FEBS Letters 486 (2000) 14–18 FEBS 24333

Preferential interaction of loach DNA polymerase δ with DNA duplexes containing single-stranded gaps

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Received 27 September 2000; revised 3 November 2000; accepted 6 November 2000

First published online 9 November 2000

Edited by Ned Mantei

Abstract We studied the interaction of DNA polymerase δ (pol δ) purified from the eggs of the teleost fish *Misgurnus fossilis* (loach) with DNA duplexes containing single-stranded gaps of 1–13 nucleotides (nt). In the absence of processivity factors (PCNA, RF-C, and ATP), pol δ elongated primers on single-stranded DNA templates in a distributive manner. However, the enzyme was capable of processive synthesis by filling gaps of 5–9 nt in DNA duplexes. These data suggest that, upon filling a small gap, pol δ interacts with the 5′-terminus downstream of the gap as well as with the 3′-terminus of the primer. Interaction of pol δ with the proximal 5′-terminus restricting the gap was confirmed by electrophoretic mobility shift assay. Analysis of the enzyme binding to DNA duplexes containing gaps of various sizes showed a much higher affinity of pol δ for duplexes with gaps of about 10 nt than for DNA substrates with primers annealed to single-stranded templates. The most efficient pol δ binding was observed in experiments with DNA substrates containing unpaired 3′-tails in primers. The data obtained suggest that DNA molecules with small gaps and single-stranded tails may serve as substrates for direct action of pol δ in the course of DNA repair.

Key words: Replication; DNA repair; Gapped DNA; DNA polymerase δ; Oocyte; Teleost fish

1. Introduction

The excision repair of nuclear DNA is initiated by elimination of a fragment around a lesion by specific endonucleases and exonucleases. This is followed by resynthesis of the fragment by DNA polymerase thus filling the gap at the position of lesion. The repair synthesis in the cell nucleus is carried out by DNA polymerases β (pol β), δ (pol δ), and, probably, ε [1–4]. Pol β showed a maximum efficiency on DNA duplexes containing 1-nt gaps in in vitro experiments [5,6]. In agreement with these data, the primary function of pol β in the nucleus was related to the short-patch repair when only one nucleotide (nt) was replaced in damaged DNA [7]. Purified pol β catalyzes a distributive synthesis on single-stranded templates while it is capable of filling small gaps in duplexes in a processive manner polymerizing up to six residues without dissociation from DNA [8]. Interaction with 5′-terminus of a fragment downstream the gap stabilizes the productive complex of pol β with the primer–template and allows a processive synthesis [9–11]. The processive filling of small gaps permits pol β to participate in the long-patch repair of nuclear DNA, as was confirmed by some recent observations [12,13]. However, the major role in the long-patch repair is generally attributed to pol δ [14–21]. Pol δ is essential for replication of nuclear DNA and cell survival [22–24]. In association with processivity factors (PCNA, RF-C, and RP-A), pol δ performs processive synthesis of the leading strand in a replication fork. The function of pol δ in repair synthesis is less well understood. Isolated pol δ has low processivity in the absence of processivity factors and autonomous action of this enzyme in DNA repair is questioned.

In a search for factors essential for DNA repair in fish oocytes, we have recently isolated loach pol δ [25]. The loach pol δ is a candidate for the primary repair polymerase in the oocytes and early embryos because the activity of pol β in the loach oocytes is extremely low [26]. Like other δ-type DNA polymerases, the loach pol δ possesses the $3' \rightarrow 5'$ exonuclease activity specific for single-stranded DNA. The polymerase activity is sensitive to aphidicolin, but resistant to BuPhedGTP, and correlates with the presence of a 120–130-kDa polypeptide, which specifically reacted with polyclonal antibodies to the calf thymus pol δ in Western blots. In this paper, we show that the loach pol δ performs a distributive elongation of primers on single-stranded DNA, whereas the enzyme was capable of processive filling of small gaps in DNA duplexes. Preferential binding of pol δ to gapped DNA and molecules with unpaired 3′-tails suggest a variety of possible substrates for pol δ action inside the nucleus.

