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Poly(ADP-ribose) Polymerase-1 Inhibits Strand-Displacement Synthesis of DNA Catalyzed by DNA Polymerase β

M. V. Sukhanova, S. N. Khodyreva, and O. I. Lavrik*

Novosibirsk Institute of Bioorganic Chemistry, Siberian Division, Russian Academy of Sciences, pr. Lavrentieva 8, Novosibirsk 630090, Russia; fax: (3832) 333-677; E-mail: lavrik@niboch.nsc.ru

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Abstract—Poly(ADP-ribose) polymerase-1 (PARP-1), a eucaryotic nuclear DNA-binding protein that is activated by breaks in DNA chains, may be involved in the base excision repair (BER) because DNAs containing single-stranded gaps and breaks are intermediates of BER. The effect of PARP-1 on the DNA synthesis catalyzed *in vitro* by DNA polymerase β (pol β) was studied using analogs of DNA substrates produced during BER and imitating intermediates of the short patch and long patch subpathways of BER. Oligonucleotide duplexes of 34 bp that contained a mononucleotide gap or a single-strand break with tetrahydrofuran phosphate or phosphate at the 5′-end of the downstream oligonucleotide were taken as DNA substrates. The efficiency of DNA synthesis was determined at various ratios of pol β and PARP-1. The efficiency of gap filling was decreased in the presence of PARP-1, but strand-displacement DNA synthesis was inhibited significantly stronger, which seemed to be due to competition between PARP-1 and pol β for DNA. In the presence of NAD^+ and single-strand breaks in DNA, PARP-1 catalyzes the synthesis of poly(ADP-ribose) covalently attached to the enzyme, and this automodification is thought to provide for dissociation of PARP-1 from DNA. The effect of PARP-1 automodification on inhibition of DNA synthesis was studied, and efficiency of mononucleotide gap filling was shown to be restored, but strand-displacement synthesis did not revert to the level observed in the absence of PARP-1. PARP-1 is suggested to regulate the interaction between pol β and DNA, in particular, via its own automodification.

Key words: poly(ADP-ribose) polymerase-1, DNA polymerase β, base excision repair, poly(ADP-ribosyl)ation

Poly(ADP-ribose) polymerase-1 (PARP-1) is a eucaryotic nuclear protein responsible for two catalytic functions: synthesis of poly(ADP-ribose) and NAD^+ glycohydrolase activity [1]. This enzyme recognizes single- and double-stranded breaks generated in DNA under the influence of genotoxic agents (free radicals, ionizing radiation, monofunctional alkylating agents) and as a result of activity of repair enzymes [2]. PARP-1 is thought to protect DNA ends produced as a result of DNA repair or by its direct damage [2]. PARP-1 binds undamaged DNA with rather high affinity, but its enzymatic activity is displayed only in the presence of single- and double-stranded breaks in DNA [3, 4]. In response to DNA damage, PARP-1 poly(ADP-ribosyl)ates a number of nuclear proteins, and this causes an intracellular signal, which either activates the repair or starts the cell death program (apoptosis, necrosis) [5]. Although various enzymes involved in DNA metabolism (replication, transcription, repair) and proteins involved in formation of the chromatin structure (histones) are poly(ADP-ribosyl)ated both *in vitro* and *in vivo*, PARP-1 is the main acceptor of poly(ADP-ribose) [6]. In the case of local damages to DNA, PARP-1 seems to be indispensable for cell survival because it activates proteins responsible for DNA repair. In the case of multiple damages to DNA, a "hyperactivation" of the enzymatic activity of PARP-1 decreases the concentration of NAD^+ and, as a consequence, depletes the ATP store in the cell that can cause cell death [7]. Thus, PARP-1 itself and poly(ADP-ribosyl)ation of proteins caused by it are involved in vitally important processes in the cell: replication [8, 9], repair [10, 11], transcription [12, 13], recombination [7], cell proliferation and death [14]. The idea of the PARP-1 involvement in DNA repair is based on the direct correlation between the degree of DNA damage and the level of synthesis of poly(ADP-ribose) [10]. Data on PARP-1 functions in the cell have been mainly obtained in experiments on mouse embryo fibroblasts with inactivated PARP-1 gene (PARP-$1^{-/-}$ cells) [2]. The PARP-$1^{-/-}$ cells are highly sensitive to γ-radiation and monofunctional alkylating agents, which suggests a possible involvement of PARP-1 in base excision repair (BER)

Abbreviations: PARP-1) poly(ADP-ribose) polymerase-1; pol β) DNA polymerase β; BER) base excision repair; AP-sites) apurinic/apyrimidinic sites.

* To whom correspondence should be addressed.

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[15]. BER is one of the principal pathways of DNA repair responsible for replacement of modified nucleotides and restoration of spontaneous apurinic/apyrimidinic sites [16]. BER occurs either by the short patch subpathway (insertion of one nucleotide) or by the long patch subpathway (insertion of several nucleotides) [17]. With the first subpathway, DNA polymerase β, after incorporation of one nucleotide, excises the deoxyribosophosphate (dRP) residue due to its lyase activity and forms the duplex ready for ligation [18]. The dRP residue is excised by the β-elimination mechanism, which is sensitive to chemical modification. Thus, oxidized or reduced AP sites are resistant to β-elimination. AP sites with modified dRP residues are repaired by the long patch subpathway of BER via the action of other enzymes [19, 20]. According to the literature, pol β in the long patch subpathway incorporates the first nucleotide [21]. Studies on PARP-1 functions in BER mainly concern repair of damaged plasmid DNA by activities of enzymes of the extract from the PARP-$1^{-/-}$ cells. The findings are often contradictory and suggest both a negative [10] and neutral [22] role of PARP-1. Disorders in the repair in such systems are thought to be associated either with a decreased expression of enzymes of the long patch subpathway of BER in the PARP-$1^{-/-}$ cells [23], or with the absence of this enzyme among proteins of the BER complex [24-26]. The formation of protein-protein PARP-1 complexes with pol β, XRCC1, and DNA ligase III (proteins involved in BER) is shown by immunoprecipitation and dihybrid analysis [27]. Based on detection of these complexes, PARP-1 may be considered to be a component of the BER system. Moreover, by photoaffinity modification PARP-1 was shown to directly interact with photoreactive DNA intermediates of the long patch subpathway of BER, which had been synthesized *in situ* using enzymes of the extract of mouse fibroblasts [28]. The effect of PARP-1 on BER was studied in the system reconstructed from individual proteins, and PARP-1 itself failed to change the efficiency of the DNA synthesis catalyzed by pol β, but in the combined presence of PARP-1 and FEN-1 the repair synthesis of DNA by the long patch subpathway of BER was very effectively stimulated [29]. Although the role of PARP-1 in BER has been intensively studied in numerous works, the functional significance of this enzyme in BER is still not elucidated and discussed in different aspects. Because the role of PARP-1 during BER is not unambiguously determined, it is interesting to assess the effect of PARP-1 on repair synthesis during BER that is catalyzed by pol β and also on the effect of poly(ADP-ribosyl)ation in this system.

