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Poly(ADP-ribose) polymerase 1 regulates activity of DNA polymerase β in long patch base excision repair

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ABSTRACT

Poly(ADP-ribose)polymerase 1 (PARP1), functioning as DNA nick-sensor, interacts with base excision repair (BER) DNA intermediates containing single-strand breaks. When bound to DNA breaks, PARP1 catalyzes synthesis of poly(ADP-ribose) covalently attached to itself and some nuclear proteins. Autopoly(ADP-ribosyl)ation of PARP1 facilitates its dissociation from DNA breaks and is considered as a factor regulating DNA repair. In the study, using system reconstituted from purified BER proteins, bovine testis nuclear extract and model BER DNA intermediates, we examined the influence of PARP1 and its autopoly(ADP-ribosyl)ation on DNA polymerase β (Pol β)-mediated long patch (LP) BER DNA synthesis that is accomplished through a cooperation between Pol β and apurinic/apyrimidinic endonuclease1 (APE1) or flap endonuclease 1 (FEN1) and gap-filling activity of Pol β. PARP1 upon interaction with nicked LP BER DNA intermediated, formed after gap-filling, was shown to suppress the subsequent steps in LP pathway. PARP1 interferes with APE1-dependent stimulation of DNA synthesis by Pol β via strand-displacement mechanism. PARP1 also represses Pol β/FEN1-mediated LP BER DNA synthesis via a "gap translation" mechanism inhibiting FEN1 activity on the nicked DNA intermediate. Poly(ADP-ribosyl)ation of PARP1 abolishes its inhibitory influence on LP BER DNA synthesis catalyzed by Pol β both via APE1-mediated strand-displacement and FEN1-mediated "gap translation" mechanism. Thus PARP1 may act as a negative regulator of Pol β activity in LP BER pathway and poly(ADP-ribosyl)ation of PARP1 seems to play a critical role in enablement of Pol β-mediated DNA synthesis in this process. In contrast, interaction of PARP1 with one nucleotide gapped DNA mimicking the intermediate of short patch (SP) BER slightly inhibits the gap-filling activity of Pol β and the overall efficiency of SP BER is practically unaffected by PARP1. Thus, PARP1 differentially influences DNA synthesis in SP- and LP BER pathways.

1. Introduction

Base excision repair (BER) is one of the main strategies of cells for defense against single-base lesions in DNA [1]. The generally accepted model of base excision repair in mammalian cells involves two pathways [2]. In each pathway, the repair process can be initiated by DNA glycosylase action to produce an apurinic/apyrimidinic (AP) site [3]. Then apurinic/apyrimidinic endonuclease (APE1) cleaves the phosphodiester bond 5′ to the abasic site, generating a nick with 5′-deoxyribose phosphate (dRP) and 3′ hydroxyl group [4]. Following this incision of DNA backbone, addition of a nucleotide in the gap and removal of the dRP group is catalyzed by DNA polymerase β (Pol β) [5,6]. The nick is sealed by a DNA ligase III [7]. This stage is completed the short patch (SP) BER pathway. Oxidized or reduced 5′-dRP groups cannot be removed by the dRP lyase activity of Pol β and such modified residues will block downstream steps in SP BER [5,8]. Repair of such blocked intermediates may proceed by the long patch (LP) BER pathway [2,8,9]. DNA synthesis in LP BER is performed by Pol β or Pol δ(ε) and the modified 5′-dRP group is displaced as part of a 5′-flap structure formed upon strand-displacement DNA synthesis [2,8,10]. The pathway requires several DNA replication factors, including flap endonuclease 1 (FEN1) and proliferating cell nuclear antigen (PCNA) [8,9]. The final nicked DNA product is sealed by DNA ligase I or III [11]. Initially, Pol β has been characterized as a major gap-filling DNA polymerase that catalyzes incorporation of one nucleotide during SP BER [5,6,12]. Later, increasing evidence indicated that this protein could function as the primary DNA polymerase in LP BER [9,10]. However, Pol β alone does not effectively catalyze strand-displacement DNA synthesis in LP BER and has no 3′ → 5′ exonuclease activity [13]. The present biochemical studies indicate that the functions of Pol β in LP BER may be modulated by other proteins involved in the BER process, among them APE1, FEN1 and PARP1 [14–20]. Using *in vitro* reconstitution