2. Materials and methods

2.1. Enzymes

Pol δ (fractions δ1 and δ2) was purified from eggs of the teleost fish, *Misgurnus fossilis* (loach), as previously described [25]. The enzyme was stored at −20°C in a buffer containing 50% glycerol, 10 mM potassium phosphate, pH 7.5, 5 mM 2-mercaptoethanol, 1 mM EDTA, 10 mM $Na_2S_2O_5$, 0.2 mM phenylmethylsulfonyl fluoride, 1 μM pepstatin, 1 μg/ml leupeptin, and 1 μg/ml aprotinin. One unit of DNA polymerase activity was defined as the incorporation of 1 nmol dNMP into activated DNA in 1 h at 37°C. Phage T4 polynucleotide kinase was purchased from Boehringer Mannheim.

2.2. DNA substrates

The following oligonucleotides were used for preparation of partial duplexes: 17-mer (TGCCGGGATCATAGAAG), 21-mer (AGAGTGCCGGGATCATAGAAG), 25-mer (ACACAGAGTGCCGGG-

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PII: S0014-5793(00)02232-8

ATCATAGAAG), 29-mer (AGCCACACAGAGTGCCGGGATCATAGAAG), standard 21-mer primer (AAGCGGAGTGTATGTGCAGTG), (17+4)-mer primer with unpaired 3′-tail (AAGCGGAGTGTATGTGCCTGA), 51-mer template (CTTCTATGATCCCGGCACTCTGTGTGGCTACACTGCACATACACTCCGCTT). All oligonucleotides were purified by electrophoresis in polyacrylamide gels. The oligonucleotides were 5′-end-labeled with ^{32}P by using T4 polynucleotide kinase and [γ-^{32}P]ATP. To prepare partial duplexes containing gaps of 1 nt (*21:51:29-mer), 5 nt (*21:51:25-mer), 9 nt (*21:51:21-mer), and 13 nt (*21:51:17-mer), the labeled 21-mer primer, 51-mer template and respective downstream oligonucleotide were annealed at a ratio 1:1.2:1.3. To prepare the partial duplexes 21:*51-mer, (17+4):*51-mer, 21:*51:21-mer and (17+4):*51:21-mer, the primers (21-mer or (17+4)-mer), the labeled 51-mer template and the downstream 21-mer oligonucleotide were annealed at a ratio 1.5:1:1.5.

2.3. Elongation assay

Reaction mixtures (20 μl) contained 0.42 pmol ^{32}P-labeled DNA probes: *21:51-mer, *21:51:25-mer (5-nt gap) or *21:51:21-mer (9-nt gap), 30 mM Tris–HCl, pH 7,5, 6 mM $MgCl_2$, 100 μg/ml BSA, 2 mM DTT, and 50 μM each of dATP, dGTP, dCTP, TTP. The reaction was started by addition of pol δ (0.015–0.05 units). After incubation for 1, 2 and 4 min at 30°C, 5-μl portions were removed, mixed with a formamide loading solution and subjected to electrophoresis in 10% polyacrylamide, 8 M urea gel.

2.4. Electrophoretic mobility shift assay (EMSA)

Binding reactions were carried out in a buffer containing 10 mM Tris–HCl, pH 8.0, 2 mM DTT, and 100 μg/ml BSA. Reaction mixtures (10 μl) contained 0.005–0.04 pmol of DNA probe and 3 μl (0.004–0.03 units) of pol δ. After mixing of the components on ice, reactions were incubated for 15–20 min at 22–23°C, unless noted otherwise. After incubation, the binding reactions were directly loaded onto 6% polyacrylamide (acrylamide:bisacrylamide, 60:1) slab gel (60×100×0.75 mm), prepared in a buffer containing 20 mM HEPES, pH 8.0, and 0.1 mM EDTA. Electrophoresis was performed at 80 V in a buffer containing 20 mM HEPES, pH 8.0, 0.1 mM EDTA, and 2 mM thioglycolic acid at room temperature. The gels were dried onto Whatman DE81 paper under vacuum and then exposed to X-ray film.

