ISSN 0006-2979, Biochemistry (Moscow), 2008, Vol. 73, No. 3, pp. 261-272. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © N. S. Dyrkheeva, S. N. Khodyreva, O. I. Lavrik, 2008, published in Biokhimiya, 2008, Vol. 73, No. 3, pp. 322-335.
Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM07-259, January 7, 2008.

Interaction of APE1 and Other Repair Proteins with DNA Duplexes Imitating Intermediates of DNA Repair and Replication

N. S. Dyrkheeva, S. N. Khodyreva, and O. I. Lavrik*

Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, pr. Lavrentieva 8, 630090 Novosibirsk, Russia; fax: (383) 333-3677; E-mail: lavrik@niboch.nsc.ru

Received July 24, 2007
Revision received August 20, 2007

Abstract—Interactions of APE1 (human apurinic/apyrimidinic endonuclease 1) and DNA polymerase β with various DNA structures imitating intermediates of DNA repair and replication were investigated by gel retardation and photoaffinity labeling. Photoaffinity labeling of APE1 and DNA polymerase β was accomplished by DNA containing photoreactive group at the 3′-end in mouse embryonic fibroblast (MEF) cell extract or for purified proteins. On the whole, modification efficiency was the same for MEF-extract proteins and for purified APE1 and DNA polymerase β depending on the nature of the 5′-group of a nick/gap in the DNA substrate. Some of DNA duplexes used in this work can be considered as short-patch (DNA with the 5′-phosphate group in the nick/gap) or long-patch (DNA containing 5′-sugar phosphate or 5′-flap) base excision repair (BER) intermediates. Other DNA duplexes (3′-recessed DNA and DNA with the 5′-hydroxyl group in the nick/gap) have no relation to intermediates forming in the course of BER. As shown by both methods, APE1 binds with the highest efficiency to DNA substrate containing 5′-sugar phosphate group in the nick/gap, whereas DNA polymerase β binds to DNA duplex with a mononucleotide gap flanked by the 5′-p group. When APE1 and DNA polymerase β are both present, a ternary complex APE1–DNA polymerase β–DNA is formed with the highest efficiency with DNA product of APE1 endonuclease activity and with DNA containing 5′-flap or mononucleotide-gapped DNA with 5′-p group. It was found that APE1 stimulates DNA synthesis catalyzed by DNA polymerase β, and a human X-ray repair cross-complementing group 1 protein (XRCC1) stimulates APE1 3′-5′ exonuclease activity on 3′-recessed DNA duplex.

DOI: 10.1134/S0006297908030048

Key words: APE1, base excision repair, photoaffinity labeling

Base excision repair (BER) of DNA (Scheme) is a multistage process with participation of certain proteins. This process removes DNA damages such as modified nitrogen bases and AP (apurinic/apyrimidinic) sites [1, 2] and is initiated on spontaneous nitrogen base loss or on formation of an AP site by the action of DNA glycosylases, which cleave the N-glycoside bond in nucleotides with the damaged base (Scheme, pathway I) [1, 3, 4]. AP endonucleases, e.g. human AP endonuclease 1 (APE1), cleave the DNA sugar phosphate backbone at the 5′-position in relation to the AP site, forming a nick with the hydroxyl group at the 3′-end and deoxyribose phosphate (dRp) at the 5′-end [5]. Subsequent action of β polymerase results in insertion of one nucleotide and excision of the dRp residue by the lyase function via the β-elimination mechanism [6, 7]. This is a short-patch (mononucleotide insertion) BER pathway (pathway Ia on Scheme). The BER mechanism is sensitive to chemical modification of dRp: oxidized or reduced AP sites are refractory to the lyase activity, and β polymerase cannot excise the dRp residue. As a result, repair switches to the long-patch pathway (pathway Ib on Scheme): polymerase inserts several (from 2 to 7) nucleotides into the elongating strand; this is accompanied by displacement of the

Abbreviations: AP site) apurinic/apyrimidinic site; AP-dsDNA) double-strand DNA containing AP site in the middle of one of strands; AP-ssDNA) single-strand DNA containing AP site in the middle of strand; APE1) human apurinic/apyrimidinic endonuclease 1; BER) base excision repair; β polymerase) DNA polymerase β; FAPdCTP (FAPdCMP)) exo-N-[2-N-(N-(4-azido-2,5-difluoro-3-chloropyridin-6-yl)-3-aminopropionyl)-aminoethyl]-2′-deoxycytidine-5′-triphosphate (monophosphate); MEF extract) mouse embryonic fibroblast cell extract; tetrahydrofuran or F) 3′-hydroxy-2′-hydroxymethyltetrahydrofuran; 5′-pF) 5′-tetrahydrofuran phosphate; XRCC1) human X-ray repair cross-complementing group 1 protein.

* To whom correspondence should be addressed.

DNA strand flanking a gap. Flap endonuclease 1 (FEN1) participates in the long-patch pathway: it excises a flap of 2-7 nucleotides containing the 5′-dRp group [8]. DNA repair is finished by restoration of sugar phosphate backbone integrity catalyzed by DNA ligase (Scheme). Functioning of all BER proteins is coordinated by the "passing the baton" mechanism: repaired DNA, which goes from one protein to another, acts as a "baton" [9]. APE1 is one of the candidates for the role of BER coordinator, which controls the whole process [5, 9]. This suggestion is based on data showing that APE1 participates in stimulation of activity of BER enzymes [10-15].

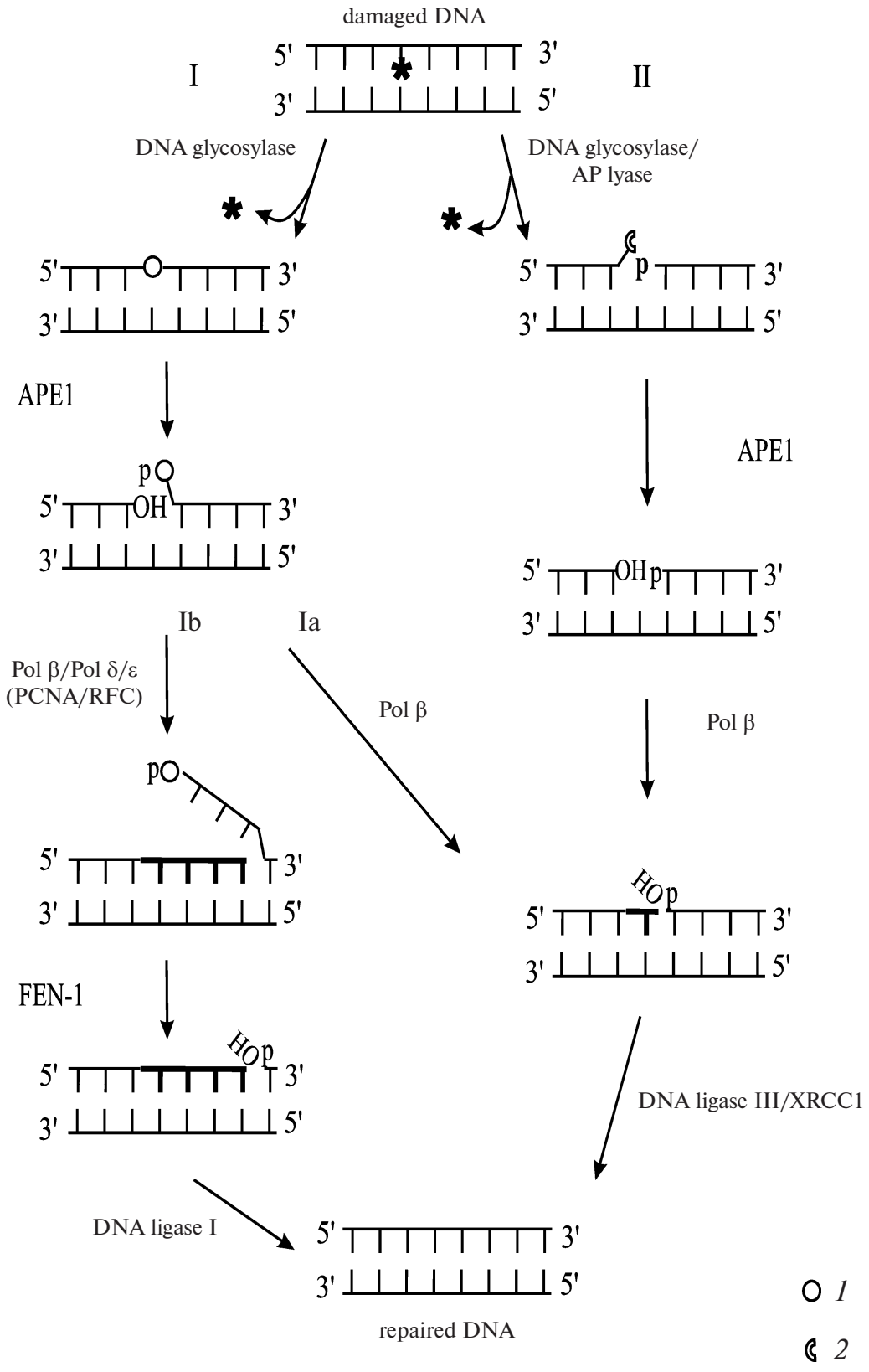
β Polymerase is the main polymerase of BER synthesis in mammalian cells [16]. Being located on an AP site, APE1 promotes β polymerase binding to noncleaved AP site of DNA and also stimulates lyase activity of this enzyme [12]. A suggestion about coordinated actions of APE1 and β polymerase at the initial stages of short-patch BER was based on these data [12]. APE1 also stimulates strand-displacement DNA synthesis catalyzed by β polymerase, which is typical of long-patch repair [14]. Along with endonuclease activity, APE1 also exhibits 3′-phosphodiesterase (Scheme, pathway II), 3′-phosphatase, and 3′-5′-exonuclease activities [17], and the latter manifests itself more efficiently against noncanonical pairs; this is an additional argument for a concerted action of APE1 and β polymerase. DNA synthesis catalyzed by β polymerase is rather erroneous, because the latter does not possess its own corrective 3′-5′-exonuclease activity [18]. However, it is known that fidelity of DNA repair synthesis *in vivo* is significantly higher than *in vitro* [1].

