Identification of Minimal Size Requirements of DNA for Activation of Poly(ADP-ribose) Polymerase†

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ABSTRACT: Poly(ADP-ribose) polymerase requires DNA as an essential enzyme activator. Using enzyme purified from lamb thymus and double-stranded deoxynucleotide oligomers of defined length, we conducted studies to identify the smallest size DNA fragment capable of successfully activating poly(ADP-ribose) polymerase. These studies revealed that a double-stranded hexadeoxynucleotide activated the enzyme 30% as effectively as highly polymerized calf thymus DNA and a double-stranded octadeoxynucleotide activated the enzyme even more effectively than calf thymus DNA. When histone H1 was also included in the reaction system, the enzyme could be activated by even smaller DNA fragments. Thus, in the presence of histone H1, a double-stranded tetradeoxynucleotide activated the enzyme 25% as effectively as calf thymus DNA, and a double-stranded hexadeoxynucleotide was equally as effective as calf thymus DNA. The time courses for activation and the stabilities of the products were identical when the enzyme was activated by a double-stranded hexadeoxynucleotide or by calf thymus DNA. Double-stranded oligodeoxynucleotides containing dephosphorylated termini were more effective activators than those containing 3′-phosphorylated termini which in turn were more effective than those containing 5′-phosphorylated termini.

Poly(ADP-ribose) polymerase is a chromatin-bound enzyme activated in vivo by the formation of DNA strand breaks (Miller, 1975; Benjamin & Gill, 1980a; Berger & Sikorski, 1981). In vitro, the purified enzyme has an absolute requirement for exogenous DNA as an essential enzyme activator (Yoshihara & Koide, 1973; Petzold et al., 1981; Kawaichi et al., 1981). In some laboratories, the enzyme has been purified in association with DNA fragments which eliminates the need for exogenous DNA to achieve activation (Hashida et al., 1979; Niedergang et al., 1979). These fragments, designated sDNA, are smaller than bulk DNA and are in the range of 150–300 base pairs long (Hashida et al., 1979; Niedergang et al., 1979). sDNA is more effective than either bulk cellular DNA or calf thymus DNA in stimulating enzyme activity (Hashida et al., 1979; Niedergang et al., 1979). This increased efficiency of sDNA may be due to its relatively small size or to some sequence, composition, or structural specificity (Hashida et al., 1979; Niedergang et al., 1979).

Electron micrographs show that several molecules of poly(ADP-ribose) polymerase can be associated with a single sDNA molecule (de Murcia et al., 1983), suggesting that only a portion of each DNA is required for enzyme binding and activation. Hashida et al. (1979) estimated that a maximum number of 15 enzyme molecules could be activated by a fragment of double-stranded sDNA, 300 base pairs in length. These results suggest that the enzyme may be activated by a DNA segment as short as 20 base pairs in length. Calculations based on kinetic studies with sDNA and poly(ADP-ribose) polymerase suggest that enzyme activation requires a 16 base pair stretch of sDNA (Niedergang et al., 1979). It is, however, unknown whether a DNA fragment 16–20 base pairs long can activate the enzyme or whether such an activating segment must be part of a longer piece of DNA. It is also unknown whether any specific sequences are required for enzyme activation. The present studies were initiated to identify the smallest double-stranded DNA fragment capable of activating purified poly(ADP-ribose) polymerase.

Materials and Methods

Poly(ADP-ribose) polymerase, purified from lamb thymus, was the same as previously described (Petzold et al., 1981). Highly polymerized calf thymus DNA (CT DNA) was obtained from Sigma Chemical Co., St. Louis, MO. Other double-stranded oligodeoxynucleotides were from Pharmacia P-L Biochemicals, Piscataway, NJ. Stock solutions of DNA and oligodeoxynucleotides were prepared either in 0.1 M NaCl and 0.01 M potassium phosphate, pH 7.0, or in 0.015 M NaCl. The different solvents for the stock solutions did not affect the ability of the DNA to activate enzyme. The absorbance at 260 nm was determined on all solutions of DNA and oligodeoxynucleotides, and concentrations were standardized by using the value 1 A_{260} unit = 50 μg/mL DNA. Histone H1 was from Boehringer-Mannheim. Adenosine-[2,8-^{14}C]NAD (specific activity 554 mCi/mmol) was from New England Nuclear. The standard enzyme assay system contained 10 μg of DNA or oligodeoxynucleotide, 0 or 10 μg of histone H1, 0.45 mM [^{14}C]NAD$^+$ (specific activity 5.2 dpm/pmol), 1.14 mM dithiothreitol, 114 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 8.0, and 30 μL of enzyme solution (23 μg/mL) in a final volume of 100 μL. Reaction components were combined in an ice–water bath, and reactions were started by transferring tubes to a 37 °C water bath where they were incubated with gentle shaking for 30 min. Reactions were stopped by addition of an excess of cold 20% trichloroacetic acid. Samples were processed for scintillation counting on Whatman GF/C disks as previously described (Petzold et al., 1981).

