Inhibition of poly(ADP-ribose)polymerase binding to DNA by thymidine dimer

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Abstract The ability of poly(ADP-ribose)polymerase to bind damaged DNA was assessed by electrophoretic mobility shift assay. DNA binding domain of poly(ADP-ribose)polymerase (PARPDBD) binds to synthetic deoxyribonucleotide duplex 10-mer. However, the synthetic deoxyribonucleotide duplex containing *cys-syn* thymidine dimer which produces the unwinding of DNA helix structure lost its affinity to PARPDBD. It was shown that the binding of PARPDBD to the synthetic deoxyribonucleotide duplex was not affected by O^6-Me-dG which causes only minor distortion of DNA helix structure. This study suggests that the stabilized DNA helix structure is important for poly(ADP-ribose)polymerase binding to DNA breaks, which are known to stimulate catalytic activity of poly(ADP-ribose)polymerase.

Key words: Poly(ADP-ribose)polymerase; Thymidine dimer; DNA binding

1. Introduction

Poly(ADP-ribose)polymerase (PARP) is a chromatin-associated nuclear enzyme in eukaryotic cells. The binding of PARP to DNA breaks is known to activate catalytic activity of the enzyme. The DNA bound, activated PARP transfers ADP-ribose moiety from nicotinamide adenine dinucleotide (NAD) to numerous nuclear proteins including PARP itself, histones, RNA polymerase II, topoisomerase I, DNA polymerase α and DNA polymerase β [1,2].

PARP is composed of three functional domains: an N-terminal DNA binding domain that binds to DNA strand breaks [3], a central automodification domain and a C-terminal catalytic domain [4,5]. PARP has been thought to play multifunctional roles in various cellular events including DNA repair, mitotic cell division, stress responses, cytotoxicity, apoptosis and maintenance of chromosomal stability [6]. The free DNA end is known as a strong activator of PARP and PARP activity is increased by DNA damaging agents that introduce DNA breaks [7]. N-terminal fragment of PARP containing DNA binding domain (PARPDBD) comprises two zinc fingers that are involved in the recognition of DNA strand breaks [8]. Work by Benjamin and Gill [9] has shown that the ability of DNA to stimulate the catalytic activity of PARP is solely dependent upon the number and type of strand breaks, not upon the sequence at the binding sites. They suggested that double-stranded DNA conformation is important for the activation of PARP. For example, double-stranded DNA with flush end stimulates PARP activity approximately 3-fold more than the DNA fragment with unpaired nucleotide extending from 3′ strand. They also found that the duplex structure at 5′ end is more important for the PARP activation than the unpaired extension structure at 5′ end.

There are numerous studies showing that DNA damage disrupts the helical structure of DNA duplex. The UV induced photoadducts in DNA were typically known to destabilize the helical structure [10–12]. In this study, we report that PARP binding to DNA is inhibited by UV induced photoadduct.

2. Materials and methods

2.1. Synthetic deoxynucleotide oligomers

The oligonucleotide, d(CGCATTACGC), was synthesized by the phosphoramidite method using a DNA synthesizer (Applied Biosystem Model 391, USA). The crude 5′-dimethoxytritylated oligonucleotide was isolated by treating the support with concentrated aqueous ammonia for 48 h at room temperature. Purification of the product was carried out using reverse-phase high performance liquid chromatography followed by desalting in Sephadex G-25 column. *Cis-syn* thymidine dimer was produced by irradiating deoxyribonucleotide 10-mer with UV as previously described [13]. Duplex forms were prepared by dissolving the photochemically modified strand and complementary strand (adjusted as a stoichiometric 1:1 ratio) in a solution containing 20 mM Na_2PO_4 (pH 7.0), 100 mM NaCl. Deoxyribonucleotide 10-mer containing O^6-Me-dG was purchased from Biosynthesis Inc. (USA). The sequences of the synthetic deoxyribonucleotide oligomers employed in this study were presented in Table 1. The oligomers were end-labeled using [γ-^{32}P]ATP (Amersham Corp., UK) and T4 polynucleotide kinase (Boehringer Mannheim, Germany). The ^{32}P-labeled oligomers were collected using Sephadex G-25 spun column chromatography (Pharmacia LKB Biotechnology Inc., USA). Single- and double-stranded deoxyribonucleotide oligomers used as competitor DNA in the electrophoretic mobility shift assay were supplied by Genotech Inc. (South Korea). The competitor supercoiled pGEM3 plasmid DNA was purified using QIAGEN plasmid Maxi Kit (USA). Care was taken during the preparation of plasmid DNA to avoid the generation of nicked circular or linear plasmid DNA. The preparation contained routinely >90% supercoiled form of DNA as assessed by relative band intensities after electrophoresis in agarose gel through ethidium bromide staining.