Thus, a search for factors increasing the fidelity of repair synthesis is of interest. APE1 is one of the potential correctors of β polymerase functioning [19-22]. The nature of the 5′-terminal group of nick/gap markedly influences the efficiency of APE1 exonuclease activity [13, 21-25]. As found in most investigations, if the tetrahydrofuran phosphate residue (pF) is at the 5′-end of an oligonucleotide flanking a gap or nick, efficiency of excision of the 3′-terminal dNMP of oligonucleotide is lower than that of the phosphate group in this position [13, 22-25]. Tetrahydrofuran (F) is an analog of deoxyribose in the composition of a synthetic AP site. Such residue cannot be removed by lyase activity of β polymerase, so a DNA duplex with 5′-pF group flanking a gap or nick can be considered as a model of the DNA structure formed when BER switches from the short-patch to the long-patch pathway. The maximal efficiency of exonuclease activity is observed when the 5′-hydroxyl group is present in a gap or nick compared with phosphate or pF [13, 21]. We also noted the same tendencies [22, 25]. However, for various DNA structures, a real contribution of APE1 exonuclease activity to the BER process is probably defined by the reaction rate as well as efficiency of APE1 interaction with such structure. For MEF extract proteins, it was shown by photoaffinity modification [23, 26] that APE1 more efficiently binds to photoreactive DNA substrates bearing the 5′-pF or 5′-dRp group in the nick as compared with substrates with the 5′-p group. Such DNA structures with the 5′-pF or 5′-dRp group arise at the stage of switching from the short-patch to the long-patch BER pathway.

In this work, we studied the efficiency of APE1 and β polymerase interaction with various DNA structures. Some of the studied DNA duplexes may be considered as intermediates of short-patch (DNA^X-p, DNA^X-gap) or long patch (DNA^X-pF, DNA^X-flap) BER pathway (Table 1). Interaction of APE1 and β polymerase with DNA was studied by gel retardation and photoaffinity labeling. DNA structures used for photoaffinity labeling bore a modified nucleotide with a photoreactive group at the 3′-end of the primer (Table 1). Using our earlier data on the effect of reaction conditions and the nature of 5′-terminal group in the nick on APE1 exonuclease activity [22, 25], we compared the efficiency of interaction of recombinant purified APE1 and enzyme from cell extract with various DNA structures. A set of structures bearing various groups at the 5′-end of the nick (Table 1) was extended compared with those used earlier [23, 26] and included not only BER intermediate models but also a partial DNA duplex with the recessed 3′-end (DNA^X-rec) and DNA with 5′-OH group (DNA^X-OH).

MATERIALS AND METHODS

The following reagents were used in this study: Tris and reagents for electrophoresis from Sigma (USA); Hepes from Fluka (Switzerland); [γ-^{32}P]ATP (>3000 Ci/mmol) produced in the Laboratory of Biotechnology, Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of the Russian Academy of Sciences; phage T4 polynucleotide kinase from Biosan (Russia); protein G-agarose from Roche Applied Science (Germany). Other reagents were produced in Russia and were of extra pure or analytically pure grade. Recombinant proteins: APE1, rat β polymerase, and XRCC1 (human X-ray repair cross-complementing group 1 protein) were isolated as described in [24, 27, 28]. Mouse embryonic fibroblast cell extract was obtained according to [23]. Synthesis and photochemical properties of dCTP analog (FAPdCTP) were described in [29]. Oligonucleotides were synthesized by Genset (France) or in the Laboratory of Medical Chemistry, Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of the Russian Academy of Sciences. ^{32}P-label was incorporated into the 5′-end of oligonucleotides using T4 polynucleotide kinase. Labeled oligonucleotides were purified by electrophoresis under denaturing conditions: PAGE in the presence of 7 M urea in TBE buffer (0.089 M Tris-HCl, pH 8.0, 0.089 M



Routes of DNA base excision repair. * Damaged nitrogen base; p, phosphate group at the 5′-end of the nick; *1*) AP site; *2*) 3′-blocking group, the DNA glycosylase/AP lyase product

Table 1. DNA structures and sequences

DNA structure and sequence	Notation
5'-*GG-CGA-TTA-AGT-TGG-G-3' 3'- CC-GCT-AAT-TCA-ACC-C - **G** - T-TGC-AGT-CCC-AGA-AGG -5'	DNA1
5'-*GG-CGA-TTA-AGT-TGG-G- **U** - A-ACG-TCA-GGG-TCT-TCC -3'	ssDNA
5'-*GG-CGA-TTA-AGT-TGG-G- **U** - A-ACG-TCA-GGG-TCT-TCC -3' 3'- CC-GCT-AAT-TCA-ACC-C - **G** - T-TGC-AGT-CCC-AGA-AGG -5'	dsDNA
5'-*GG-CGA-TTA-AGT-TGG-G- **F** - A-ACG-TCA-GGG-TCT-TCC -3'	AP-ssDNA
5'-*GG-CGA-TTA-AGT-TGG-G- **F** - A-ACG-TCA-GGG-TCT-TCC -3' 3'- CC-GCT-AAT-TCA-ACC-C - **G** - T-TGC-AGT-CCC-AGA-AGG -5'	AP-dsDNA
5'-*GG-CGA-TTA-AGT-TGG-G- **X** 3'- CC-GCT-AAT-TCA-ACC-C - **Y** - T-TGC-AGT-CCC-AGA-AGG -5'	DNA^X-rec
p-5' 5'-*GG-CGA-TTA-AGT-TGG-G- **X** A-ACG-TCA-GGG-TCT-TCC -3' 3'- CC-GCT-AAT-TCA-ACC-C - **Y** - T-TGC-AGT-CCC-AGA-AGG -5'	DNA^X-p
pF-5' 5'-*GG-CGA-TTA-AGT-TGG-G- **X** A-ACG-TCA-GGG-TCT-TCC -3' 3'- CC-GCT-AAT-TCA-ACC-C - **Y** - T-TGC-AGT-CCC-AGA-AGG -5'	DNA^X-pF
5'-*GG-CGA-TTA-AGT-TGG-G- **X** 5'A-ACG-TCA-GGG-TCT-TCC -3' 3'- CC-GCT-AAT-TCA-ACC-C – **Y** - T-TGC-AGT-CCC-AGA-AGG -5'	DNA^X-OH
p-5' 5'-*GG-CGA-TTA-AGT-TGG-G- **X** ACG-TCA-GGG-TCT-TCC -3' 3'-CC-GCT-AAT-TCA-ACC-C - **Y** - T-TGC-AGT-CCC-AGA-AGG -5'	DNA^X-gap
5'-CATCCACA 5'-*GG-CGA-TTA-AGT-TGG-G-**X** A-ACG-TCA-GGG-TCT-TCC -3' 3'- CC-GCT-AAT-TCA-ACC-C - **Y** - T-TGC-AGT-CCC-AGA-AGG -5'	DNA^X-flap
p-5' 5'-*GG-CGA-TTA-AGT-TGG-G A-ACG-TCA-GGG-TCT-TCC -3' 3'- CC-GCT-AAT-TCA-ACC-C - **A** - T-TGC-AGT-CCC-AGA-AGG -5'	2DNA-p (gap)
pF-5' 5'-*GG-CGA-TTA-AGT-TGG-G A-ACG-TCA-GGG-TCT-TCC -3' 3'- CC-GCT-AAT-TCA-ACC-C - **A** - T-TGC-AGT-CCC-AGA-AGG -5'	2DNA-pF
5'-*GG-CGA-TTA-AGT-TGG-G 5'A-ACG-TCA-GGG-TCT-TCC -3' 3'- CC-GCT-AAT-TCA-ACC-C - **A** - T-TGC-AGT-CCC-AGA-AGG -5'	2DNA-OH
p-5' 5'-*GG-CGA-TTA-AGT-TGG-G ACG-TCA-GGG-TCT-TCC -3' 3'- CC-GCT-AAT-TCA-ACC-C - **A**-T -TGC-AGT-CCC-AGA-AGG -5'	2DNA-gap

Note: The following combinations of nucleotide pairs (X/Y) are given, where X denotes a native or modified nucleotide at the 3′-end of the primer and Y designates an opposite nucleotide in the template chain. X = FAP (FAPdCMP), Y = G (dGMP); X = C (dCMP), Y = G (dGMP); X = G (dGMP), Y = C (dCMP); X = T (dTMP), Y = A (dAMP), C (dCMP), T (dTMP), G (dGMP). Asterisk designates phosphate group bearing ^{32}P-label; F, 3′-hydroxy-2′-hydroxymethyltetrahydrofuran (tetrahydrofuran) in the middle of the non-incised strand; pF, tetrahydrofuranophosphate; p, phosphate.

H_3BO_3, 0.002 M EDTA) [30]. Polyclonal rabbit antibodies against APE1 and β polymerase and also plasmids containing cDNA APE1 and β polymerase were kindly provided by Drs. S. H. Wilson and R. Prasad (National Institute of Environmental Health Sciences, USA) and rabbit preimmune serum by P. P. Laktionov (Institute of Chemical Biology and Fundamental Medicine).

DNA duplexes (Table 1) were formed by mixing oligonucleotides as follows: ^{32}P-labeled primer/template/downstream primer in 1 : 1.2 : 1.5 ratio in buffer containing 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA. The reaction mixtures were heated to 90°C and then slowly cooled to room temperature.

Synthesis of DNA structures bearing FAPdCMP at the 3′-end of the primer using β polymerase. The reaction mixture contained the following standard components: 50 mM Tris (pH 8.0), 50 mM KCl, 10 mM $MgCl_2$, and also 1.0 μM DNA duplex (DNA1, Table 1), 80 μM FAPdCTP, and 3.0 μM β polymerase. The products were analyzed and purified by electrophoresis under denaturing conditions (PAGE in the presence of 7 M urea in 1× TBE buffer [30]); the resulting ^{32}P-labeled photoreactive oligonucleotide was used for construction of DNA duplexes (Table 1).

Photoaffinity modification of pure APE1 and β polymerase was performed in the reaction mixture containing 50 nM DNA substrate and components of buffers 1-3 (Table 2). APE1 and/or β polymerase were added to the final concentration 0.5 μM. The reaction mixture was incubated for 5 min at 37°C. Photomodification of MEF extract proteins was performed in the reaction mixture (10 μl) containing MEF extract proteins (3.0 mg/ml), 100 nM DNA substrate, 50 mM Tris-HCl (pH 7.5). The mixture was incubated for 5 min at 37°C and UV-irradiated using a Bio-Link BLX cross-linker from Vilber Lourmat (France), wavelength 312 nm, light intensity 0.12 J/cm^2·sec (0.115 J/min, irradiation time 5 min). Immunoprecipitation was performed according to [23]. In brief, after UV irradiation 20 μl of antibodies against APE1 or β polymerase was added to the reaction mixture (100 μl), and the resulting mixture was incubated for 1 h at 4°C. Then the complex was adsorbed on protein G-agarose (20 μl) equilibrated with the buffer recommended by the producer. The reaction mixtures were then treated according to the protein G-agarose producer's protocol. The products were separated by SDS-PAGE (15% polyacrylamide gel) according to Laemmli [31] with subsequent gel scanning using the Molecular Imager from BioRad (USA).

Table 2. Buffer systems used for photoaffinity modification

Buffer	pH	[KCl], mM	[$MgCl_2$], mM
1	8.0	50	10
2	7.5	0	10
3	7.5	50	0

Evaluation of stability of various complexes of DNA substrates with APE1 and/or β polymerase by gel retardation. The reaction mixtures contained components of buffer 3, 1 mM EDTA, and 50 nM DNA. APE1 to final concentration 0.2 μM (if another concentration is not given) and/or β polymerase to final concentration 0.1 μM were added to the mixtures. The latter were then incubated in an ice bath for 5 min and electrophoresed at 12.5 V/cm and 4°C in 10% polyacrylamide gel under nondenaturing conditions in 0.25× TBE buffer with subsequent gel scanning using the Molecular Imager. The dissociation constant (K_d) of APE1 complex with double-stranded DNA with tetrahydrofuran (F) in the middle of one of the strands was determined via dependence on concentration of a complex ([ES]) in the reaction mixture on the initial enzyme concentration (e_0) (at constant substrate concentration (s_0)). K_d was calculated using the formula

$$K_d = \frac{(s_0 - [ES])(e_0 - [ES])}{[ES]}$$

and Origin Pro software.

DNA synthesis on DNA-rec catalyzed by β polymerase. The reaction mixtures contained 20 nM DNA, 0.06 μM β polymerase, 1 μM APE1 (where noted), and 5 μM mixture of four dNTP. Reaction was performed in buffer 1 at 37°C for 20 min or for a time given in the figure. The reaction products were separated by electrophoresis in 20% polyacrylamide gel under denaturing conditions in TBE buffer [30] with subsequent autoradiography or scanning using the Molecular Imager.

APE1 exonuclease activity. The reaction mixtures contained 10 nM DNA, 4 nM APE1, 20 nM (or at various concentrations where noted) XRCC1, and buffer components: 25 mM Hepes (pH 7.0), 25 mM KCl, and 2 mM $MgCl_2$. The reaction proceeded for 20 min at 37°C. The reaction products were separated by electrophoresis in 20% polyacrylamide gel under denaturing conditions in TBE buffer [30] with subsequent autoradiography or scanning using the Molecular Imager.

RESULTS AND DISCUSSION

Photoaffinity modification of APE1 and β polymerase recombinant proteins by DNA duplexes bearing FAPdCMP at the 3′-end of the primer. DNA substrates bearing a photoreactive nucleotide at the 3′-end of the primer used in this work were obtained from purified 5′-

^{32}P-labeled primer presynthesized *in vitro* by β polymerase (see "Materials and Methods"). APE1 is able to efficiently excise not only mismatch but also modified nucleotide analogs from the 3′-end of a nick [20, 25, 32]. Of four photoreactive derivatives investigated by us earlier [25], for photoaffinity modification in APE1-containing systems we chose the dCMP analog (FAPdCMP) excised with the lowest efficiency. The structure of this analog in the form of nucleotide-5′-triphosphate is presented in Fig. 1a. Based on earlier data [25], we chose such conditions of photoaffinity modification that FAPdCMP incorporated opposite dGMP in the template was negligibly excised by the 3′-5′-exonuclease function of APE1 at the 3′-end of the primer. The level of FAPdCMP excision at the 3′-end of the primer was analyzed in parallel with SDS-PAGE separation of the products of photochemical cross-linking of proteins and DNA. Efficiency of 3′-FAPdCMP excision was not more than 4% (for DNA duplex with the 5′-OH group in the nick (DNA^{FAP}-OH)).

APE1 or β polymerase were incubated with photoreactive DNA substrates and then DNA was covalently bound to purified enzymes by the action of UV irradiation. The products of photochemical protein binding to DNA were separated and analyzed by SDS-PAGE.

Photoinduced binding of DNA substrates to APE1 without a downstream oligonucleotide (DNA^{FAP}-rec, Table 1) or with various 5′-groups in the nick (DNA^{FAP}-pF, DNA^{FAP}-p, DNA^{FAP}-OH, DNA^{FAP}-flap; Table 1) was performed under various reaction conditions (buffers 1-3, Table 2). Our results and literature data [33] indicate that conditions under which activity of BER enzymes is studied (e.g. DNA polymerase and DNA ligase reactions) are optimal for the main endonuclease activity of APE1. Under such conditions (buffer 1, Table 2) efficiency of photoinduced APE1 modification (% ratio of DNA substrate covalently bound to protein relatively to the total amount of DNA) was not more than 5%. APE1 is most efficiently (5% yield) modified by DNA substrate bearing the 5′-pF group (DNA^{FAP}-pF), which flanks a nick. This DNA structure is a model of a short-patch BER intermediate or a long-patch BER product and is an analog of the product of endonuclease or substrate of the exonuclease APE1 activity (Scheme). APE1 covalently binds to other DNA structures (DNA^{FAP}-rec, DNA^{FAP}-p, DNA^{FAP}-OH, and DNA^{FAP}-flap) with lower and nearly equal efficiency (2%, Fig. 1b).

DNA structures with a nick and DNA-rec (Table 1) used for photoaffinity modification are substrates for APE1 3′-5′-exonuclease activity, that is more efficient at decreased salt concentrations. When KCl was removed from the reaction mixture (buffer 2, Table 2), but 10 mM $MgCl_2$ was present, efficiency of photoinduced cross-linking of APE1 to DNA^{FAP}-rec and DNA^{FAP}-p increased to 3.4 and 2.8%, respectively. Modification was most efficient (8%) for DNA^{FAP}-pF (Fig. 1c), the same as in the presence of KCl (buffer 1).

In order to cleave phosphodiester bonds in the course of endonuclease and exonuclease reactions catalyzed by APE1, Mg^{2+} or Mn^{2+} is needed [20, 34-37].

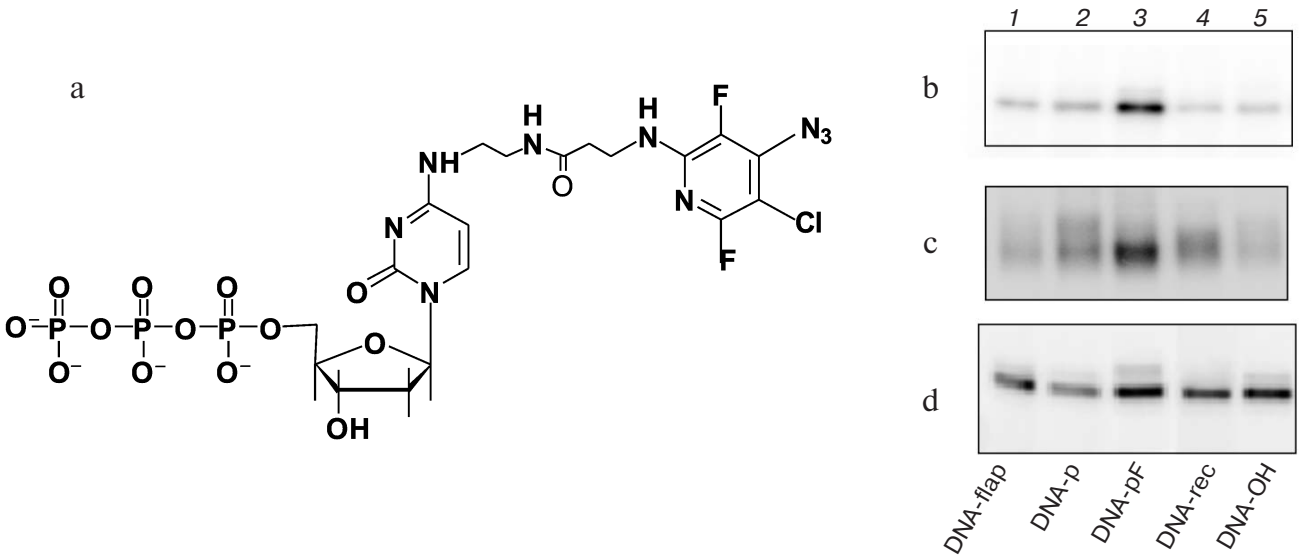


Fig. 1. a) Structural formula of FAPdCTP analog. b-d) APE1 photoaffinity modification (buffers 1-3, respectively, Table 2). Reaction products were separated according to Laemmli in 15% polyacrylamide gel. Reaction mixtures contained 50 nM DNA and 0.5 μM APE1. Effectiveness of modification (formation of covalent photoinduced bond APE1–DNA substrate) for various DNA substrates in three different buffers (Table 2) is given for lanes *1-5* (in buffer 1 – 2.1, 2.2, 5.1, 2.1, 2.1%; in buffer 2 – 1.9, 2.8, 8.0, 3.4, 1.8%; in buffer 3 – 28.3, 26.6, 31.7, 24.1, 28.5%, respectively).

When photoaffinity modification proceeds in the absence of $MgCl_2$, modification defined as the fraction of photoreactive DNA covalently bound to DNA increases to 15-30% (buffer 3, Table 2). Efficiency of APE1 covalent binding to various DNA structures (Table 1) in this buffer changes as follows: DNA^{FAP}-pF (31.7%) > DNA^{FAP}-OH (28.5%) ≥ DNA^{FAP}-flap (28.3%) > DNA^{FAP}-p (26.6%) > DNA^{FAP}-rec (24.1%) (Fig. 1d).

Photochemical cross-linking of β polymerase to DNA substrates with various 5′-groups in the nick was performed under two different conditions: in buffer 1 optimal for DNA polymerase reaction and in buffer 3 not containing $MgCl_2$ (Table 2). Analogously to APE1, efficiency of β polymerase–DNA covalent binding in buffer 3 was significantly higher than in buffer 1. We probably observed this distinction owing to stabilization of protein–DNA complex at the lower ionic strength of solution and inhibition of the complex dissociation in the absence of $MgCl_2$. Along with five different DNA duplexes (Table 1 and Fig. 1b) used for APE1 modification, for photoaffinity modification of β polymerase we used DNA structures bearing FAPdCMP at the 3′-end of the mononucleotide gap and the phosphate group at the 5′-end (DNA^{FAP}-gap, Table 1). Such structure was chosen because this substrate imitates a DNA intermediate forming in the course of DNA repair and being a substrate for β polymerase (Scheme). It was shown by photoaffinity modification that β polymerase is modified by this DNA duplex with the maximal efficiency of 26.2% (buffer 3). This protein covalently binds to DNA^{FAP}-rec and DNA^{FAP}-OH with the efficiency 24.8 and 24.3%, respectively. Efficiency of modification of this protein by the action of three DNA duplexes was nearly equal (DNA^{FAP}-p, 25.3%; DNA^{FAP}-pF, 25.1%; DNA^{FAP}-flap, 25.4%) and somewhat lower than by DNA^{FAP}-gap.

Photoaffinity modification of APE1 and β polymerase in the simultaneous presence of purified proteins and in cell extract. Proteins of mouse embryonic fibroblast (MEF) cell extract, in which APE1 and β polymerase are present, were modified by six different DNA structures (Table 1) bearing a photoreactive group at the 3′-end of the primer. Results of electrophoretic separation of the reaction products are presented in Fig. 2b. Earlier six proteins were detected in MEF extract by photoaffinity modification [23, 26]; these proteins most efficiently bind to photoreactive DNA substrates imitating BER intermediates (Scheme) and bearing the phosphate, pF, or dRp group at the 5′-end of the nick. Analogously to [23, 26], photoinduced DNA cross-linking to MEF extract proteins resulted in labeling of a limited number of proteins. Among the main products detected, two proteins labeled with the maximal efficiency were identified by immunoprecipitation as β polymerase and APE1 (Fig. 2a).

For APE1 in MEF extract, efficiency of formation of protein–DNA cross-linking changed as follows depending on the nature of 5′-group flanking the nick in DNA structure (Table 1): DNA^{FAP}-pF (100%) > DNA^{FAP}-OH (68.2%) > DNA^{FAP}-flap (54.6%) > DNA^{FAP}-gap (44.2%) > DNA^{FAP}-rec (41.3%) ~ DNA^{FAP}-p (41.1%). The analogous series for β polymerase was as follows: DNA^{FAP}-gap (100%) > DNA^{FAP}-p (72.9%) ~ DNA^{FAP}-pF (71.7%) > DNA^{FAP}-flap (63.9%) > DNA^{FAP}-rec (44.4%) > DNA^{FAP}-OH (40.6%). Numerical evaluation of the relative efficiency of protein–DNA cross-linking is given above for various DNA structures in comparison with the maximal efficiency taken as 100% (DNA^{FAP}-pF for APE1 and DNA^{FAP}-gap for β polymerase, respectively). On the whole, the same dependence of modification efficiency on the nature of the 5′-group in DNA substrate was observed for MEF extract proteins and purified APE1 and

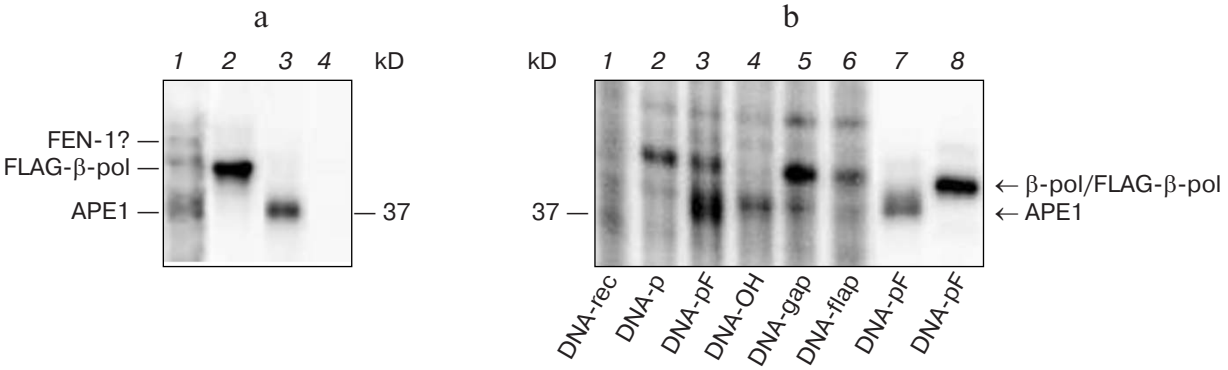


Fig. 2. a) Identification of MEF extract proteins APE1 and β polymerase among DNA–protein adducts by immunoprecipitation. Presynthesized DNA^{FAP}-pF bearing a photoreactive group was incubated with MEF extract. The reaction mixture was UV irradiated (λ = 312 nm). Products of photomodification are shown on lane *1*. The mixture was immunoprecipitated with pre-immune serum (lane *4*) and antibodies against β polymerase and APE1 (lanes *2* and *3*, respectively). Position of molecular mass marker is shown on the right. b) Photoaffinity modification of MEF extract proteins by photoreactive DNA structures. The reaction mixtures (10 μl) contained 50 nM DNA^{FAP}-pF and 0.5 μM APE1/β polymerase (lanes *7* and *8*) or 100 nM DNA and MEF extract proteins (3.0 mg/ml) (lanes *1*-*6*). The reaction mixture was incubated with MEF extract and UV irradiated (λ = 312 nm). The reaction products were electrophoretically separated in 15% polyacrylamide gel according to Laemmli. Position of molecular mass marker is shown on the left.

β polymerase (in buffer 3). The data confirm earlier results [23, 26] that APE1 in MEF extract most efficiently binds to DNA bearing the 5′-pF in the nick.

On simultaneous incubation of purified recombinant proteins APE1 and β polymerase with various DNA structures (DNA^{FAP}-pF, DNA^{FAP}-rec, DNA^{FAP}-p, DNA^{FAP}-OH, DNA^{FAP}-flap, and DNA^{FAP}-gap; Table 1) in buffers 1-3 we did not observe mutual effect of these proteins on efficiency of photoinduced protein–DNA cross-linking (data not presented here). Earlier it was shown for DNA substrate with the 5′-pF group that if exogenous APE1 was added to MEF extract before UV irradiation, efficiency of β polymerase labeling did not change, whereas efficiency decreased for FEN1 and poly(ADP-ribose)-polymerase-1 (PARP1). Thus, PARP1 and FEN1 compete with APE1 for binding to such DNA substrate, and APE1 and β polymerase do not compete [23]. For all DNAs we used (Fig. 2b), addition of exogenous recombinant proteins APE1 and β polymerase to MEF extract also did not affect the degree of modification of β polymerase and APE1, respectively. However, addition of excess exogenous proteins caused drastic increase in efficiency of excess protein labeling and decreased labeling of other MEF extract proteins (data not presented here). Such effect was already observed during the study of proteins of cell/nucleus extracts by photoaffinity modification [38].

APE1 binding to various oligonucleotide structures. We determined by gel retardation APE1 ability for binding to a wider set of DNA structures in order to compare it with data on photoaffinity modification. Numerical data on APE1 binding to DNA having an AP site in the middle of one of the strands prevail in the literature [21, 39-41]. Under the conditions we used, K_d of such complex is 75 nM (Fig. 3a); on the whole this is in accord with the literature data. A histogram illustrating efficiency of complex formation of various DNA duplexes with APE1 and β polymerase is presented in Fig. 3b. As expected, APE1 most efficiently binds to DNA substrate bearing tetrahydrofuran in the middle of one of the strands (AP-dsDNA, Table 1). For each DNA substrate, binding efficiency was initially determined as a ratio of substrate bound in substrate–protein complex relative to the total amount of DNA. The value thus obtained was then compared with efficiency of AP-dsDNA–APE1 complex formation (100%, Fig. 3b). It is known that APE1 with comparable affinity binds to un-excised DNA with AP site (AP-dsDNA, Table 1) and to the product of endonuclease reaction (2DNA-pF, Table 1) [39]. This fact was a basis for presentation of the BER mechanism as passing a baton (DNA) from protein to protein in conversion sequence [5]. Our data indicate that APE1 binds with affinity somewhat lower but still high enough with 2DNA-pF structure (Table 1) imitating a product of endonuclease reaction catalyzed by APE1.

APE1 binds to other DNA structures: dsDNA, 2DNA-OH, DNA-pF, 2DNA-p(gap), DNA-gap, and DNA-flap with nearly the same efficiency as to 2DNA-pF (Table 1). This enzyme interacts with 2DNA-gap, DNA-p, DNA-OH with lower efficiency and with DNA-rec and single-stranded oligonucleotides AP-ssDNA and ssDNA with the minimal efficiency (Fig. 3b). Preferential binding of APE1 to DNA with a mononucleotide gap (2DNA-OH, 2DNA-p(gap), DNA-gap) compared with such DNAs as 2DNA-gap, DNA-p, and DNA-rec was noted in [13, 39]. As shown in Figs. 1d and 2b, such binding tendency corresponds well with the series of efficiency of photoinduced cross-linking of APE1 to DNA structures bearing FAPdCMP/G pair at the 3′-end (in these duplexes C/G or T/A group was at the 3′-end of the primer (Table 1)). Rather high APE1 affinity to dsDNA can be explained as follows: APE1 binds to undamaged DNA and slides along it, "searching" for a damage—an AP site [42].

For β polymerase, the series of binding efficiency of DNA structures looks as follows (Fig. 3b): β polymerase binds with the maximal efficiency to the two DNA structures bearing a mononucleotide gap with the 5′-p group (2DNA-p(gap) and DNA-gap), and with lower efficiency to DNA-p (a nick with the 5′-p group) and 2DNA-pF structure. DNA duplex 2DNA-pF imitates the product of APE1 endonuclease activity "transferred" to β polymerase. Structures with the maximal affinity bearing a gap imitate the product of β polymerase lyase activity, without inserting one nucleotide (Scheme). Our data indicate that β polymerase complex with the elongated product bearing the 5′-pF group (DNA-pF) is less stable than the β polymerase complex with substrate having a mononucleotide gap flanked by the 5′-pF group (2DNA-pF). It should be noted that β polymerase complexes with DNA-flap and 2DNA-gap (with a dinucleotide gap with 5′-p group) structures are more stable than β polymerase–DNA-pF complex. The following structures complete the series of binding efficiency with β polymerase: 2DNA-OH > DNA-OH > DNA-rec > AP-dsDNA, dsDNA, AP-ssDNA, ssDNA. Similar to APE1, the series of β polymerase binding efficiency to various DNA obtained by gel retardation is well agreed with the series of binding efficiency obtained by photoaffinity modification of β polymerase (Fig. 2b). Thus, in this case efficiency of photoaffinity modification may reflect the enzyme affinity to substrate. Efficiency of photoinduced cross-linking may be used for evaluation of complex stability under the given conditions and correctly chosen enzyme and DNA substrate concentrations.

Figure 3c presents the results of electrophoretic separation of a mixture of APE1 and β polymerase with various DNA substrates under non-denaturing conditions. A histogram of quantitative evaluation of the ratio of substrate bound in a double or ternary complex with proteins and the total amount of DNA substrate is presented below. Interaction of APE1 and β polymerase in the course of short-patch BER on DNA structures imitating

intermediates of this repair pathway is discussed in [43]. Using gel retardation, Liu and coworkers also observed APE1–β polymerase–DNA complex, but only for DNA bearing the 5′-pF group, whereas this complex was not observed for AP-DNA and structures with a nick/gap bearing 5′-p groups [43]. In contrast, we observed more efficient formation of ternary complex for DNA with a mononucleotide gap with the 5′-p group (2DNA-p(gap), DNA-gap) than that with DNA duplexes containing the 5′-pF group (2DNA-pF and to a higher extent, DNA-pF) (Fig. 3c). According to a commonly accepted concept, in the course of short-patch BER β polymerase firstly fills a mononucleotide gap by its polymerase function and then excises 5′-dRp by the lyase activity [13, 44]. It is suggested [43] that under conditions of the ternary complex formation, stages of gap filling and dRp removal catalyzed by β polymerase may be occasional or simultaneous, not strictly successive (Scheme). It was noted [43] that faster removal of the 5′-dRp group from BER intermediate with 3′-mismatch may increase the correcting capacity of APE1, because its exonuclease activity is higher on a structure without the 5′-dRp group than on the intermediate with the 5′-dRp group. Along with this, discrimination between match and mismatch 3′-base exerted by APE1 is less efficient for a structure with the 5′-pF group [14].

Earlier we showed that APE1 most efficiently exhibits its 3′-5′-exonuclease activity against DNA with a flap (DNA-flap) and DNA with the recessed 3′-end (DNA-rec) and also against DNA with the 5′-OH group in a nick (DNA-OH) (Table 1) [22]. It should be noted that DNA duplexes imitating intermediates of the short-

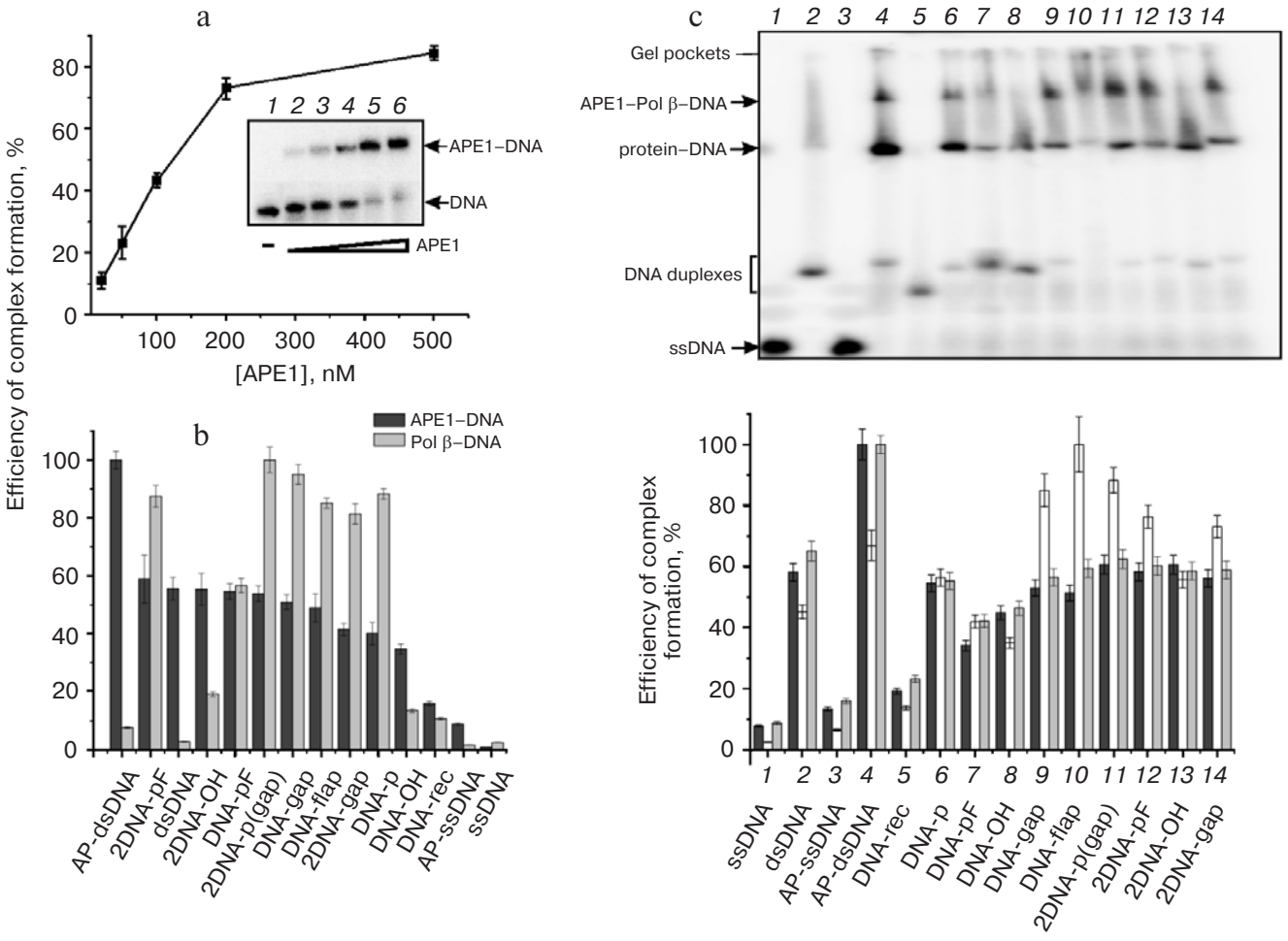


Fig. 3. a) Evaluation of the dissociation constant of APE1–AP-DNA complex (K_d = 75 ± 8 nM). The reaction mixtures contained components of buffer 3 (Table 2), 1 mM EDTA, 50 nM AP-DNA (Table 1), and APE1 in the concentration range 10-500 nM (lanes *2-6*). b) Evaluation of stability of complexes of various DNA substrates with APE1 or β polymerase. The reaction mixtures contained components of buffer 3, 1 mM EDTA, 50 nM DNA, and also 0.2 μM APE1 or 0.1 μM β polymerase. c) Evaluation of stability of complexes of various DNA substrates with APE1 and β polymerase in their simultaneous presence. The reaction mixtures contained components of buffer 3, 1 mM EDTA, 50 nM DNA, and also 0.2 μM APE1 or 0.1 μM β polymerase. The reaction products were separated by electrophoresis in 10% polyacrylamide gel under non-denaturing conditions. Dark columns, APE1–DNA and β polymerase–DNA complexes with the same electrophoretic mobility under these conditions; light columns, ternary complex APE1–β polymerase–DNA; gray columns, total binding of DNA substrate in binary and ternary complexes.

patch BER pathway (DNA-p and DNA-pF, Table 1) are not preferential substrates for APE1 3′-5′-exonuclease activity [13, 22-24]. A ternary complex APE1–β polymerase–DNA is most effectively formed with DNA-flap. Such complex is also formed with significant efficiency (>50%) with DNA product of APE1 endonuclease activity (2DNA-pF), DNA with a dinucleotide gap (2DNA-gap), AP-dsDNA, DNA with a nick (DNA-p) and a gap flanked by 5′-OH group (2DNA-OH) (Fig. 3c). DNA duplexes used by us (Table 1) may be considered as intermediates of the short-patch (DNA-p, DNA-gap) or long-patch (DNA-pF, DNA-flap) BER pathways. DNA-rec and DNA-OH do not belong to structures arising in the course of BER. Literature data on efficiency of APE1 exonuclease activity [22, 25] and our data on formation of APE1–DNA and ternary complexes indicate that APE1

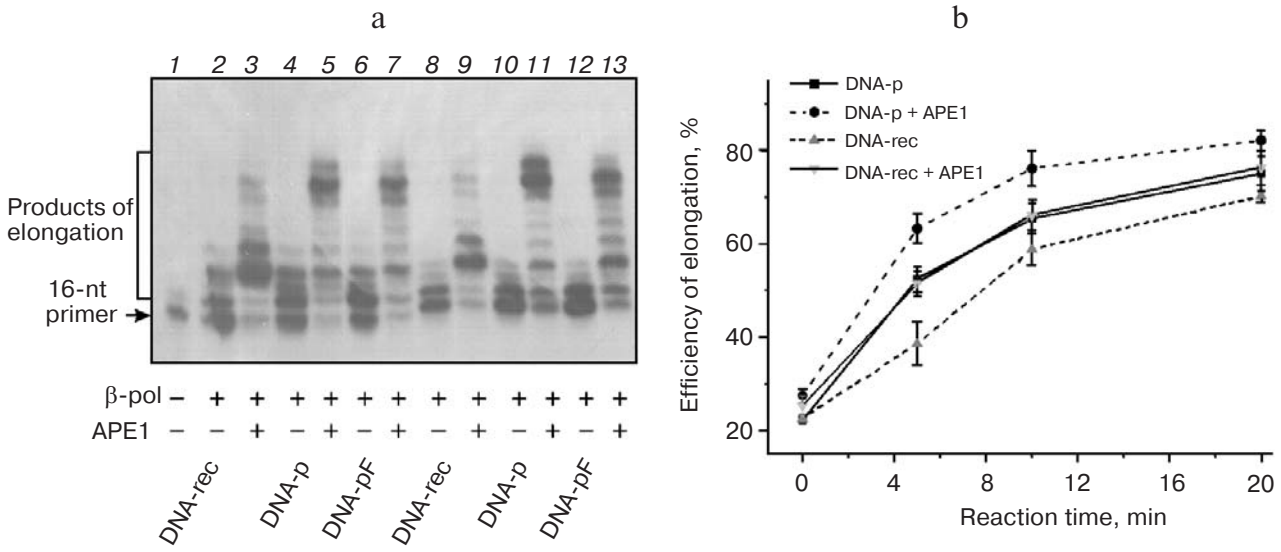


Fig. 4. Stimulation of DNA synthesis by β polymerase in the presence of APE1 on DNA-rec. a) Separation of the products of polymerase reaction by electrophoresis in 20% denaturing polyacrylamide gel [30]. Position of 16-nt long initiating primer is shown on the left. Reaction mixtures contained 20 nM DNA with C/G (lanes *1-7*) or G/C pairs (lanes *8-13*) at the 3′-end of the primer, 0.06 μM Pol β, 1 μM APE1 where given, and 5 μM mixture of four dNTP. b) Kinetic dependence of DNA synthesis in the presence and in the absence of APE1 studied in the reaction mixture as in (a) on DNA-p or DNA-rec bearing 3′-T/A pair.

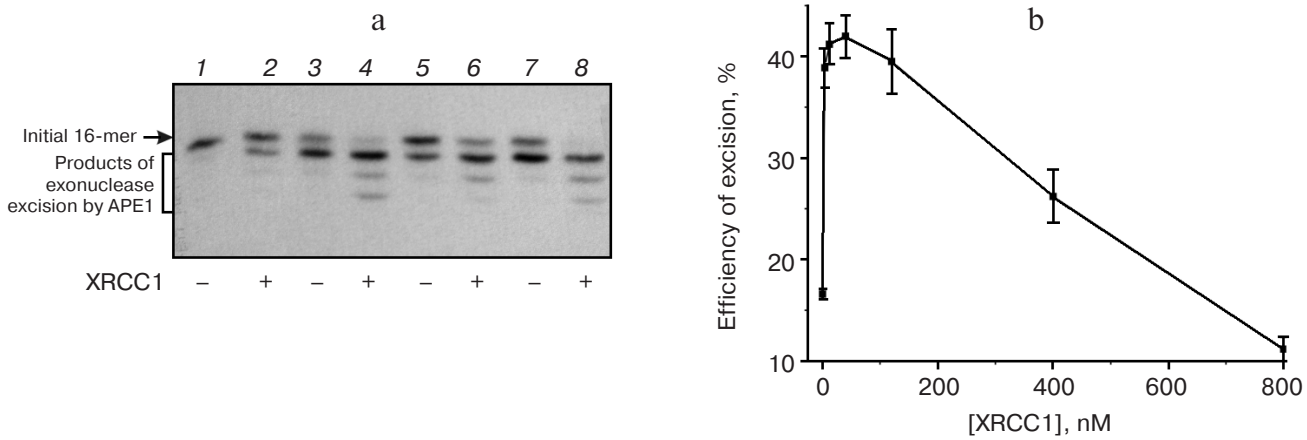


Fig. 5. Stimulation of APE1 3′-5′-exonuclease activity in the presence of XRCC1 on DNA-rec. a) Reaction mixtures contained buffer components (25 mM Hepes (pH 7.0), 25 mM KCl, and 2 mM $MgCl_2$), 4 nM APE1, 20 nM XRCC1, and 10 nM DNA with 3′-dTMP at the end of the primer (within the nucleotide pairs T/A (lanes *1* and *2*), T/C (lanes *3* and *4*), T/G (lanes *5* and *6*), and T/T (lanes *7* and *8*)). b) Reaction mixture contained the same buffer components as in (a), as well as 10 nM DNA with 3′-T/G pair at the end of the primer, 4 nM APE1, and XRCC1 at given concentrations. The reaction products were separated by electrophoresis in 20% polyacrylamide gel under denaturing conditions [30]. Products of hydrolysis less than 16 nucleotides in length were not observed in the presence of XRCC1 and in the absence of APE1. Increased APE1 exonuclease activity detected on addition of XRCC1 at the range of concentrations 4-400 nM was not observed in control in the presence of BSA.

more efficiently interacts with DNA structure with the 5′-OH group in the nick and with the long-patch BER intermediates than with the short-patch BER structures.

Interaction of APE1 with β polymerase and XRCC1 on DNA structure with the recessed 3′-end (DNA-rec). Earlier we showed [22] that DNA with the recessed 3′-end (DNA-rec) is one of the preferential substrates for APE1 3′-5′-exonuclease activity, as compared with DNA-p and DNA-pF for example. However, according to data presented here, APE1–DNA complex with such substrate is less stable than with DNA-p and DNA-pF, as well as the ternary complex APE1–β polymerase–DNA and a complex of this substrate with β polymerase (Fig. 3, b and c). It is considered that structures like DNA-rec do not arise in the course of BER. DNA-rec duplex can imitate a structure arising in the course of other processes of DNA metabolism, e.g. DNA replication. It is unclear whether the suggestion that enzyme participating in BER is able to interact by some way with DNA structure with a single-stranded part more than 10 nucleotides in length is justified. According to [45], β polymerase efficiently synthesizes in a gap not more than six nucleotides in length in the presence of 5′-p group. We suggested that APE1 and β polymerase can mutually effect their enzymatic activities on interaction with DNA-rec. Figure 4a presents results of electrophoretic separation of the products of elongation by β polymerase in the presence and in the absence of APE1 on DNA with C/G or G/C pair at the 3′-end of the primer. Figure 4b presents the kinetic dependence of DNA synthesis also in the presence/absence of APE1 on DNA-rec bearing the 3′-T/A pair. As shown earlier, APE1 stimulates strand-displacement DNA synthesis catalyzed by β polymerase on oligonucleotide DNA substrates with the 5′-p and 5′-pF groups flanking a nick [12, 14, 43]. Our data demonstrate that APE1 stimulates DNA synthesis by β polymerase also on DNA-rec (Fig. 4). However, efficiency of polymerase activity of β polymerase on this substrate is lower than on DNA with the 5′-p group in the nick (Fig. 4b) as well as efficiency of stimulation (Fig. 4). APE1 affinity to DNA-rec is low, but high APE1 3′-5′-exonuclease activity on DNA-rec may be caused by higher mobility of the 3′-end of this duplex compared with duplexes with a nick [22], although participation of APE1 in DNA replication cannot be excluded.

The presence of β polymerase in the reaction mixture either does not influence efficiency of APE1 exonuclease activity [43] or inhibits it at equimolar ratio of enzyme concentrations [14]. We found that the presence of XRCC1 influences efficiency of 3′-dNMP excision from DNA-rec by APE1 3′-5′-exonuclease activity (Fig. 5). XRCC1 does not possess enzymatic activity, although it plays an important role in BER coordination. This protein increases BER efficiency, forming a stable complex with DNA ligase III and stimulating β polymerase activity [46]. It is also known that XRCC1 stimulates AP-endonuclease and 3′-phosphodiesterase activities of APE1 [47] and increases efficiency of 3′-5′-exonuclease excision of the match (Fig. 5a, lanes *1* and *2*) as well as non-canonical (Fig. 5a, lanes *3-8*) pairs by APE1. Dependence of stimulation of APE1 exonuclease activity by XRCC1 on concentration of the latter has a bell shape (Fig. 5b). At high XRCC1 concentrations, inhibition of APE1 exonuclease activity is observed (Fig. 5b, the last point).

So, since a role of APE1 as a corrector of β polymerase errors during short-patch BER is still unclear; our data suggest that APE1 3′-5′-exonuclease activity is needed for correction of errors during long-patch BER or other processes of DNA metabolism. Stimulation of APE1 exonuclease activity in the presence of XRCC1 on DNA-rec may also indicate that along with BER, APE1 participates in other processes, e.g. in replication.

The authors are grateful to Zh. K. Nazarkina for kindly donated XRCC1 preparation and to Y. Liu for help and giving advice on gel retardation assay.

This work was financially supported by the Russian Foundation for Basic Research (grant No. 07-04-00178), the Lavrent'ev grant of the Siberian Branch of the Russian Academy of Sciences for young scientists (No. 96), and Integration Project of the Siberian Branch of the Russian Academy of Sciences (grant No. 110).

REFERENCES

1. Lindahl, T. (2000) *Mutat. Res.*, **462**, 129-135.
2. Memislogu, A., and Samson, L. (2000) *Mutat. Res.*, **451**, 39-51.
3. Krokan, H. E., Standal, R., and Slupphaug, G. (1997) *Biochemistry*, **68**, 255-285.
4. Lantsov, V. A. (1998) *Mol. Biol.*, **32**, 757-772.
5. Wilson, D. M., III, and Barsky, D. (2001) *Mutat. Res.*, **484**, 283-307.
6. Matsumoto, Y., and Kim, K. (1995) *Science*, **269**, 699-702.
7. Piersen, C. E., Prasad, R., Wilson, S. H., and Lloyd, R. S. (1996) *J. Biol. Chem.*, **271**, 17811-17815.
8. Klungland, A., and Lindahl, T. (1997) *EMBO J.*, **16**, 3341-3348.
9. Wilson, S. H., and Kunkel, T. A. (2001) *Nature Struct. Biol.*, **7**, 176-178.
10. Hill, J. W., Hazra, T. K., Izumi, T., and Mitra, S. (2001) *Nucleic Acids Res.*, **29**, 430-438.
11. Vidal, A. E., Hickson, I. D., Boiteux, S., and Radicella, J. P. (2001) *Nucleic Acids Res.*, **29**, 1285-1292.
12. Bennet, R. A. O., Wilson, D. M., III, Wong, D., and Demple, B. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 7166-7169.
13. Wong, D., deMott, M. S., and Demple, B. (2003) *J. Biol. Chem.*, **278**, 36242-36249.
14. Sukhanova, M. V., Khodyreva, S. N., Lebedeva, N. A., Prasad, R., Wilson, S. H., and Lavrik, O. I. (2005) *Nucleic Acids Res.*, **33**, 1222-1229.
15. Ranalli, T. A., Tom, S., and Bambara, R. A. (2002) *J. Biol. Chem.*, **277**, 41715-41724.

16. Singhal, R. K., Prasad, R., and Wilson, S. H. (1995) *J. Biol. Chem.*, **270**, 949-957.
17. Evans, A. R., Limp-Foster, M., and Kelley, M. R. (2000) *Mutat. Res.*, **461**, 83-108.
18. Chagovetz, A. M., Sweasy, J. B., and Preston, B. D. (1997) *J. Biol. Chem.*, **272**, 27501-27504.
19. Chou, K.-M., and Cheng, Y.-C. (2002) *Nature*, **415**, 655-659.
20. Chou, K.-M., and Cheng, Y.-C. (2003) *J. Biol. Chem.*, **278**, 18289-18296.
21. Wilson, D. M., III (2003) *J. Mol. Biol.*, **330**, 1027-1037.
22. Dyrkheeva, N. S., Lomzov, A. A., Pyshnyi, D. V., Khodyreva, S. N., and Lavrik, O. I. (2006) *Biochim. Biophys. Acta*, **1764**, 699-706.
23. Cistulli, C., Lavrik, O. I., Prasad, R., Hou, E., and Wilson, S. H. (2004) *DNA Repair*, **3**, 581-591.
24. Lebedeva, N. A., Khodyreva, S. N., Favre, A., and Lavrik, O. I. (2003) *Biochem. Biophys. Res. Commun.*, **300**, 182-187.
25. Dyrkheeva, N. S., Khodyreva, S. N., Sukhanova, M. V., Safronov, I. V., Dezhurov, S. V., and Lavrik, O. I. (2006) *Biochemistry (Moscow)*, **71**, 200-210.
26. Lavrik, O. I., Prasad, R., Sobol, R. W., Horton, J. K., Ackerman, E. J., and Wilson, S. H. (2001) *J. Biol. Chem.*, **276**, 25541-25548.
27. Drachkova, I. A., Petruseva, I. O., Safronov, I. V., Zakharenko, A. L., Shishkin, G. V., Lavrik, O. I., and Khodyreva, S. N. (2001) *Bioorg. Khim.*, **27**, 179-204.
28. Marsin, S., Vidal, A. E., Sossou, M., Menissier-de Murcia, J., Le Page, F., Boiteux, S., de Murcia, G., and Radicella, J. P. (2003) *J. Biol. Chem.*, **278**, 44068-44074.
29. Dezhurov, S. V., Khodyreva, S. N., Plekhanova, E. S., and Lavrik, O. I. (2005) *Bioconj. Chem.*, **16**, 215-222.
30. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Edn., Cold Spring Harbor, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
31. Laemmli, U. K. (1970) *Nature*, **227**, 680-685.
32. Chou, K.-M., Kukhanova, M., and Cheng, Y.-C. (2000) *J. Biol. Chem.*, **275**, 31009-31015.
33. Dyrkheeva, N. S., Khodyreva, S. N., and Lavrik, O. I. (2007) *Mol. Biol.*, **41**, 450-466.
34. Mol, C. D., Izumi, T., Mitra, S., and Tainer, J. (2000) *Nature*, **430**, 451-455.
35. Mol, C. D., Hosfield, D. J., and Tainer, J. A. (2000) *Mutat. Res.*, **460**, 211-229.
36. Kane, C. M., and Linn, S. (1981) *J. Biol. Chem.*, **256**, 3405-3414.
37. Beernink, P. T., Segelke, B. W., Hadi, M. Z., Erzberger, J. P., Wilson, D. M., III, and Rupp, B. (2001) *J. Mol. Biol.*, **307**, 1023-1034.
38. Sukhanova, M. V., Khodyreva, S. N., and Lavrik, O. I. (2006) *Biochemistry (Moscow)*, **71**, 736-748.
39. Wilson, D. M., III, Takeshita, M., and Demple, B. (1997) *Nucleic Acids Res.*, **25**, 933-939.
40. Masuda, Y., Bennet, R. A. O., and Demple, B. (1998) *J. Biol. Chem.*, **273**, 30360-30365.
41. Erzberger, J. P., Barsky, D., Scharer, O. D., Colvin, M. E., and Wilson, D. M., III. (1998) *Nucleic Acids Res.*, **26**, 2771-2778.
42. Strauss, P. R., Beard, W. A., Patterson, T. A., and Wilson, S. H. (1997) *J. Biol. Chem.*, **272**, 1302-1307.
43. Liu, Y., Prasad, R., Beard, W. A., Kedar, P. S., Hou, E. W., Shock, D. D., and Wilson, S. H. (2007) *J. Biol. Chem.*, **282**, 13532-13541.
44. Prasad, R., Beard, W. A., Strauss, P. R., and Wilson, S. H. (1998) *J. Biol. Chem.*, **273**, 15263-15270.
45. Singhal, R. K., and Wilson, S. H. (1993) *J. Biol. Chem.*, **268**, 15906-15911.
46. Kubota, Y., Nash, R. A., Klungland, A., Schar, P., Barnes, D. E., and Lindahl, T. (1996) *EMBO J.*, **15**, 6662-6670.
47. Vidal, A. E., Boiteux, S., Hickson, I. D., and Radicella, J. P. (2001) *EMBO J.*, **2**, 6530-6539.