2.5. Recovery of DNA polymerase activity in polyacrylamide gel

Pol δ (0.3 units) was incubated in the standard reaction mixture for binding (10 μl) with 0.4 pmol of unlabeled partial duplexes 21:51:21-mer (9-nt gap) or 21:51:17-mer (13-nt gap) for 20 min on ice. The samples were loaded onto a native 6% polyacrylamide gel prepared as described above. Electrophoresis was performed at 4°C in a buffer containing 20 mM HEPES, pH 8.0, 0.1 mM EDTA, and 2 mM thioglycolic acid. A portion of the gel was placed in a reaction mixture (15 ml) containing 50 pmol (750 Ci/mmol) of [α-^{32}P]dATP, 5 μM each of dCTP, dGTP, and TTP, 10 mM Tris–HCl, pH 7.5, 8 mM $MgCl_2$, 2 mM DTT, 100 μg/ml BSA, and incubated at 30°C for 10 min, while shaking gently. The gel was then washed for 4 h with several changes of 50 ml of cold 5% trichloroacetic acid, 1% sodium pyrophosphate, and dried onto DE81 paper.

2.6. Exonuclease assay

Labeled oligonucleotides (3′-[^3H]dC_1)dT_{100}, (3′-[^3H]dT_1)dT_{100}, and (5′-[^{32}P])dT_{100} were prepared as described earlier [27]. The oligonucleotides were annealed with a twofold quantity of poly(dA). Reaction mixtures (100 μl) contained labeled dT_{100}:poly(dA) (0.025:0.05 optical units), 20 mM Tris–HCl, pH 7,5, 10 mM $MgCl_2$, 100 μg/ml BSA, and 1 mM DTT. Reaction was started by addition of pol δ (0.07 unit) and carried out at 30°C. At time points 0, 30, 60, and 120 min, 20-μl portions were removed and processed to determine the acid-insoluble radioactivity.

3. Results

To analyze the interaction of the loach pol δ with gapped DNA, a set of 51-mer duplexes containing gaps of 1, 5, 9, and 13 nt was constructed. The duplexes were constructed as follows: the 21-mer primer was labeled at the 5′-end with ^{32}P and annealed to the 51-mer template together with one of the downstream oligonucleotides thereby producing a defined gap in the duplex. Elongation of the primer by pol δ on a single-stranded template and in the partial duplexes was monitored by sequencing gel electrophoresis. Two fractions of pol δ (δ1 and δ2) obtained in the course of the enzyme purification from loach eggs [25] showed the same properties in the experiments on primer elongation or the binding to DNA substrates (described below) and were used interchangeably.

In the first experiment, the *21:51-mer primer–template and the duplex *21:51:21-mer containing a gap of 9 nt were used