MATERIALS AND METHODS

Materials. The following reagents were used: EDTA, β-NAD^+, N,N′-methylenebisacrylamide, Tris, isopropyl-thio-β-D-galactoside (IPTG), and glycerol (Sigma, USA); β-mercaptoethanol, bromophenol blue, xylene cyanole, and formamide (Fluka, Switzerland); acrylamide (Helicon, Russia); dATP, dCTP, dGTP, dTTP, and [γ-^{32}P]ATP (110 TBq/mmol) (Biosan, Russia); TEMED and urea (Merck, Germany); phenylmethylsulfonyl fluoride (PMSF) and imidazole (Serva, Germany); AntPrt-Coctail, a mixture of protease inhibitors (Roche, Germany); benzamidine (ICN, USA); heparin-Sepharose (Pharmacia, Sweden); single-stranded DNA-cellulose (USB-Amersham, USA); Ni-NTA (Quiagen, USA); [^{32}P]NAD^+ (Amersham Pharmacia Biotech, Sweden); other reagents produced in Russia were of special purity.

DNA substrates. All synthetic oligonucleotides obtained from Oligos Etc, Inc. (USA) were labeled with ^{32}P-phosphate at the 5′-end using T4-polynucleotide kinase [30] and purified by electrophoresis in denaturing polyacrylamide gel supplemented with 7 M urea [31]. DNA duplexes were prepared by equimolar mixing of its three oligonucleotides in buffer containing 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA; then the mixture was heated to 90°C, and slowly cooled to room temperature. The resulting DNA duplexes were tested by electrophoresis in 10% polyacrylamide gel under native conditions.

Enzyme preparations. Recombinant rat DNA polymerase β (EC 2.7.7.7) was isolated as described in [32]. Recombinant human poly(ADP-ribose) polymerase-1 (EC 2.4.2.30) was expressed in the *Escherichia coli* system (strain BL21 DE3 (pLys E)). The plasmid pET 32 containing the human PARP-1 gene was kindly presented by M. S. Satoh (Laval University, Canada). The synthesis of PARP-1 was induced by addition of IPTG to 1 mM, and the cell culture was incubated for 3 h at 37°C. Then the cells were precipitated by centrifugation, and the resulting precipitate was suspended in buffer containing 50 mM Tris-HCl (pH 8.0), 10% glycerol, 1 mM β-mercaptoethanol, 1 mM PMSF, 1 mM benzamidine (buffer A), AntPrt-Coctail in the amount recommended by the producer, and 2 M NaCl. The cells were destroyed by ultrasonication. The further purification included a number of successive chromatographies with Ni-NTA (elution with 250 mM imidazole in buffer A), heparin-Sepharose (elution by a linear gradient of NaCl (0.1-0.8 M) in buffer A), and single-stranded DNA-cellulose (elution by a linear gradient of NaCl (0.1-1 M) in buffer A). The purity of the resulting PARP-1 preparations was monitored at all stages of the purification by electrophoresis according to Laemmli [33]. The final product was dialyzed against buffer A supplemented with 0.1 M NaCl. The final preparation was stored in 20-μl aliquots at −70°C.

DNA repair synthesis. The DNA synthesis catalyzed by DNA polymerase β was performed in a standard reaction mixture containing 50 mM Tris HCl (pH 8.0), 50 mM NaCl, 7 mM β-mercaptoethanol, 10 mM $MgCl_2$, dATP, dGTP, dCTP (each in concentration of 10 μM),

and 50 nM DNA substrate. The concentration of DNA polymerase β was varied from 50 nM to 1 μM, and the concentration of PARP-1 was varied from 50 to 500 nM. The reaction was initiated by addition of the four dNTP. The effect of NAD^+ on the DNA synthesis was studied after the reaction was initiated by simultaneous addition of NAD^+ to 1 mM and the four dNTP. The reaction mixtures of 10 μl volume were incubated at 37°C for 30-300 sec, and the reaction was stopped by addition of 20 μl of mixture containing 90% formamide, 50 mM EDTA, 0.1% bromophenol blue, and 0.1% xylene cyanole. The reaction mixtures were heated for 5 min, and products of the DNA synthesis were analyzed by electrophoresis in 20% polyacrylamide gel supplemented with 7 M urea, 89 mM Tris, 89 mM boric acid, and 2 mM EDTA (pH 8.0), with subsequent autoradiography. The electrophoresis was performed in plates (20 × 20 × 0.04 cm) at the voltage drop of 50 V/cm and stabilization in voltage. Radioactive regions of the gel were cut out and counted by the method of Cherenkov.

RESULTS

To study the effect of PARP-1 on the repair synthesis catalyzed by pol β, DNA duplexes were chosen that contained a mononucleotide gap in the middle of one of the chains. The oligonucleotide flanking the gap at the 5′-end contained in its 5′-end either phosphate group (DNA-P) or tetrahydrofuran phosphate (DNA-F) residue. DNA-P can be considered as an analog of the substrate of the short patch subpathway of BER, whereas DNA-F seems to be an intermediate of the long patch subpathway because tetrahydrofuran phosphate residue (analog of deoxyribosophosphate) cannot be excised by the lyase activity of pol β [18]. To study the DNA synthe-

Table 1. Structures and short-cuts of DNA substrates

Nucleotide sequence	Short-cut
5'-F\ 5'-[^{32}P]CTGCAGCTGATGCGC GTACGGATCCCCGGGTAC-3' 3'-GACGTCGACTACGCG**G**CATGCCTAGGGGCCCATG-5'	DNA-F
5'-P\ 5'-[^{32}P]CTGCAGCTGATGCGC GTACGGATCCCCGGGTAC-3' 3'-GACGTCGACTACGCG**G**CATGCCTAGGGGCCCATG-5'	DNA-P
5'-F\ 5'-[^{32}P]CTGCAGCTGATGCGCC GTACGGATCCCCGGGTAC-3' 3'-GACGTCGACTACGCG**G**CATGCCTAGGGGCCCATG-5'	DNA-F'
5'-P\ 5'-[^{32}P]CTGCAGCTGATGCGCC GTACGGATCCCCGGGTAC-3' 3'-GACGTCGACTACGCG**G**CATGCCTAGGGGCCCATG-5'	DNA-P'

Note: F) 3-hydroxy-2-hydroxymethyltetrahydrofuran with 5′-phosphate; P) phosphate.

