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Activation of Poly(adenosine diphosphate ribose) Polymerase by SV 40 Minichromosomes: Effects of Deoxyribonucleic Acid Damage and Histone H1†

Jonathan J. Cohen, Donna M. Catino, Shirley J. Petzold, and Nathan A. Berger*

ABSTRACT: Poly(ADP-ribose) polymerase is a chromosomal enzyme that is completely dependent on added DNA for activity. The ability of DNA molecules to activate the polymerase appears to be enhanced by the presence of DNA damage. In the present study, we used SV 40 DNA and SV 40 minichromosomes to determine whether different types of DNA damage and different chromosomal components affect stimulation of polymerase activity. Treatment of SV 40 minichromosomes with agents or conditions that induced single-strand breaks increased their ability to stimulate poly(ADP-ribose) synthesis. This stimulation was enhanced by addition of histone H1 at a ratio of 1 μg of histone H1 to 1 μg of DNA. Higher ratios of histone H1 to DNA suppressed the ability of SV 40 minichromosomes containing single-strand breaks to stimulate enzyme activity. Treatment of SV 40 minichromosomes or SV 40 DNA with *Hae*III restriction endonuclease to produce double-strand breaks markedly stimulated poly(ADP-ribose) polymerase activity. The stimulation of poly(ADP-ribose) polymerase by double-strand breaks occurred in the absence of histone H1 and was further enhanced by adding histone H1 up to ratios of 2 to 1 relative to DNA. At higher ratios of histone H1 to DNA, the presence of the histone continued to enhance the poly(ADP-ribose) synthesis stimulated by double-strand breaks.

Poly(adenosine diphosphate ribose) polymerase is a chromosomal enzyme that catalyzes the polymerization of ADP-ribose moieties from NAD^+ (Hayaishi & Ueda, 1977; Purnell et al., 1980). In some cases, the polymers of ADP-ribose are covalently linked to chromosomal proteins (Hayaishi & Ueda, 1977; Purnell et al., 1980). Many studies now indicate that this enzyme has a role in the recognition and repair of DNA damage. For example, studies with nuclei isolated from HeLa cells and *Physarum polycephalum* show that DNA damage induced by alkylating agents results in increased poly(ADP-ribose) polymerase activity (Smulson et al., 1975; Whish et al., 1975; Sudhakar et al., 1979). Permeabilized lymphocytes with DNA damage induced by UV irradiation, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, *N*-acetoxy-2-(acetylamino)-fluorene, and bleomycin have increased poly(ADP-ribose) polymerase activity (Berger et al., 1979), 3T3 cells depleted of NAD^+, the substrate for poly(ADP-ribose) polymerase, are unable to undergo *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced unscheduled DNA synthesis (Jacobson et al., 1980),

† From the Hematology/Oncology Division, The Department of Medicine, Washington University School of Medicine at the Jewish Hospital of St. Louis, St. Louis, Missouri 63110. *Received March 17, 1982.* This work was supported by National Institutes of Health Grants GM26463 and CA23986 and American Cancer Society Grant CH-134. Cell culture medium and electron micrographs were prepared in a cancer center facility funded by National Institutes of Health Grant 2 P30 CA16217. J.J.C. is the recipient of Postgraduate Training in Molecular Hematology Grant T32 H107088-06. N.A.B. is a Leukemia Society of America Scholar.

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and L-1210 cells with DNA damaged by dimethyl sulfate are inhibited from rejoining DNA strand breaks by inhibitors of poly(ADP-ribose) polymerase (Durkacz et al., 1980).

Purified poly(ADP-ribose) polymerase has an absolute requirement for DNA for activity, and the ability of DNA to activate the enzyme is enhanced by the presence of strand breaks (Ito et al., 1979; Yoshihara et al., 1978; Benjamin & Gill, 1980; Ohgushi et al., 1980; Cohen & Berger, 1981). One approach to understanding the function of this enzyme and its potential role in DNA repair is to study enzyme activation with well-characterized DNA molecules containing defined types of DNA damage. Recently, Benjamin & Gill (1980) utilized plasmid pBR322 DNA to show that restriction endonuclease fragments were more effective than the plasmid with single-strand nicks, which in turn was more effective than the undamaged plasmid in stimulating poly(ADP-ribose) polymerase. Ohgushi et al. (1980) demonstrated that increasing the number of single-strand nicks in plasmid ColE1 DNA increased poly(ADP-ribose) polymerase activity.

We used isolated SV 40 minichromosome preparations to probe the types of DNA damage and chromatin conformations that affect polymerase activity (Cohen & Berger, 1981). The SV 40 minichromosome isolated from purified virus is a well-defined entity with a precisely sequenced DNA genome of 5224 base pairs (Reddy et al., 1978; Fiers et al., 1978) and a supercoiled nucleosome structure (Kornberg, 1974; Felsenfeld, 1978; Griffith, 1975; Muller et al., 1978) containing only histones H2A, H2B, H3, and H4, but no histone H1 (Griffith & Christiansen, 1977). Since the SV 40 minichromosome contains nucleosomes arranged in a similar manner to eukaryotic chromatin, it may be used as a model to probe both the effects of specific DNA lesions and the effects of chromosomal structure on the activity of poly(ADP-ribose) polymerase.

We recently showed that treatment of SV 40 minichromosomes with UV irradiation did not affect their ability to stimulate poly(ADP-ribose) polymerase activity; however, when subsequently treated with UV endonuclease, an enzyme that nicks DNA at the site of pyrimidine dimers (Riazuddin & Grossman, 1977), the nicked DNA could then increase polymerase activity (Cohen & Berger, 1981). This latter stimulation was suppressed when histone H1 was added to the polymerase assay. This result was in contrast to the usual stimulation found when histone H1 is added to the poly(ADP-ribose) polymerase assays (Yoshihara et al., 1978; Kawaichi et al., 1980). Some workers have noted, however, that histone H1 interferes with polymerase activity and therefore leave histone H1 out of their assays (Benjamin & Gill, 1980; Ohgushi et al., 1980). Because our previous experiments showed that histone H1 enhanced polymerase activity with calf thymus DNA, and suppressed polymerase activity with SV 40 minichromosome DNA (Cohen & Berger, 1981), we decided to use SV 40 minichromosomes, SV 40 DNA, and histone H1 to detail the precise roles of each in the poly(ADP-ribose) polymerase assay.

Materials and Methods

Chemicals. [*Adenosine*-U-^{14}C]NAD^+ was purchased from New England Nuclear, Boston, MA. NAD^+, highly polymerized calf thymus DNA I, pancreatic DNase I, and phenylmethanesulfonyl fluoride were purchased from Sigma, St. Louis, MO. Histone H1 was obtained from Boehringer Mannheim, Indianapolis, IN. Restriction endonuclease *Hae*III was purchased from Bethesda Research Laboratory, Rockville, MD.

