Coenzymatic Activity of Randomly Broken or Intact Double-stranded DNAs in Auto and Histone H1 Trans-poly(ADP-ribosylation), Catalyzed by Poly(ADP-ribose) Polymerase (PARP I)*

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The enzymatic transfer of ADP-ribose from NAD to histone H1 (defined as trans-poly(ADP-ribosylation)) or to PARP I (defined as auto-poly(ADP-ribosylation)) was studied with respect to the nature of the DNA required as a coenzyme. Linear double-stranded DNA (dsDNA) containing the MCAT core motif was compared with DNA containing random nicks (discontinuous or dcDNA). The dsDNAs activated trans-poly(ADP-ribosylation) about 5 times more effectively than dcDNA as measured by $V_{\text{max}}$. Activation of auto-poly(ADP-ribosylation) by dcDNA was 10 times greater than by dsDNA. The affinity of PARP I toward dcDNA or dsDNA in the auto-poly(ADP-ribosylation) was at least 100-fold lower than in trans-poly(ADP-ribosylation) ($K_a = 1400 \text{ versus } 3-15$, respectively). Mg$^{2+}$ inhibited trans-poly(ADP-ribosylation) and so did dcDNA at concentrations required to maximally activate auto-poly(ADP-ribosylation). Mg$^{2+}$ activated auto-poly(ADP-ribosylation) of PARP I. These results for the first time demonstrate that physiologically occurring dsDNAs can serve as coenzymes for PARP I and catalyze preferentially trans-poly(ADP-ribosylation), thereby opening the possibility to study the physiologic function of PARP I.

The activity of poly(ADP-ribose) polymerase (PARP I), EC 2.4.2.30) was originally considered to represent a novel form of covalent protein modification analogous to phosphorylation, acetylation, etc. (1). Immunochemical analysis of normal adult rat tissues for poly(ADP-ribose) (pADPR) showed that 99% of covalently bound polymer was found in the non-histone fraction of nuclear proteins, with histone H1 being the major acceptor among the remaining 1% (2). The actual quantities of protein-bound pADPR in normal tissues, however, are small (200–250 ng/g, dry weight) as compared with the large abundance of PARP I protein itself (0.5 × 10$^6$ copies/cell (cf. Ref. 4). This difference suggests that PARP I activity in vivo is highly regulated. The secondary structure of pADPR (3) predicts that binding of modified proteins to other proteins or to DNA will be altered by poly(ADP-ribosylation). Furthermore, unmodified PARP I avidly binds to a large number of nuclear proteins (4), an association that is completely blocked by added histone H1. In addition, an assortment of transcription factors was shown to bind to PARP I (cf. Ref. 5), and a transcriptional regulatory role of PARP I in muscle differentiation has been identified (6). The MCAT element derived from this work (cf. Ref. 6) served as a basic DNA sequence for the construction of dsDNAs studied in the present experiment. Recently the topology of the direct binding of PARP I to Topo 1 has been demonstrated in detail (7). The binding of PARP I to histone H1 induces conformational changes in the latter coinciding with new phosphorylation sites for Cdc2-kinase (8).

Although the enzymatic transfer of ADP-ribose from NAD to PARP I and to several nuclear proteins has been well established (1), it has remained unclear what factors determine protein auto- or heteromodification by poly(ADP-ribose). The large activation of PARP I by randomly broken dcDNA, both in vitro and in vivo, created the prevalent impression that only apparently nonphysiologic forces, which cause DNA damage, regulate the intracellular activity of PARP I (9). These results fail to explain a potentially physiologic cellular role of PARP I in the absence of such DNA damage (e.g., Refs. 5–8 and 10). A more detailed knowledge of the enzymatic mechanism involved in auto- and trans-poly(ADP-ribosylation), as outlined in this report, is prerequisite to the understanding of the cellular physiology of PARP I.

The research leading to the present studies is summarized as follows. We have shown in 1990 (11) that dimerization of PARP I molecules is a prerequisite to auto-poly(ADP-ribosylation), a conclusion supported by enzyme kinetic analyses (12). The possibility of the formation of higher oligomers of PARP I was deduced from enzyme kinetics of the activation of Topo 1 by PARP I (13). The distinct role of a catalytic function of PARP I monomer and the exclusive acceptor role of the second associating PARP I molecule in the homodimer were further clarified in 1994 (14). The transfer of ADP-ribose from PARP I to an acceptor protein was shown to occur via an unstable imidazolyl ADP-ribose intermediate (15). The unique role of randomly broken dcDNA (coDNA) to serve as a coenzyme of PARP I (9) was challenged by our previous results, which show a strong binding of PARP I to unusual DNA structures (16–18). Finally, the recent demonstration of sequence-specific DNA binding interactions among PARP I, members of the transcription enhancer factor 1 (TEF-1) family of transcription factors, and the MCAT 1 transcriptional regulatory element (6) suggested physiologic circumstances in which linear double-stranded DNA may serve as coenzyme for PARP I. These results are expanded...
in the present study, which demonstrates that dsDNAs containing neither free termini nor discontinuities (breaks) activate trans-poly(ADP-ribosylation) more effectively than randomly damaged “coenzyme” DNA. Thus the unique role of artificially damaged DNA in the regulation of PARP I is questioned, and the basis for a physiologic role for PARP I through binding to linear, double-stranded chromosomal DNA is supported by these studies.