Table 2. Effect of PARP-1 (0, 50, 100, and 500 nM) on weighted-mean length of products and efficiency of primer elongation by DNA polymerase β

DNA substrates	Weighted-mean length of product (synthesis efficiency)			
	0	50 nM	100 nM	500 nM
DNA-F	17.3 (93)	17.4 (92)	16.8 (92)	15.6 (60)
DNA-P	16.3 (96)	16.2 (96)	16.0 (96)	15.8 (78)

Note: Synthesis efficiency was determined as the fraction of the initial primer (%) elongated during the reaction. The reaction was performed for 5 min, the length of the initial primer was 15 nt. Data of three independent determinations are presented; the error was less than 1.5%. In parentheses, synthesis efficiency, %.

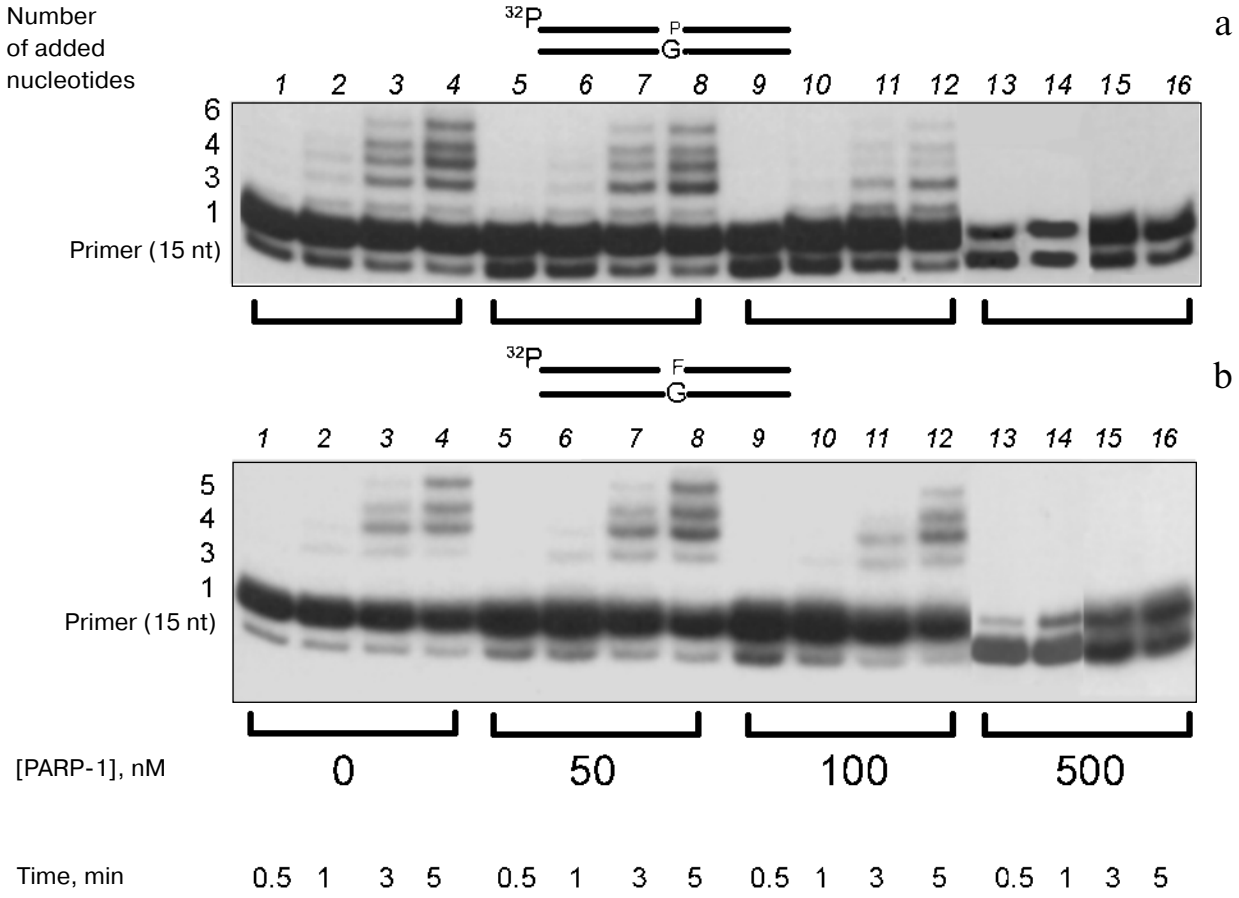

Fig. 1. Effect of PARP-1 on the synthesis on substrates DNA-P and DNA-F catalyzed by pol β. The DNA synthesis catalyzed by pol β was performed for 0.5-5 min in the standard mixture containing 50 nM DNA-P (a) or 50 nM DNA-F (b) and 100 nM pol β. The concentration of PARP-1 was varied from 0 to 500 nM.

sis with the strand-displacement of the downstream primer catalyzed by pol β, DNA duplexes were chosen that contained a single-strand break in the middle of the chain. The oligonucleotide flanking the break at the 5′-end had on its end exposed into the break either phosphate group (DNA-P′) or a "dangling" tetrahydrofuran phosphate (DNA-F′) residue. DNA-P′ can be considered to be the end product of the short patch subpathway, whereas DNA-F′ seems to be a substrate of the long patch subpathway. Sequences of the DNA duplexes used and their designations are presented in Table 1.

The effect of PARP-1 on DNA synthesis catalyzed by pol β was assessed by comparing elongation products of a 15-meric primer in various DNA duplexes in the presence of the four dNTP. Figure 1 presents an autoradiograph of the gel after separation of the primer elongation products in DNA-P and DNA-F catalyzed by pol β at different concentrations of PARP-1. In the presence of 100-nM pol β, the synthesis mainly results in the mononucleotide elongation of the primer that corresponds to gap filling. The further synthesis occurs with displacement of the gap-flanking oligonucleotide, and the synthesis results in oligonucleotides with length corresponding to elongation of the initial primer to six nucleotide units. The addition of PARP-1 to the reaction mixture influences both the first stage of the synthesis (lanes *1* and *13* in Fig. 1, a and b) and the subsequent stages of the synthesis accompanied by strand-displacement of the downstream primer. Results of the quantitative determination of the PARP-1 effect on the weighted-mean length of products of the DNA synthesis catalyzed by pol β and efficiency of the synthesis are presented in Table 2.