Results

Table I shows that double-stranded oligodeoxynucleotides can replace calf thymus DNA as the essential enzyme activator

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Table I: Poly(ADP-ribose) Polymerase Activity[a]

activator	% reaction with CT DNA activator	
	-H1	+H1
CT DNA	100	100
no DNA	0	0
d(AGCT)	0.1	25
d(GTTAAC)	30	108
d(GGAATTCC)	385	396
d(CCGAATTCGG)	212	278
d(CCCGAATTCGGG)	215	287

[a] Poly(ADP-ribose) polymerase activity was measured by incorporation from [^{14}C]NAD in a 30-min reaction as described under Materials and Methods. The oligodeoxynucleotides, listed under activators, were double stranded. A, G, C, and T stand for adenine, guanine, cytosine, and thymine, and their sequence refers to the base sequence of one strand of each double-stranded oligomer. The oligomers all contained dephosphorylated termini. In a typical experiment, incorporation in the absence of histone H1 was 1000 dpm, and incorporation in the presence of histone H1 was 10000 dpm. All values in the first column are expressed relative to the poly(ADP-ribose) synthesis in the presence of CT DNA but without added histone H1. All values in the second column are expressed relative to the poly(ADP-ribose) synthesized in the presence of CT DNA with 10 μg of histone H1 added.

for purified poly(ADP-ribose) polymerase. The enzyme activity measured in the presence of 10 μg of calf thymus DNA, without added histone, was set at 100% for comparison to the other reactions listed in the same column, and all were run in the absence of histone H1. As previously shown, the enzyme activity measured in the presence of both 10 μg of calf thymus DNA and 10 μg of histone H1 was approximately 10 times greater than the value obtained in the absence of H1 (Petzold et al., 1981). This value, obtained in the presence of DNA and histone H1, was also set at 100% for purposes of comparison to other reactions listed in the second column of Table I, and all were run in the presence of histone H1. The 10-fold increase in poly(ADP-ribose) synthesis in the reaction containing histone H1 is associated with both the histone and the polymerase serving as acceptors for poly(ADP-ribosylation) whereas in the absence of H1, only the polymerase serves as acceptor for poly(ADP-ribosylation) (Carter & Berger, 1982). In the absence of added DNA, there was no poly(ADP-ribose) synthesis, confirming the requirement for DNA as an essential enzyme activator. This requirement for DNA was not affected by the presence or absence of histone H1.

When the oligodeoxynucleotides were evaluated, we obtained the surprising result that the enzyme could be activated by a double-stranded fragment, 4 base pairs in length. Thus, when the double-stranded tetramer d(AGCT) was employed as activator in the presence of histone H1, poly(ADP-ribose) was synthesized at 25% of the level that occurred in the presence of CT DNA. When the same double-stranded tetramer was evaluated in the absence of histone H1, there was negligible poly(ADP-ribose) synthesis. This reaction shows that a fragment of DNA, 4 base pairs long, is capable of serving as activator for poly(ADP-ribose) polymerase. At this length, however, the presence of an accessory protein such as histone H1 is required for the fragment to serve as an effective activator.

The double-stranded hexadeoxynucleotide d(GTTAAC) served as an effective enzyme activator in the presence of histone H1. Under these conditions, the activation by the hexadeoxynucleotide was essentially equivalent to that which occurred with CT DNA. More importantly, the double-stranded hexamer was able to activate the enzyme, even in the absence of H1. This contrasts to the results with the tetramer where activation occurred in the presence of H1 but

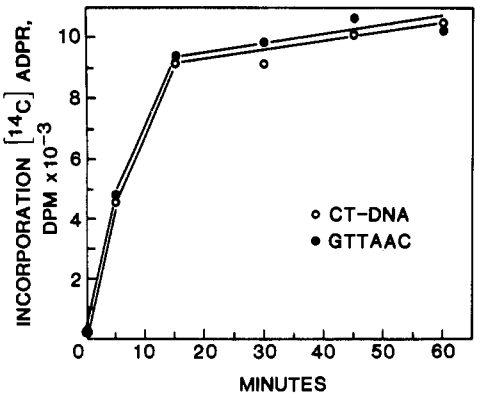

FIGURE 1: Time course of poly(ADP-ribose) synthesis using either 10 μg of CT DNA (O) or 10 μg of the double-stranded oligomer d(GTTAAC) (●) as enzyme activator. Reactions measure incorporation from [^{14}C]NAD, and all contained 10 μg of histone H1 as described under Materials and Methods.

Table II: Poly(ADP-ribose) Polymerase Activity[a]

chain length of activator	% reaction with CT DNA activator			
	3′-phosphates		5′-phosphates	
	-H1	+H1	-H1	+H1
2	0	1	0	0
3	0	2.3	0	0
4	3.2	10.1	0	0.5
5	8.4	70.1	0.4	0.9
6			0	1.3

[a] Poly(ADP-ribose) polymerase activity was measured as described under Materials and Methods. The chain lengths (number of deoxynucleotides) of the double-stranded deoxynucleotide activators are listed in the first column. The oligomers contained 3′-phosphorylated termini or 5′-phosphorylated termini as indicated. All values are expressed relative to reactions run simultaneously with calf thymus DNA as activator as indicated in Table I. The columns labeled -H1 were run in the absence of histone H1; those labeled +H1 were run in the presence of 10 μg of histone H1 as in Table I.

not in its absence. Thus, a double-stranded hexadeoxynucleotide is able to activate poly(ADP-ribose) polymerase, even in the absence of accessory proteins. Double-stranded oligomers of 8, 10, and 12 nucleotides in length were even more effective than CT DNA as activators. The highest activity was associated with the octadeoxynucleotide. In this size range, the ability of the oligomers to fully activate the enzyme, relative to CT DNA, was not dependent on the presence or absence of histone H1.

It is interesting to note that the oligomers of 8 base pairs and above are more effective activators than calf thymus DNA. This comparison of the activation properties of oligomers and CT DNA is based on equivalent weights but not equivalent moles. Thus, for solutions containing equivalent weights of DNA, those composed of short oligomers have many more strand ends than those composed of highly polymerized CT DNA. Thus, once the size of the oligomers reaches a critical length of 8 base pairs, the degree of enzyme activation is dependent on the number of available strand ends.

Figure 1 shows that the time courses of enzyme activity and the stabilities of the products were identical when the enzyme was activated by CT DNA or the hexadeoxynucleotide d(GTTAAC).

To further define the ability of short DNA oligomers to activate poly(ADP-ribose) polymerase, we tested a series of random-sequence oligodeoxynucleotides, purified by high-pressure liquid chromatography (HPLC) separation of nuclease digests of CT DNA. These oligomers were available with 3′- or 5′-phosphorylated termini. As shown in Table II,

Table III: Poly(ADP-ribose) Polymerase Activity[a]

	% reaction with CT DNA activator			
	dephospho		5′-phosphates	
activator	−H1	+H1	−H1	+H1
d(AGCT)	0.1	25	0	6
d(GTTAAC)	30	108	6	76

[a] Poly(ADP-ribose) polymerase activity was measured as described under Materials and Methods. d(AGCT) refers to the base sequence of one strand of a double-stranded deoxynucleotide tetramer, and d(GTTAAC) refers to the base sequence of one strand of a double-stranded deoxynucleotide hexamer. The oligomers were examined with termini that were dephosphorylated or with termini that contained 5′-phosphates as indicated. All values are compared to reactions run with CT DNA as activator and histone H1 either absent (−H1) or present (+H1) as described in Table I.

double-stranded dimers and trimers with either 3′- or 5′-phosphates were unable to significantly activate the enzyme. This inability to activate poly(ADP-ribose) polymerase was not affected by the presence or absence of histone H1. The 3′-phosphorylated, double-stranded tetramers in this series were weak activators when tested in the presence of histone H1. By comparison, the 5′-phosphorylated tetramers were inactive in both the presence and absence of histone H1. The 3′-phosphorylated pentamers were able to activate the enzyme in the absence of histone H1 and produced a high level of activity in the presence of histone H1. In contrast, pentamers and hexamers with 5′-phosphorylated termini were ineffective enzyme activators, in either the presence or the absence of histone H1.

In these studies, the double-stranded tetramer d(AGCT) was a more effective enzyme activator than the 3′-phosphorylated, random-sequence tetramers which in turn were more effective than the 5′-phosphorylated, random-sequence tetramers. The results in Tables I and II suggest that deoxynucleotide oligomers with dephosphorylated termini are more effective enzyme activators than fragments with 3′-phosphorylated termini which in turn are more effective than fragments with 5′-phosphorylated termini. However, these comparisons are indirect since the dephosphorylated compounds were oligodeoxynucleotides of defined sequence whereas the 5′- and 3′-phosphorylated oligomers contained variable and random base compositions. In order to perform a more direct comparison, we were able to obtain deoxynucleotide tetramers and hexamers of defined base sequences with 5′-phosphorylated termini comparable to those used in Table I. Table III shows that enzyme activity obtained with the dephosphorylated oligomers was greater than with the oligomers containing the 5′-phosphorylated termini. It is interesting to note that in the presence of histone H1, the 5′-phosphorylated hexamer was able to activate the enzyme almost as effectively as CT DNA whereas, as shown in Table II, under the same conditions, the 5′-phosphorylated hexamer of variable sequence only resulted in 1.3% activation. These latter results suggest that base sequence and/or composition may also be important in determining the ability of a DNA fragment to activate poly(ADP-ribose) polymerase.

Discussion

Purified poly(ADP-ribose) polymerase has been shown to require DNA as an essential enzyme activator (Yoshihara & Koide, 1973; Petzold et al., 1981; Kawaichi et al., 1981). It has also been shown that DNA strand ends are required for this activation, since closed-circular double-stranded DNA will not activate the enzyme whereas such a molecule will become an effective enzyme activator when single- or double-stranded cuts are introduced (Cohen & Berger, 1981; Benjamin & Gill, 1980b). This has been shown by treating closed-circular plasmids with UV irradiation followed by treatment with UV endonuclease to create strand breaks and consequently increase their ability to activate poly(ADP-ribose) polymerase (Cohen & Berger, 1981). It has also been shown that treating closed-circular plasmids with restriction endonucleases will increase their ability to activate poly(ADP-ribose) polymerase (Benjamin & Gill, 1980b). Using this approach, with large molecular weight restriction fragments of pBR322, it was also demonstrated that poly(ADP-ribose) polymerase is activated more effectively by dephosphorylated termini than by termini with 5′-phosphates (Benjamin & Gill, 1980b). Our present studies indicate that the abilities of the small oligodeoxynucleotide fragments to activate poly(ADP-ribose) polymerase are similar to the restriction fragments in that dephosphorylated oligomers are more effective enzyme activators than 5′-phosphorylated oligomers. In addition, our studies indicate that 3′-phosphorylated oligomers are intermediate between nonphosphorylated and 5′-phosphorylated fragments with respect to their ability to activate the enzyme.

Recent studies indicate that poly(ADP-ribose) polymerase protects a segment of double-stranded DNA, 60–90 base pairs in length, from nuclease digestion (Ittel et al., 1985). This suggests that an enzyme molecule bound to DNA covers a segment 60–90 base pairs long. Further studies show that even more base pairs can be wrapped around the enzyme in a fashion similar to nucleosomes (Ittel et al., 1985). Since the enzyme binds such long strands of DNA, we were surprised to find that very small deoxynucleotide oligomers were capable of significant activation. The role of histone H1 in facilitating this activation is unknown. It is, however, possible that the histone alters the binding properties of the enzyme. Alternatively, it is possible that the histone helps to align multiple oligomers, thereby providing, in effect, a longer stretch of DNA for enzyme binding. The demonstration that hexamers can activate poly(ADP-ribose) polymerase, even in the absence of histone H1, indicates that such an association of multiple fragments is not required and that small fragments can directly activate the enzyme without the need for accessory binding proteins.

Eight base pairs appears to be the critical length for a double-stranded fragment to serve as a full activator of poly(ADP-ribose) polymerase. Above this length, the ability of an oligomer to activate the enzyme is independent of histone-like proteins.

All studies of purified poly(ADP-ribose) polymerase to date show a requirement for DNA strand ends as essential enzyme activators (Benjamin & Gill, 1980a,b; Berger & Sikorski, 1981; Cohen & Berger, 1981). This observation correlates with the occurrence of DNA strand breaks as the stimulus for enzyme activation at the cellular level (Miller, 1975; Benjamin & Gill, 1980a; Berger & Sikorski, 1981). The present demonstration that poly(ADP-ribose) polymerase can be activated by double-stranded DNA fragments, smaller than 10 base pairs in length, now provides the means to study activation without binding multiple enzyme molecules to large DNA fragments. Thus, it should be possible to use the DNA oligomers to perform kinetic studies in which each enzyme molecule is activated by its own precisely defined DNA oligomer. Kinetic studies of poly(ADP-ribose) polymerase are also complicated by the fact that auto-poly(ADP-ribosylation) of the active enzyme results in a charge repulsion between the modified enzyme and the associated DNA, causing dissociation of the enzyme and consequent autoinactivation (Kawaichi et al., 1981; Niedergang et al., 1979; Zahradka & Ebisuzaki,

1982). It may now be possible to conduct studies with the oligomers to eliminate the charge repulsion that occurs between the auto-poly(ADP-ribosylated) enzyme and other portions of the DNA strand not essential for enzyme activation.

The results of this study also suggest that caution should be taken in interpreting some results of enzyme activation at the cellular level. It has recently been reported that certain metabolic or solvent perturbations increase the activity of poly(ADP-ribose) polymerase without changing the number of DNA strand breaks (Juares-Salinas et al., 1984). Such increases in activity are probably due to modulation of enzyme molecules that are already activated by existing strand breaks. However, the possibility must now be considered that very small DNA fragments, difficult to detect by standard methods, may become associated with the enzyme and involved in its activation.

Registry No. d(AGCT), 84520-45-6; d(GTTAAC), 94052-52-5; d(GGAATTCC), 70755-49-6; d(CCGAATTCGG), 63734-81-6; d(CCCGAATTCGGG), 96492-36-3; poly(ADP-ribose) polymerase, 9055-67-8.

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Infrared Spectroscopic Study of the Gel to Liquid-Crystal Phase Transition in Live *Acholeplasma laidlawii* Cells†

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ABSTRACT: The temperature dependences of the infrared spectra of deuterium-labeled plasma membranes of live *Acholeplasma laidlawii* B cells and of the isolated plasma membranes demonstrate that the profiles of the gel to liquid-crystal phase transitions are very different. At temperatures within the range of the phase transition, the live mycoplasma is able to keep the "fluidity" of its plasma membrane at a much higher value than that of the isolated plasma membrane at the same temperature. The difference is particularly pronounced at and around the temperature of growth. Live *Acholeplasma laidlawii*, grown at 37 °C on a fatty acid depleted medium supplemented with myristic acid (C14:0), pentadecanoic acid (C15:0), or palmitic acid (C16:0), are highly "fluid"; i.e., at the temperature of growth, the fractional population of the liquid-crystalline phase is 95–100% at 37 °C, whereas in the case of the isolated plasma membranes the fractional population of the liquid-crystalline phase at 37 °C is only 58% (C14:0), 36% (C15:0), or 38% (C16:0).

Few biological membranes have been more thoroughly studied to date than the plasma membrane of the microorganism *Acholeplasma laidlawii* (*A. laidlawii*).[1] The procaryotic *Acholeplasma laidlawii* belongs to the subbacterial family of mycoplasmas, the simplest microorganisms capable of autonomous growth and reproduction in cell-free media (Razin, 1979, 1982). *A. laidlawii* cells have no cell wall and possess only a single membrane system, the limiting or plasma membrane, which contains practically all the cellular lipid and a large fraction of the cellular protein as well. The plasma membrane can be easily isolated from the rest of the cell content by mild osmotic lysis and washing procedures. Furthermore, *A. laidlawii* cells readily incorporate exogenous fatty acids (including deuterated fatty acids) into the endogenous lipid pool of the plasma membrane (Silvius & McElhaney, 1978; Silvius et al., 1980; Jarrell et al., 1982). If the protein avidin is added to the growth medium, the de novo fatty acid

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[1] Abbreviations: C14:0-d_{27}, perdeuteriomyristic acid; C15:0-d_{29}, perdeuteriopentadecanoic acid; C16:0-d_{31}, perdeuteriopalmitic acid; PG, phosphatidylglycerol; MGDG, monoglucosyl diglyceride; DGDG, diglucosyl diglyceride; *A. laidlawii*, *Acholeplasma laidlawii* B; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; IR, infrared; TLC, thin-layer chromatography.

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