Complementation of the xeroderma pigmentosum DNA repair synthesis defect with *Escherichia coli* UvrABC proteins in a cell-free system

Johan Hansson, Lawrence Grossman¹, Tomas Lindahl and Richard D.Wood*

Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts EN6 3LD, UK and ¹Department of Biochemistry, The Johns Hopkins University, School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore, MD 21205, USA

Received November 2, 1989; Revised and Accepted November 30, 1989

ABSTRACT

A newly developed cell-free system was used to study DNA repair synthesis carried out by extracts from human cell lines in vitro. Extracts from a normal human lymphoid cell line and from cell lines established from individuals with hereditary dysplastic nevus syndrome perform damage-dependent repair synthesis in plasmid DNA treated with cis- or trans-diamminedichloroplatinum(II) or irradiated with ultraviolet light. Cell extracts of xeroderma pigmentosum origin (complementation groups A, C, D, and G) are deficient in DNA repair synthesis. When damaged plasmid DNA was pretreated with purified *Escherichia coli* UvrABC proteins, xeroderma pigmentosum cell extracts were able to carry out DNA repair synthesis. The ability of *E. coli* UvrABC proteins to complement xeroderma pigmentosum cell extracts indicates that the extracts are deficient in incision, but can carry out later steps of repair. Thus the in vitro system provides results that are in agreement with the incision defect found from studies of xeroderma pigmentosum cells.

INTRODUCTION

Soluble extracts from normal human cells were recently shown to be capable of mediating repair synthesis in plasmid DNA that has been damaged by ultraviolet light (UV), psoralen derivatives plus long wave UV, and platinating agents (1, 2, 3, 4). In contrast, cell extracts made from xeroderma pigmentosum (XP) cells were found to be deficient in repair synthesis in response to such DNA damage.

XP is a rare autosomal recessive disease characterized by severe photosensitivity, development of skin tumors on sun-exposed body surfaces, and neurological abnormalities (5). Cells from individuals with XP are hypersensitive to a large number of DNA damaging agents including UV and many chemical carcinogens (6). This sensitivity is associated with a defect in the ability to incise damaged DNA during nucleotide excision repair (5, 7, 8, 9). At least eight different complementation groups, designated XP-A through XP-G and XP-V, have been identified by cell fusion studies (10, 11, 12, 13).

Since the repair defect in most XP complementation groups is thought to lie in the incision step, it follows that provision of incision activity from an exogenous source should allow XP cells to complete nucleotide excision repair and perform DNA repair synthesis. Following this reasoning, a number of investigators have introduced cyclobutane pyrimidine dimer-DNA glycosylase/endonucleases into XP cells, and observed a stimulation of DNA repair synthesis after UV irradiation. These enzymes cleave UV-irradiated DNA specifically at cyclobutane pyrimidine dimers, producing a chain scission with a 5' pyrimidine dimer and a 3' baseless sugar (14). The glycosylase/endonuclease from *Micrococcus luteus* or from bacteriophage T4 has been introduced by microinjection into XP cell lines (15, 16), and by stable transfection of the gene for the T4 enzyme into a XP group D-derived cell line (17). Using a cell-free system to monitor repair synthesis, we have noted that XP cell extracts can mediate repair synthesis in UV irradiated DNA when supplemented with pyrimidine dimer-DNA glycosylase from *M. luteus* (1, 2).

However, DNA repair enzymes in human cells do not cleave UV-irradiated DNA by the same mechanism as used by the *M. luteus* or bacteriophage T4 enzymes. Instead, pyrimidine dimers are released as part of short oligonucleotides (18, 19). Consequently, repair synthesis stimulated by the *M. luteus* enzyme is not wholly appropriate as a model for the normal human excision repair process. Human cells are more likely to introduce nicks in damaged DNA by a mechanism analogous to that in *E. coli* (1). In *E. coli*, the UvrA, UvrB, and UvrC proteins act together as a complex to incise DNA containing a wide variety of lesions. Incision occurs as a dual event on both sides of the lesion, resulting in release of the adduct in a dodecanucleotide. The DNA adducts incised by the UvrABC protein complex are essentially the same as those repaired by nucleotide excision repair in human cells (20). It is therefore expected that the introduction of incisions in damaged DNA by *E. coli* UvrABC proteins should more closely simulate the initial stages of DNA repair in human cells.

* To whom correspondence should be addressed
We report here on the ability of purified *E. coli* UvrABC proteins to complement the repair defect in extracts made from XP cells in response to DNA damage produced by UV and by the cancer chemotherapeutic drug *cis*-diaminedichloroplatinum(II) (cis-DDP), and its clinically inactive isomer *trans*-diaminedichloroplatinum(II) (trans-DDP).