MATERIALS AND METHODS

Synthesis of DNAs as single strands was carried out by Operon Technologies and purified (to more than 95% purity) by high pressure liquid chromatography, and homogeneity was assayed by PAGE. Single-strand DNA oligonucleotides were annealed in 100 mM NaCl, 1 mM Tris, 1 mM EDTA (pH 8.0). Single-strand nicks resulting from annealing of loop-stem-loops were repaired with T-4 ligase (overnight at 4 °C), and traces of incomplete ligation were removed by Exo III digestion and checked in native acrylamide gels.

Table I illustrates the composition of dsDNAs used in enzymatic assays. The MCAT core motif is underlined. Homogeneous calf thymus PARP I was isolated as reported (7). Biotinylated NAD and human PARP I were purchased from Trevigen Co. Electrophoretically homogeneous histone H1 and H3 were obtained from Roche, and ultrapure NAD PARP I were purchased from Trevigen Co. Electrophoretically homogeneous PARP I was isolated as reported (7). Biotinylated NAD and human PARP I were purchased from Trevigen Co. Electrophoretically homogeneous histone H1 and H3 were obtained from Roche, and ultrapure NAD was from Sigma.

Auto-poly(ADP-ribosylation) of PARP I was assayed as reported (4) by the incorporation of [32P]labeled ADP-ribose (from NAD) into acid-precipitable PARP I-bound polymers. Trans-poly(ADP-ribosylation) from NAD to histone H1-PARP I heterodimers was assayed by two methods described in the following paragraphs.

The first method is a colorimetric technique for the determination of biotinylated poly(ADP-ribose) bound to histone H1 coats (a modification of the assay described by Trevigen Inc.). Histone H1 was coated to the wall of 96-well plates by adding 0.1 mg/ml histone solution (in 50 mM Tris, 1 mM EDTA (pH 8.0). Single-strand nicks resulting from annealing of loop-stem-loops were repaired with T-4 ligase (overnight at 4 °C), and traces of incomplete ligation were removed by Exo III digestion and checked in native acrylamide gels.

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The second method is a trans- and auto-poly(ADP-ribosylation) with [32P]NAD as ADP-ribose donor. Histone H1 was added to a concentration of 5 μg/50 μl of reaction volume, which was a 100-fold excess over PARP I concentration. The formation of PARP I-histone heterodimers was complete within 10 min at room temperature. The 50-μl reaction system contained the same buffer as described in the previous paragraph. The poly(ADP-ribosylation) reaction was initiated by [32P]-labeled NAD (400 μM final concentration) and terminated by 1 ml of 20% trichloroacetic acid followed by the addition of 0.3 mg of bovine serum albumin (in 30 μl); then after three centrifugal washings (1 ml of 20% trichloroacetic acid-precipitable material were carried out as described under “Materials and Methods” (first method). The coenzyme DNase-treated DNA was obtained from Trevigen. Incubation time was 10 min.

![Fig. 1. Trans-poly(ADP-ribosylation) of histone H1 using various coenzymic DNAs. Curves 1–4, trans-poly(ADP-ribosylation) using double-stranded DNA oligomers. Curve 1, loop-stem-loop DNA (open circles); curve 2, double-stranded DNA with free ends (closed circles); curve 3, double-stranded DNA with chemical block on one end and single-stranded loop at the other (closed boxes); curve 4, double-stranded DNA with chemical block at both ends (open circles); curve 5, histone H1 trans-poly(ADP-ribosylation) using DNase I-treated salmon sperm DNA as coenzyme (closed diamonds) was measured as described under “Materials and Methods” (first method). The coenzyme DNase-treated DNA was obtained from Trevigen. Incubation time was 10 min.](image)

![Fig. 2. Poly(ADP-ribosylation) assays consisting of the incorporation of [32P]ADP-riboside from radiolabeled NAD into 20% trichloroacetic acid-precipitable material were carried out as described under “Materials and Methods” (second method). Compositions of the 50-μl reaction mixtures are shown (second method). Reaction times (at room temperature) were 10 min in all cases. All measurements were done in triplicate with an S.D. of ±20%.](image)
TABLE II

| Coenzymic DNA type | \( V_{\text{max}} \) | \( K_a \) |
|-------------------|----------------|-------|
| A. Trans-poly(ADP-ribosylation) | | |
| 1. Loop-stem-loop dsDNA | 1.25 | 17 |
| 2. Unmodified dsDNA | 5.0 | 15 |
| 3. Stem-loop-block dsDNA | 3.3 | 7 |
| 4. End-blocked dsDNA | 4.0 | 3 |
| 5. DNase-treated dcDNA | 1.0 | 20 |
| B. Auto-poly(ADP-ribosylation) | | |
| dsDNA (DNase-treated salmon sperm) | 5.0 | 1400 |
| dsDNA (modified or unmodified) | 0.5 | 1400 |

The activation of histone \( H_1 \) trans-poly(ADP-ribosylation) and PARP I auto-poly(ADP-ribosylation) by both dcDNA and by the end-blocked synthetic polydeoxyribonucleotide 4 (see Fig. 1) was also analyzed by the second method, which permits the simultaneous determination of both poly(ADP-ribosylation) by kinetic analysis that were linear over 20 min \((n = 3)\). As illustrated in Fig. 2, \( v_{\text{init}} \) as 10-min rates are shown in all assays for comparison.