In the case of both duplexes, the presence of PARP-1 (50 and 100 nM) in the reaction mixture mainly decreases the length of the resulting products, without changing the fraction of the elongated primer. On increase in the PARP-1 concentration to 500 nM the strand-displacement synthesis of DNA is virtually fully inhibited, the efficiency of gap filling significantly decreases, and the DNA synthesis is inhibited stronger in the case of DNA-F.

Note, that in the absence of PARP-1 and in the presence of its low concentrations the strand-displacement

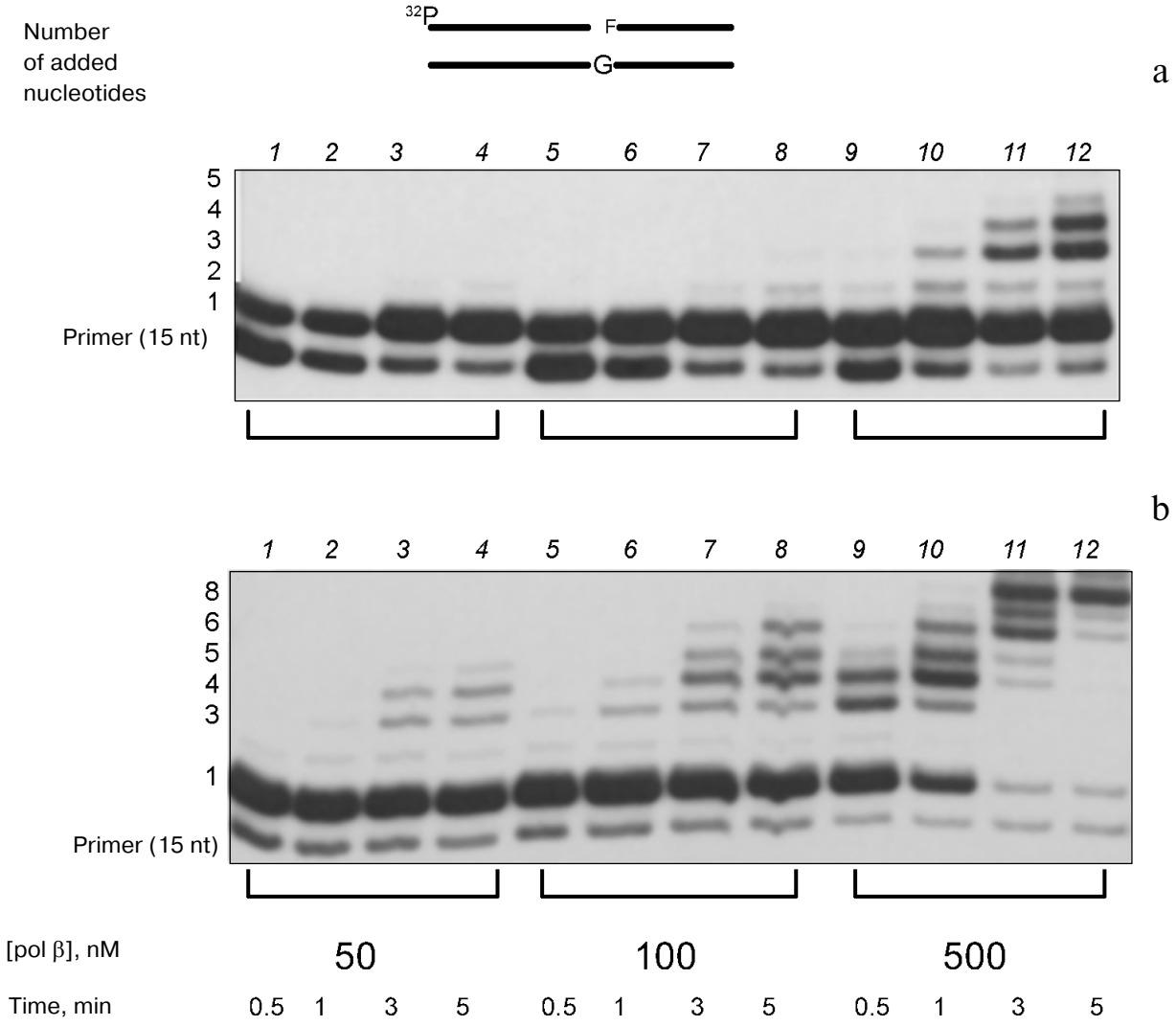

Fig. 2. Effect of pol β concentration on primer elongation in DNA-F in the presence (a) and in the absence of PARP-1 (b). The DNA synthesis catalyzed by pol β was performed for 0.5-5 min in standard mixture containing 50 nM DNA-F and 500 nM PARP-1 (a). The pol β concentration was varied from 50 to 500 nM.

DNA synthesis is more efficient in the case of DNA-F (the weighted-mean length of the products is about one nucleotide more) than in the case of DNA-P, whereas the gap filling efficiency is comparable for both substrates.

The inhibitory effect of PARP-1 is most likely due to its competition with pol β for binding to DNA; therefore, it was interesting to determine the effect of increase in the concentration of pol β on the synthesis efficiency at fixed concentration of PARP-1 (500 nM), i.e., under conditions of significantly inhibited strand-displacement DNA synthesis.

Figure 2 presents an autoradiograph of the gel after separation of the primer elongation products in the DNA-F duplex catalyzed by pol β (at concentrations of 50, 100, and 500 nM) in the presence of PARP-1 (Fig. 2a) and in its absence (Fig. 2b). In the absence of PARP-1, increase in the concentration of pol β was associated with a significant increase in the intensity of the strand-displacement synthesis, and at 500-nM pol β after 5 min of the reaction, 78% of the synthesis products were represented by the primer elongated by eight nucleotides (Fig. 2b, lane *12*). Increase in the concentration of pol β in the presence of PARP-1 promoted the gap filling (Fig. 2a, lanes *1*, *5*, *9*) and partially restored the strand-displacement DNA synthesis (Fig. 2a, lanes *4*, *8*, *12*). However, in this case the main product of the reaction corresponded to the primer elongation by one nucleotide and was 60% of all products (Fig. 2a, lanes *11*, *12*).