2.2. Preparation of DNA binding domain of PARP

The entire DNA binding domain of PARP (PARPDBD) was prepared by PCR using the primers of 5′-GGGCGGATCCTGGCGGAGTCTTCGG-3′ (primer A, *Bam*HI site was underlined) and 5′-GGGCGGATCCCCATCCACCTCATCG-3′ (primer B, *Bam*HI site was underlined). The pGEM7 plasmid (Promega, USA) in which full length human PARP cDNA was inserted in *Xba*I site was used as DNA template. The cDNA fragment encoding two zinc finger domains of PARP was approximately 700 bp in length and was amplified by PCR using the primers A and B. The resulting fragment was cloned into *Bam*HI site of pET-21c expression vector (Novagen, USA) to construct pET-739 and *E. coli* BL21(DE3) cells were transformed with the vector construct. The expression of the cloned gene was induced by incubating cells in the media containing 0.4 mM isopropyl-beta-D-thiogalactopyranoside (IPTG) for 3 h. PARPDBD

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was purified using Ni-NTA column (Qiagen, USA). The purity of human PARPDBD isolated from *E. coli* cells was examined by 12% SDS-PAGE [14]. Immunoblot analysis was also carried out using polyclonal antibody against N-terminal fragment of human PARP (Santa Cruz Biotechnology Inc., USA) as a probe [15].

2.3. Electrophoretic mobility shift assay

The electrophoretic mobility shift assay was performed as described by Blier et al. [16] with slight modification. DNA binding was performed in a final volume of 20 μl of a reaction buffer containing 10 mM Tris (pH 7.5), 150 mM NaCl, 1 mM DTT, 0.01% Nonidet P-40 and 2% polyvinyl alcohol. The ^{32}P-labeled deoxyribonucleotide oligomers (approximately 20 000 cpm, 50 femtomole) were added to the reaction mixture together with 3.25 pmole of competitor oligomers. To the reaction mixture, 4 μg of PARPDBD protein was then added and the preparation was incubated for 30 min at room temperature. Each 10 μl of sample preparation was loaded directly onto premade 8% non-denaturing polyacrylamide minigels (BioRad, USA) and the electrophoresis was performed at 4°C. The mobility shift of ^{32}P-labeled DNA after its binding to PARPDBD was monitored by autoradiography.

3. Results and discussion

The recombinant protein, PARPDBD containing two zinc finger domains (amino acids 1–215) migrated to the distance corresponding to its molecular weight (~29 kDa) on 12% SDS-polyacrylamide gels (Fig. 1A and B). Immunoblot analysis identified the band as PAPRDBD (Fig. 1C). In Fig. 2A, the ability of different forms of DNAs to compete with ^{32}P-labeled oligomer duplex was assessed by electrophoretic mobility shift assay. It was shown that PARPDBD preferentially binds to double-stranded DNA. Double-stranded deoxyribonucleotide oligomers apparently competed with the labeled deoxynucleotide oligomer duplex and inhibited the complex formation between the labeled duplex and PARPDBD. Single-stranded oligomer and supercoiled DNA did not compete with the labeled oligomer duplex. The double-stranded deoxyribonucleotide 10-mer was known to be sufficient in length for the activation of PARP since the double-stranded DNA fragment longer than 8 base pairs was reported to act as a full activator of PARP [17].

While the direct participatory role of PARP during DNA repair process is not available, it has been suggested that PARP binding to DNA breaks produced by DNA damaging agents may have other functions important for the survival of damaged cells. The binding of PARP to the sites of DNA strand breaks may protect the nicked sites from nucleases, DNA binding proteins and other small DNA molecules. PARP may protect DNA by preventing recombination events before DNA repair. The PARP binding to DNA may as well facilitate survival responses in damaged cells by association with nuclear proteins necessary for DNA repair such as DNA ligase III, DNA polymerases, p53, XRCCI and DNA dependent protein kinase [18]. This study was initiated to examine the effects of DNA damages on the binding ability of PARP to DNA. With such a study, we attempted to evaluate the effects of DNA damages on PARP activation during cellular recovery from DNA damage.