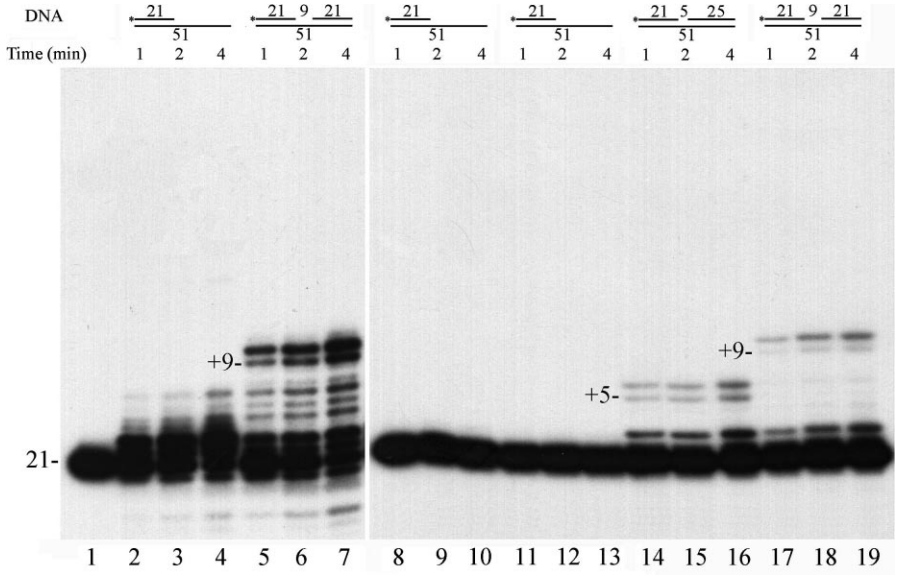

Fig. 1. Elongation of ^{32}P-labeled 21-mer primers catalyzed by DNA pol δ on single-stranded template and on gapped DNA. Reaction mixtures (20 μl) contained 0.42 pmol of following DNA substrates: *21:51-mer (lanes 1–4, 8–13), partial duplex *21:51:25-mer with 5-nt gap (lanes 14–16) or partial duplex *21:51:21-mer with 9-nt gap (lanes 5–7, 17–19), and other components (see Section 2). Reactions were started by addition of pol δ, 0.05 units (lanes 2–7) or 0.015 units (lanes 8–19), to the mixtures. After incubation for 1, 2, and 4 min at 30°C, 5-μl portions were taken from the reactions and analyzed by electrophoresis in 10% polyacrylamide, 8 M urea gel. Pol δ was omitted from the reaction shown in lane 1. dCTP was omitted from the reactions shown in lanes 11–13. The asterisk indicates the radioactive label.

as substrates to investigate primer elongation by pol δ. To measure enzyme processivity, the reactions were conducted using an excess of DNA substrate. Pol δ showed a low level of activity on *21:51-mer primer–template. The reaction products were predominantly primers elongated by 1 or 2 nt (Fig. 1, lanes 2–4) indicating a distributive mode of elongation on a single-stranded template. However, in the reaction with gapped DNA a large portion of primers were elongated by 9 or 10 nt, in addition to those elongated by 1 or 2 nt (lanes 5–7). Hence, pol δ is capable of filling the gap in a processive manner when the template site is restricted by the proximal 5′-terminus of the downstream oligonucleotide.

Substitution of gapped DNA for the single-stranded template caused a more pronounced stimulation of primer elongation when a limited amount of pol δ was added into the reaction. Very few primers were elongated by pol δ in the reaction on *21:51-mer primer–template shown in lanes 8–10. Subtraction of one precursor (dCTP) from the reaction mixture should terminate synthesis opposite the first dG in the template thus limiting the elongation products to the addition of only three bases to the 21-mer primer. However, the dCTP subtraction did not change the pattern of reaction products in the gel (lanes 11–13) indicating the absence of any long products in the complete reaction. In contrast to inefficient synthesis on single-stranded template, pol δ showed much higher activity on gapped DNA (lanes 14–19). The enzyme was able to add processively 5 or 6 nt to the 21-mer primer on the *21:51:25-mer duplex (5-nt gap) and 9 or 10 nt upon synthesis on the *21:51-21-mer duplex (9-nt gap). Thus, pol δ filled small gaps completely and, probably, could displace the 5′-terminal residue of the downstream fragment upon filling the gap. A several-fold increased amount of pol δ added into the reaction and an increased reaction time (from 4 to 30 min) raised the overall amount of primers utilized in the reaction, but did not affect the maximum size of products (data not shown). Hence, pol δ is unable to enter deep into the duplex and perform DNA synthesis with displacement of an extra strand.

