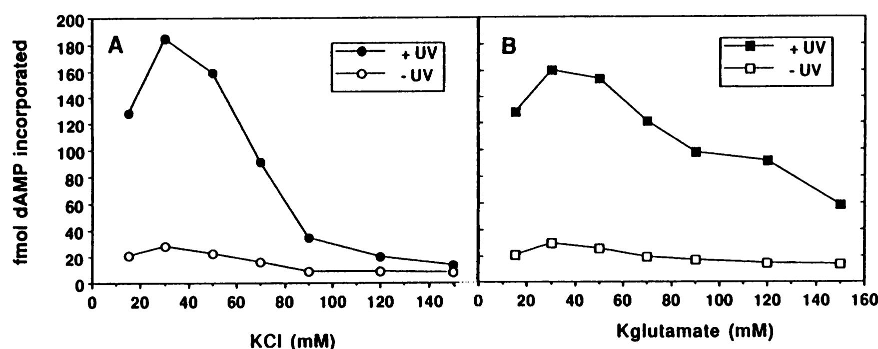

Figure 5. Effect of KCl and potassium glutamate concentrations on damage-dependent DNA nicking activity by HeLa extracts. 300 ng each of UV-treated pBluescript KS^+ and untreated pHM14 (pHM) control plasmids were incubated for 2 h at 30°C with 150 μg HeLa protein extracts and the experiment was performed as in Fig. 3. The figure shows fmol dAMP incorporated into damaged (+UV) and undamaged (−UV) plasmids by Klenow polymerase. For each sample, incorporation was normalized for the amount of DNA recovered.

Salt, pH and metal ion optima

Lesion-dependent incision proceeded with a KCl concentration up to 50 mM (Fig. 5A). At KCl concentrations above 90 mM, the damage-specific incision reaction was strongly reduced. Potassium glutamate (Kglu) could substitute for KCl (Fig. 5B) The lowest concentrations tested were limited by the buffer used for preparing whole cell extracts, which contained 0.1 M KCl or Kglu; lower concentrations could not be explored since extracts prepared without KCl or Kglu were completely deficient in repair activity and no activity could be recovered during further incubation in the presence of KCl or Kglu (data not shown). The incision reaction appeared to be sensitive to elevated choride concentrations, since concentrations of Kglu higher than KCl were tolerated; specific incision in the UV-damaged plasmid yielded 60% of the optimum at 90 mM Kglu and still 35% at 150 mM Kglu compared to <5% of the optimum at 150 mM KCl.

Significant incision in the UV-damaged plasmid occurred at pH 7.2−8.1 (data not shown). This activity tended to disappear for lower values of pH, while the background incision activity in the undamaged control plasmid increased. Over the full range of pH tested, no significant variation of plasmid DNA recovery was observed.

Repair synthesis *in vitro* requires $MgCl_2$ (22, 23). Since $MgCl_2$ is likely to contribute to the DNA resynthesis step *per se* (54), it was of interest to analyze the requirement for divalent cations at the incision stage. Fig. 6 shows the effect of varying the $MgCl_2$ concentration on the damage-specific incision activity of cell-free extracts. Extracts prepared without $MgCl_2$ and incubated in a repair reaction without divalent cations are almost completely deficient in lesion-dependent incision. However, contrary to extracts prepared without KCl or Kglu, incision activity could be efficiently recovered on addition of $MgCl_2$ to the repair reaction. There is a sharp Mg^{2+} optimum at 4.5−7 mM and a complete inhibition at 18 mM (Fig. 6). In the optimum concentration range, Mn^{2+} could substitute for Mg^{2+} and yielded an ~80% extent of specific incision activity. However Ca^{2+}, Co^{2+}, Zn^{2+} or Ni^{2+} could not replace Mg^{2+} efficiently (Fig. 6 and data not shown). There is a strong decrease in damage-specific incision activity for Mn^{2+} concentrations higher than 10 mM. High Mg^{2+} concentrations reduced specifically the incision activity on the UV-damaged plasmid; in contrast, high Mn^{2+} concentrations provoked a high random nicking activity on both damaged and undamaged plasmids and a similar effect was observed for various concentration ranges of the other divalent cations tested (data not shown).

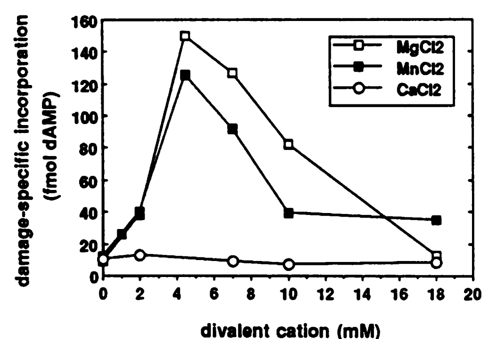

Figure 6. Effect of various divalent cation concentrations on damage-dependent DNA nicking activity by HeLa extracts. 300 ng each of UV-treated pBluescript KS^+ and untreated pHM14 (pHM) control plasmids were incubated for 2 h at 30°C with 150 μg HeLa protein extracts prepared whithout divalent cations and the experiment was performed as in Fig. 3. The figure shows fmol dAMP incorporated into damaged plasmids by Klenow polymerase. For each sample, incorporation was normalized for the amount of DNA recovered and the amount of background incorporation in pHM14 control plasmid was subtracted from incorporation in pBluescript KS^+ plasmid.

The requirement for Mg^{2+}, already known for the polymerization step, is also demonstrated for the incision reaction. Recently, the properties of a UV DNA damage binding protein implicated in XP-E have been analyzed (55). Optimal binding required Mg^{2+}, which might be one reason for the Mg^{2+} dependence of the incision reaction. Interestingly, the XP-G repair protein which was recently isolated exhibited a magnesium-dependent DNA endonuclease activity (56); the nuclease activity was also efficient in the presence of Mn^{2+}, but neither Ca^{2+} nor Zn^{2+} could be substituted, which ressembles the properties that we found for the incision reaction. In yeast, the Rad1/Rad10 and Rad2 endonucleases, which are both indispensable for incision of damaged DNA by NER also have a strict requirement for Mg^{2+} (57, 58). Finally, since several helicases possibly participate in the excinuclease complex, a requirement for Mg^{2+} as well as

for ATP might be expected (3). For example, the DNA helicase encoded by the XP-D gene exhibits an ATPase activity which is also Mg^{2+}-dependent, and Mg^{2+} can be substituted by Mn^{2+} but not by Ca^{2+} (59).

Our biochemical assay that examines the incision reaction might help in the re-construction of the different steps that sequentially take place during the NER reaction. For analysis of the initial steps of the NER repair mechanism, this assay might allow the detailed description of the role of the various proteins involved in this process. Since we observed the complementation of damage-specific DNA incision by mixing xeroderma pigmentosum cell extracts (1), analysis of the particular role of protein factors deficient in this repair disease might also be facilitated.

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