A similar experiment was performed with DNA-P duplex as a substrate, and its results (Fig. 3) show that on increase in the concentration of pol β the synthesis efficiency increases in total, similarly to the case of DNA-F.

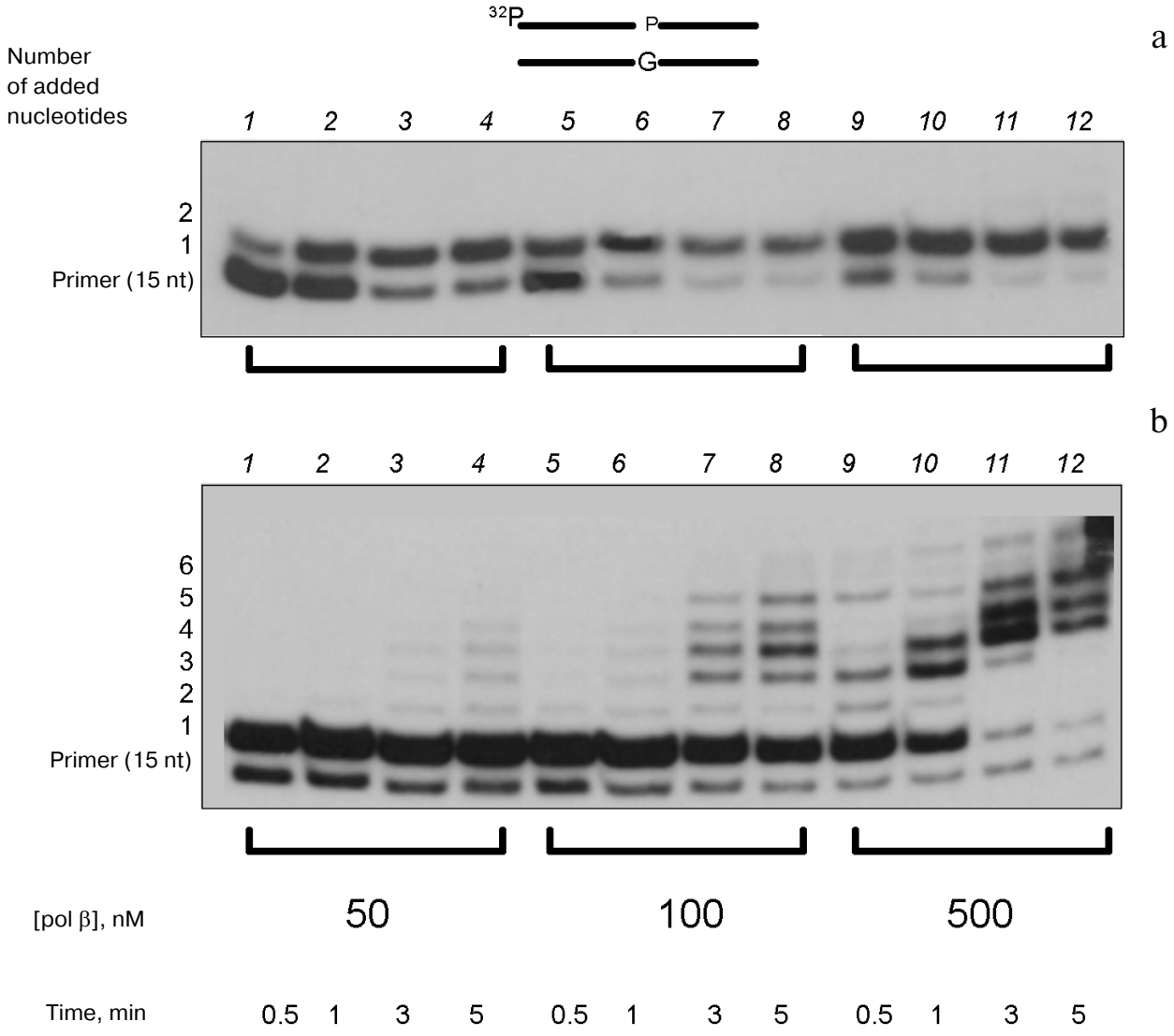

Fig. 3. Effect of pol β concentration on primer elongation in DNA-P in the presence (a) and in the absence of PARP-1 (b). DNA synthesis catalyzed by pol β was performed for 0.5-5 min in the standard mixture containing 50 nM DNA-P and 500 nM PARP-1 (a). The pol β concentration was varied from 50 to 500 nM.

But unlike the case of DNA-F, in the presence of PARP-1 the strand-displacement synthesis is completely inhibited in the case of DNA-P. In high concentrations pol β efficiently catalyzes the strand-displacement synthesis on both substrates, DNA-P and DNA-F, sets of the primer elongation products are comparable, but in the case of DNA-F, the main elongation product is longer (Figs. 2b and 3b, lanes *11*, *12*). Thus, the effect of PARP-1 on the primer elongation depends on both the type of DNA duplex and the stage of the synthesis. The effect of PARP-1 on gap filling is significantly less pronounced than on strand-displacement synthesis for both DNA duplexes; however, in the case of DNA duplex imitating the intermediate of the short patch subpathway, PARP-1 completely inhibits the strand-displacement synthesis.

The effect of PARP-1 on the stage of the strand-displacement DNA synthesis was studied using as substrates structures with a single-strand break (DNA-P′) and the "dangling" furan phosphate at the 5′-end of the flanking primer (DNA-F′). The synthesis efficiency and the length of the primer elongation products were compared depending on the pol β concentration in the reaction mixtures in the presence or in the absence of PARP-1. Figure 4 presents an autoradiograph of the gel after separation of products of the strand-displacement DNA synthesis in the absence of PARP-1 (lanes *1-6*) and in its presence (lanes *7-12*) for different concentrations of pol β. In the reaction mixtures without PARP-1, increase in the pol β concentration from 0.1 to 1 μM was associated with a sharp increase in the fraction of the elongated primer and in the