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experiments with purified proteins, APE1 was found to physically interact with Pol β stimulating the 5′-dRP lyase activity of the DNA polymerase [21,22]. APE1 also has 3′–5′ exonuclease activity [23] and removes some 3′ mismatches 10–20 times more efficiently than 3′ matched nucleotides [24]. Thus, it has been speculated that APE1 could coordinate the gap-filling and 5′-dRP lyase activities of Pol β in SP BER and have a proofreading role in the repair pathway, removing mismatched nucleotides arising from Pol β action [22,25]. Under certain circumstance APE1 could also enhance the strand-displacement activity of Pol β in LP BER and increase the fidelity of DNA synthesis catalyzed by Pol β, suggesting cooperation between the proteins in LP BER DNA synthesis [20]. Recent studies have also demonstrated functional interaction between Pol β and FEN1 in the LP BER DNA synthesis [17,18]. In the system reconstituted from purified proteins it has been shown that FEN1 stimulates the Pol β-mediated LP BER DNA synthesis via a "gap translation" [18]. According to the mechanism, Pol β fills one-nucleotide gap producing a nick with 5′-reduced/oxidized sugar flap, then FEN1 removes a single nucleotide with the modified sugar residue from the 5′-end of the nick and generates again a one-nucleotide gap [18]. Thus, FEN1 can create one-nucleotide gap containing DNA substrates where Pol β has the highest catalytic efficiency and fidelity. Hence the data obtained in systems reconstituted from purified proteins indicate that activity of Pol β in LP BER may be strongly enhanced by APE1 or FEN1 and the cooperation between Pol β and APE1 (or FEN1) could be important feature of the Pol β-dependent LP BER pathway.

However, the functions of APE1 and FEN1 in the Pol β-mediated LP BER DNA synthesis may be modulated by other proteins involved in the BER process, among them poly(ADP-ribose) polymerase1 (PARP1) [26,27]. PARP1 is thought to be involved in BER and its interaction with enzymes and DNA intermediates of BER still remains under active investigation [27–33]. PARP1 can interact with single-strand breaks in DNA caused by genotoxic agents (free radicals, ionizing radiation) and also in the course of BER [28–30]. When bound to DNA breaks, PARP1 catalyzes synthesis of poly(ADP-ribose) resulting in covalent modification of itself and other nuclear proteins [27,28–30]. Autopoly(ADP-ribosyl)ation of PARP1 results in decrease of DNA-binding activity of this enzyme and facilitates its dissociation from DNA [27,28–30]. Poly(ADP-ribosyl)ation of PARP1 seems to be a factor regulating DNA repair [28–30]. It has been demonstrated that PARP1 binds to the incised AP site [34] and physically interacts with components of the multiprotein SP BER machinery such as XRCC1, Pol β, and DNA ligase III, suggesting a functional interaction and cooperation among these proteins in SP BER [31,35,36]. Studies involving photoaffinity labeling of mouse embryonic fibroblast extract proteins have shown that PARP1 interacts with LP BER DNA intermediate carrying photoreactive dCMP moiety at the 3′-end and reduced sugar phosphate group at the 5′-end of a nick [37,38]. Thus, along with Pol β, APE1 and FEN1, PARP1 has been identified in the cell extracts as a major factor that binds DNA intermediate of LP BER [37,38]. Overall, the results indicate that APE1, PARP1, and FEN1 could interact with the same nicked LP BER DNA, which can be considered as intermediate prior to the strand-displacement DNA step in LP BER. This suggests that PARP1 may participate in LP BER and modulate APE1/FEN1 functions in Pol β-dependent DNA synthesis. In our previous studies, we observed that PARP1 differentially interferes with DNA synthesis catalyzed by Pol β, insignificantly decreases the efficiency of gap-filling (SP BER), but inhibits strand-displacement DNA synthesis (LP BER) [39]. Using conditions of the BER enzyme excess over substrate DNA, we also found that PARP1 can interfere with APE1-mediated stimulation of the strand-displacement activity of Pol β [20]. The observed inhibition of the BER step by PARP1 appears to be a result of competition between PARP1 and these proteins in binding to the nicked DNA substrates formed after gap-filling. This conclusion was partly supported by the observation that poly(ADP-ribosyl)ation of PARP1 decreases its inhibitory action on DNA synthesis catalyzed by Pol β alone [39]. Thus, it is expected that PARP1 and its poly(ADP-ribosyl)ation could have important implication in proceeding of the Pol β-mediated LP BER DNA synthesis.