SV 40 Production. African green monkey kidney cells from American Type Culture Collection, Rockville, MD, were grown in Corning 490-cm^2 roller bottles in α-modified Eagle's medium with 25 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (Hepes) buffer (pH 7.2), 100 units/mL penicillin, 100 μg/mL streptomycin, and 10% fetal calf serum until confluent. Growth medium was then replaced with medium containing 2% fetal calf serum, and cells were infected with SV 40 virus at a dosage of 12.5–25 plaque forming units/cell. The SV 40 was a gift from Dr. L. Gelb, Washington University Medical School, and was descendent from SV 40 NIH 776-777 plaque purified by Danna & Nathans (1971). After complete cell lysis, approximately 7 days after infection, the cells were frozen and thawed 3 times, and virus was purified on CsCl gradients by a modification of the Khoury & Lai (1979) technique. Briefly, the frozen and thawed contents of 8–12 roller bottles were pelleted for 10 min at 10000*g*, 4 °C, the supernatant was discarded, and the pellet was frozen overnight. The cell pellet was adjusted to 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 8.0), 130 mM NaCl, 1.3% deoxycholate, and 0.025% trypsin in a total volume of 20 mL, suspended by rapid pipetting, and then incubated at 37 °C for 20 min. The suspension was centrifuged for 10 min at 10000*g*, 4 °C, and the supernate was layered on 10 mL of CsCl (ρ = 1.3723) in 50 mM Tris-HCl (pH 8.0) and 130 mM NaCl in 1 × 3 in. cellulose nitrate tubes and centrifuged in a Beckman SW 25.1 rotor at 23 000 rpm, 4 °C, for 3 h. At completion of the centrifugation, the tubes were illuminated in the dark with a high-intensity light source, and a blue-white band of virus could be located about 1 cm into the CsCl shelf. This band was collected in 0.5 × 2 in. cellulose nitrate tubes and adjusted to CsCl (ρ = 1.3660), 50 mM Tris-HCl (pH 8.0), and 130 mM NaCl. The second banding was done in a Beckman SW 50.1 rotor at 35 000 rpm, 4 °C, for 16 h. The blue-white virus band was collected and dialyzed overnight against 50 mM Tris-HCl (pH 8.0), 130 mM NaCl, and 0.1 mM phenylmethanesulfonyl fluoride. The virus was then stored at −40 °C until use.

Production of SV 40 Minichromosomes. Minichromosomes were prepared from isolated virus by a modification of the method of Beard (1978). SV 40 virus was pelleted in a 0.5 × 2 in. cellulose nitrate tube in a Beckman SW 50.1 rotor at 35 000 rpm, 4 °C, for 1 h. The virus was then disrupted in 1 mL of 0.1 M glycine–NaOH (pH 9.8), 75 mM NaCl, 5 mM ethylenediaminetetraacetic acid (EDTA), and 1 mM dithiothreitol (DTT), at 37 °C, for 5 min. The suspension was transferred to an ice bath, chilled, and then loaded on a discontinuous sucrose gradient in a 0.5 × 2 in. polyallomer tube composed of 1 mL of 50% (w/v) sucrose in 0.1 M Tris-HCl (pH 7.9 at 25 °C), 0.15 M NaCl, 1 mM EDTA, 0.1% (v/v) Nonidet P40, and 0.5 mM DTT, then 1.7 mL of 10% (w/v) sucrose in the same buffer, and finally 1 mL of 5% (w/v) sucrose in the same buffer, containing 0.1 M NaCl. After centrifugation in a Beckman SW 50.1 rotor at 43 000 rpm and 4 °C for 2.5 h, the SV 40 minichromosomes could be seen as a band over the 50% sucrose shelf. This band was collected and dialyzed overnight against the appropriate buffer.

Production of Form I SV 40 DNA. The SV 40 virus preparations were adjusted to 1% sodium dodecyl sulfate ($NaDodSO_4$) and 1 mM EDTA (pH 7.0) in a glass tube, incubated for 30 min in a 50 °C water bath, placed on ice, adjusted to contain 1 g/mL CsCl, and centrifuged in a Sorvall SS-34 rotor at 7750 rpm at 4 °C for 10 min. The supernatant was transferred to a 0.5 × 2 in. polyallomer tube and adjusted to a CsCl concentration of 1.56 g/mL, 30 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 100 μg/mL ethidium bromide. The refractive index was adjusted to 1.3880, and then the sample

FIGURE 1: Gel electrophoresis of untreated and *Hae*III-digested SV 40 minichromosomes. SV 40 minichromosomes were either (a) untreated or (b) pretreated with *Hae*III as described under Materials and Methods. 2.5-μg samples of the minichromosomes were adjusted to 1% $NaDodSO_4$ and 1% 2-mercaptoethanol, 2-mercaptoethanol, into 2% agarose gels in 36 mM Tris-HCl (pH 7.5), 30 mM NaH_2PO_4, and 1 mM EDTA at 40 V (Tegtmeyer & Macasset, 1972), then stained with 1 μg/mL ethidium bromide, and photographed with Polaroid P/N type 55 film by using a yellow filter and a 254-nm ultraviolet light source.

was centrifuged in a Beckman SW 50.1 rotor at 35 000 rpm at 20 °C for 24 h. The lower band identified with ultraviolet light was form I supercoiled DNA (Tegtmeyer & Macasset, 1972). This band was collected, and the ethidium bromide was extracted by two washes with equal volumes of 2-propanol saturated with CsCl. The form I DNA was then dialyzed against the appropriate reaction buffer and confirmed to be form I DNA by 2% agarose gel electrophoresis (Tegtmeyer & Macasset, 1972). DNA concentration was determined by the flurometric diaminobenzoic acid method described by Setaro & Morley (1976).

Restriction Endonuclease Digestion of Minichromosome DNA. SV 40 minichromosomes were dialyzed overnight against 20 mM Tris-HCl (pH 7.45), 7 mM $MgCl_2$, 60 mM NaCl, and 0.5 mM DTT. A 180-unit sample of *Hae*III (1 unit of enzyme degrades 1 μg of λ DNA in 1 h at 37 °C in a reaction mixture of 0.05 mL) was used to hydrolyze 16 μg of minichromosome DNA in a total reaction volume of 250 μL for 1 h at 37 °C in plastic tubes. In each case, the hydrolysis products were analyzed by 2% agarose gel electrophoresis. All SV 40 DNA molecules were completely hydrolyzed by *Hae*III endonuclease. SV 40 minichromosome hydrolysis by *Hae*III was incomplete, as demonstrated by more than 19 bands in lane b (Figure 1). Complete hydrolysis of SV 40 DNA produces only 19 bands on gel electrophoresis (Roberts, 1980). This is in agreement with previous reports showing that restriction endonucleases do not completely hydrolyze all of the molecules in minichromosome preparations due to the presence of nucleosomes (Shelton et al., 1980; Jakobovits et al., 1980).

DNase I Digestion of the SV 40 Minichromosome. Purified SV 40 minichromosomes were dialyzed overnight against 20 mM Tris-HCl (pH 7.45), 7 mM $MgCl_2$, 60 mM NaCl, and 0.5 mM DTT. The minichromosome preparation was then divided into six 90-μL samples each containing 3 μg of DNA and treated with 1, 5, 10, 50, or 100 ng/mL DNase I or mock treated for 30 min at 25 °C. The hydrolysis was stopped by immersing the tubes in ice, and the DNA was used immediatedly for poly(ADP-ribose) polymerase assays. Samples of DNase-treated DNA were also adjusted to contain 1% $NaDodSO_4$ and 1% β-mercaptoethanol and then subjected to 2% agarose gel electrophoresis (Tegtmeyer & Macasset, 1972). The gels were stained with 1 μg/mL ethidium bromide, illuminated with long-wave UV light, and photographed through a Wratten 9 filter by using Polaroid type 55 P/N film. Negatives were analyzed with a Zeineh soft laser scanning densitometer to determine the amount of DNA in each peak.