**MATERIALS AND METHODS**

**Cell extracts**

Human lymphoid cell lines transformed with EB-virus were obtained from the N.I.G.M.S. Human Genetic Mutant Cell Repository (Coriell Institute for Medical Research, Camden, N.J.): GM1953 established from a healthy donor; GM2345 from a donor with XP belonging to complementation group A; GM2248 and GM2249 from two individuals belonging to complementation group C; and GM2253 from a donor belonging to complementation group D. XP3BR(SV40 clone 15), an SV40 transformed fibroblast cell line, established from an individual with XP belonging to complementation group G, was obtained from Drs. B. Klein and A. van der Eb (University of Leiden), through the courtesy of Dr. J. Hoeijmakers. CoBo and PeCh are EB-virus transformed lymphoid cell lines established from two melanoma patients with dysplastic nevus syndrome and were obtained from Drs. W. Wakeling and B. Ponder (Dept. of Pathology, University of Cambridge). Cells were cultured in RPMI 1640 medium supplemented with 15% fetal calf serum. All cell cultures were free from contamination with *Mycoplasma*.

Whole cell extracts were made according to the method of Manley et al. (21) with minor modifications, as previously described (1). For each preparation, 1 to 2 liter cultures of cells in late exponential growth phase (5—8×10^6 cells/ml) were used. After preparation, the cell extracts were immediately frozen and stored at −80°C.

**Plasmids containing DNA adducts**

Plasmids pAT153 and pBR322 were grown in *E. coli* host strain DH5α (*recA*, *hsdR*). Purified plasmid pAT153 was UV-irradiated (450 J/m^2), treated with *E. coli* Nth protein, and repurified on sucrose gradients as described (1). Plasmid pAT153 was treated with cis-DDP or trans-DDP at concentrations that induce averages of 13 cis-DDP or 16 trans-DDP adducts per plasmid molecule (3).

**In vitro repair reactions**

Standard 50 μl reaction mixtures contained 300 ng each of damaged plasmid pAT153 and nondamaged plasmid pBR322, 45 mM Hepes-KOH (pH 7.8), 60 mM KCl, 7.5 mM MgCl₂, 0.9 mM dithiothreitol, 0.4 mM EDTA, 2 mM ATP, 20 μM each of dGTP, dCTP, and TTP, 8 μM dATP, 2 μCi of α-[³²P]dATP (3000 Ci/mmol), 40 mM phosphocreatine, 2.5 μg creatine phosphokinase (Type I, Sigma), 3.4% glycerol, 18 μg bovine serum albumin, and 60 to 100 μg of extract protein. Reactions were incubated for 6 h at 30°C, and plasmid DNA was purified from the reactions as described (1). The DNA from each reaction was linearized with EcoRI and electrophoresed overnight on a 1% agarose gel, cast and run in buffer containing 0.5 μg/ml ethidium bromide. Data were quantified by autoradiography and densitometry (1). The amount of [³²P]dAMP incorporated per reaction was corrected for the amount of DNA recovered from the reaction, as measured by densitometry of the photographic negative.

**Complementation of repair reactions with *E. coli* UvrABC proteins**

*Escherichia coli* UvrA, UvrB, and UvrC proteins were purified as previously described (22). In cases where DNA was pretreated with *E. coli* UvrABC proteins before repair reactions, UvrA (24—72 nM) and UvrB (20—59 nM) proteins were added simultaneously to complete reaction mixtures without cell extract, which were incubated for 15 min at 30°C. UvrC protein (6.9 nM) was then added and the mixtures were incubated for another 15 min at 30°C. These conditions resulted in the introduction of nicks into approximately 10% of the UV-irradiated pAT153 plasmid molecules, with minimal nicking of undamaged pBR322 molecules. Cell extract was added and incubations were continued...
at 30°C for 6 h. In other experiments, UvrA, UvrB, and UvrC proteins, or M. luteus pyrimidine dimer-DNA glycosylase/endonuclease, were added simultaneously with the cell extract at the beginning of repair incubations. Purified M. luteus enzyme (700 U/µg protein) was obtained from Applied Genetics Inc., Freeport, NY, USA.

RESULTS
Repair synthesis in UV-damaged DNA by cell extracts and stimulation of repair by E. coli UvrABC proteins
Cell extracts made from the normal lymphoid cell line GM1953 perform repair synthesis in UV-irradiated DNA, whereas extracts made from the XP-C cell line GM2249 are defective in such repair synthesis (Figure 1, lanes 1 and 7). The repair synthesis defect observed in vitro with extracts from this cell line (1) is more pronounced than that observed in vivo for most XP-C cell lines as judged by UV-induced unscheduled DNA synthesis (6). As previously reported (1), when M. luteus pyrimidine dimer-DNA glycosylase/endonuclease is added to repair reactions, it stimulates repair synthesis in UV-damaged DNA by extract from XP-C cells, and also enhances the repair synthesis performed by normal cell extract (Figure 1, lanes 2 and 8).

When UV-irradiated DNA was incubated with E. coli UvrABC proteins before repair reactions, damage-dependent repair synthesis was obtained with XP-C cell extract (Figure 1, lanes 11, 12). DNA repair synthesis was also stimulated when UvrABC proteins were added simultaneously with cell extract at the start of reaction (Figure 1, lane 13). The ability of UvrABC proteins to complement the XP-C extract implies that this cell extract is deficient in incision at UV-induced DNA damage, but can carry out later steps in repair, once incisions are produced. Incubation of UV-irradiated DNA with UvrABC proteins enhanced the repair synthesis obtained with extract from normal GM1953 cells (Figure 1, lanes 5, 6); the damage-dependent synthesis stimulated by UvrABC proteins was superimposed on synthesis initiated by incision enzymes in the human cell extract. Addition of UvrA and UvrB proteins without UvrC, or of UvrC protein only, did not enhance damage-dependent repair synthesis by XP-C or normal cell extracts (Figure 1, lanes 3, 4, 9, 10). Thus, neither the UvrAB protein combination nor UvrC protein can substitute for the defective XP-C gene product. In fact, repair synthesis carried out by the GM1953 cell extract was partially inhibited by the addition of UvrAB only; this inhibition may have been caused by the formation of UvrAB complexes at damaged sites that block incision by proteins in the human cell extract.

DNA repair synthesis in DNA damaged by cis-DDP or trans-DDP by extracts from a normal cell line and cell lines from individuals with dysplastic nevus syndrome
To extend these observations to another class of DNA adducts, we investigated in vitro repair synthesis of DNA damaged by cis-DDP or trans-DDP. The two isomers differ in the types of lesions that are formed upon treatment of purified DNA. While DNA intrastrand crosslinks between adjacent purine residues are the most frequent lesions induced by cis-DDP, trans-DDP is unable to form such lesions and instead causes drug-DNA monoadducts and cross-links between purine residues separated by one or more bases (23). Such adducted DNA is cleaved by the UvrABC enzyme (24). Extracts made from normal GM1953 cells performed repair synthesis in DNA containing trans-DDP or cis-DDP adducts (Figures 2 and 3, lanes 1, 2; Table 1), in agreement with previously reported findings (3). Extracts from cell lines derived from two individuals with hereditary dysplastic nevus syndrome (DNS) were also able to perform repair synthesis in plasmids damaged by trans-DDP or cis-DDP (Figures 2 and 3, lanes 3, 4; Table 1). In addition, extracts made from the two DNS cell lines were found to be proficient in performing repair synthesis in UV-damaged DNA (data not shown).

Defective DNA repair synthesis by XP cell extracts in DNA damaged by cis-DDP or trans-DDP, and complementation of repair synthesis by E. coli UvrABC proteins
In contrast to the results obtained with repair-proficient cell lines, extracts made from XP cell lines belonging to complementation groups A, C, D, and G showed variable levels of background incorporation of radioactive material into both undamaged and damaged plasmids, but no or very low damage-specific repair synthesis in plasmids with trans-DDP or cis-DDP adducts (Figures 2 and 3, lanes 5–8; Table 1).

When cis-DDP damaged DNA was incubated with E. coli UvrABC proteins before repair reactions, significant stimulation of repair synthesis was seen with extracts prepared from XP cells (Figure 3, lanes 13–16; Table 1). As observed with UV-irradiated DNA, incubation with UvrABC proteins also enhanced the repair synthesis performed in cis-DDP-damaged plasmids by...
Figure 3. Deficient repair synthesis by extracts from XP cells in cis-DDP-damaged DNA, and stimulation of repair synthesis by UvrABC proteins. Standard repair reactions included approximately 300 ng pAT153 DNA containing 13 cis-DDP adducts per plasmid molecule, 300 ng nondamaged pBR322 DNA and 100 μg cell extract protein. Upper panel: DNA fluorescence. Lower panel: autoradiograph. Lanes 1–8: cell extract only. Lanes 9–16: DNA preincubated in reaction buffer with 72 nM UvrA and 59 nM UvrB proteins for 15 min at 30°C, then with 6.9 nM UvrC protein for 15 min at 30°C before addition of cell extract. Lanes 1, 2, 9, 10: reactions with two different preparations of GM1953 normal cell extract. Lanes 3, 11: extract from CoBo (DNS) cell line. Lanes 4, 12: extract from PeCh (DNS) cell line. Lanes 5, 13: extract from GM2345 (XP-A) cells line. Lanes 6, 14: extract from GM2248 (XP-C) cell line. Lanes 7, 15: extract from GM2253 (XP-D) cell line. Lanes 8, 16: extract from XP3BR-SV40 clone 15 (XP-G) cell line.

DISCUSSION

Extracts from XP cells showed defective DNA repair synthesis in DNA damaged by cis- or trans-DDP. This was previously noted for an extract made from XP-A cells (3); in the present study these observations have been extended to several other XP complementation groups. Repair synthesis carried out by normal cell extracts in DNA damaged with cis-DDP or trans-DDP is principally due to removal of a fraction of frequently occurring drug adducts, rather than repair of a rare subclass of lesions (3). Therefore, the defective repair synthesis by XP cell extracts in response to cis- or trans-DDP DNA damage implies that the extracts are unable to incise both short-range DNA intrastrand cross-links (induced by cis-DDP) and DNA monoadducts or long-range intrastrand cross-links (induced by trans-DDP).

The results obtained in the cell-free system are consistent with a number of cellular studies. For example, XP-A (and XP-F) cells are known to be hypersensitive to cis-DDP DNA adducts (25, 26, 27, 28). XP-A cells are defective in reactivating SV40 DNA damaged by cis-DDP (26), and XP cells from groups A, C, and F are less efficient than normal cells in restoring expression of a marker gene after transfection of cis-DDP damaged plasmids (29). Direct evidence for deficient repair of DNA damage was recently obtained by Dijt et al., who showed that XP-A fibroblasts remove cis-DDP adducts from genomic DNA more slowly than normal fibroblasts (28). It has also been reported that XP cells have a decreased ability to remove DNA-protein cross-links induced by trans-DDP adducts from genomic DNA (30).

Studies of the interaction of purified UvrABC proteins with damaged DNA have shown that the protein complex binds at damaged sites and forms incisions on both sides of the lesions. Incision is catalyzed as a dual event at the eighth phosphodiester bond 5’ and the fourth or fifth bond 3’ to UV-induced pyrimidine dimers (31, 32), cis-DDP-induced intrastrand cross-links (24), and many other DNA adducts (20).

In the present experiments, cleavage of UV-irradiated or cis-DDP damaged DNA with UvrABC proteins allowed XP-A, C,
and also provide no evidence for a DNA repair defect associated with the syndrome. Some other metabolic abnormality might cause the hypersensitivity of DNS cells to DNA damaging agents. In the case of the carcinogen 4-nitroquinoline 1-oxide, the hypersensitivity of DNS cells has been linked to an increased metabolic activation of this compound, resulting in increased levels of DNA adducts (43, 44).

The present results show for the first time the ability of E. coli UvrABC proteins to complement the XP repair defect. There have been previous attempts to use E. coli UvrABC proteins to enhance DNA repair synthesis in XP cells. The uvrA gene was expressed in XP-A cells, but did not convert the cells to a repair-proficient state (45). Notably, careful efforts have been made to introduce active UvrA, UvrB, UvrC, and UvrD proteins into XP cells by microinjection (46). Introduction of the proteins separately or together, into either the cytoplasm or nucleus of XP-A, XP-C, or normal fibroblasts, did not stimulate DNA repair synthesis in any instance. Several possibilities for this lack of stimulation were considered, including the instability of the Uvr proteins and the possible inability of UvrABC to gain access to chromatin. Subsequent investigations have shown that the UvrC protein is particularly unstable (22, 47, 48). In the present study, we have used UvrABC in a simpler, soluble system, with purified plasmid DNA as substrate. Potential barriers caused by intracellular compartmentalization and the presence of DNA in higher-order chromatin structures are absent in the soluble extracts. Thus, a result was obtained that is exactly in concordance with cellular studies that have suggested a damage incision defect in XP (6). This demonstration of complementation of the XP DNA repair defect by purified UvrABC proteins suggests the possibility of using a similar complementation approach to isolate proteins from normal cell extracts that would allow XP extracts to incise damaged DNA.

ACKNOWLEDGEMENTS

This work was supported by the Imperial Cancer Research Fund. J.H. was partially supported by the Swedish Cancer Society. L.G.’s stay at the Imperial Cancer Research Fund was supported by the Burroughs Wellcome Travel Fund.

REFERENCES

40  Nucleic Acids Research