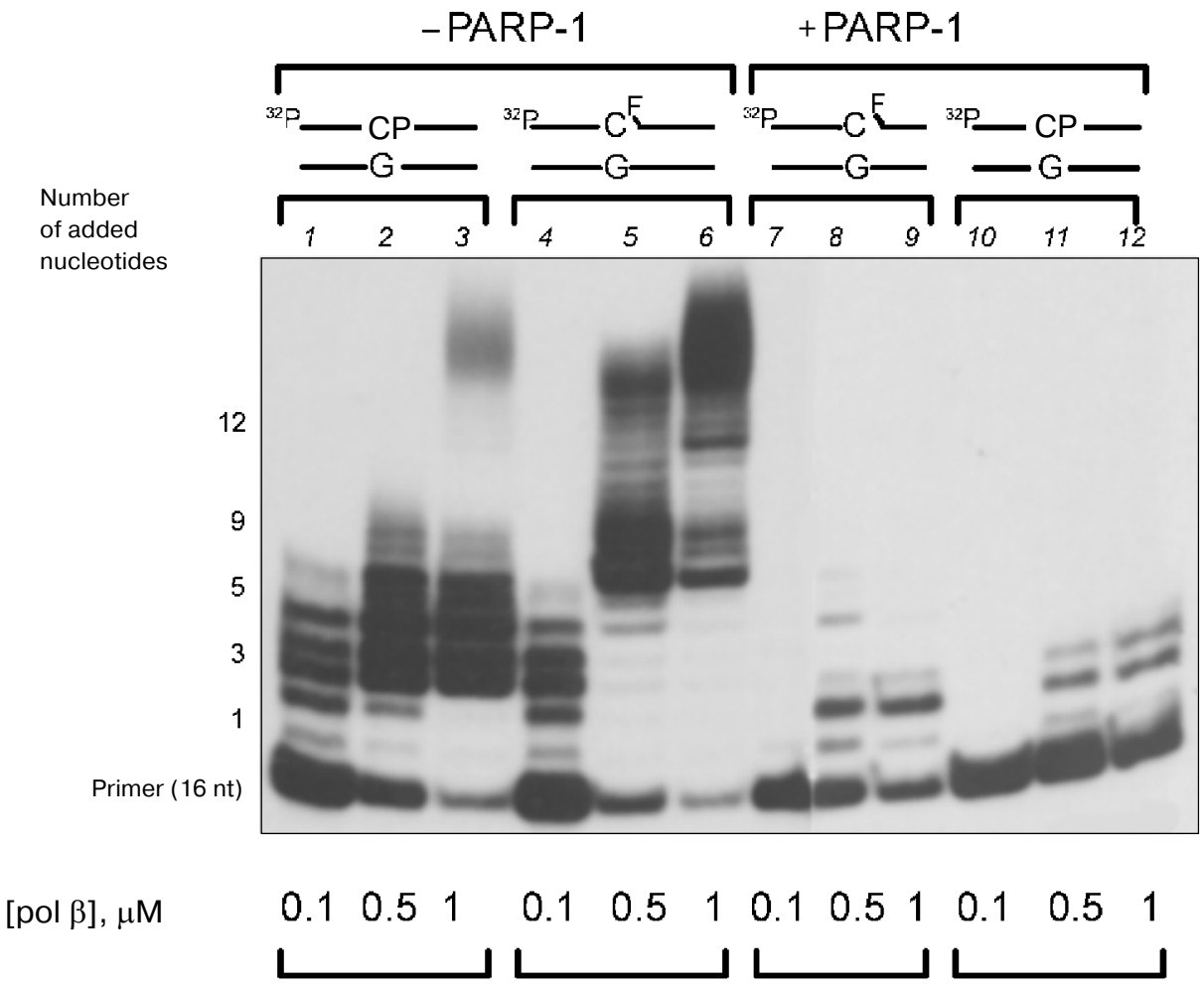

Fig. 4. Effect of pol β concentration on strand-displacement synthesis performed using substrates containing a single-strand break with phosphate (DNA-P′) and one-strand break with the "dangling" furan phosphate (DNA-F′) in the presence and in the absence of PARP-1. The primer elongation catalyzed by pol β was performed for 10 min in standard mixture containing 50 nM DNA-P′ (lanes *1-3*, *10-12*) or DNA-F′ (lanes *4-9*), and 500 nM PARP-1 (lanes *7-12*). The pol β concentration was varied from 0.1 to 1 μM.

length of the products (Fig. 4, lanes *1-6*). In the reaction mixtures containing 500 nM PARP-1, on increase in the pol β concentration to 1 μM, the primer was maximally elongated by 40% and the longest product corresponded to the primer completion by two nucleotides (Fig. 4, lanes *8*, *9* and *11*, *12*). Thus, in the case of DNA duplexes when only the strand-displacement synthesis can occur, the increase in the pol β concentration did not prevent the inhibitory effect of PARP-1 as it did for the gap filling, and, consequently, PARP-1 displayed a certain specificity with respect to the strand-displacement synthesis.

According to the literature, PARP-1 displays enzymatic activity, i.e., catalyzes the synthesis of poly(ADP)-ribose on the interaction of PARP-1 with DNA breaks in the presence of NAD^+. ADP residues are transferred from NAD^+ onto PARP-1 with production of a covalently attached poly(ADP)-ribose, a branched negatively charged polymer, and this is believed to cause dissociation of PARP-1 complexes with DNA due to electrostatic repulsion [10]. The automodification of PARP-1 resulting in suppression of the DNA-binding activity of this enzyme seems to be a factor regulating DNA repair [11]. Because PARP-1 effectively inhibits strand-displacement synthesis, it was interesting to assess the effect of poly(ADP-ribosyl)ation of PARP-1 on this process. The level of PARP-1 automodification was determined by electrophoresis in polyacrylamide gel under denaturing conditions by difference in electrophoretic mobilities of the poly(ADP-rybosyl)ated and initial forms of PARP-1 [34]. Incubation of the enzyme in the presence of 1 mM NAD^+ and DNA with breaks decreased the electrophoretic mobility of PARP-1, which suggested its automodification (data not presented). Automodification of PARP-1 was also confirmed by its radioactive labeling after incubation in a mixture containing [^{32}P]NAD^+ and DNA with breaks (data not presented).