Poly(ADP-ribose) Polymerase. Poly(ADP-ribose) polymerase was purified from sheep thymus by a modification of the method of Yoshihara et al. (1978). The purified sheep thymus enzyme preparation showed a single protein band and had an apparent molecular weight of 135 000 as determined by $NaDodSO_4$–polyacrylamide gel electrophoresis; it was free of DNA and was absolutely dependent on added DNA for its activity (Petzold et al., 1981). Poly(ADP-ribose) polymerase activity was assayed in a reaction mixture containing 82 mM Tris-HCl (pH 8.0), 0.42 mM [*adenosine*-U-^{14}C]NAD^+ (4.3 dpm/pmol), 8 mM $MgCl_2$, 0.8 mM DTT, variable amounts of histone H1 as indicated, 1–2.5 μg of appropriately treated SV 40 minichromosomes or SV 40 DNA or 7 μg of highly polymerized calf thymus DNA, and 0.25 unit of poly(ADP-ribose) polymerase (1 unit = 1 nmol of ADP-ribose incorporated/min) for a total reaction volume of 120 μL (Cohen & Berger, 1981). 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. The acid-insoluble material was collected on Whatman GF/C glass fiber filters and washed 6 times with 20% trichloroacetic acid and 3 times with 95% ethanol. The filters were dried and counted in a toluene-based scintillation fluid with a Tracor Mark III scintillation spectrometer (Berger et al., 1978). Background for control assays conducted in the absence of DNA was never higher than 200 dpm. This background was subtracted from experimental values prior to the calculation of incorporation of picomoles of ADP-ribose per microgram of DNA.

Autoradiographs of ADP-Ribosylated Proteins. For autoradiographic experiments, systems containing 20 μg of untreated or *Hae*III-cut minichromosome DNA were incubated in the presence or absence of 40 μg of histone H1, 300 μM [^{32}P]NAD (specific activity 65 Ci/mol), 100 mM Tris-HCl, pH 8.0, 1 mM DTT, and 10 mM EDTA in a final volume of 0.5 mL. The reactions were run at 37 °C for the indicated times and stopped by addition of 4.4 mL of ice-cold 20% trichloroacetic acid and 100 μL of 1 mg/mL carrier DNA. Trichloroacetic acid precipitates were collected by centrifugation at 12000*g* for 15 min, 4 °C, and washed once with cold 95% ethanol. Pellets were dried, and then 100 μL of a solution containing 2% $NaDodSO_4$, 0.1 M DTT, 10 mM potassium phosphate buffer, pH 7.6, 20% glycerol, and 0.032% bromophenol blue was added and the suspension boiled for 5 min. The entire solubilized system was applied to a 7.5–20% exponential gradient acrylamide $NaDodSO_4$ slab gel with a 5% acrylamide stacking gel. Electrophoresis was in a buffer containing 50 mM Tris, pH 8.3, 400 mM glycine, 2 mM EDTA, and 0.1% $NaDodSO_4$ at 25 mA/slab for 3.5 h. The gel was stained for 4 h in 0.25% Coomassie blue in methanol–deionized water–acetic acid (5:5:1) and destained in

Table I: Effects of DNA Modifications and Histone H1 on Poly(ADP-ribose) Polymerase Activity[a]

enzyme activator	treatment	poly(ADP-ribose) polymerase activity (pmol of ADP-ribose incorporated/μg of DNA)	
		no additives	histone H1 added
calf thymus DNA		14	134
SV 40 DNA (form I)	none	0	1
	*Hae*III	30	86
SV 40 minichromosome	none	73	11
	*Hae*III	208	415

[a] Poly(ADP-ribose) polymerase activity was measured as the incorporation of [^{14}C]ADP-ribose from [^{14}C]NAD$^+$ by using standard assay conditions described under Materials and Methods without histone H1 or with 10 μg of histone H1 and with either 7.5 μg of calf thymus DNA or 2.5 μg of SV 40 DNA or SV 40 minichromosome DNA; incubations were for 5 min at 37 °C. All treatments of the different DNA molecules are also given under Materials and Methods. All experimental points are the means of assays performed in duplicate or triplicate and are typical of results obtained in three separate repeats of each experiment.

deionized water–methanol–acetic acid (16:2:2). The gels were dried, and autoradiography was performed by using Kodak XAR-5 X-ray film with a Du Pont Cronex Lightning-Plus AC intensifier screen in the cassette.

Results

Characterization of the SV 40 Minichromosome. SV 40 virus particles were isolated from cellular components (Khoury & Lai, 1979), and the SV 40 minichromosomes were purified from the isolated virus particles (Beard, 1978). Analysis of the minichromosome preparations by acid–urea (Panyim & Chalkely, 1969) and NaDodSO$_4$–polyacrylamide (Bhorjee & Peterson, 1973) gel electrophoresis showed that the final preparation contained the typical patterns of histones H2A, H2B, H3, and H4, with traces of VP 1, 2, and 3 (Griffith & Christiansen, 1977). As previously noted, histone H1 was completely absent from the minichromosome preparations (Griffith & Christiansen, 1977). Electron microscopy (de-Murcia et al., 1978) of the preparations showed the beaded circular nucleosomal appearance typical of SV 40 minichromosomes (Griffith & Christiansen, 1977). There were no protein bands on the gel electrophoretogram in the region of poly(ADP-ribose) polymerase. When SV 40 minichromosomes were incubated in the polymerase assay system with or without calf thymus DNA, there was no incorporation of [^{14}C]ADP-ribose into acid-precipitable counts. Thus, SV 40 minichromosomes were judged to be free of poly(ADP-ribose) polymerase, in contrast to the finding that poly(ADP-ribose) polymerase is present in nucleosomes isolated from HeLa cells (Mullins et al., 1977).

SV 40 DNA and SV 40 Minichromosomes as Activators of Poly(ADP-ribose) Polymerase. As shown in Figure 2, untreated minichromosomes activated poly(ADP-ribose) polymerase. *Hae*III-hydrolyzed minichromosomes stimlated activity even more. Incorporation of ADP-ribose in both assays continued to increase for 10 min and then plateaued. On the basis of these curves, we selected reaction times of 5 min to compare the ability of different treatments of SV 40 minichromosomes and SV 40 DNA to stimulate activity of poly(ADP-ribose) polymerase. In Figure 3, untreated minichromosomes and *Hae*III-digested minichromosomes showed a concentration-dependent ability to activate poly(ADP-ribose) polymerase. Subsequent experiments were performed with 1.0–2.5 μg of DNA in each reaction, and results are expressed per microgram of added DNA.