Table 1
Sequences of synthetic deoxyribonucleotide oligomers[a]

Name	Sequence
10-mer duplex I	5′-(CGCATTACGC)-3′ 3′-(GCGTAATGCG)-5′
Double-stranded 10-mer competitor	5′-(GCGTTAACGC)-3′ 3′-(CGCAATTGCG)-5′
Single-stranded 10-mer competitor	5′-(CGCATTACGC)-3′
10-mer duplex I w/*cis-syn*[b]	5′-(CGCATTACGC)-3′ 3′-(GCGTAATGCG)-5′
10-mer duplex II w/O^6-Me-dG[c]	5′-(GCGTCGACGC)-3′ 3′-(CGCAGCTGCG)-5′
10-mer duplex II	5′-(GCGTCGACGC)-3′ 3′-(CGCAGCTGCG)-5′

[a]All the synthetic oligomers except cold competitor oligomers were 5′ end-labeled with ^{32}P.
[b]*cis-syn* denotes cyclobutane pyrimidine photodimer of thymidylyl (3′-5′) thymidine in which *cis* refers to the relative stereochemistry of the two methyl groups and *syn* to the relative orientation of the two C_4 carbonyl groups.
[c]O^6-Me-dG denotes methylated guanosine at O^6 position.

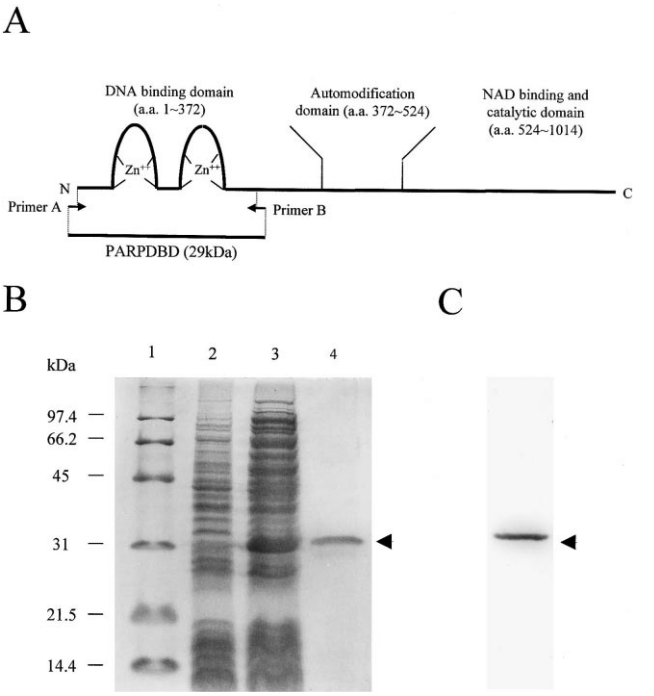

Fig. 1. Purification of 29 kDa poly(ADP-ribose)polymerase DNA binding domain (PARPDBD). A: Schematic representation of PARPDBD and zinc fingers. B: Affinity purified PARPDBD was analyzed in 12% SDS-PAGE. Lane 1, size markers (Sigma); lane 2, cell lysate of *E. coli* cells before the induction of cloned human PARPDBD gene; lane 3, cell lysate of IPTG induced *E. coli* cells expressing human PARPDBD; lane 4, PARPDBD was affinity purified using Ni-NTA affinity column. C: The purified PARPDBD was transferred on the nitrocellulose membrane and probed with polyclonal antibody against N-terminal fragment of PARP for immunoblot analysis. Arrowheads in B and C indicate 29 kDa PARPDBD.

Our data showed that the PARPDBD forms a specific complex with ^{32}P-labeled deoxyribonucleotide oligomer duplex. However, the binding of PARP to the duplex was suppressed if the deoxyribonucleotide oligomer duplex contained *cis-syn* thymidine dimer, a major class of cytotoxic, mutagenic and carcinogenic DNA lesion induced by UV light (Fig. 2C). It is unlikely that 10-mer duplex containing *cis-syn* thymidine dimer denatured into single-stranded 10-mer during the electrophoretic mobility shift assay and lost its affinity with PARPDBD. In an independent control experiment, we found that the structural stability of the modified duplex was not altered during electrophoretic mobility shift assay. The electrophoretic mobility of modified duplex under the same experimental condition as in Fig. 2C was compared with that of