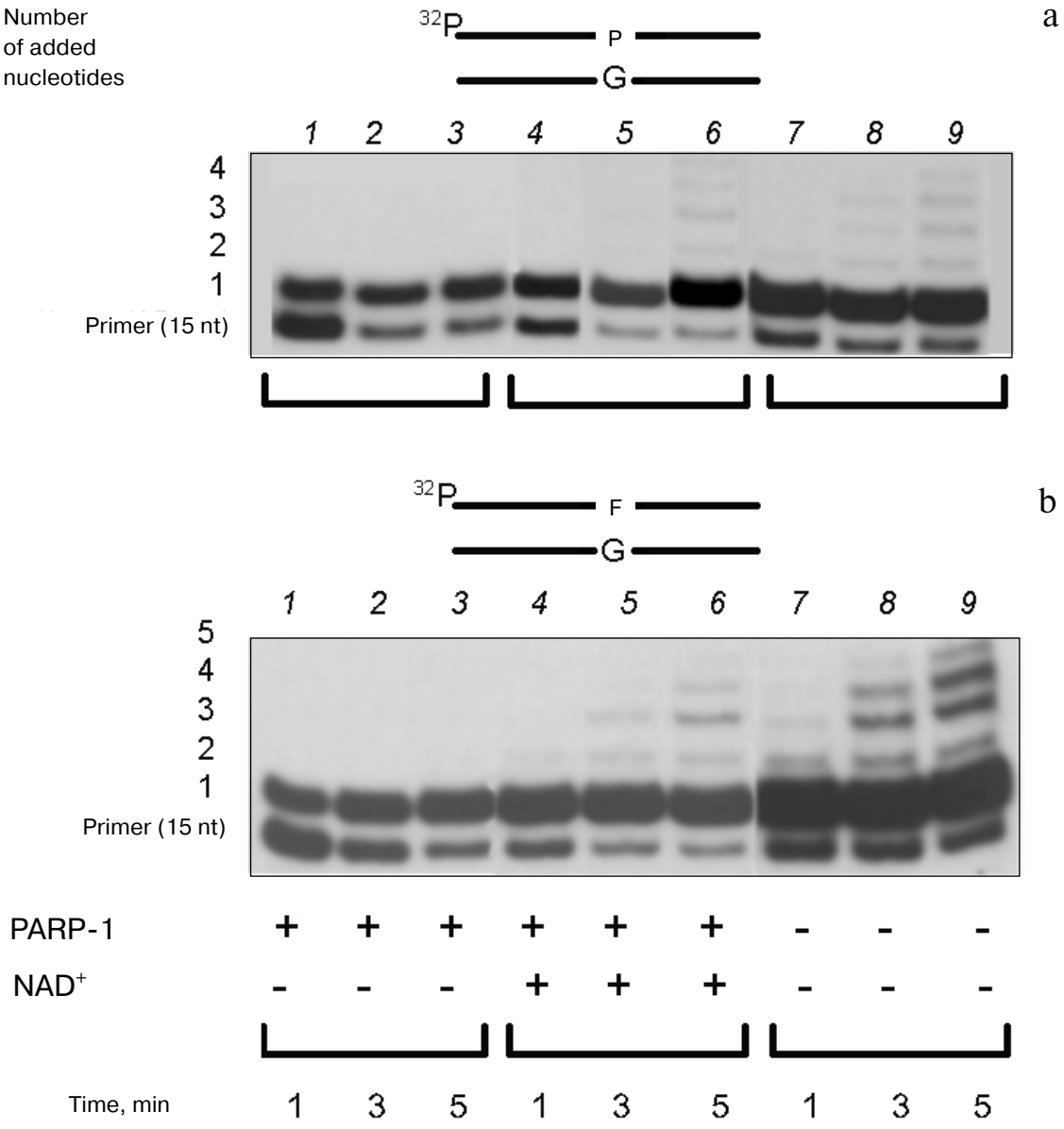

Fig. 5. Effect of poly(ADP-ribosyl)ation of PARP-1 on the synthesis catalyzed by pol β with DNA-P and DNA-F as substrates. The DNA synthesis catalyzed by pol β was performed for 1-5 min in standard mixture containing 50 nM DNA-P (a) and 50 nM DNA-F (b) and pol β (50 nM) in the presence of PARP-1 (500 nM) (lanes *1-3*), PARP-1 (500 nM) and NAD^+ (1 mM) (lanes *4-6*), and in the absence of PARP-1 and NAD^+ (lanes *7-9*).

To determine the effect of PARP-1 automodification on the DNA synthesis catalyzed by pol β, the primer elongation by DNA polymerase β was compared in reaction mixtures, which in addition to the main components contained either PARP-1 or PARP-1 and NAD^+ or neither of them. Data of these experiments with two duplexes used are presented in Fig. 5. The primer elongation products were analyzed, and it was found that addition of NAD^+ to the reaction mixtures where the DNA synthesis was performed in the presence of PARP-1 restored the efficiency of the gap filling to the level observed in the absence of PARP-1 (Fig. 5, a and b, lanes *1*, *4*). However, the strand-displacement DNA synthesis was less effective than in the reaction mixture without PARP-1 (Fig. 5, a and b, lanes *6*, *9*). In the control experiment, NAD^+ (1 mM) itself had no effect on the DNA synthesis catalyzed by pol β (data not presented). Thus, the addition of NAD^+ to the reaction mixture failed to completely abolish the inhibition of the strand-displacement synthesis caused by the presence of PARP-1. It seems that under these conditions pol β is poly(ADP-ribosyl)ated that decreases its activity. Incorporation of poly(ADP-ribose) residues into pol β accompanied by decrease in its activity was shown in [35].

DISCUSSION

The role of PARP-1 in the base excision repair is intensively studied by various methods, and by now there is a large volume of information concerning this problem. During the interaction of PARP-1 with single-strand breaks in DNA some proteins, including PARP-1 itself, are poly(ADP-ribosyl)ated [4, 6]. It is suggested that the automodification of PARP-1 results in its dissociation from the site of the break in DNA and, thus, allows access of the repair enzymes for the break to be repaired [10, 11]. It seems that PARP-1 is drawn into BER not only via binding to breaks in DNA, but is also involved in formation of the repair complex or directly regulates activities of the enzymes responsible for BER by poly(ADP-ribosyl)ation. Studies on the repair process *in vivo* in mice and in cell lines with inactivated PARP-1 gene allow us to assess the consequences of the inactivation of the protein for both the organism as a whole and the individual cell. Thus, the PARP-$1^{-/-}$ mice display an increased sensitivity to γ-radiation and injections of alkylating agents, such as *N*-methyl-*N*-nitrosourea (NMU). In PARP-$1^{-/-}$ cell lines the repair of breaks in DNA is decelerated that increases the sensitivity of such cells to DNA-damaging agents [2, 15]. Data on the interaction of PARP-1 with single-strand breaks in DNA and formation of protein-protein contacts of PARP-1 with the BER-catalyzing proteins (XRCC1, pol β, DNA ligase III) suggest PARP-1 as an enzyme, which is a constituent of the protein complex responsible for BER [27]. However, data on the role of PARP-1 in BER obtained in experiments in cell extracts are very inconsistent. Thus, in [22] the level of repair of DNA damages caused by γ-radiation and alkylating agents was unchanged in mouse fibroblasts of the PARP-$1^{-/-}$ type, and the authors suggest that PARP-1 has no effect on DNA repair both *in vivo* and *in vitro*. But in [26] the efficiency of repair of plasmids containing dUMP or 8-oxo-dGMP was decreased twofold in the short-patch subpathway and virtually by an order of magnitude in the long-patch subpathway. Decreased efficiency of repair of plasmids containing single-strand breaks was recently found in extracts from the PARP-$1^{-/-}$ cells [23]. More careful studies have shown that in these cells the synthesis of enzymes of the long patch subpathway of BER (flap endonuclease 1 and DNA ligase I) is decreased, and the authors suggest that this is the cause of the less efficient repair. Thus, experiments in PARP-$1^{-/-}$ cells not always result in an unambiguous conclusion that PARP-1 has a direct effect on BER. Therefore, studies on systems reconstructed from the purified proteins are promising for obtaining additional information about separate stages of BER.

In the present work, the primer elongation catalyzed by pol β was analyzed on substrates that modeled different stages of repair synthesis, and the effect of PARP-1 on this process was determined. The findings suggest that, in the presence of the four dNTP, pol β efficiently fills in the gap, whereas further strand-displacement synthesis is less effective. This is consistent with the role in the cell that is ascribed to pol β, as it is thought to fulfill the inclusion of one nucleotide in the short patch subpathway of BER [36, 37]. Pol β, being the most error-prone eucaryotic DNA polymerase, has maximal catalytic efficiency and accuracy during the filling of mononucleotide gap with phosphate at the 5′-end of the downstream primer [38]. In [39], pol β was shown to processively fill gaps of one to six nucleotides in the presence of phosphate at the 5′-end of the downstream primer. However, lengthy regions of DNA are synthesized distributively, and during this synthesis the accuracy of pol β is low [40]. Therefore, it is reasonable for the cell to limit the synthesis of elongated regions of DNA varying the pol β activity. It was earlier shown that XRCC1, which can form a complex with pol β and is considered to be a protein factor of the short patch BER, had no effect on the gap filling but inhibited the strand-displacement synthesis. These data were obtained in the reconstituted system in studies on the repair of dUMP-containing DNA, i.e., under conditions of the short patch subpathway of BER [41]. The further synthesis of DNA after the gap filling can be, in particular, limited by displacement of pol β from DNA by competition of protein factors and/or repair enzymes with pol β for binding to DNA. It seems that PARP-1, which has a high affinity for DNA with single-strand breaks (i.e., DNA-F′ or DNA-P′), can be involved in this process. And in fact data on the efficiency of DNA synthesis catalyzed by pol β at different ratios of pol β and PARP-1 concentrations are totally consistent with this hypothesis, because the strand-displacement synthesis is inhibited by PARP-1 stronger than the gap filling. Moreover, the decreased efficiency of gap filling in the presence of 500 nM PARP-1 is less pronounced in the case of DNA-P, i.e., during the formation of a duplex ready for ligation. Note that pol β itself has different efficiency in the primer elongation in DNA duplexes with the 5′-end of the primer exposed into the gap and containing phosphate (DNA-P) or furan phosphate (DNA-F), which seem to be intermediates of the short- and long-patch subpathways of BER, respectively. Thus, the weighted-mean length of the product of the strand-displacement DNA synthesis is in one nucleotide more in the case of DNA-F (Table 2).

Interaction of PARP-1 and other enzymes and factors of BER with the site in DNA to be repaired can be also regulated by poly(ADP-ribosyl)ation mediated by PARP-1 *in vitro* and *in vivo* [6]. Poly(ADP-ribosyl)ation of PARP-1 is considered to be a mechanism providing for the dissociation of its complexes with DNA [10, 11]. Data on the effect of poly(ADP-ribosyl)ation of PARP-1 on inhibition of the DNA synthesis suggest that automodification of PARP-1 really decreases its inhibitory effect. But under our conditions, the strand-displacement synthesis is restored incompletely in the presence of NAD^+

in the reaction mixture, and this seems to be due to the decreased activity of pol β as a result of its poly(ADP-ribosyl)ation [35]. It is also likely that poly(ADP-ribosyl)ation of PARP-1 did not completely abolish the affinity of this enzyme for break-containing DNA. We have shown in experiments on PARP-1 modification by photoreactive intermediates of the long-patch subpathway of BER that poly(ADP-ribosyl)ation of PARP-1 only slightly decreases the level of its modification and does not prevent appearance of its modification products [42]. Such a change in the modification more likely suggests a decrease in the affinity of poly(ADP-ribosyl)ated PARP-1 for DNA than the inability of this form of the enzyme to form complexes with DNA. Note that during the affinity modification of PARP-1 in the presence of NAD^+ the electrophoretic mobility of covalent adducts PARP-1-DNA was decreased, and just this should occur in the case of poly(ADP-ribosyl)ation of the protein. Products of the photoaffinity modification of PARP-1 with molecular weight corresponding to the enzyme without poly(ADP-ribose) were absent, and this showed that all molecules of the enzyme were automodified. Similar changes in the electrophoretic mobilities of products of the modification caused by photoreactive intermediates of the long-patch subpathway of BER were similarly observed in the presence of NAD^+ in experiments with the extract from mouse fibroblasts [28]. The effect of PARP-1 on efficiency of the repair synthesis catalyzed by pol β was studied in the system reconstituted from the purified proteins, which models the long-patch subpathway of BER, and PARP-1 itself had no effect on efficiency of the DNA synthesis but in combination with flap endonuclease 1 it noticeably stimulated the repair synthesis of DNA [29]. In work [29], other conditions were used. Thus, PARP-1 was added to the reaction mixtures in the fixed concentration of 50 nM. But in the present work this concentration of PARP-1 was the lowest concentration used. This concentration of PARP-1 had no effect on the efficiency of DNA synthesis, and the inhibitory effect was observed at the higher concentrations.

Thus, PARP-1 can regulate the interaction between pol β and DNA (in particular, via its own automodification) and also inhibit the strand-displacement DNA synthesis catalyzed by pol β.

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