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ACTIVATION OF POLY(ADENOSINE DIPHOSPHATE RIBOSE) POLYMERASE WITH UV IRRADIATED AND UV ENDONUCLEASE TREATED SV 40 MINICHROMOSOME

Jonathan J. Cohen and Nathan A. Berger

The Hematology/Oncology Division of The Department of Medicine,
Washington University School of Medicine at
The Jewish Hospital of St. Louis
216 South Kingshighway
St. Louis, MO 63110

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SUMMARY: SV 40 minichromosomes were used as a molecular model of eukaryotic chromatin to probe the nature of the lesion responsible for UV stimulation of poly (ADPR) polymerase. UV irradiation of the minichromosomes with doses between 50 and 1000 J/m^2 did not increase their ability to stimulate the activity of purified poly(ADPR) polymerase. In contrast, when the minichromosomes were UV irradiated and then treated with M. luteus UV endonuclease, there was a marked increase in their ability to stimulate poly(ADPR) polymerase. This stimulation was completely suppressed when histone H1 was added to the poly(ADPR) polymerase assay. These studies demonstrate in a purified in vitro system that damage caused by UV irradiation alone is not sufficient to stimulate poly(ADPR) polymerase activity. Only when DNA is nicked at the site of UV damage by UV endonuclease is there stimulation of poly(ADPR) polymerase.

INTRODUCTION: Poly(ADPR) polymerase[1] is a chromosomal enzyme that catalyzes the polymerization of ADP-Ribose moieties derived from NAD$^+$ (1,2). Many studies now indicate that this enzyme has a role in recognition and repair of DNA damage (3-10). It was recently demonstrated that UV irradiation stimulates the activity of poly(ADPR) polymerase in human lymphocytes but not in cells from patients with xeroderma pigmentosum (XP) (10). The failure of UV irradiation to stimulate poly (ADPR) synthesis in the XP cells has been attributed to their inability to initiate DNA strand breaks at the sites of UV damage (10,11). However, no well defined in vitro models have been used to clearly show whether UV damage or breakage of the DNA at the site of the UV damage is responsible for poly(ADPR) polymerase stimulation. In this paper, we report the use of the SV 40 minichromosome reconstituted with the appropriate purified components to provide a precisely de-

Abbreviations used: ADPR, adenosine diphosphate ribose; XP, xeroderma pigmentosum; UV, ultraviolet; SDS, sodium dodecyl sulfate; DTT, dithiothreitol.

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fined in vitro system with which to investigate the aspect of UV induced DNA damage responsible for stimulating poly(ADPR) synthesis.

METHODS: [Adenine-U-^{14}C]NAD^+ was purchased from New England Nuclear, Boston. NAD^+ and highly polymerized calf thymus DNA type I were purchased from Sigma, St. Louis. Histone H1 was obtained from Boehringer Mannheim, Indianapolis. Poly (ADPR) polymerase was purified from sheep thymus to apparent homogeneity on SDS polyacrylamide gels by a modification of the method of Yoshihara (12,13). The purified sheep thymus enzyme has an apparent molecular weight of 135,000 as determined by SDS polyacrylamide gel electrophoresis; it is free of DNA and is absolutely dependent on added DNA for its activity (13). M. luteus UV endonuclease purified through the Sephadex G-75 stage was a gift of Dr. L. Grossman, Johns Hopkins University School of Hygiene and Public Health (14). SV 40 was propagated in African green monkey kidney cells and purified by the Khoury and Lai technique (15). SV 40 minichromosomes were prepared from purified virus by the method of Beard (16) using 0.15 M NaCl in the sucrose gradients. The protein components of the SV 40 minichromosomes were analyzed by two different gel electrophoresis systems including acid urea 15% polyacrylamide gels and SDS 5% polyacrylamide gels and were shown to contain the typical components of SV 40 minichromosomes (17) including viral proteins 1, 2, and 3, and histones H2A, H2B, H3, and H4. Histone H1 was completely absent from these preparations, a finding which is also typical for SV 40 minichromosomes (17). Electron microscopy showed the typical circular beaded nucleosomal appearance of the SV 40 minichromosomes (17).

Purified minichromosomes were dialyzed overnight against 10 mM Tris/HCl (pH 7.9), 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 0.5 mM dithiothreitol (DTT), and 50 mM KCl. They were then UV irradiated as a 250 µl droplet in open plastic petri dishes with a General Electric 15 watt germicidal tube (principal irradiation 254 nm) at an incident dose of 1.2 J/(m^2-s) to a total dose of 50, 100, 500, or 1000 J/m^2, or mock irradiated with the UV lights turned off. For treatment with UV endonuclease, 20 units of M. luteus UV endonuclease (14) (1.5 units/µl in 100 mM potassium phosphate (pH 7.6), 5 mM 2-mercaptoethanol, 5 mM EDTA, 10% glycerol) were added to 120 µl of irradiated or control minichromosomes (4 µg of DNA) and incubated for 1 h at 37°C. Non-UV endonuclease treated minichromosomes were treated in parallel with the same buffer and incubation conditions.

Poly(ADPR) polymerase activity was assayed in a reaction mixture containing 82 mM Tris/HCl (pH 8.0), 0.42 mM [adenine-U-^{14}C]NAD^+ (4.3 dpm/pmol), 8 mM $MgCl_2$, 0.8 mM DDT, 9.2 µg of histone H1 (where indicated), 30 µl of appropriately treated SV 40 minichromosome (1 µg of DNA) or 10 µl highly polymerized calf thymus DNA (7 µg of DNA), and 30 µl of purified poly(ADPR) polymerase containing 0.25 units of enzyme activity (one unit = 1 nM ADP-Ribose incorporated/min) for a total reaction volume of 120 µl. The reaction mixtures were incubated at 37°C for 5 min, and the reactions were terminated by the addition of 2 ml of ice-cold 20% trichloroacetic acid (TCA). The acid insoluble material was collected on Whatman GF/C glass fiber filters and washed 6 times with 20% TCA and 3 times with 95% ethanol. The filters were dried and counted in toluene-PPO-POPOP scintillation fluid with a Tracor Mark III scintillation spectrometer (18).

Two percent agarose gels were made according to the method of Tegtmeyer and Macasat (19). Samples were adjusted to contain 1% SDS, 1% 2-mercaptoethanol and allowed to incubate at 37°C for 1 h. The gel buffer was 36 mM Tris/HCl (pH 7.5), 30 mM NaH_2PO_4, 1 mM EDTA. After completion of the electrophoresis, the gels were stained in 1 µg/ml ethidium brominde for 30 min and photographed with Polaroid type 55 P/N film illuminated by a long wave ultraviolet light source. DNA was measured by the fluorometric diaminobenzoic acid method described by Setaro and Morley (20).

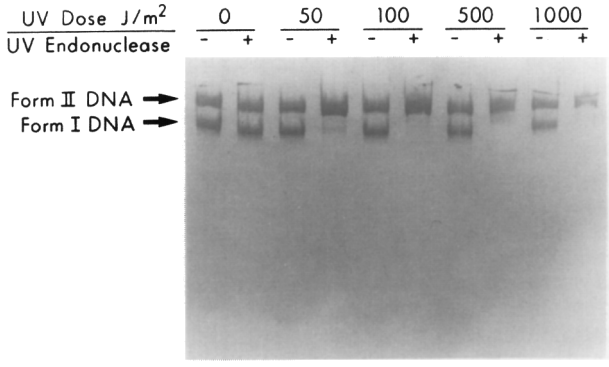

FIGURE 1: SV 40 minichromosomes were treated with either UV irradiation alone or UV irradiation and UV endonuclease as described in Methods. 30 µl (1 µg of DNA) was then adjusted to contain 1% SDS and 1% 2-mercaptoehtanol and incubated at 37°C for 1 h as described in Methods. The samples were then electrophoresed for 5 h at 40 volts in a 2% agarose gel. Bands of form I DNA (supercoiled) and for II DNA (nicked circular) are shown with arrows. The treatments with UV irradiation and the subsequent incubations with and without UV endonuclease are indicated. Note that the form I DNA bands are lost in the 500 and 1000 J/m² irradiated minichromosomes only when they are subsequently treated with UV endonuclease.

RESULTS: The DNA in SV 40 minichromosome preparations exists in two forms: form I DNA, or supercoiled, and form II DNA, or relaxed circles with at least one single strand nick (Fig 1) (19). No linear forms were seen on the gel, although they can be clearly found by this technique when both strands of the minichromosome are cut by the restriction enconuclease Eco Rl. When the minichromosomes were treated with increasing doses of UV irradiation from 50 to 1000 J/m², there was no change in the gel pattern or distribution of the species of DNA present. This shows that UV irradiation did not break the minichromosomes and furthermore that none of the proteins present in the minichromosomes were capable of nicking them at the sites of UV induced DNA damage. When the UV endonuclease was added to the unirradiated minichromosomes there was no change in the DNA distribution demonstrating that the UV endonuclease had no effect in the absence of UV irradiation. However, when UV irradiated minichromosomes were treated with UV endonuclease the form I DNA shifted to the form II DNA. This shift from supercoiled to nicked circular DNA occurred because the UV endonuclease cut the DNA strands at the sites of pyrimidine dimers (21). The effect of the UV endonuclease increased with the

TABLE I: Activation of Poly(ADPR) Polymerase with UV Irradiated and UV Endonuclease Treated SV 40 Minichromosome

	Poly(ADPR) Synthesis Incorporation from [^{14}C]NAD$^+$ (dpm)			
	Treatment and Incubation Conditions			
	No UV Endonuclease		With UV Endonuclease	
	Without H1	With H1	Without H1	With H1
DNA PRIMER:				
Control				
No DNA	200	238		
CT DNA	638	4238		
SV 40 Minichromosome				
No UV	495	371	516	304
50 J/m^2	448	273	666	356
100 J/m^2	515	298	1000	393
500 J/m^2	530	262	1677	264
1000 J/m^2	392	310	1539	256

SV 40 minichromosomes were treated with UV irradiation or mock irradiation as outlined in Methods. Minichromosomes were then treated with UV endonuclease or buffer without UV endonuclease for 60 min at 37°C. 1 µg of minichromosome DNA was then incubated in the poly(ADPR) polymerase assay containing [^{14}C] NAD$^+$ with and without histone H1 for 5 min at 37°C as described in Methods. Controls without DNA were used to determine the background of the assay. Controls with calf thymus DNA (CT DNA) (7 µg of DNA) were included to determine the effect of highly polymerized CT DNA on the stimulation of poly(ADPR) polymerase in the presence and absence of histone H1. All values are the mean of duplicate reactions, and are typical of the results obtained in three separate repeats of this experiment.

UV dose such that at 500 and 1000 J/m^2 virtually all of the DNA was converted to the nicked circular form.

The effects of the different treatments on the ability of the SV 40 minichromosomes to stimulate the activity of purified poly(ADPR) polymerase are shown in Table I. In the absence of added DNA there was no enzyme activity and the radioactivity detected represents the background of this assay. The level of the background was not affected by the presence or absence of histone H1. As noted with other purified preparations of this enzyme (12,22), incubation with highly polymerized calf thymus DNA results in a slight stimulation of purified poly(ADPR) polymerase activity. Incubation with calf thymus DNA and histone H1

resulted in marked stimulation of enzyme activity. When the SV 40 minichromosomes were used as DNA primer there was a small but reproducible stimulation of poly (ADPR) polymerase activity. In contrast to the results obtained with calf thymus DNA, addition of histone H1 to the SV 40 minichromosomes did not stimulate any further increase in poly(ADPR) polymerase activity. In fact, the presence of H1 caused a slight suppression of poly(ADPR) synthesis.

Treatment of the SV 40 minichromosomes with increasing doses of UV irradiation did not alter their ability to stimulate activity of poly(ADPR) polymerase. When minichromosomes were UV irradiated and then incubated with UV endonuclease there was a dose dependent increase in their ability to stimulate poly (ADPR) polymerase activity. This increase in ability of the minichromosome to stimulate poly(ADPR) polymerase activity was completely suppressed by adding histone H1 to the final incubation system. In all cases the incubation with UV endonuclease was performed before histone H1 was added to the system, therefore the presence of histone H1 in the final poly(ADPR) polymerase assay system could not interfere with the ability of the UV endonuclease to cut the DNA at the sites of pyrimidine dimers. Thus, only when SV 40 minichromosomes were treated with UV irradiation and then with UV endonuclease were they able to significantly stimulate the activity of poly(ADPR) polymerase. This stimulation was markedly suppressed when the reaction was performed in the presence of histone H1.

DISCUSSION: In these studies we used a well defined molecular structure, the SV 40 minichromosome, to determine which aspect of a particular type of DNA damage stimulates poly(ADPR) polymerase activity. The purified SV 40 minichromsome is ideal for this analysis since it has a well defined nucleosome structure (23-26). It also contains well defined protein components (16) which can be used to confirm the purity of the minichromosome preparations. Moreover, untreated minichromosome preparations cause very low levels of poly(ADPR) polymerase stimulation, thus providing a low background against which the effects of DNA damage can be examined.

Using purified poly(ADPR) polymerase that was absolutely dependent on added DNA for activity, we clearly demonstrated that UV irradiation of the SV 40 minichromosome was not sufficient to stimulate poly(ADPR) polymerase activity. Only when UV endonuclease was used to make incisions at the sites of pyrimidine dimers was there an increase in the ability of the UV irradiated SV 40 minichromosome to stimulate the activity of the poly(ADPR) polymerase. Thus, the incision phase of processing UV induced DNA damage was a definite prerequisite for the stimulation of poly(ADPR) polymerase activity. These studies provide an explanation for the differences observed in the ability of UV irradiation to stimulate poly(ADPR) polymerase in normal and XP cells (10). Stimulation of poly(ADPR) polymerase activity by UV irradiation of normal cells can be attributed to the strand break phase of the DNA repair process (10). In contrast, the failure of UV irradiation to stimulate poly(ADPR) polymerase activity in XP cells can be attributed to their inability to perform the incision step required for the repair of UV induced DNA damage (11). It is interesting to note that addition of histone H1 to the minichromosomes, after they were UV irradiated and treated with histone H1, suppressed their ability to stimulate poly(ADPR) polymerase activity. While the mechanism of this effect remains to be determined, its occurrence suggests that the in vivo processes involved in DNA repair may be profoundly affected by the proximity and concentration of different chromosomal components.

SV 40 has already proved a useful model for analyzing some of the steps in the processes of eukaryotic replication (27,28) and transcription (29,30). Our present studies suggest that SV 40 will be useful to study the components and sequence of events involved in eukaryotic DNA repair systems as well as the different chromosomal components that modulate these processes.

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