Table I shows that when highly polymerized calf thymus DNA was used to activate poly(ADP-ribose) polymerase there was a slight stimulation of activity in the absence of histone H1 and a 10-fold greater increase in activity in the presence of histone H1. Form I or supercoiled SV 40 DNA, free of all protein and nucleosomal structure, was unable to stimulate

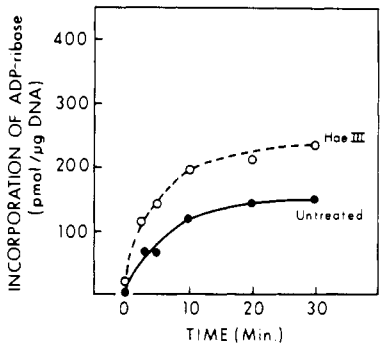

FIGURE 2: Time course of poly(ADP-ribose) synthesis with SV 40 minichromosomes. Poly(ADP-ribose) polymerse activity was measured with untreated SV 40 minichromosome preparations (●) or after treatment with *Hae*III (○) by using the standard assay described under Materials and Methods except that histone H1 was omitted. Incubations were at 37 °C. Reactions were stopped at the indicated times by rapidly transferring the tubes to an ice–water bath and simultaneously adding 2 mL of ice-cold 20% trichloroacetic acid. All experimental points are the means of duplicate assays and are typical of results obtained in two separate repeats of this experiment.

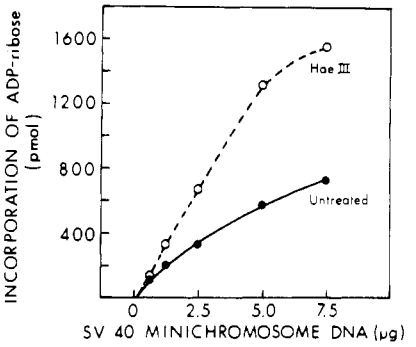

FIGURE 3: Dependence of poly(ADP-ribose) polymerase activity on concentration of added DNA. Increasing concentrations of SV 40 minichromosomes that were either untreated (●) or treated with *Hae*III (○) as in Figure 2 were added to the standard poly(ADP-ribose) polymerase assay, without added histone H1. Incubations were for 5 min at 37 °C. All experimental points are the means of duplicate assays.

polymerase activity, indicating that undamaged supercoiled DNA does not activate poly(ADP-ribose) polymerase. Addition of histone H1 to this reaction did not alter the ability of form I DNA to activate poly(ADP-ribose) synthesis. *Hae*III-hydrolyzed SV 40 DNA stimulated poly(ADP-ribose) polymerase activity, and this stimulation was increased almost 3-fold by the addition of histone H1. Untreated SV 40 minichromosomes caused a stimulation of poly(ADP-ribose) synthesis, and this stimulation was further enhanced by hydrolysis of the minichromosomes with *Hae*III. Two percent agarose gel electrophoresis of the SV 40 minichromosome

Table II: Effects of SV 40 Minichromosome Digestion with *Hae*III on Poly(ADP-ribose) Polymerase Activity[a]

enzyme activator	preincubation conditions	poly(ADP-ribose) polymerase activity (pmol of ADP-ribose incorporated/ μg of DNA)
SV 40 minichromosome	(a) untreated	105
	(b) EDTA	71
	(c) EDTA + *Hae*III	93
	(d) *Hae*III (EDTA after 60 min)	220

[a] SV 40 minichromosomes (6 μg of DNA in each sample) were either (a) untreated, (b) pretreated with 25 mM EDTA, (c) pretreated with 25 mM EDTA and 120 units of *Hae*III, or (d) pretreated with 120 units of *Hae*III for 60 min at 37 °C. (d) was then adjusted to 25 mM EDTA prior to its use as an activator of the polymerase. A 1.5-μg sample of minichromosome DNA preincubated in each of the four conditions above was tested for ability to stimulate poly(ADP-ribose) synthesis from [^{14}C]NAD$^+$ by using the standard assay conditions described under Materials and Methods except for omission of histone H1. All experimental points are the means of assays performed in triplicate.

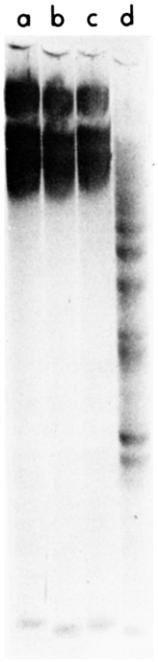

FIGURE 4: Gel electrophoresis of untreated, EDTA-treated, and *Hae*III-digested SV 40 minichromosomes. SV 40 minichromosomes were treated as described in Table II and subjected to gel electrophoresis as described in Figure 1: (a) untreated; (b) pretreated with 25 mM EDTA; (c) pretreated with 25 mM EDTA and *Hae*III; (d) pretreated with *Hae*III for 60 min at 37 °C and then adjusted to 25 mM EDTA.

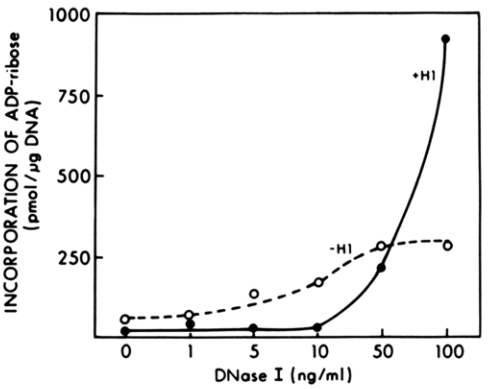

FIGURE 5: Effects of histone H1 on poly(ADP-ribose) polymerase activity stimulated by minichromosomes treated with DNase I. SV 40 minichromosomes (3 μg of SV 40 DNA) were treated with 1, 5, 10, 50, or 100 ng/mL DNase I or mock treated for 30 min at 25 °C, and then minichromosome samples containing 1 μg of DNA were incubated in the polymerase assay containing [^{14}C]NAD$^+$ in the presence (●) or absence (○) of 10 μg of histone H1 for 5 min at 37 °C. All points are the means of assays performed in duplicate and are typical of two separate repeats of this experiment.

preparations showed that they contained a combination of form I supercoiled and form II single-strand nicked circular DNA (Figure 1). These nicks were apparently sufficient to account for the activation of poly(ADP-ribose) polymerase by the untreated minichromosomes (Cohen & Berger, 1981). Under the conditions of this experiment, the enzyme activity stimulated by the untreated SV 40 minichromosomes was suppressed by the addition of histone H1. In contrast, the enzyme activity stimulated by *Hae*III-treated SV 40 minichromosomes was even further enhanced by the addition of histone H1 to the assay system.

The increase in poly(ADP-ribose) synthesis that occurred with *Hae*III-treated minichromosomes was not due to the presence of the restriction endonuclease itself but was dependent upon hydrolysis of the minichromosomes. This was demonstrated by preincubating the minichromosomes with the restriction endonuclease in the presence of EDTA to prevent the enzyme from performing any hydrolysis (Figure 4). Table II compares the effects of preincubating the minichromosomes for 60 min at 37 °C with either no additions, 25 mM EDTA, 25 mM EDTA and 120 units of *Hae*III, or 120 units of *Hae*III alone. In the latter case, 25 mM EDTA was added at the end of the 60-min incubation period. Incubations of the minichromosomes with *Hae*III in the presence of EDTA did not increase their ability to stimulate the subsequent synthesis of poly(ADP-ribose). Only when the minichromosomes were hydrolyzed by *Hae*III was there an increase in their ability to stimulate poly(ADP-ribose) polymerase activity.

