Human DNA Topoisomerase I-mediated Cleavages Stimulated by Ultraviolet Light-induced DNA Damage

(Received for publication, October 19, 1995, and in revised form, January 6, 1996)

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DNA topoisomerases have been proposed as the proteins involved in the formation of the DNA-protein cross-links detected after ultraviolet light (UV) irradiation of cellular DNA. This possibility has been investigated by studying the effects of UV-induced DNA damage on human DNA topoisomerase I action. UV lesions impaired the enzyme's ability to relax negatively supercoiled DNA. Decreased relaxation activity correlated with the stimulation of cleavable complexes. Accumulation of cleavable complexes resulted from blockage of the rejoining step of the cleavage-religation reaction. Mapping of cleavage sites on the pAT153 genome indicated UV-induced cleavage at discrete positions corresponding to sites stimulated also by the topoisomerase I inhibitor camptothecin, except for one. Subsequent analysis at nucleotide level within the sequence encompassing the UV-specific cleavage site revealed the precise positions of sites stimulated by camptothecin with respect to those specific for UV irradiation. Interestingly, one of the UV-stimulated cleavage sites was formed within a sequence that did not contain dimerized pyrimidines, suggesting transmission of the distortion through the enzyme's DNA strand passage cleavage/religation equilibrium. Treatment of cellular DNA with these inhibitors results in cryptic single and double strand breaks associated with enzyme's covalent attachment to DNA (reviewed in Ref. 11).

UV photoproducts cause alterations of the DNA conformation that can affect the activity of DNA processing enzymes. It has been shown that UV irradiation of the substrate inhibits digestion of recognition sequences containing dimerizable pyrimidines by restriction enzymes (12). Moreover, the catalytic reaction of bacterial DNA topoisomerase I (13, 14) and Drosophila melanogaster DNA topoisomerase II (15) is inhibited by UV damage in the target DNA. The molecular mechanism by which damage can affect the enzyme's DNA strand passage step is unknown. However, it has been proposed that the inhibitory lesions may be present in the enzyme's active site at the time of strand passage (15) or that the helical distortion induced by photodamage to DNA can slow down the diffusion of the helix through the DNA-protein bridge (13).

In an effort to verify whether eukaryotic DNA topoisomerases might be the proteins involved in the formation of UV-induced DNA-protein cross-links, the effects of short wave UV-induced photoproducts on the enzymatic activity of human DNA topoisomerase I were investigated. Our results indicate that the enzyme's relaxation reaction is inhibited by the presence of UV damage in the substrate. Reduced relaxation activity correlated with alteration of the cleavage-religation equilibrium of the reaction, resulting in the stimulation of cleavable complexes. This observation supports the notion that DNA topoisomerase I may be the protein involved in UV-induced DNA-protein cross-linking of cellular DNA.

EXPERIMENTAL PROCEDURES

Materials—Human DNA topoisomerase I, purified from HeLa cell nuclei according to Ishii et al. (16), was diluted to the appropriate concentration in DNA topoisomerase I diluent (40 mM Hepes, pH 8.0, 0.5 mM KCl, 5 mM MgCl₂, 1 mM DTT, 0.25 mM EDTA, 1 mM 2-mercaptoetanol). The incubation was performed at 37°C for 10 min. Reactions were stopped by adding 50 μl of an equal volume of 10% trichloroacetic acid. The DNA-protein complexes were collected on Millipore filters (0.45 μm) and washed at least five times with 5 ml of 5% trichloroacetic acid and further once with ethanol. The filters were air-dried and then counted for radioactivity. The reaction mixtures were also fractionated on agarose gels, and the DNA was stained with ethidium bromide to visualize bands by UV light. The assay was carried out in the presence of increasing concentrations of camptothecin.

Cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts are the most prevalent lesions produced in DNA by ultraviolet (UV) light (1). However, pyrimidine dimers are not the only photochemical effect of UV light on cellular DNA. It has been shown that UV radiation induces also the formation of DNA-protein cross-links (2-4). Proteinase K treatment abolishes the cross-linking effect and reveals the presence of cryptic DNA strand breaks. Since this cross-linking is partially repaired, it has been suggested that this non-dimer DNA damage may play an important role in the biological effect of UV radiation (2, 4). DNA topoisomerases have been proposed as possible candidates for the protein(s) involved in UV-induced DNA-protein cross-linking (4). This proposal is consistent with the formation of transient single and double strand breaks during DNA topoisomerase reactions, with covalent attachment of the enzyme to one terminus of the DNA nick (reviewed in Ref. 5).

DNA topoisomerases are ubiquitous enzymes involved in a number of crucial cellular processes, including replication, transcription, and recombination. A relationship between cellular responses to DNA damage and topoisomerases has been proposed (6–9). The catalytic cycle of DNA topoisomerases can be divided into several steps: 1) enzyme-DNA binding; 2) DNA cleavage, resulting in a covalent attachment between the protein and one terminus of the DNA nick; 3) DNA strand passage; 4) poststrand passage DNA religation concerted with the enzyme turnover (reviewed in Ref. 10). Treatment with strong protein denaturants arrests the catalytic cycle after the cleavage event by trapping the transient DNA-protein intermediate of step 2, termed “cleavable complex.” Several topoisomerase inhibitors stabilize the cleavable complex by interfering with the strand passage cleavage-religation equilibrium. Treatment of cellular DNA with these inhibitors results in cryptic single and double strand breaks associated with enzyme's covalent attachment to DNA (reviewed in Ref. 11).

UV photoproducts cause alterations of the DNA conformation that can affect the activity of DNA processing enzymes. It has been shown that UV irradiation of the substrate inhibits digestion of recognition sequences containing dimerizable pyrimidines by restriction enzymes (12). Moreover, the catalytic reaction of bacterial DNA topoisomerase I (13, 14) and Drosophila melanogaster DNA topoisomerase II (15) is inhibited by UV damage in the target DNA. The molecular mechanism by which damage can affect the enzyme's DNA strand passage step is unknown. However, it has been proposed that the inhibitory lesions may be present in the enzyme's active site at the time of strand passage (15) or that the helical distortion induced by photodamage to DNA can slow down the diffusion of the helix through the DNA-protein bridge (13).
Inhibition of Human Topoisomerase I by UV Damage

0.2 mg/ml bovine serum albumin, 2 mM dithiothreitol, 0.5 mM EDTA, pH 8.0, 40% glycerol, 6% poly(ethylene glycol)]. One unit of enzyme is defined as the amount of topoisomerase I required to relax 50% of 0.25 μg of pAT153 DNA after a 10-min incubation at 30 °C. Micrococcus luteus PD-endonuclease was obtained by purification to the CM-cellulose step according to Grafstrom et al. (17).

Each DNA labeling kit was purchased from Boehringer Mannheim (Mannheim, Germany). calf thymus intestinal alkaline phosphatase, and DNA 3'-end-labeling kit were purchased from New England Biolabs (Beverly, MA). [γ-32P]ATP and [α-32P]dATP were obtained from Amersham (Buckinghamshire, United Kingdom). Maxam and Gilbert sequencing kit was obtained from Merck (Darmstadt, Germany). Low melting Sea Plague agarose GTG was obtained from FMC BioProducts (Rockland, ME) and Gelase enzyme from Epigence Technologies Inc. (Madison, WI). Camptothecin, camptothecin, polynucleotide kinase was from New England BioLabs (Beverly, MA).

The samples were kept on ice to avoid heating and evaporation. Under the conditions employed, the band intensity in the negative of the gel photograph was directly proportional to the amount of DNA. Regression coefficients were consistently near 0.99. Each autoradiography lane was analyzed using the same reference line, and the size of each DNA fragment, induced by topoisomerase I cleavage, was determined. Fragment size determination was usually within 50 bp for a given fragment analyzed in different gels.

Localization at nucleotide level of topoisomerase I-mediated cleavage sites was obtained by linearization of pAT153 DNA with PvuII or Asp700 restriction enzymes. Protruding and blunt 3'-ends were labeled using terminal deoxynucleotidyl transferase and [α-32P]dATP according to Brash (20) and then digested with SspI or EcoRI enzymes, respectively. The two 3'-end-labeled fragments (435 and 398 bp, respectively) were purified by 5% acrylamide gel electrophoresis, followed by electrophoresis and ethanol precipitation. Mapping of the phosphodiester bonds cleaved by topoisomerase I was obtained by electrophoresis of the reaction products on sequencing gel alongside with the Maxam and Gilbert sequence ladder of the same fragment (18).

RESULTS

Effect of UV-induced Photoproducts on DNA Topoisomerase I-catalyzed DNA Relaxation—A DNA relaxation assay was uti-
lized to examine the effects of UV-induced damage in the sub-
strate on the catalytic activity of human DNA topoisomerase I. This assay measured the conversion of naturally supercoiled pAT153 DNA (RFI) into relaxed covalently closed circular DNA (RFIV). RFI and RFIV DNAs were separated from the nicked molecules (RFII) by inclusion of ethidium bromide in the agarose gel. In this condition the relaxed topoisomers migrate as a single band ahead of the RFI band allowing an accurate quan-
titation of the reaction products by densitometric scanning of gel photographs. A time course of relaxation was carried out with catalytic amount of enzyme and pAT153 DNA molecules irradiated at increasing UV doses from 0 to 1750 J/m² (Fig. 1, A and B). Under these conditions, the relaxation velocity of supercoiled DNA was linear for 20 min. Following a 20-min incubation, 0.7 unit of topoisomerase I relaxed about 65% of nonirradiated pAT153 DNA. As shown in Fig. 1 C, the initial DNA relaxation rate linearly decreased as the level of UV-dose increased. At the highest UV dose, the initial velocity was reduced to about 20%. These results indicate a dose-dependent inhibition of topoisomerase I activity by UV-induced damage.

Effect of UV-induced Photoproducts on the DNA Cleavage/Religation Equilibrium of Topoisomerase I—The catalytic cycle of topoisomerase I can be divided into several discrete steps involving DNA binding, DNA cleavage, DNA strand passage, religation of the DNA break, and enzyme turnover. The cleavage reaction involves a transient single-stranded break in the DNA backbone containing the enzyme covalently bound at the 3'-side of the cleaved strand. This transient intermediate, termed cleavable complex, can be evidenced by freezing the cleavage/religation equilibrium with strong protein denatur-
ants. In order to determine which specific reaction step(s) of the normal catalytic cycle is impaired by UV light-induced lesions in the target DNA, the effect of this damage on the DNA cleavage/religation equilibrium was examined.

Cleavage was studied by reacting 5'-end-labeled EcoRI-cut pAT153 DNA with topoisomerase I for 5 min at 37 °C; the transiently nicked enzyme-DNA intermediate was trapped as a single band ahead of the RFI band allowing an accurate quantita-
tion of the reaction products by densitometric scanning of gel photographs. A time course of relaxation was carried out with catalytic amount of enzyme and pAT153 DNA molecules irradiated at increasing UV doses from 0 to 1750 J/m² (Fig. 1, A and B). Under these conditions, the relaxation velocity of supercoiled DNA was linear for 20 min. Following a 20-min incubation, 0.7 unit of topoisomerase I relaxed about 65% of nonirradiated pAT153 DNA. As shown in Fig. 1 C, the initial DNA relaxation rate linearly decreased as the level of UV-dose increased. At the highest UV dose, the initial velocity was reduced to about 20%. These results indicate a dose-dependent inhibition of topoisomerase I activity by UV-induced damage.

Cleavage was studied by reacting 5'-end-labeled EcoRI-cut pAT153 DNA with topoisomerase I for 5 min at 37 °C; the transiently nicked enzyme-DNA intermediate was trapped by addition of SDS and digestion with proteinase K followed by separation of reaction products on alkaline agarose gel and visualization by autoradiography. Cleavable complex stimulation was evaluated by measuring the decrease of full-length linear molecules as a function of UV dose. As shown in the gel photograph (Fig. 2A), there is a continuous decrease of uncleaved molecules with the increase of UV irradiation. As summarized in the graph (Fig. 2B), where results were plotted as residual uncleaved DNA versus UV dose, there is linear dependence of global cleavage with increasing amount of photoproducts. This finding clearly indicates that the presence of UV-induced DNA lesions affects the DNA cleavage/religation equilibrium by causing the accumulation of cleavable complexes. This effect is analogous to that described for the specific topoisomerase I inhibitor camptothecin (CPT) (22), and for
mismatches adjacent to a topoisomerase I cleavage site (23).

Genomic Localization of Topoisomerase I-mediated DNA Breaks Stimulated by UV Photoproducts in Plasmid pAT153 DNA—Eukaryotic topoisomerase I-mediated cleavages are nonrandomly introduced in the DNA helix. Cleavage sites are characterized by a weak sequence-specificity (24, 25) and a loose conformational consensus (26) with preference for curved DNA, be it stably (27, 28) or dynamically bent (29). Although CPT has only a minimal effect on the sequence selectivity of the enzyme (reviewed in Refs. 30 and 31), the degree of stimulation differs among sites (32). Experimental and modeling studies have shown that UV damage is characterized by a small but significant deformation of the DNA double helix, which can affect protein-DNA interaction (12). Thus, it is possible to envisage that the modifications of the helical parameters produced by UV photoproducts may interfere with the sequence specificity of topoisomerase I-mediated cleavage. Therefore, it is of interest to study topoisomerase I cleavage specificity on UV-irradiated DNA and to compare this specificity with that described for CPT.

![Fig. 2. Topoisomerase I-mediated cleavable complexes stimulation by UV-induced photoproduc.ts. BamHI-cut duplex pAT153 DNA (30 ng), 32P-end-labeled at the 5' termini, was reacted with 250 units of topoisomerase I (lanes 2–7), treated with SDS-proteinase K and analyzed on alkaline agarose gel. In A is shown the autoradiogram of a gel containing the full-length molecules. Lane 1, DNA alone; lane 2, topoisomerase I and unirradiated DNA; lanes 3–7, topoisomerase I and DNA irradiated at 440, 880, 1320, 1750, and 2200 J/m², respectively. B illustrates the results plotted as residual uncleaved DNA versus UV dose on the basis of densitometric analysis of autoradiograms from three independent experiments. Error bars represent the standard errors. Assay conditions and quantitation of uncleaved DNA were described under "Experimental Procedures."](http://www.jbc.org/)

![Fig. 3. Localization of topoisomerase I-mediated DNA cleavages stimulated by UV damage on pAT153 genome. A, the BamHI/SalI pAT153 restriction fragment (3382 bp), uniquely 5'-end-labeled at the BamHI site, was reacted with topoisomerase I and analyzed as in the legend of Fig. 2. The autoradiogram of a typical gel is shown. Lane 1, DNA alone; lane 2, DNA and topoisomerase I in the presence of 2.5 μM CPT; lane 3, DNA and topoisomerase I and DNA irradiated at 440, 880, 1320, 1750, and 2200 J/m², respectively. The positions and sizes of coelectrophoresed marker DNAs and the corresponding genomic positions (in base pairs) are shown on the left. The site uniquely stimulated by UV photodamage is indicated by an arrow. B shows the approximate genomic positions of topoisomerase I-mediated DNA cleavage sites on the amp' coding strand of plasmid DNA, obtained by computer analysis of six independent experiments using BamHI/SalI and ScaI/Asp700 restriction fragments. Bold indicates the map position of the break site uniquely stimulated by UV photoproducts.](http://www.jbc.org/)
The EcoRI/PstI restriction fragment (752 bp, genomic positions 3655–2903), spanning the region containing the uniquely UV-stimulated site, 5'-end-labeled at the EcoRI terminus (16 ng), was reacted with 100 units of topoisomerase I after irradiation at increasing UV doses or in the presence of increasing amount of CPT. Reaction products were processed and analyzed on acrylamide gel as described under "Experimental Procedures." Lane 1, DNA alone; lanes 2 and 7, DNA and topoisomerase I; lanes 3–6, DNA and topoisomerase I with 0.75, 1.25, 2.50, and 5.0 μM CPT, respectively; lanes 8–11, topoisomerase I and DNA irradiated at 880, 1320, 1750, and 2200 J/m², respectively; lane 12, UV-irradiated DNA (220 J/m²) digested with PD-endonuclease as described under "Experimental Procedures." Numbers on the left side correspond to the approximate genomic positions (in base pairs) in the plasmid sequence obtained with appropriate size markers. The lower-case letters and the bars on the right indicate the positions of topoisomerase I-mediated cleavages stimulated: by CPT and UV damage (a), by CPT only (b), and uniquely by photodamage (c).

The cleavage pattern of the labeled strand irradiated at increasing UV doses is shown. UV-stimulated topoisomerase I-mediated cleavages appeared nonrandomly distributed. Damage-induced bands (lanes 4–8) corresponded to breaks at pre-existing topoisomerase I sites (lane 3) or at CPT-induced sites (lane 2) except for one site (indicated by arrow).

Using two different uniquely 5'-end-labeled fragments, (5'- BamHl/Sall and 5'-Sal/Asp700), genomic localization of cleavage sites was obtained. The position of these sites on the pAT153 map was determined by densitometric scanning of the autoradiograms and computer analysis of six independent experiments with an average standard error of ± 25 nucleotides (Fig. 3B). The break site uniquely stimulated by UV damage (Fig. 3A, arrow) was at position 3134 (±11 bp) of the plasmid map. The same analysis performed on the complementary strand did not reveal any breakage site stimulated only by UV damage (data not shown).

Higher Resolution Analysis of Cleavage Site Specificity—The influence of UV damage on the cleavage properties of human topoisomerase I was further characterized by analysis, on denaturing acrylamide gel, of the break sites previously mapped within the region encompassing the site preferentially stimulated by UV photolesions. The position of UV-induced breakages was compared with the position of breaks stimulated by CPT and with CPDs distribution, obtained by running in parallel the same UV-irradiated fragment digested with the cyclobutane dimer-specific endonuclease from M. luteus (21).

The 752-bp EcoRI/PstI fragment (5'-end-labeled at the EcoRI restriction site) was incubated with topoisomerase I in the presence of increasing amount of CPT or after irradiation at increasing UV dose (Fig. 4). Cleavages with the enzyme alone are limited (lanes 2 and 7), while several additional cleavage sites were visible on UV-irradiated DNA (lanes 8–11) or in the presence of CPT (from 0.75 to 5.0 μM, lanes 3–6). Cleavage stimulation by CPT appeared concentration-dependent (lanes 3–6). No major change in band intensities and in cleavage pattern could be observed as a consequence of increase in UV irradiation (from 880 up to 2200 J/m², lanes 8–11).

Breakage sites within this fragment were tentatively classified into three categories according to the response elicited by UV or CPT. Topoisomerase I cleavages at a sites were stimulated by UV damage and by CPT, those at b sites only by CPT, and those at c sites specifically by UV irradiation of the target DNA. Among the c sites, the c1 site corresponded to the uniquely UV-stimulated site identified by alkaline agarose gel electrophoresis. Interestingly, c3 and c4 sites were formed at some distance from the major CPDs clusters (lane 12). To examine whether c sites were UV-specific, we increased the molar ratio of enzyme to DNA. We found that a 3-fold increase in the molar ratio of enzyme to DNA resulted in very weak breakage at c1 and c2 sites on nonirradiated DNA. These sites appeared slightly stimulated also by CPT. No effect was observed at the c3 and c4 sites even with more enzyme (data not shown).
The cleavage sites stimulated by UV damage (c and a sites) were mapped at nucleotide level by running the reaction products on sequencing gels in parallel with Maxam-Gilbert chemical degradation reactions and PDE-endo nuclease digestion products of the same end-labeled fragment. The Sphi-PvuI restriction fragment (3'-end-labeled at the PvuI restriction site) was used to characterize c1 and c2 sites and the EcoRI-XmnI fragment (3'-end-labeled at the XmnI restriction site) to study c3 and c4 sites.

In Fig. 5, the portion of the gel containing the topoisomerase I cleavage products at the a1, a2, and c2 sites, obtained after stimulation with UV damage (lane 6), with CPT (lane 7) or with CPT on an irradiated substrate (lane 5), is shown. Control experiments, in which DNA was irradiated and topoisomerase I was omitted (lane 3), excluded the possibility that cleavages were caused by UV light. The two a sites, previously identified as single bands (Fig. 4, lanes 8–11), appeared to be flanked by two b sites that were named b1 and b2. Interestingly, cleavage intensity at a1, a2, b1, and b2 sites was markedly suppressed, when CPT stimulation was performed on an irradiated substrate (compare lanes 5 and 7). Inspection of the nucleotide sequences at the 5' terminus of the topoisomerase I cleavages showed the presence of CPDs on the irradiated substrate (lane 1). This effect was not detected for other b sites that were instead located within sequences that did not contain photodamage in the scissible strand, suggesting that the presence of CPDs in the sequences adjacent to the break sites can interfere with CPT action (data not shown).

Fig. 6 summarizes the nucleotide sequences containing the UV-stimulated sites, the positions of the UV-stimulated topoisomerase I-mediated cleavage sites with respect to the distribution of CPDs and (6-4) photoproducts and the relative frequency of CPDs. Comparisons of the base sequences upstream and downstream from the UV-stimulated sites has not revealed any apparent specific elements that could explain the stabilization of cleavable complexes observed in the presence of UV damage. Analysis of the relative distribution of UV lesions on the scissile strand indicate a difference between a and c sites. All three a sites were positioned at the 5' side of pyrimidines runs that have an high probability to dimerize. With the exception of the c2 site, the c sites were formed within sequences that have a very low probability to contain damage in the scissile strand. This is particularly evident for the c3 site located at least 10 bases from two dimerized thymines.

Dissociation Kinetics of Enzyme-DNA Complexes by Heating—Cleavable complexes stimulation by camptothecin has been shown to be reversed by several treatments such as elevated temperature (33, 34). To investigate the mechanism of topoisomerase I breakage stimulated by UV photodamage, the stability of preformed UV- or CPT-induced cleavable complexes was measured. The dissociation kinetics were followed by heating the reaction mixtures at 65 °C before treating samples with SDS. The electrophoretic analysis of the resulting DNA fragments is shown in Fig. 7A. It is clear that the UV- and CPT-stimulated cleavable complexes showed widely different sensitivities to heating. The enzyme-DNA complexes formed in the presence of CPT (a and b sites) dissociated very rapidly, while complexes formed after UV irradiation (a and c sites) decayed more slowly, with broken complexes still detectable after 15 min of heating. To quantify the differences, the residual cleavage, i.e. the cleavage frequency at a given time normalized to the cleavage frequency obtained in a sample from the same experimental series subjected to SDS immediately before heating, was plotted as a function of the incubation time at 65 °C (Fig. 7B). The residual breakage at the CPT-stimulated a and b sites (shaded area) was reduced to less than 1% after 1 min at 65 °C and cleavage was not detected at later times. In contrast, the complexes formed at UV-stimulated sites decayed...
more slowly and remained incomplete.

It is noteworthy that the UV-stimulated break sites appeared to have two dissociation rates; an initial rapid rate in the first min of incubation was followed by a very slow rate. The basis for the discontinuity in the decay curves is unclear but it might involve a rapid inactivation of the enzyme during the kinetic analysis or an inability to complete break religation. In the latter case, it might depend from the position of photodamage with respect to the break sites. In fact, the irradiated substrate is constituted by an heterogeneous population with respect to the position where damage is created. Thus, in the fraction of molecules, where damage was present at some distance from cleavage sites, resealing may occur at a much higher rate (initial rate), while in the fraction of molecules, where photodamage were formed close to the break sites, religation may be very slow or even prevented (slow rate). The latter explanation is supported by the observation that among the complexes stimulated by UV damage, there is a good correlation between the religation rate and the relative position with respect to damage. For example, the a1 and a2 sites, located in the scissile strand close to a run of thymines with high probability to dimerize, decayed very slowly. The c3 site, formed within a sequence without dimers, appeared instead to be reclosed more rapidly and to a greater extent.

**DISCUSSION**

Cyclobutane pyrimidine dimers and (6–4) photoproducts, the most common DNA damage induced by exposing DNA to short wave UV radiation (1), produce a small but significant local distortion of the double helix that can interfere with the proper functioning of enzymes acting on DNA, such as restriction endonucleases (12) and DNA topoisomerases. We have reported previously the inhibition of prokaryotic DNA topoisomerase I catalysis by UV damage in the target substrate (13, 14). Analogous effect has been described for D. melanogaster DNA topoisomerase II (15). In the present study we report the consequences of UV damage on the activity of human DNA topoisomerase I.

Our results indicate that the presence of UV photodamage in the irradiated substrate inhibited topoisomerase I action under steady state conditions. As determined by DNA relaxation assay, the initial rate of topoisomerase I catalysis decreased by approximately 50% when 40 CPDs were present per plasmid pAT153 molecule. This level of inhibition was lower than that previously reported for the prokaryotic type I topoisomerase from M. luteus (13) and eukaryotic type II from D. melanogaster (15).

Decreased relaxation activity by UV photodamage correlated with an interference of the enzyme's cleavage/religation equilibrium resulting in the stabilization of the cleavable complex. Cleavable complexes stimulation, measured as induction of single strand breaks in linear substrates, increased linearly with the dose. UV-dependent enhancement of topoisomerase I-mediated breakages appeared to be due to a reduction in the closure rate of the broken complexes. Thus, as established for

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**Fig. 7.** Dissociation kinetics of cleavable complexes. Two parallel reactions (120 µl), containing EcoRI/PstI restriction fragment (112 ng), 5'-end-labeled at the EcoRI site, were incubated with 672 units of topoisomerase I at 37°C for 5 min after irradiation with 1750 J/m² or in the presence of 2.5 µM CPT. The reaction mixture was then heated to 65°C, and aliquots (20 µl) were withdrawn at various times after the treatment. Reaction products were processed and analyzed by denaturing acrylamide gel electrophoresis as described under "Experimental Procedures." A, lanes 2–7, samples containing UV-irradiated DNA withdrawn at 0, 0.5, 1, 5, 10, and 15 min, respectively; lanes 8–13, samples treated with CPT withdrawn at the same times as lanes 2–7. In lane 1 is shown a control sample of DNA treated with topoisomerase I and processed after 5 min at 37°C. B, in each of the experimental series, the cleavage frequency of the indicated cleavage sites at any point were expressed as a percentage of the cleavage frequency in the sample taken before heating. This percentage was plotted against the time of sampling. The quantification is based on densitometric scanning of the autoradiogram shown in A. Residual cleavage frequency at a (dashed lines) and c (continuous lines) sites in the presence of UV damage; shaded area, residual cleavage frequency at a and b sites in the presence of CPT.
Camptothecin, the molecular mechanism by which UV damage inhibited DNA topoisomerase I catalytic activity seems to depend on its effects on the religation step. However, we cannot exclude the possibility that the presence of photolesions in the passing strand at the time of strand passage may slow down strand diffusion through the DNA-protein bridge.

Several authors have demonstrated that DNA topoisomerase I acts at preferred sequences lacking a clear consensus and that camptothecin has only minor effects on the enzyme's nucleotide specificity (30, 31). Initial low resolution mapping of UV-stimulated cleavage sites on the pAT153 genome did not reveal major differences with respect to those stimulated by camptothecin except for one position in the promoter of the amp" gene. Subsequent localization at nucleotide level of the breakage sites in the region encompassing the uniquely UV-stimulated site and in the neighboring sequences was carried out and a comparison between the UV-stimulated sites with those induced by camptothecin was done. Some sites were uniquely stimulated by UV damage (c sites) and appeared at some distance from CPT-stimulated sites except for one (c4 site). Most CPT-stimulated breakages were not stimulated also by the presence of photoproducts (b sites) except for few sites (a sites). The relative position of b sites with respect to CPDs distribution did not reveal any special features that could give some clues to understanding why they were not stimulated also by UV damage. One exception is offered by the two b sites flanking sites a1 and a2 where breakages at both sites were within a thymines run that dimerized with high efficiency. In this case, it is possible to speculate that the cyclobutane bond between the two thymines flanking the break at the b sites may affect either the cleavage and/or the binding of the enzyme to the substrate. This possibility is supported by the observation that cleavage stimulation by CPT on an UV-irradiated substrate was severely reduced at these two sites, while it was not affected at b sites formed at sequences without dimers in the scissible strand. Also the neighbor a1 and a2 sites showed a marked reduction in cutting frequency when cleavage was examined in the above condition, suggesting that UV damage stimulation was predominant at these sites and that CPT-stimulated cleavage was effective only on the fraction of molecules without damage in the flanking sequences. However, an alternative explanation to this latter observation can be formulated based on the model of the camptothecin-topoisomerase I-DNA ternary complexes proposed by Pommier (reviewed in Ref. 31). According to this model, the planar ring of camptothecin should stick with the base at the 5'-terminus of the DNA breaks within the cavity formed at the topoisomerase I cleavage sites. Thus, the presence of UV lesions on the 5'-terminus of the breaks produces conformational deformations that may alter the camptothecin receptor sites and consequently affect the action of the drug.

Numerous studies have shown that steric factors determine the interaction of topoisomerase I active site with DNA (35-37). Cleaved sites are characterized by a set of distinct local helical parameters (twisting) (26, 38), and cleavage efficiency is modulated by stable or dynamic curvature of the DNA molecule (writhing) (28, 38, 39). Evidences that photodimers cause alterations in DNA structure are offered by numerous physicochemical, biochemical, and modeling studies. Measurements of the shift of phased A-tract multimers containing site-specific CPDs (40) and of changes in band pattern of UV-irradiated topoisomers (41, 42) have shown that CPDs cause a topological unwinding due to a combination of actual duplex unwinding (twisting) and negative supercoiling (writhing) resulting from bending of DNA (40). Thus, we hypothesize that the alteration in the twist and writh consequent to damage formation can determine changes in the structural context that may either dislocate the enzyme-DNA interacting sites of few bases (c2 and c4 site) or drive into the optimal conformation for topoisomerase I activity sequences normally poorly (c1 site) or not recognized by the enzyme (c3 site). This effect may resemble that described in supercoiled DNA, where the activation of new and active sites results in the DNA conformational transition in this respect the c3 site, formed in a sequence with very low content of pyrimidine dimers in the strands surrounding the cleavage site, is of special interest. In fact, if the above hypothesis is correct, at least in this case one has to assume that CPD conformational changes are not only localized at the damaged pyrimidines but may be propagated into neighboring sequences as postulated by Pearlman et al. (43).

It is well established in the literature (1) and visible in Fig. 6 that the frequency of dimer formation varies at different potential dimer sites. Therefore, at the UV dose employed in the mapping experiments (Fig. 5), the irradiated fragments consist of an heterogeneous population of molecules with respect to UV damage frequency and position. Because of that, it is quite difficult to assess which of the lesions, that can be formed in the sequences surrounding the UV-stimulated sites, is effective in stimulating cleavage. In this respect, it cannot be excluded the possibility that also UV photodamage present on the complementary strand can determine a structural variation that may be reflected on the scissible strand.

Although camptothecin does not seem to change significantly the breakage specificity of the enzyme in vitro, it has a greater stabilizing effect on some breakage sites than on others (reviewed in Refs. 30 and 31). The enhancement breakage factor inversely correlates with the reclosure rate (32). UV-induced cleavage appeared to have different characteristics. The enhancement factor did not seem to vary significantly from one site to another. In addition, cleavage frequency at the sites identified in the EcoRI/PstI pAT153 fragment did not seem to be UV dose-dependent. Nevertheless, from the dose-response curve of overall cleavages, measured with the full-length DNA, there was a linear increase of cleavable complexes stimulation up to a dose that introduced approximately 100 CPDs per pAT153 molecule. This contradiction may be explained by assuming that as the UV dose increases damage is formed also at less probable positions. This results in additional deformation of DNA structure that activates many (potential) cleavable sites. However, each of them, being formed in low amount, is cleaved with a very low frequency so that they cannot be seen as discrete bands but as an increased background (Fig. 3). Furthermore, the religation rate, reduced with respect to that measured with CPT, did not correlate with the extent of cleavage but rather with the position with respect to UV photodimers. When CPDs were present within the sequence surrounding the cleavage site, the religation step afterwards was very slow. This case is particularly evident for the a1 and a2 sites that are flanked on the 5' side of the break site by runs of thymines with an high probability to dimerize. These sites showed a much lower religation rate when stimulated by UV with respect to CPT stimulation. This type of kinetic can be explained in terms of the extent of misalignment that the two ends at the site of the nick have acquired as result of the helical distortion imposed by the damage. Analogous explanation has been proposed for topoisomerase I cleavage at a mismatch when present in one enzyme's recognition sequence (23).

The UV doses employed in this study exceed the level of radiation to which cells commonly are exposed. However, the presence of UV lesions in critically located topoisomerase I recognition sites could have physiological consequences.

DNA topoisomerases have been proposed as the enzymes...
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responsible for sister chromatid exchange (44, 45) and chromosomal aberrations (46). Several lines of evidence suggest a role for eukaryotic DNA topoisomerases in mediating “illegitimate recombination” of genetic material (reviewed in Ref. 47). The mechanistic link derives from the capacity of topoisomerase interactive drugs to stimulate these forms of genotoxicity (Ref. 48 and reviewed in Refs. 5 and 46). The biochemical basis for such a function comes from the ability of topoisomerases to mediate cleavage and religation in two half-reactions separated by the cleavable complex that gives the enzymes the capacity to catalyze intra- and intermolecular DNA transfer reactions (36, 49). Moreover, it has been speculated that also the generation of chromosomal rearrangements by DNA damage could derive from their effects on topoisomerases (44, 50). Distortion of the DNA structure in the vicinity of unrepaired DNA damage might be sufficient stimulus to alter the correct function of DNA topoisomerase I by stabilizing the DNA-protein intermediate necessary for strand exchange, supports the notion that UV-induced chromosome rearrangements. Conversely, our finding that UV damage interfered with the activity of DNA topoisomerase I-mediated cross-links and consequently responsible for UV-induced chromosome rearrangements. Conversely, our finding that UV damage interfered with the activity of DNA topoisomerase I by stabilizing the DNA-protein intermediate necessary for strand exchange, supports the notion that UV-stimulated DNA-protein cross-links may be mediated by topoisomerase I and that this cross-linking may account for at least part of the chromosomal rearrangements induced by UV light.

Acknowledgment—We thank Dr. G. Caprano for critical reading of the manuscript.

REFERENCES

1. Sage, E. (1993) Photochem. Photobiol. 57, 163–174
2. Fornace, A. J., Jr., and Kohn, K. W. (1976) Biochim. Biophys. Acta 435, 95–103
3. Fornace, A. J., Jr., Kohn, K. W., and Kann, H. J. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 39–43
4. Rosenorn, B. S., and Lai, L. (1991) in Photobiology (Riklis, E., ed) pp. 27–34, Plenum Press, New York
5. Liu, L. F. (1989) Annu. Rev. Biochem. 58, 351–375
6. Downes, C. S., and Johnson, R. T. (1988) BioEssays 8, 179–184
7. Kaufmann, W. K. (1989) Carcinogenesis 10, 1–11
8. Stevens, T., and Bohr, V. A. (1939) Carcinogenesis 14, 1841–1850
9. Thébelin, H. W., Popanda, O., Gersbach, H., and Gilberg, F. (1939) Carcinogenesis 14, 2341–2351
10. Maxwell, A., and Gelse, M. (1986) Adv. Protein Chem. 38, 69–107
11. Liu, L. F. (1990) in DNA Topology and Its Biological Functions (Cazzarelli, N. R., and Wang, J. C., eds) pp. 371–389, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
12. Cleaver, J. E. (1983) J. Mol. Biol. 170, 305–317
13. Pedrini, A. M., and Ciarrocchi, G. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 1787–1791
14. Pedrini, A. M. (1984) in Proteins Involved in DNA Replication (Hubshuber, U., and Spadari, S., eds) pp. 449–454, Plenum Press, New York
15. Corbett, A. H., Zehiedrich, E. L., Lloyd, R. S., and Osherson, N. (1991) J. Biol. Chem. 266, 19666–19671
16. Ishi, K., Hasegawa, T., Fujisawa, K., and Andoh, T. (1983) J. Biol. Chem. 258, 12726–12732
17. Graffstrom, R. H., Park, L., and Grossman, L. (1982) J. Biol. Chem. 257, 13465–13474
18. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) in Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
19. Roddoff, C., Lanza, A., Tornatafli, and Pedrini, A. M. (1994) Nucileic Acids Res. 22, 314–320
20. Brash, D. E. (1988) in DNA Repair: A Laboratory Manual of Research Procedures (Friedberg, E. C. and Hanawalt, P. C., eds) pp. 327–345, Marcel Dekker, Inc., New York
21. Haseltine, W. A., Gordon, L. K., Lindan, C. P., Graffstrom, R. H., Shaper, N. L., and Grossman, L. (1980) Nature 285, 634–641
22. Hsiangi, Y. H., Hertzberg, R., Hecht, S., and Liu, L. F. (1985) J. Biol. Chem. 260, 14873–14876
23. Yeh, Y. C., Liu, H. F., Ellis, C. A., and Lu, A. L. (1994) J. Biol. Chem. 269, 15498–15504
24. Edwards, K. A., Halligan, B. D., Davis, J. L., Nivera, N. L., and Liu, L. F. (1982) Nucleic Acids Res. 10, 2565–2576
25. Been, M. D., and Champoux, J. J. (1984) J. Biol. Chem. 259, 515–531
26. Shen, C. C., and Shen, C. K. (1990) J. Mol. Biol. 212, 67–78
27. Caserta, M., Amadici, A., and Camilloni, G. (1989) Nucleic Acids Res. 17, 8463–8474
28. Krogh, S., Mortensen, U. H., Westergaard, O., and Bonven, B. J. (1991) Nucleic Acids Res. 19, 1235–1241
29. Camilloni, G., Di Martino, E., Di Mauro, E., and Caserta, M. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 3080–3084
30. Champoux, J. J. (1994) Adv. Pharmacol. 29A, 71–82
31. Pommier, Y., Tanizawa, A., and Kohn, K. W. (1994) Adv. Pharmacol. 29B, 73–91
32. Porter, S. E., and Champoux, J. J. (1989) Nucleic Acids Res. 17, 8521–8532
33. Hsiangi, Y. H., and Liu, L. F. (1988) Cancer Res. 48, 1722–1726
34. Cederoni, S., Paparelli, M., and Gianfranceschi, G. L. (1993) Mol. Cell. Biol. 17, 129–134
35. Champoux, J. J. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 3800–3804
36. Andersen, A. M., Portnoy, J. Q., and Westergaard, O. (1994) Adv. Pharmacol. 29A, 83–101
37. Caserta, M., Camilloni, G., Venditti, S., Venditti, P., and Di Mauro, E. (1994) J. Cell. Biochem. 55, 93–97
38. Camilloni, G., Caserta, M., Amadici, A., and Di Mauro, E. (1991) Biochim. Biophys. Acta 1129, 73–82
39. Perini, R., Caserta, M., and Di Mauro, E. (1993) J. Mol. Biol. 213, 634–645
40. Wang, C. L., and Taylor, J. S. (1993) Proc. Natl. Acad. Sci. U. S. A. 88, 9072–9076
41. Ciarrocchi, G., and Pedrini, A. M. (1982) J. Mol. Biol. 155, 177–183
42. Pedrini, A. M., Tornatali, S., Menichini, P., and Abdabando, A. (1986) in Mechanisms of DNA Damage and Repair (Simic, M. G., Grossman, L., and Upton, A. C., eds) pp. 295–301, Plenum Press, New York
43. Pearlman, D. A., Holbrook, S. R., Pirke, D. H., and Kim, S. H. (1985) Science 227, 1304–1308
44. Cleaver, J. E. (1981) Exp. Cell Res. 136, 27–30
45. Dillehay, L. E., Jacobson, K. D., and Williams, J. R. (1989) Mutat. Res. 215, 15–23
46. Anderson, R. D., and Berger, N. A. (1994) Mutat. Res. 309, 109–142
47. Ikeda, H. (1994) Adv. Pharmacol. 29A, 147–165
48. Degrassi, F., De Salvia, R., Tannarella, C., and Palitti, F. (1989) Mutat. Res. 211, 125–130
49. Christiansen, K. F., Svejstrup, A. B., Andersen, A. H., and Westergaard, O. (1993) J. Biol. Chem. 268, 9690–9701
50. Holden, H. E., Baret, J. F., Huntington, C. M., Muehlbauer, P. A., and Wahrenburg, M. G. (1989) Environ. Mol. Mutagen. 13, 238–252
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J. Biol. Chem. 1996, 271:6978-6986.
doi: 10.1074/jbc.271.12.6978

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