The present study was undertaken to further examine *in vitro* the interplay between recombinant proteins, namely, Pol β, APE1, FEN1 and PARP1 in LP BER DNA synthesis on model BER intermediates. First, we analyzed the influence of PARP1 and its poly(ADP-ribosyl)ation on Pol β/APE1- or Pol β/FEN1-mediated LP BER DNA synthesis. In addition, using a photocrosslinking assay, we examined the interaction of PARP1 with nick-containing BER DNA intermediates. We observed that PARP1 strongly competes with Pol β and APE1 in binding to LP BER DNA intermediate carrying a nick with 3-hydroxy-2-hydroxymethyltetrahydrofuran with 5′-phosphate (pF) flap groups at the margin that results in inhibition of strand-displacement DNA synthesis catalyzed by Pol β in cooperation with APE1. We also demonstrated that PARP1 suppresses Pol β/FEN1-mediated LP BER DNA synthesis via a "gap translation" mechanism by blocking of FEN1 cleavage of the nicked 5′-pF flap-containing DNA intermediate. Poly(ADP-ribosyl)ation of PARP1 decreases its inhibitory action on the Pol β-mediated LP BER DNA synthesis both via strand-displacement and "gap translation" mechanisms. Importantly, under conditions applied in the DNA synthesis assay, we detected a moderate inhibition of the gap-filling activity of Pol β by PARP1. In summary, our data suggest that interaction of PARP1 with nicked LP BER DNA intermediate could suppress the subsequent steps of the pathway and poly(ADP-ribosyl)ation of PARP1 plays a critical role in regulation of Pol β-mediated LP BER DNA synthesis.

2. Materials and methods

2.1. Materials

Synthetic oligonucleotides were from Oligos Etc. Inc. (Wilsonville, OR, USA). [γ-^{32}P] ATP (5000 Ci/mmol) was from the Laboratory of Biotechnology (Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia). T4 polynucleotide kinase was from Biosan (Russia). Protein molecular mass markers were from Helicon (Russia). The photoreactive nucleotide analog FABGdCTP (exo-N-[4-(4-azido-2,3,5,6,-tetrafluorobenzylidenehydrazinocarbonyl)-butylcarbamoyl]-2′-deoxycytidine-5′-triphosphate) synthesized as described [40] was a kind gift of Dr. I. V. Safronov (Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia).

2.2. Proteins and extract

The plasmid bearing the cDNA of the human PARP1 was kindly provided by Dr. M. Satoh (Laval University, Canada). The plasmids containing cDNA of mammalian FEN1, APE1 and Pol β were a kind gift of Dr. S.H. Wilson (National Institute of Environmental Health Sciences, NIH, NC, USA). Recombinant proteins human PARP1, FEN1, APE1 and rat Pol β were purified as described previously [39,41–43]. Protein concentrations were measured by the method of Bradford. Bovine testis nuclear extract was prepared as described previously [44] and was a kind gift of Dr. R. Prasad (National Institute of Environmental Health Sciences, NIH, NC, USA).

2.3. DNA substrates and 5′-end labeling

Oligodeoxyribonucleotides were 5′-^{32}P-phosphorylated with T4 polynucleotide kinase and purified by 20% polyacrylamide/7.0 M urea gel electrophoresis as described [45] followed by electro-elution and precipitation with 2% solution of $LiClO_4$ in acetone. The precipitated oligodeoxyribonucleotides were dissolved in 10 mM Tris–HCl (pH 8.0) and 1 mM EDTA. Complementary oligonucleotides were annealed by heating a solution of equimolar amounts at 90 °C for 5 min followed by slow cooling-down to room temperature to form the gapped double-stranded DNA substrates. The structures of DNA duplexes used are summarized in Table 1.

2.4. Photoreactive DNA synthesis and photocrosslinking assay

Standard reaction mixtures (100 μl) contained 50 mM Tris–HCl (pH 8.0), 50 mM NaCl, 10 mM $MgCl_2$, 0.1 μM 5′-[^{32}P] DNA substrate (DNApF or DNAp), 0.1 μM Pol