DNase I Digested SV 40 Minichromosomes as Activators of Poly(ADP-ribose) Polymerase. Figure 5 shows that in the range of 1–10 ng/mL, DNase I treatment increased the ability of the SV 40 minichromosome to stimulate poly(ADP-ribose) synthesis; however, the amount of poly(ADP-ribose) synthesis was suppressed when histone H1 was added to the final assay system. After treatment with 100 ng/mL DNase I, the minichromosomes stimulated more poly(ADP-ribose) polymerase activity, and this activity was further enhanced by the addition of histone H1. Riley (1980) showed that at low concentrations, DNase I produced single-strand breaks whereas at higher concentrations multiple double-strand breaks were formed. To determine whether these different breaks might be associated with the levels of activation and differential histone effects on poly(ADP-ribose) polymerase, we analyzed the results of the digestion by using 2% agarose gel electrophoresis, ethidium bromide staining, and densitometry as detailed under Materials and Methods. In general, the SV 40 minichromosomes showed increased single-strand nicks due to digestion with 1–50 ng/mL DNase I and marked double-strand breaks due to digestion with 100 ng/mL DNase I. Before treatment with DNase I, the SV 40 minichromosome

Table III: Effects of SV 40 Minichromosome Digestion with DNase I on Poly(ADP-ribose) Polymerase Activity[a]

enzyme activator	preincubation conditions	poly(ADP-ribose) polymerase activity (pmol of ADP-ribose incorporated/ μg of DNA)
SV 40 minichromosome	(a) untreated	34
	(b) EDTA	28
	(c) EDTA + DNase I	23
	(d) DNase I (EDTA after 60 min)	195

[a] SV 40 minichromosomes (6 μg of DNA in each sample) were either (a) untreated, (b) pretreated with 25 mM EDTA, (c) pretreated with 25 mM EDTA and 50 ng/mL DNase I, or (d) pretreated with 50 ng/mL DNase I for 30 min at 25 °C. (d) was then adjusted to 25 mM EDTA prior to its use as an activator of the polymerase. A 1.75-μg sample of minichromosome DNA preincubated in each of the four conditions above was tested for ability to stimulate poly(ADP-ribose) synthesis from [^{14}C]NAD$^+$ by using the standard assay conditions described under Materials and Methods except for omission of histone H1. All experimental points are the means of assays performed in triplicate.

preparations showed a similar pattern to those in Figure 1, lane a, and Figure 4, lane a, with 36% in form II and 64% in form I DNA. Thus, 36% of the minichromosomes contained single-strand breaks, and this apparently accounts for their ability to cause a low level of activation of poly(ADP-ribose) polymerase. After treatment with 5 ng/mL DNase I, 61% of the DNA was in form II and 32% in form I. Thus, the increase in the ability to stimulate poly(ADP-ribose) synthesis was associated with the increase in minichromosomes containing single-strand nicks. After the SV 40 minichromosomes were treated with 100 ng/mL DNase I, only 6.5% of the DNA was in form II DNA, and 14% remained in form I DNA. The rest of the DNA was broadly spread throughout the molecular weight range below form I DNA. This pattern indicates extensive fragmentation, and the presence of these low molecular weight DNA fragments correlated with the greatest ability of the minichromosomes to stimulate poly(ADP-ribose) synthesis. Furthermore, it is under these conditions, where the minichromosomes were extensively fragmented, that histone H1 had a stimulating rather than suppressive effect on poly(ADP-ribose) synthesis.

As with the *Hae*III hydrolysis, the increase in poly(ADP-ribose) synthesis that occurred with DNase I treatment was not simply due to the presence of the nuclease itself, but was dependent upon hydrolysis of the minichromosomes. Table III shows the results of pretreating minichromosomes with either no enzyme, 25 mM EDTA, 25 mM EDTA and 50 ng/mL DNase I, or 50 ng/mL DNase I alone for 30 min at 25 °C prior to incubation in the polymerase assay. In the latter case, 25 mM EDTA was added at the end of the 30-min incubation period. Similar gel electrophoresis to Figure 4 demonstrated that 25 mM EDTA prevented DNase I from performing hydrolysis of the SV 40 minichromosomes. Only when the minichromosomes were hydrolyzed by DNase I was their ability to stimulate poly(ADP-ribose) polymerase activity increased. When the minichromosomes were incubated with DNase I under conditions that prevented hydrolysis, there was no increase in their ability to stimulate activity of poly(ADP-ribose) polymerase.

Concentration Dependence of Histone H1 Effects on Poly(ADP-ribose) Synthesis and Identification of Acceptors for Poly(ADP-ribosylation). The effect of increasing concentrations of histone H1 relative to DNA concentration on the ability of the minichromosomes to stimulate poly(ADP-ribose) polymerase is shown in Figure 6. Addition of histone H1 to untreated minichromosomes, or to minichromosomes treated with *Hae*III, resulted in a biphasic effect on polymerase activity. For the untreated minichromosomes, maximal stimulation of polymerase activity occurred at a ratio of 1 μg of histone H1 to 1 μg of DNA. At a ratio of 2 μg of histone H1 to 1 μg of DNA, the ability of the untreated minichromosome

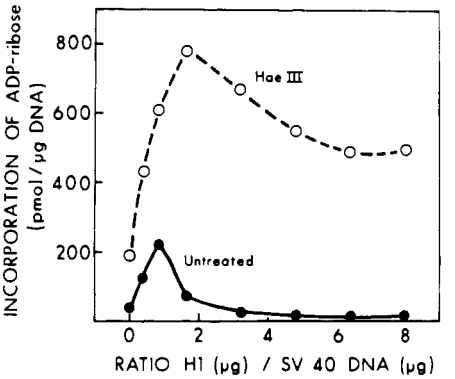

FIGURE 6: Effects of histone H1 concentration on activity of poly(ADP-ribose) polymerase. SV 40 minichromosomes were treated with *Hae*III (○) or left untreated (●) as described in Figure 2. Appropriately treated samples of SV 40 minichromosomes containing 1.25 μg of DNA were incubated in the polymerase assay containing [^{14}C]NAD$^+$ with 0, 0.5, 1, 2, 4, 6, 8, or 10 μg of histone H1 for 5 min at 37 °C. All points are the means of assays performed in duplicate and are similar to four separate repeats of this experiment.

to stimulate poly(ADP-ribose) synthesis was suppressed, and at higher ratios, the activity was suppressed to one-fourth the level of activity stimulated by the minichromosome alone. In contrast, addition of histone H1 to the reaction stimulated by *Hae*III-treated minichromosomes did not achieve maximal stimulation until the ratio of histone H1 to DNA reached approximately 2. Further addition of histone H1 resulted in reduced levels of poly(ADP-ribose) synthesis; however, even at a ratio of 8 μg of histone H1 to 1 μg of DNA, the level of poly(ADP-ribose) synthesis in the presence of histone H1 was greater than in its absence.