The specificity of histone \( H_3 \) as poly(ADP-ribose) acceptor in the trans-poly(ADP-ribosylation) reaction was tested by replacing histone \( H_3 \) with \( H_4 \) (4). No transfer of ADP-ribose from NAD to histone \( H_3 \) occurred (not shown), confirming earlier results (4) which demonstrated that incorporation of histone \( H_3 \) into nucleosomes was a prerequisite for poly(ADP-ribosylation) of histone \( H_3 \) by PARP I. The cell biological importance of trans-poly(ADP-ribosylation) of histones contained in nucleosomes is the subject of further research.

Since a significant catalytic activation of poly(ADP-ribosylation) by Mg\(^{2+}\) has been described in 1979 (19), we re-investigated the role of Mg\(^{2+}\) in both trans- and auto-poly(ADP-ribosylation) reactions. As shown in Fig. 2A, histone \( H_1 \) trans-poly(ADP-ribosylation) was dramatically inhibited by Mg\(^{2+}\). There was also a large inhibition of trans-poly(ADP-ribosylation) by randomly broken “coenzymic” DNA (dcDNA) at a concentration that is required to maximize auto-poly(ADP-ribosylation). This explains the apparent absence of trans-poly(ADP-ribosylation) in results obtained by most experimentalsists in this field (cf. Ref. 9) who used high concentrations of artificially fragmented DNA as a coenzyme to detect poly(ADP-ribose) polymerase activity.

Auto-poly(ADP-ribosylation), on the other hand, was markedly activated by Mg\(^{2+}\), an effect observed with either 7 or 70 ng of dsDNA per test (Fig. 2B) but was maximal with the artificially fragmented dcDNA as a coenzyme. Based on these results (compare Fig. 2, A and B) we conclude that Mg\(^{2+}\) can discriminate between trans- and auto-poly(ADP-ribosylation) by inhibiting the former and activating the latter.

From results obtained in this study the specific activity of PARP I can be calculated for both auto and histone \( H_3 \) trans-poly(ADP-ribosilation) reactions. The specific activity for trans-poly(ADP-ribosilation) (picomoles of ADP-ribose incorporated into polymers per pmol of PARP I \( \times \) minutes) was 106 (+30%) \((n = 3)\) and for auto-poly(ADP-ribosilation) 50 (+20%) \((n = 3)\).

CONCLUSIONS

According to prevailing opinion (cf. Ref. 9), the complex interplay of poly(ADP-ribosilation) of proteins may regulate, e.g., transcription of cell-specific genes, or influence availability of genes for transcription via chromatin modifications. These events were previously presumed to be initiated by some random DNA damage. That scenario seems counterintuitive if it is assumed that an organized cell function reflects normal cell physiology. This difficulty is alleviated by results reported here because linear and stem-loop DNA structures that are known to exist in normal cells provide a much more powerful activator of PARP I than damaged DNA without invocation of pathophysiology. Although the unique structural features of these activating DNAs and their exact molecular mode of action on PARP I are still under investigation, the generation of PARP I binding DNA structures (e.g. Ref. 18) during DNA replication has been established (20); thus the assumption of their physiologic existence is feasible. Present results lend support to an early observation reported in 1987 (21), which illustrates different modalities of activation of PARP I by octodeoxyribonucleotides of differing base sequence.

The nucleotide sequence of the dsDNA-based paired region used in these experiments is found in the MCAT 1 transcriptional regulatory element that controls cell-selective expression of the chicken gene encoding cardiac troponin T (22, 23). The core motif of the MCAT 1 element is recognized by members of the TEF-1 family of DNA-binding proteins (24–27), the activity of which is altered through introduction of the nucleotides immediately upstream of the TEF-1 binding site (see 2b in Table I) disrupts cell-selective repression (28) and PARP-TEF-1 cooperative binding to DNA (6), no differences in either \( V_{\text{max}} \) or \( K_a \) were observed in the experiments reported here. Research in progress is concerned with how PARP I-dsDNA binding events are related to PARP I-mediated trans-poly(ADP-ribosilation). The catalytic activity of unusual (18) but physiologically occurring DNA structures either derived from DNA replication (20) or from physiologic DNA sequences (6, 22–28) is also part of this study.

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PARP I Activation by Double-stranded DNA

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