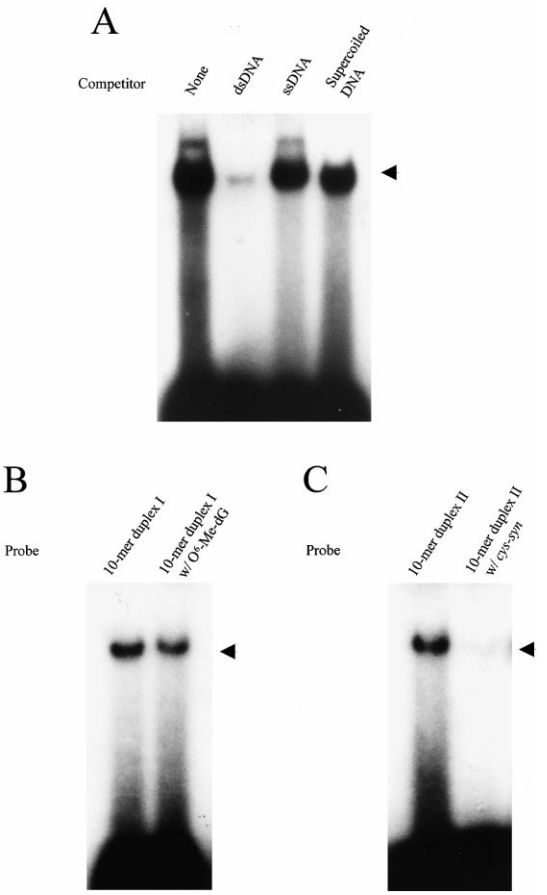

Fig. 2. Binding of poly(ADP-ribose)polymerase DNA binding domain (PARPDBD) to double-stranded DNA. A: PARPDBD was incubated with ^{32}P-labeled 10-mer duplex I with different forms of unlabeled competitors. B: The ability of PARPDBD binding to deoxyribonucleotide 10-mer duplex containing O^6-Me-dG. ^{32}P-labeled 10-mer duplex II with or without O^6-Me-dG was incubated with PARPDBD. The binding of PARPDBD with labeled 10-mer duplexes was examined by electrophoretic mobility shift assay and ^{32}P-labeled oligomer-PARPDBD complex was visualized by autoradiography. C: ^{32}P-labeled 10-mer duplex I with or without *cis-syn* thymidine dimer was incubated with PARPDBD. Arrowheads in B and C indicate ^{32}P-labeled oligomer-PARPDBD complex.

the same duplex under a known condition that maintains the structural stability of the duplex. The stable solution structure of the control duplex containing *cis-syn* thymidine dimer has been shown in the previous study using NMR [13]. The modified duplex showed the same electrophoretic mobility as the control duplex, while the single-stranded deoxyoligonucleotide 10-mer migrated farther distance on polyacrylamide gels (data not shown). Thus mobility shift of the labeled 10-mer duplex II containing *cis-syn* thymidine dimer does not seem to be related with the structural change of duplex stability during the assay. The suppression of PARP binding to the labeled oligomer probe seemed to be correlated with the unwinding of deoxynucleotide oligomer duplex in the vicinity of *cis-syn* thymidine dimer. Recent NMR studies have shown that Watson-Crick duplex is destabilized by *cis-syn* thymidine dimer that disrupts the pi-stacking to the 5′ side of the thymidine dimer [10] and distort the phosphodiester backbone at 3′ to the dimer [11]. Thus it was known that the helical structure of the duplex containing the *cis-syn* thymidine dimer was unwound by ~15° [12] and bent by 9° [13].

In contrast, PARP forms a stable complex with self-complementary deoxyribonucleotide oligomer duplex containing O^6-Me-dG (Fig. 2B). The O^6-Me-dG is one of the lesions introduced into DNA after treatment with methylating agents [19]. Molecular mechanical studies have shown that O^6-Me-dG exhibits only minor distortion of DNA helix except a dispersed phosphodiester backbone [20].

PARP binds to DNA interruptions produced by DNA damaging agents and undergoes a rapid automodification with the synthesis of long branched polymers of highly negative charged poly(ADP-ribose). The relevant function of PARP in the cellular recovery from DNA damage is still unknown. Our finding might provide some insight into the mechanism of different cellular responses to different DNA damages.

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