To further evaluate the effect of histone H1 on the synthesis of poly(ADP-ribose) stimulated by untreated or *Hae*III-cut minichromosomes, we incubated these components along with [^{32}P]NAD and then used autoradiography to determine which proteins acted as acceptors for poly(ADP-ribose) in the presence and absence of histone H1. For these studies, histone H1 was present in the appropriate reactions at a ratio of 2 to 1 with respect to DNA. This ratio of H1 to DNA was selected since, as shown in Figure 5, it allowed maximal stimulation of poly(ADP-ribose) synthesis by the *Hae*III-cut minichromosome and also produced marked suppression of the poly(ADP-ribose) synthesis stimulated by the untreated minichromosome. As shown in the time course in Table IV, the untreated minichromosomes stimulated poly(ADP-ribose) synthesis, and this reaction was significantly suppressed by the presence of a 2 to 1 ratio of histone H1. The *Hae*III-cut minichromosomes showed a greater ability to stimulate poly(ADP-ribose) synthesis, and in this case, the addition of H1 resulted in a marked stimulation of poly(ADP-ribose)

Table IV: Effect of H1 and SV 40 Minichromosome Status on Level of Poly(ADP-ribose) Synthesis[a]

reaction components		[^{32}P]ADPR incorporated (cpm) at		
minichromosome (μg)	H1 (μg)	5 s	1 min	5 min
10, untreated	0	2136	5540	10572
10, untreated	20	908	1242	2795
10, *Hae*III cut	0	4753	9383	18708
10, *Hae*III cut	20	1387	6330	79560

[a] The time course of poly(ADP-ribose) synthesis was measured in reactions parallel to those used for the autoradiographs in Figure 7. These reactions were similar to those described under Materials and Methods except that they contained 300 μM [^{32}P]-NAD (specific activity 65 Ci/mol). H1 was present in the indicated reactions at a ratio of 2 to 1 relative to DNA. Reactions were incubated at 37 °C for the indicated times, terminated with cold 20% trichloroacetic acid, and then collected for scintillation counting on GF/C discs as described under Materials and Methods. ADPR is ADP-ribose.

synthesis, the latter being apparent at the 5-min incubation point. Figure 7 shows the autoradiographs of these reactions. The top panel shows the acceptors for poly(ADP-ribose) in the reaction activated by the intact minichromosome; the bottom panel shows the acceptors in the reactions activated by the *Hae*III-cut minichromosomes. Lanes A–C show that in the absence of H1 the minichromosome activated the synthesis of poly(ADP-ribose) which was primarily attached to the enzyme poly(ADP-ribose) polymerase. This is seen most clearly in lane A where the 5-s reaction shows that the major ADP-ribosylated protein coincides with the purified enzyme band seen on protein staining of the same gels (Petzold et al., 1981). This auto-ADP-ribosylation reaction in which the enzyme acts as the acceptor protein for the covalent attachment of poly(ADP-ribose) has also been demonstrated for the enzymes purfied from rat liver, calf thymus, and human tonsils (Ogata et al., 1980a; Kawaichi et al., 1981; Yoshihara et al., 1981; Carter & Berger, 1982). Lanes A–C show that with progressively longer incubation times some of the ADP-ribosylated enzyme does not penetrate as far into the gels. In fact, some of the ADP-ribosylated enzyme does not even penetrate into the 5% stacking gel. This apparent increase in size is due to the progressively larger size of the newly synthesized polymer attached to the enzyme. In addition to the poly(ADP-ribose) attached to the polymerase, lanes B and C also show that some ADP-ribose residues electrophoresed in the same position as viral proteins 1 and 2. Lane C also shows that there is a small amount of ADP-ribosylated material in the regions of the core histones H2A, H2B, H3, and H4. Lanes D–F show the effect of H1 in the reaction stimulated by the untreated minichromosomes. It is clear that the major effect of H1 is to suppress the ADP-ribosylation of all proteins including the polymerase. As shown in lanes D′, E′, and F′, greater exposure of these lanes indicates that despite its suppressive effect, some poly(ADP-ribose) is in fact synthesized in attachment to H1.

Lanes G–I show the synthesis of poly(ADP-ribose) stimulated by *Hae*III-cut minichromosomes in the absence of H1. This reaction also shows extensive ADP-ribosylation of the enzyme itself as well as some ADP-ribose in association with viral protein 1. When H1 was added to the *Hae*III-cut minichromosomes as in lanes J–L, it was apparent that in addition to ADP-ribosylation of the enzyme there was also extensive ADP-ribosylation of H1. The heavy background in lanes K and L is probably due to the cleavage of some polymer chains from their attachment to proteins during the boiling used to

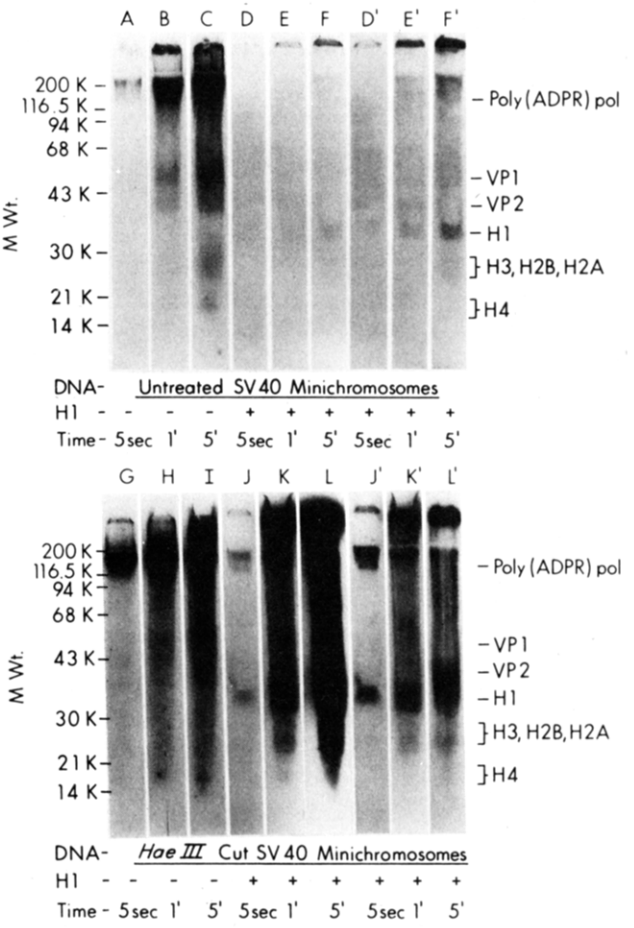

FIGURE 7: Autoradiographs of ADP-ribosylated proteins in the presence and absence of histone H1. Each reaction system contained 20 μg of SV 40 minichromosome DNA and 300 μM [^{32}P]NAD (specific activity 65 Ci/mol) as indicated under Materials and Methods. The minichromosomes used in the upper row were untreated; those used in the lower row were pretreated with restriction endonuclease *Hae*III. The reaction systems shown in lanes A, B, C, G, H, and I contained no histone H1. The reaction systems shown in lanes D, E, F, J, K, and L each contained 40 μg of histone H1. Reactions were run for the indicated times at 37 °C and terminated by precipitation with cold trichloroacetic acid. The pellets were washed with alcohol and solubilized, and then the entire sample was analyzed on 7.5–20% exponential NaDodSO$_4$–acrylamide slab gels as described under Materials and Methods. Gels were stained for proteins with Coomassie blue, dried, and autoradiographed as described under Materials and Methods. The autoradiographs of lanes A–L were obtained from one exposure lasting 48 h. Lanes D′, E′, F′, and J′ are the same as lanes D, E, F, and J except they were exposed with an intensifier in the cassette for 16 h. Lanes K′ and L′ are the same as K and L except that they were exposed with an intensifier in the cassette for 8 and 1 h, respectively. Assignment of protein bands was from Coomassie blue staining patterns of purified components included with each gel. VP 1 and 2 are viral proteins 1 and 2, respectively, as shown by Christiansen et al. (1977); histones are indicated as H1, H2A, H2B, H3, and H4. Molecular weight standards included with each gel were myosin (200 000), β-galactosidase (116 500), phosphorylase *b* (94 000), bovine serum albumin (68 000), ovalbumin (43 000), carbonic anhydrase (30 000), soybean trypsin inhibitor (21 000), and lysozyme (14 300).

solubilize samples for application to gels. As shown in lanes K′ and L′, shorter exposure of this area of the gel clearly shows that the major acceptors for ADP-ribosylation are the enzyme and H1. These experiments show that when untreated SV 40 minichromosomes were used to stimulate poly(ADP-ribose) polymerase, addition of a 2 to 1 ratio of histone H1 relative to DNA markedly suppressed the synthesis of polymer. However, when histone H1 was added to *Hae*III-cut minichromosomes at a similar ratio, the histone stimulated poly-

(ADP-ribose) synthesis, in part, by acting as an acceptor itself.

Discussion

Several techniques have been used to demonstrate that DNA damage stimulates poly(ADP-ribose) synthesis in eukaryotic cells (Smulson et al., 1975; Whish et al., 1975; Sudhakar et al., 1979; Berger et al., 1979; Jacobson et al., 1980; Durkacz et al., 1980). For example, isolated cell nuclei and permeable cell preparations have been used to show that treatment of cells with UV irradiation, γ irradiation, alkylating agents, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, bleomycin, and DNase I all cause an increase in activity of poly(ADP-ribose) polymerase (Berger et al., 1979; Miller, 1975; Skidmore et al., 1979). This increase in enzyme activity has been demonstrated in intact cells by showing that treatment with the DNA-damaging agents noted above results in decreases in cellular NAD^+ levels due to its consumption in the poly(ADP-ribose) polymerase reaction (Skidmore et al., 1979; Goodwin et al., 1978; Rankin et al., 1980). The recent development of a high-pressure liquid chromatography technique and fluorescent detection method to measure small quantities of poly(ADP-ribose) has made it possible to actually show an increase in polymer levels when cells are treated with DNA-damaging agents (Sims et al., 1980). Using this technique, it was shown that treatment of 3T3 cells with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine produced a 150-fold increase in cellular levels of poly(ADP-ribose) (Juarez-Salinas et al., 1979). Thus, treatment of cells with a variety of DNA-damaging agents causes an increase in activity of poly(ADP-ribose) polymerase, a decrease in its substrate NAD^+, and an increase in the product poly(ADP-ribose). On the basis of these cellular studies, it has been suggested that poly(ADP-ribose) polymerase is stimulated by the strand-break phase of DNA damage (Smulson et al., 1975; Whish et al., 1975; Sudhakar et al., 1979; Berger et al., 1979, 1980; Jacobson et al., 1980; Durkacz et al., 1980). Further confirmation of this relation has been obtained by showing that UV irradiation of xeroderma pigmentosum cells does not result in the activation of poly(ADP-ribose) synthesis (Berger et al., 1980), or lowering of cellular NAD^+ levels (McCurry & Jacobson, 1981). This is presumably due to the failure of xeroderma pigmentosum cells to make incisions at the sites of pyrimidine dimers (Cleaver, 1978). When this defect is corrected by supplying the cells with UV endonuclease, the formation of strand breaks at the site of pyrimidine dimers is associated with an increase in poly(ADP-ribose) synthesis (Berger & Sikorski, 1981).

The studies outlined above were all performed in preparations of whole cells and are potentially complicated by the multiple components present in such systems. The availability of purified preparations of poly(ADP-ribose) polymerase that require added DNA as an essential enzyme activator made it possible to use well-defined in vitro systems to study the types of lesions and mechanisms by which DNA damage stimulates activity of poly(ADP-ribose) polymerase. Benjamin & Gill (1980) and Ohgushi et al. (1980) have used plasmid pBR322 and ColE1 DNA to study activation of the polymerase. We have used SV 40 minichromosomes and SV 40 DNA for this purpose. Unlike the plasmid DNA, the DNA in minichromosomes is associated with histones in a nucleosomal arrangement that provides a useful model of eukaryotic chromatin (Kornberg, 1974; Felsenfeld, 1978; Griffith, 1975; Muller et al., 1978). While studies with the isolated plasmids are useful for identifying specific alterations in the DNA that affect polymerase activity, those with minichromosomes should provide a representative model with which to evaluate factors in eukaryotic chromatin that affect polymerase activity.

Mullins et al. (1977) have used oligonucleosomes purified from HeLa cells to measure some of the effects of chromatin on poly(ADP-ribose) polymerase. These preparations contain endogenous poly(ADP-ribose) polymerase, histone H1, and numerous other chromosomal proteins and have the advantage of having all of these chromosomal components present in a similar fashion to their intracellular relationship. Using these oligonucleosome preparations from HeLa cells, they have shown that poly(ADP-ribose) polymerase appears to occupy the internucleosomal region of the chromatin. Our studies show that the SV 40 minichromosomes isolated from mature virus particles contain no histone H1 or poly(ADP-ribose) polymerase. Thus, purified poly(ADP-ribose) polymerase and histone H1 can be added individually to the SV 40 minichromosome to determine their effects on the activity of poly(ADP-ribose) polymerase.

In our studies, supercoiled SV 40 DNA, free of all protein and nucleosomal structure, showed little ability to stimulate activity of poly(ADP-ribose) polymerase, indicating that undamaged supercoiled DNA will not activate poly(ADP-ribose) polymerase. This is similar to the observations of Benjamin & Gill (1980) which showed that intact pBR322 molecules did not stimulate enzyme activity. When SV 40 DNA was hydrolyzed with *Hae*III producing 19 flush double-strand breaks, the preparation was then able to stimulate polymerase activity. In this case, the ability of the molecule with double-strand breaks to stimulate poly(ADP-ribose) synthesis was enhanced by the addition of histone H1 to the polymerase assay.

When these studies were done with SV 40 minichromosomes which contain chromosomal proteins and nucleosomal structures, the results differed from those obtained by using form I SV 40 DNA. SV 40 minichromosomes were found to stimulate poly(ADP-ribose) polymerase activity. Under these conditions, it was presumably the single-strand nicks in form II DNA that stimulated polymerase activity. Single-strand nicks produced by low concentrations of DNase I also stimulated polymerase activity, and we have previously shown that polymerase activity can also be stimulated by the single-strand nicks produced by UV irradiation and UV endonuclease (Cohen & Berger, 1981). In all cases, the ability of single-strand nicks to stimulate poly(ADP-ribose) polymerase activity could be suppressed by addition of histone H1 to a ratio of greater than 2 to 1 relative to the SV 40 DNA in the polymerase assay.

When SV 40 minichromosomes or SV 40 DNA was treated with *Hae*III, the hydrolyzed minichromosomes were able to stimulate much greater enzyme activity than the hydrolyzed SV 40 DNA. Although the minichromosomes were incompletely hydrolyzed (Figure 1), their greater activity indicated that either chromosomal proteins or nucleosomal conformation enhanced the activity of poly(ADP-ribose) polymerase. In contrast to untreated SV 40 minichromosomes, *Hae*III-hydrolyzed minichromosomes not only had greater activity in the polymerase assay but also this activity was enhanced even further by the presence of histone H1. In the case of the untreated minichromosomes, it was apparent that the poly(ADP-ribose) polymerase served as the major acceptor for polymer synthesis, and histone H1 suppressed this reaction. In the case of the *Hae*III-cut minichromosomes, poly(ADP-ribose) polymerase also served as a major acceptor for polymer synthesis, and histone H1 augmented this reaction by serving as an additional acceptor for poly(ADP-ribose) synthesis.

These studies show that poly(ADP-ribose) polymerse activity can be stimulated by both single- and double-strand

breaks in either DNA or chromatin and that the level of polymerase activity can be modulated by the different proteins associated with DNA. Addition of histone H1 to SV 40 minichromosomes has been shown by electron microscopy studies to condense the SV 40 minichromosome (Muller et al., 1978), and this may account for its ability to suppress the stimulation of poly(ADP-ribose) synthesis by single-strand breaks. Thus, when SV 40 minichromosomes contain single-strand breaks, addition of increasing concentrations of histone H1 may alter the conformation of the minichromosome so as to shield the DNA damage from stimulating the activity of poly(ADP-ribose) polymerase. When SV 40 minichromosomes are modified by large numbers of double-strand breaks as after treatment with *Hae*III or high concentrations of DNase I, addition of histone H1 no longer interferes with the ability of the DNA fragments to activate polymerase activity. This is presumably because the histone H1 can no longer condense the multiple fragments and shield the DNA damage from stimulating the activity of the polymerase. In addition, histone H1 provides acceptors for attachment of ADP-ribose polymers (Adamietz et al., 1979; Ogata et al., 1980b; Carter & Berger, 1982), and this adds to its ability to augment the stimulation of poly(ADP-ribose) synthesis by *Hae*III-cut minichromosomes. This model demonstrates how histone H1 can differentially affect the ability of single- and double-strand breaks to stimulate poly(ADP-ribose) polymerase activity and explains why some workers have found that histone H1 enhances enzyme activity (Kawaichi et al., 1980; Yoshihara et al., 1978) and others have found that histone H1 suppresses activity (Benjamin & Gill, 1980; Ohgushi et al., 1980). These studies illustrate that the activity of poly(ADP-ribose) polymerase is sensitive to DNA strand breaks and DNA conformation and also that it can be regulated by the presence and concentration of specific chromatin components.

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Specificity of Autoimmune Monoclonal Fab Fragments Binding to Single-Stranded Deoxyribonucleic Acid†

Jeremy S. Lee,* Dennise F. Dombroski, and Tim R. Mosmann

ABSTRACT: Fab fragments from hybridoma HEd 10 [Lee, J. S., Lewis, J. R., Morgan, A. R., Mosmann, T. R., & Singh, B. (1981) *Nucleic Acids Res. 9*, 1707–1721] were prepared in large amounts by papain digestion of the immunoglobulin G (IgG) fraction from ascites fluid. Binding data were generated by a fluorescence quenching technique, and binding constants [$K(0)$] were estimated from Scatchard plots. The Fab fragments bound tightly to poly(dT) [$K(0) = 12.7 \times 10^6$ M^{-1}], and analysis of binding constants for the series $p(dT)_2$ to $p(dT)_{17}$ showed that the recognition sequence consisted of four consecutive residues. The effect of ionic strength on the interaction suggested that only two phosphates were involved. Binding constants for poly(dU), poly[d(brU)], poly[d(brC)], and poly(rU) were 1.0×10^6 M^{-1}, 18.8×10^6 M^{-1}, 0.5×10^6 M^{-1}, and $<0.5 \times 10^6$ M^{-1}, respectively, implicating the involvment of the 3, 4, and 5 positions of the pyrimidine ring as well as the deoxyribose sugar in the recognition process. At high ionic strength (0.5 M) $K(0)$ for whole IgG binding to poly(dT) was 75×10^6 M^{-1} compared to a value of 1.1×10^6 M^{-1} for the Fab fragment. These results may have implications for the tissue damage caused by DNA-containing immune complexes in systemic lupus erythematosus.

From autoimmune NZB/NZW mice we recently prepared six hybridoma cell lines that secrete antibodies to single-stranded DNAs (Lee et al., 1981). Two of these cell lines (HEd 8 and HEd 10) were of paticular interest since, although they were derived from different mice, they produce antibodies that are indistinguishable by isoelectric focusing gels as well as on the basis of their DNA-binding specificity patterns (Lee et al., 1981). Thus they may represent a particularly common type of murine autoimmune antibody. The mechanism by which these antibodies cause tissue damage, particularly to the kidneys, in the disease systemic lupus erythematosus (SLE) is not well understood, but complexes between antibodies and nucleic acids are clearly implicated (Koffler et al., 1971; Hahn, 1981). Thus a detailed understanding of antibody–DNA interactions at the molecular level may give a better insight to this rather puzzling disease. Recently several other nucleic acid binding autoimmune antibodies have been prepared and some of their properties described (Lafer et al., 1981a; Tron et al., 1980; Eilat et al., 1980; Jacob & Tron, 1982), but as yet no unique features of autoimmune antibodies have become apparent.

Proteins that bind to nucleic acids are also of considerable interest to molecular biology, and several systems, for example, the lac repressor (Ogata & Gilbert, 1977; Lin & Riggs, 1972; Goeddel et al., 1978), the cro repressor (Ptashne et al., 1980; Anderson et al., 1981), and the gene 32 protein of phage T4 (Kowalczykowski et al., 1981; Newport et al., 1981), have been studied in detail. However, these systems are complex because of the multimeric nature of the proteins and/or the possession of several different modes of binding. In this paper we shall describe the properties of Fab fragment HEd 10 binding to single-stranded DNAs of defined sequence and length and show that this is a comparatively simple process. It may thus serve as a good model for protein recognition of nucleic acids in general.

Materials and Methods

Nucleic Acids. Poly(dT), poly(dU), poly(dC), poly(dA), poly(dA)·poly(dT), and the $p(dT)_x$ oligomers ($x = 2$–17) were purchased from P-L Biochemicals. The repeating-sequence pyrimidine DNAs were prepared by depurination, and poly[d(TG)] was prepared by alkaline ceasium chloride buoyant density centrifugation from the appropriate duplex DNAs as described previously (Harwood & Wells, 1970; Morgan et al., 1974, 1979; Lee et al., 1979). Concentrations were calculated from absorbance measurements with extinction coefficients listed previously (Morgan et al., 1979; Lee et al., 1979) or with those supplied by the manufacturer. Cardiolipin and glucose 1,6-diphosphate were purchased from Sigma, and solutions of known concentration were prepared by direct weighing.

Preparation of Immunoglobulin G (IgG) and Fab Fragment HEd 10. The hybridoma cell line HEd 10 was derived from autoimmune NZB/NZW mice (Lee et al., 1981), and injection into three C_3H pristane-primed mice produced large quantities of ascites fluid (10–15 mL/mouse). Clots after formation were removed by centrifugation, and the supernatant

† From the Department of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada (J.S.L.), and the Departments of Biochemistry and Immunology, University of Alberta, Edmonton, Alberta, Canada (D.F.D. and T.R.M.). *Received April 21, 1982.* Supported by the Medical Research Council of Canada.

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