The processive synthesis catalyzed by loach pol δ on gapped DNA, in spite of the distributive polymerization on single-stranded template, suggests that the 5′-terminus of the downstream oligonucleotide may stabilize the productive complex of pol δ with DNA substrate. This would allow elongation of the primer without the enzyme dissociating from the DNA after each catalytic event. To test the hypothesis of direct interaction of pol δ with the 5′-terminus at the gap boundary, we analyzed the physical binding of pol δ to 51-mer duplexes containing gaps of various sizes using EMSA. The loach pol δ showed a low affinity for *21:51-mer primer–template (Fig. 2, lanes 1–4). This result is in agreement with numerous observations that a sliding clamp formed by three PCNA molecules is required for stable binding of pol δ to the primer terminus [22–24]. pol δ showed a similar low level of binding to duplexes containing 1-nt gaps (lanes 5–8) and slightly higher binding to duplexes with 5-nt gaps (lanes 9–12). However, pol δ efficiently bound duplexes with 9-nt gaps (lanes 13–16) and slightly less efficiently the duplexes with 13-nt gaps (lanes 17–20). These data suggest that pol δ has a markedly higher affinity for duplexes with gaps of about 10 nt than for standard primer–template structures.

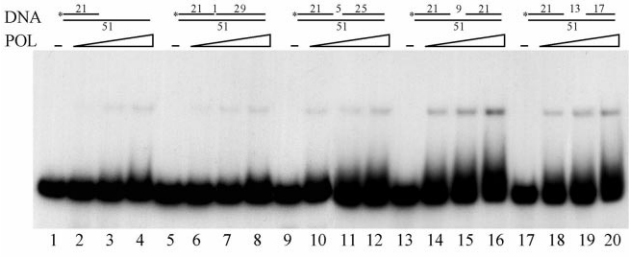

Fig. 2. Binding of DNA pol δ to a primer–template complex and partial duplexes containing gaps of various sizes analyzed by EMSA. Reaction mixtures (10 μl) containing 0.01 pmol of ^{32}P-labeled DNA substrates: *21:51-mer (lanes 1–4), *21:51:29-mer (1-nt gap) (lanes 5–8), *21:51:25-mer (5-nt gap) (lanes 9–12), *21:51:21-mer (9-nt gap) (lanes 13–16) и *21:51:17-mer (13-nt gap) (lanes 17–20) and other components (see Section 2) were incubated in the absence of pol δ (lanes 1, 5, 9, 13, 17) or in the presence of pol δ added in the following amounts: 0.004 units (lanes 2, 6, 10, 14, 18), 0.01 units (lanes 3, 7, 11, 15, 19) or 0.03 units (lanes 4, 8, 12, 16, 20). After incubation for 15 min at 22°C, the reaction mixtures were analyzed by electrophoresis in 6% polyacrylamide gel. The asterisk indicates the radioactive label.

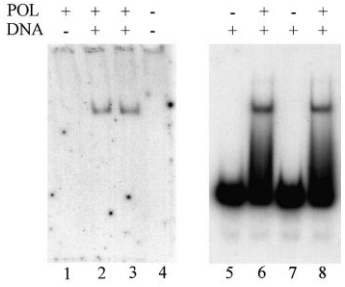

Fig. 3. In situ activity of DNA pol δ on gapped DNA following mobility shift electrophoresis. Reaction mixtures (10 μl) containing 0.04 pmol unlabeled partial duplexes 21:51:21-mer (9-nt gap) (lane 2) and 21:51:17-mer (13-nt gap) (lane 3) or 0.01 pmol of ^{32}P-labeled partial duplexes *21:51:21-mer (9-nt gap) (lanes 5, 6) and *21:51:17-mer (13-nt gap) (lanes 7, 8) and other components (see Section 2) were incubated for 15 min on ice in the absence of pol δ (lanes 4, 5, 7) or in the presence of 0.03 units pol δ (lanes 1–3, 6, 8). After electrophoresis in 6% polyacrylamide gel at 4°C, the gel region with lanes 5–8 was dried, while the other part with lanes 1–4 was placed in a reaction mixture with [α-^{32}P]dATP for an in situ assay of DNA polymerase activity as described in Section 2.

In order to confirm that the complexes of protein with gapped DNA observed under EMSA contained functional DNA polymerase, we performed a control experiment. The loach pol δ was incubated with unlabeled 51-mer duplexes containing gaps of 9 or 13 nt, and the probes were subjected to non-denaturing polyacrylamide gel electrophoresis. The gel was incubated in a reaction mixture with [α-^{32}P]dATP to detect DNA polymerase activity in situ, and then processed to measure acid-insoluble radioactivity (Fig. 3, lanes 1–4). To monitor the electrophoretic migration of the complexes under electrophoresis, another set of probes was prepared by incubation of pol δ with ^{32}P-labeled gapped duplexes and run in the same gel. A piece of gel containing the radioactive probes was dried just after electrophoresis and exposed to X-ray film (lanes 5–8). The probes with pol δ and unlabeled gapped DNA contained complexes catalyzing DNA synthesis on endogenous templates (lanes 2, 3). Electrophoretic mobility of the active complexes in the gel corresponded to that of the protein complexes with ^{32}P-labeled DNA (lanes 6, 8). Therefore, the retardation of gapped duplexes seen in Fig. 2 and in

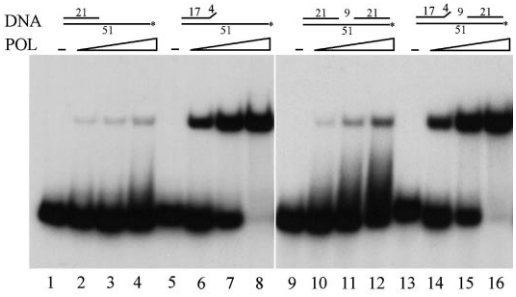

Fig. 4. Binding of DNA pol δ to a primer–template complex and partial duplex containing unpaired 3′-tails in primers analyzed by EMSA. Reaction mixtures (10 μl) containing 0.005 pmol of ^{32}P-labeled DNA substrates: 21:*51-mer (lanes 1–4), (17+4):*51-mer (lanes 5–8), 21:*51:21-mer (lanes 9–12) or (17+4):*51:21-mer (lanes 13–16) and other components (see Section 2) were incubated in the absence of pol δ (lanes 1, 5, 9, 13) or in the presence of pol δ added in the following amounts: 0.004 units (lanes 2, 6, 10, 14), 0.01 units (lanes 3, 7, 11, 15) or 0.03 units (lanes 4, 8, 12, 16). After incubation for 15 min at 22°C, the reaction mixtures were analyzed by electrophoresis in 6% polyacrylamide gel. The asterisk indicates the radioactive label.

Fig. 3, lanes 6 and 8, was indeed due to a stable binding of functional DNA polymerase.

Besides gapped DNA molecules, branched structures with unpaired single-stranded tails are frequent intermediates in DNA repair. To estimate the affinity of pol δ for 3′-tailed DNA substrates, we compared the enzyme binding to DNA probes containing properly annealed primer with the binding to analogous DNA probes but having four unpaired bases at the 3′-terminus of the primer (Fig. 4). The loach pol δ showed very strong binding to two DNA substrates that had unpaired 3′-termini in primers, *(17+4):51-mer primer–template and *(17+4):51:21-mer gapped duplex. An oligonucleotide located downstream of the primer produced little if any effect on the enzyme binding to the tailed DNA probes (compare lanes 6–8 and 14–16), although the same oligonucleotide markedly increased the enzyme binding to the structures with properly annealed primers (compare lanes 2–4 and 10–12).

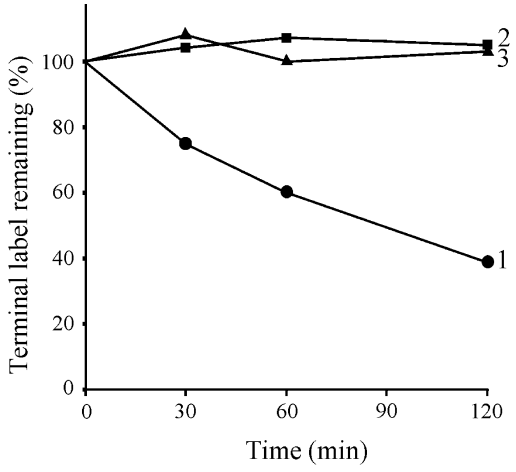

Fig. 5. Exonucleolytic hydrolysis of unpaired 3′-terminal residues by DNA pol δ. