The Zinc Fingers of Human Poly(ADP-ribose) Polymerase Are Differentially Required for the Recognition of DNA Breaks and Nicks and the Consequent Enzyme Activation

OTHER STRUCTURES REQUIRE DNA* 

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The recognition of double-stranded DNA breaks and single-stranded nicks by human poly(ADP-ribose) polymerase and the consequent enzymic activation were examined using derivatives of the enzyme expressed in Escherichia coli. The N-terminal 162 residues encompass two zinc fingers. Deletion or mutation of the first finger results in a loss of activation by DNA with either single-stranded or double-stranded damage. Destruction of the second finger reduces activation by double-stranded DNA breaks only slightly, but eliminates activation by single-stranded DNA nicks. These data suggest that activation by single-stranded DNA nicks requires two zinc fingers, but activation by double-stranded DNA breaks requires only the finger closer to the N terminus. Variant proteins that lack both zinc fingers are enzymatically inactive but still exhibit weak DNA binding, which is independent of DNA damage. Thus, other regions are also capable of binding intact DNA, but the recognition of a strand nick or break which occasions the synthesis of poly(ADP-ribose) specifically requires the zinc fingers.

Poly(ADP-ribose) polymerase is a DNA-binding protein of higher eukaryotes that synthesizes poly(ADP-ribose) when cellular DNA is damaged and is believed to be required for DNA repair (1, 2). Poly(ADP-ribose) synthesis depends on the presence of DNA nicks or breaks (3). The polymerase is highly conserved among mammals (4, 5). The human polymerase is a single polypeptide of 113 kilo daltons (1014 amino acid residues), of which the N-terminal region of 373 residues has been implicated in DNA binding and DNA dependent activation (5-8).

The N-terminal region of human polymerase contains several potential DNA-binding motifs including two potential zinc fingers (6-8). Since the polymerase molecule is known to bind two zinc ions (9, 10), the zinc coordination residues are presumed to subtend actual zinc fingers. As a first attempt to determine how the various features contribute to DNA binding and to enzymic activity, we have analyzed the properties of polymerase whose zinc fingers have been deleted or destroyed by altering zinc coordinating residues.

The experiments were made possible by our recent development of an expression system for human poly(ADP-ribose) polymerase in Escherichia coli (11). The expressed polymerase has the same electrophoretic mobility, turnover number, and dependence on DNA for activity as does polymerase isolated from human tissues. Some of the derivatives we have constructed are also expressed well. We find that alterations of the first zinc finger (closer to the N terminus) result in the loss of DNA-dependent enzymic activity. In contrast, destruction of the second zinc finger reduces double-stranded DNA break-dependent enzymic activity only slightly, but results in loss of single-stranded DNA nick-dependent enzymic activity. These data suggest that activation by single-stranded DNA nicks requires two zinc fingers, but activation by double-stranded DNA breaks requires only the first finger. Other regions of the polymerase appear to recognize unbroken DNA in a manner that does not result in enzymic activation. Features which may be involved in such recognition include two lysine clusters at residues 221 226 and 346 352 and a possible helix-turn-helix in the interval 220-280 (6).

EXPERIMENTAL PROCEDURES

Plasmids Encoding Variant Human Poly(ADP-ribose) Polymerase: The expression plasmid for poly(ADP-ribose) polymerase, pTP, is described in Ref. 11. It consists of the complete cDNA for human poly(ADP-ribose) polymerase under the control of a trp promoter. One base of the original cDNA, a C at position 24, was changed to a T in order to create a new HindIII site. The resulting protein has the same electrophoretic mobility, turnover number, and dependence on DNA for activity as does polymerase isolated from human tissues. Some of the derivatives we have constructed are also expressed well. We find that alterations of the first zinc finger (closer to the N terminus) result in the loss of DNA-dependent enzymic activity. In contrast, destruction of the second zinc finger reduces double-stranded DNA break-dependent enzymic activity only slightly, but results in loss of single-stranded DNA nick-dependent enzymic activity. These data suggest that activation by single-stranded DNA nicks requires two zinc fingers, but activation by double-stranded DNA breaks requires only the first finger. Other regions of the polymerase appear to recognize unbroken DNA in a manner that does not result in enzymic activation. Features which may be involved in such recognition include two lysine clusters at residues 221 226 and 346 352 and a possible helix-turn-helix in the interval 220-280 (6).
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Fig. 1. Amino acid sequence of zinc fingers with zinc coordinating cysteines and histidine residues circled. Arrows indicate KpnI cleavage sites in the DNA. The asterisk represents the 1st residue (Met) of P99 (the internally initiated 99-kDa protein).

The concentration of polymerase was estimated from the intensity of gel bands stained with Coomassie Brilliant Blue, using β-galactosidase as the standard. β-Galactosidase may not be a valid standard for precise protein concentration measurements, but it is sufficient for comparing the protein concentration of several different samples.

Assay for Poly(ADP-ribose) Polymerase Activity—Samples of bacterial lysate or of purified polymerase were incubated for 5 or 10 min at 25 °C in assay buffer containing 50 mM Tris-HCl (pH 8.0), 10 mM Mg(O2-C03), 1 mM dithiothreitol, 100 μM [32P]NAD (Du Pont-New England Nuclear, diluted to 0.1 Ci/mmol), 2 μg of fragmented lambda phage DNA (2 μg of calf thymus DNA), and a total volume of 0.1 ml. The fragmented DNA used ("activated DNA," Sigma) is calf thymus DNA partially digested with DNase I in the presence of magnesium ions and thus contains both single-stranded nicks and double-stranded breaks (14). The average fragment size is 20 kilobase pairs. Poly(ADP-ribose) formation was stopped by adding 1 ml of 10% trichloroacetic acid, and acid-insoluble material was collected and counted.

Immunoblot Assays—Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) was performed as described by Laemmli (15). The proteins were transferred electrophoretically to an Immobilon membrane (Millipore), and immunoblots were probed with rabbit anti-bovine poly(ADP-ribose) polymerase antibody prepared by Gerald Marschall (Tufts University), followed by peroxidase-conjugated anti-rabbit IgG (Bio-Rad). Blots were developed with 4-chloro-1-naphthol (Bio-Rad) and H2O2. The antibody against bovine polymerase cross-reacted well with human polymerase.

Activity Gels—Bacterial lysates were fractionated on 7% polyacrylamide gels containing SDS, and 100 μg/ml sonicated salmon sperm DNA was added just before the gel was poured. The separated proteins were renatured in the gels by incubation in several changes of 50 mM Tris-HCl (pH 8.0) and 3 mM 2-mercaptoethanol for 30 min, then 6 mM guanidine hydrochloride in this buffer for 30 min, and then the buffer alone for 3 h. The gel was finally incubated in 1 mM dithiothreitol, 1 μM [32P]NAD (20 Ci/mmol), 10 mM Mg(O2-C03), and 50 mM Tris-HCl (pH 8.0) at 30 °C overnight as described by Scovassi et al. (16). The gels were stained with Coomassie Brilliant Blue, destained, and processed for autoradiography.

Protein Sequencing—The polymerase fraction purified by HPLC was separated on a 7% SDS-polyacrylamide gel electrophoresis. The protein bands were transferred to an Immobilon membrane and stained by Coomassie Brilliant Blue. The region of the polymerase band was cut out and analyzed by a gas-phase protein Sequencer (Applied Biosystems 477A) (17).

Preparation of pBR322—The preparations of supercoiled, HaeIII-digested, and nicked pBR322 have been described previously (3). By agarose gel analysis, the pBR322 purified by CsCl centrifugation consisted of 90% supercoiled DNA and 10% of a slower species that co-migrated with dimeric or nicked monomeric forms. pBR322 is cut into 22 blunt-ended fragments by HaeIII. The number of nicks introduced by partial DNase I digestion of pBR322 was estimated by extrapolating from the observed rate of disappearance of supercoiled DNA (14). The digestion was stopped at about 10 nicks/plasmid. The nicked DNA preparation contained less than 3% linear molecules, which might be inevitably formed by DNase I (14). Since the number of double-stranded breaks (≤0.03 per molecule) is so much less than the number of single-stranded nicks (10 per molecule), the contribution of double-stranded DNA breaks to the enzyme activity should be negligible. The concentration of DNA was determined by A260 and the number of the double-stranded DNA was estimated from the fluorescence intensities of bands in agarose gels stained with ethidium bromide.

RESULTS

Fig. 1 shows the two zinc fingers found in human poly(ADP-ribose) polymerase (6-8). Each is of the form Cys-X(Cys)-X8 or X9-3-11 or X7-Cys, and there is a considerable similarity of sequence between the two fingers. Figure 1 (closer to the N terminus) starts with Cys31, has a finger of 28 residues, and is electrically neutral (3 basic and 3 acidic residues). Figure 2 (the second finger) starts at Cys32, has a finger 30 residues long, and is basic (7 basic and 5 acidic residues).

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of human poly(ADP-ribose) polymerase in E. coli (11). pTP is a derivative of pBR322 carrying human poly(ADP-ribose) polymerase cDNA under the control of a trp promoter. The bacterially expressed protein (P113) has the same molecular size (113 kDa) and enzymic characteristics including turnover number, dependence on DNA for activity, and sensitivity to the inhibitors thymidine and benzamidine as poly(ADP-ribose) polymerase purified from placenta. However, a large fraction of the expressed products appears as proteins that are shorter than full length (Fig. 2a, lanes 1 and 2). Prominent among our products are two immunoreactive proteins (P99 and P89), which correspond in size and N-terminal sequence to proteins translated from internal positions (18). The amino acid sequence of P99 was determined to be Met-(Glu)-Lys-Ile-Glu-

Lys-Gly-Gln-Val, which corresponds to the region starting with Met136 in the human polymerase sequence (6-8). P89 was fractionated into two peaks by hydroxylapatite HPLC (HA-1000). Their sequences were Met-(?)-Glu-Val-Ala-Lys-Lys and Ala-Lys-Lys-Lys-Lys-Lys, which correspond to the regions starting with Val116 and Ala200. The substitution of Val216 by Met also suggests that P89 is the product of an internal start (19). These internal start sites are the same as those found by Herzog et al. (19), who expressed poly(ADP-ribose) polymerase lacking its first 67 amino acids. P89 proteins lack both of the zinc fingers and more than 50 additional downstream residues. P99 lacks zinc finger 1 and enough of the finger 2 sequence (Cys131-Lys-Gly-Cys135) to prevent the formation of zinc finger structure (Fig. 1).

Deletion of One or Two Zinc Fingers Reduces or Eliminates Enzymic Activity—The activity gel assay is appropriate for analyzing the internal start products since it requires no prior purification of proteins and allows the activities of fragments and intact polymerase to be compared in the same gel track. In this technique, the enzyme is fractionated on a SDS-gel containing DNA fragments, renatured in situ, and incubated with [32P]NAD. Any [32P]poly(ADP-ribose) formed remains attached to its synthetic enzyme and is detected by autoradiography.

Full length polymerase (P113) was readily detected as an active species, but none of the truncated forms were active (Fig. 2b). In particular, P99 and P89 had no DNA-dependent activity, even though they were present at about the same abundance as P113 as determined by immunoblotting of a parallel gel (Fig. 2a). Therefore, the N-terminal region of the DNA-binding domain, which contains the two zinc fingers, appears to be required for DNA-dependent activity. Features beyond residue 182, as discussed below, bind DNA, but such binding is insufficient to activate the enzyme. Herzog et al. (19) were also unable to show DNA-dependent activity of the 99-kDa and 89-kDa internal start proteins.

We constructed deletion mutants in the N-terminal region by eliminating codons between paired restriction sites in frame. The larger of these deletions, pTP2, extended between two HincIII sites in the DNA and resulted in the elimination of residues 7-232 in the protein, encompassing both fingers and the first lysine cluster, Lys221-Lys-Lys-Ser-Lys-Lys226. The deletion product, P88A is inactive. We found no activity in a total bacterial lysate expressing pTP2, no activity by activity gel analysis, and no activity of the purified protein (Fig. 2, Table I).

To construct pTPK, a smaller region was deleted, between two KpnI restriction sites, which eliminated amino acids 53-158. The derived protein, P101A, thus contains a hybrid finger that consists almost entirely of finger 1 with only the change His158-Pro-Gly-Cys202 in place of His200-Phe-Ser-Cys204 at the C-terminal side (Fig. 1). It had lost much of its activity and could not be demonstrated on activity gels. After purification, it was found that P101A retained negligible activity. pTPK retains the second internal start position and consequently directs the production of the inactive P89 mentioned above (Fig. 2a).

Substitutions of Cysteines in the Zinc Fingers—For a finer analysis, we constructed derivatives in finger 1 (pTPZ1) and finger 2 (pTPZ2) in which the cysteines at the start of each finger were both replaced by tyrosine residues, leaving Tyr-Tyr-His-Cys motifs which are expected not to make zinc fingers. These were synthesized by guest on November 6, 2017http://www.jbc.org/Downloaded from
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TABLE I

| Plasmid       | Protein                  | Expected finger structures in N-terminal regiona | Activity gelb | Specific activity of purified polymerasec |
|---------------|--------------------------|-------------------------------------------------|---------------|------------------------------------------|
| pTP           | P113 (wild type, full length) | [ ][ ][ ]                                     | +             | 300 units/mg                             |
|               | P89 (internal initiation) | None                                            | -             | ND                                       |
| pTPH          | P88A ([Δresidues 7–232]) | None                                            | -             | 0.3 units/mg                             |
| pTPK          | P101Δ ([Δresidues 53–158])| [ ][ ][ ]                                     | -             | 1.2 units/mg                             |
| pTPZ1         | P113-Z1- ([C21Y, C24Y])  | [ ][ ]                                         | -             | ND                                       |
| pTPZ2         | P113-Z2- ([C125Y, C128Y])| [ ][ ][ ]                                     | +             | 210 units/mg                             |

a The N-terminal structures (1–177 residues) with the two expected zinc fingers drawn in proportion. Finger 1 and finger 2 are shown by closed and open boxes. The remainder of the molecule, indicated by the dashed line, is the same in every case.
b DNA-dependent production of poly(ADP-ribose) by gel-fractionated enzyme renatured in situ. The limit of detection is 1% of control.
c Enzymic activity measured on the purified proteins, which were at least 60% pure. One unit of enzyme activity was defined as the amount of enzyme which catalyzes the incorporation of 1 nmol of ADP-ribose into acid-insoluble material per min. The comparable specific activities of polymerase purified from human placenta were 490 units/mg and 4 units/mg, in the presence and in the absence of DNA, respectively. The DNA used had both single-stranded nicks and double-stranded breaks.

The nature of the minor activity in the absence of DNA is not clear. Because this DNA-independent activity is blocked by 3-aminobenzamide, an inhibitor of the polymerase, it is assumed to require at least some function of the enzyme. Polymerase-derived proteins show the same low level of DNA-independent activity (Fig. 2c) which therefore does not require the zinc finger region and cannot be ascribed, for example, to an undetected quantity of DNA fragment in the enzyme preparation.

d ND, not determined.
e The activity of P113-Z2- was enhanced only by double-stranded breaks, but not by single-stranded DNA nicks; see Fig. 4c and the text.

The products of pTP (P113), pTPZ2 (P113-Z2-), pTPH (P88A), and pTPK (P101Δ) were purified as described under "Experimental Procedures." In each case, samples were taken of crude extract and after chromatography on phosphocellulose, 3-aminobenzamide-Sepharose, and HPLC HA-1000 columns. Samples were fractionated by 7% polyacrylamide SDS-gel electrophoresis and stained with Coomassie Brilliant Blue.

FIG. 3. Purification of poly(ADP-ribose) polymerase and its derivatives expressed in E. coli. The products of pTP (P113), pTPZ2 (P113-Z2-), pTPH (P88A), and pTPK (P101Δ) were purified as described under "Experimental Procedures." In each case, samples were taken of crude extract and after chromatography on phosphocellulose, 3-aminobenzamide-Sepharose, and HPLC HA-1000 columns. Samples were fractionated by 7% polyacrylamide SDS-gel electrophoresis and stained with Coomassie Brilliant Blue.

In contrast, the destruction of zinc finger 2 resulted in an active protein (P113-Z2-), which was easily detectable on an activity gel (Fig. 2b). The activity was still remarkably dependent upon broken DNA (Table I).

Biochemical Properties of P113 and P113-Z2-—In order to compare the biochemical properties of bacterially expressed poly(ADP-ribose) polymerase and its derivatives, they were purified from 1-liter cultures to near homogeneity (Fig. 3). Their structures and activities are summarized in Table I.

Under standard assay conditions using activated DNA as described under "Experimental Procedures," the variant polymerase, P113-Z2-, has 70% of the activity of wild type polymerase, P113. The Kₐ (NAD) of P113-Z2- is 50 μM, as compared with 30 μM for P113. The activity of each protein approached a maximum with about 20 μg/ml activated DNA but could be increased by adding histone. Incorporation of ADP-ribose increased linearly for the first 10 min and more slowly for the next 50 min in both cases.

As has been shown before, supercoiled plasmid DNA has little or no ability to activate polymerase (3). The small apparent stimulation by undigested pBR322 seen in Fig. 4 may be largely attributed to a small quantity of nicked circles.
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FIG. 4. Poly(ADP-ribose) polymerase activity as a function of DNA concentration and type. Polymerase was purified from human placenta (a) and from bacteria (b and c). Portions (0.05 µg) were incubated with 50 mM Tris-HCl (pH 8.0), 10 mM Mg(O.CO.CH₃), 1 mM dithiothreitol, 100 µM [³²P]NAD, and various amounts of pBR322:histone mixture (1:1, w/w). The pBR322 was supercoiled (O), nicked (X) or digested with HaeIII which cuts with blunt ends (D). The activities using activated DNA (20 µg/ml) were a, 103; b, 43; c, 62 pmol of ADP-ribose incorporation per 5 min. In several independent experiments, we have determined that the activity of each protein approaches the same maximum at 20 µg/ml HaeIII-digested pBR322 and 40 µg/ml nicked pBR322.

FIG. 5. DNA binding by P113 and P88Δ. Supercoiled pBR322 (0.1 µg, 0.035 pmol) was used as such or digested HaeIII (thus 0.76 pmol) and incubated with various amounts of purified poly(ADP-ribose) polymerases expressed in E. coli, P113, or P88Δ, in 50 mM Tris-HCl (pH 8.0), 2 mM Mg(O.CO.CH₃), 1 mM dithiothreitol, and 0.1% Nonidet P-40 in a total volume of 15 µl at 25 °C for 30 min. The samples were mixed with 5 µl of 30% glycerol containing bromphenol blue and separated on a 5% polyacrylamide gel (a) or a 1% agarose gel (b) containing 50 mM Tris-HCl (pH 8.0). After electrophoresis at 50 V for 3 h (a) or 30 V for 16 h (b), the gels were stained with ethidium bromide.

in the plasmid preparation. However, the DNA becomes substantially more effective when it contains either single-stranded nicks or double-stranded breaks. With respect to activation by different types of DNA damage, bacterially synthesized P113 behaves like polymerase purified from human placenta (Fig. 4, a and b). In contrast, the activity of P113-Z2⁻ was enhanced only by double-stranded breaks, but not by single-stranded nicks (Fig. 4c). This result implies that the second finger is required to recognize single-stranded nicks.

Specificity of P113-Z1⁻—We have been unable to purify the protein lacking finger 1 (P113-Z1⁻) and could not therefore estimate its response to DNA in a pure system. The activity gel containing sonicated DNA suggests that P113-Z1⁻ does not respond to double-stranded breaks (Fig. 2). To determine whether P113-Z1⁻ is activated by single-stranded nicks, we repeated the activity gel using DNase I-treated calf thymus DNA as the activator. P113-Z1⁻ was not activated although wild type polymerase (P113) was (not shown). Therefore, P113-Z1⁻ is activated neither by double-stranded breaks nor by single-stranded nicks.

DNA Binding—A semiquantitative gel shift assay was employed to compare the interactions of activatable (P113) and nonactivatable (P88Δ) protein with activating (HaeIII-digested) and nonactivating (supercoiled) DNA in the presence of 50 mM Tris-HCl (pH 8.0) (Fig. 5).

There was little difference between the four combinations. In all four cases, sufficient protein could move all of the DNA to the top of the gel, presumably in some multicomponent complex. Thus, it is clear that DNA binding can occur without enzyme activation, without DNA damage, and without the zinc fingers. Indeed, this generalized binding to internal, undamaged sites is the major interaction revealed by the gel-shift assay and is not obviously influenced by zinc fingers. P88Δ shifts large HaeIII fragments preferentially, consistent with multiple internal binding.

The only apparent difference among the four combinations was that about 2-fold less P113 than P88Δ was required to give a comparable shift to HaeIII fragments. Perhaps this reflects the additional ability of P113 to recognize DNA ends.

When the gel shifts were repeated in the presence of 100 mM KCl, the binding of P88Δ to either the supercoiled or
HaeIII-digested pBR322 was reduced by a factor of 10, while the binding of P113 to both DNAs was reduced only by a factor of 2 (data not shown). In the presence of 100 mM KCl, DNA binding of P88Δ was reduced approximately one-tenth of that of P113 with either the supercoiled or HaeIII-digested pBR322. These results suggest that the productive DNA binding with zinc finger motifs is tighter than the nonproductive DNA binding by motifs other than the zinc fingers. This nonproductive binding is weak but still significant and may have a different role than the tight binding to the DNA breaks determined by zinc fingers (see "Discussion").

DISCUSSION

The activities of variant polymerases show that the zinc finger region as a whole is essential for the activation of the enzyme by fragmented DNA. Elimination or destruction of zinc finger 1 is sufficient for inactivation for double-stranded breaks and single-stranded nicks, while destruction of finger 2 leaves intact the ability to recognize double-stranded breaks (Figs. 2 and 4, Table I). Thus, we deduce that zinc finger 1 is a determining structure for the activation of the enzyme by DNA breaks and nicks. In the deletion mutant pTPPK, finger 1 is essentially moved to the position of finger 2 and the inactivity of the product probably means, not surprisingly, that finger 1 must be at its appropriate position to be effective. Presumably, the binding of the finger 1 to DNA breaks or nicks results in an allosteric change in the polymerase that translates into the production of poly(ADP-ribose). In contrast, finger 2 contributes to the recognition of single-stranded DNA nicks only and apparently not of double-stranded DNA breaks (Fig. 4). During the preparation of this paper, Gradwohl et al. (21) examined DNA binding by a bacterially expressed 26-kDa N-terminal fragment of polymerase. They determined that finger 2 is required for binding of this fragment to single-stranded DNA nicks. Our observations show that finger 2, as well as finger 1, is required for activation of enzyme by single-stranded DNA nicks (Fig. 4, Table I).

It is interesting that enzyme activation by a single-stranded DNA nick requires two fingers and enzyme activation by a double-stranded DNA break requires only one finger, finger 1. It is not clear that a zinc finger is suitable structure for recognizing a DNA end directly; we suggest that a separate site on the enzyme performs this recognition role and that the role of the zinc fingers is to stabilize the broken DNA at the activating site by interacting with the adjacent single-stranded or double-stranded DNA.

Multiple zinc fingers are often found in transcription factors which are associated with recognition of specific DNA sequences (22–24). By contrast, single finger proteins have not been shown to bind DNA in a sequence specific manner (24, 25). Although poly(ADP-ribose) polymerase has two fingers, it resembles the single-finger group, since it recognizes double-stranded DNA breaks and single-stranded nicks, while destruction of finger 2 leaves intact the ability to recognize double-stranded breaks (Figs. 2 and 4, Table I). Thus, we deduce that zinc finger 1 is a determining structure for the activation of the enzyme by DNA breaks and nicks. In the deletion mutant pTPPK, finger 1 is essentially moved to the position of finger 2 and the inactivity of the product probably means, not surprisingly, that finger 1 must be at its appropriate position to be effective. Presumably, the binding of the finger 1 to DNA breaks or nicks results in an allosteric change in the polymerase that translates into the production of poly(ADP-ribose). In contrast, finger 2 contributes to the recognition of single-stranded DNA nicks only and apparently not of double-stranded DNA breaks (Fig. 4). During the preparation of this paper, Gradwohl et al. (21) examined DNA binding by a bacterially expressed 26-kDa N-terminal fragment of polymerase. They determined that finger 2 is required for binding of this fragment to single-stranded DNA nicks. Our observations show that finger 2, as well as finger 1, is required for activation of enzyme by single-stranded DNA nicks (Fig. 4, Table I).

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The zinc finger region can be separated from the downstream portion of the DNA binding domain by digestion with plasmin or trypsin (10, 28). Each protease releases an approximately 26-kDa (sometimes reported as 29-kDa) N-terminal fragment containing the zinc fingers and the first lysine cluster at residues 221–226. The adjacent 32-kDa plasmin fragment (apparent size 36 kDa) contains the second lysine cluster, Lys246-Lys-Leu-Lys-Val-Lys-Lys259 and possibly a helix-turn-helix (6). As we would expect, the 26-kDa and 36-kDa fragments both bind to DNA-cellulose (28). The intact polymerase protects DNA termini against exonuclease digestion (29). It has been reported that the 26-kDa fragment separated by SDS-gel electrophoresis does not protect DNA termini against DNase I digestion (30). This is unexpected in view of our data, but the 26-kDa fragment may not have recovered its DNA-binding capacity after denaturation in SDS (30). In contrast, the subterminal 32-kDa plasmin fragment does recover DNA binding after SDS-gel electrophoresis and transblotting (29), possibly indicating that it has less stringent structural requirements than does the zinc finger region for DNA interaction. In agreement with our model, the 32-kDa fragment partially protects some internal DNA regions against endonuclease (29).

We have been able to distinguish different roles of the DNA binding motifs of human poly(ADP-ribose) polymerase by estimating enzymic activities and DNA-binding properties of polymerase and derivatives of polymerase expressed in E. coli. The zinc fingers are involved in the recognition of DNA breaks and nicks and the consequent formation of poly(ADP-ribose). The region C-terminal to the zinc fingers also binds DNA weakly, but this alone is not sufficient to engender enzymic activity. Rather, we think that this region adjacent to the zinc fingers may be involved in the other action of polymerase: the generalized binding to unbroken stretches of DNA. There are three features between residues 221 and 352, which might interact with DNA, two lysine clusters, and a possible helix-turn-helix, and it will be interesting to determine whether these contribute to DNA break-independent binding.

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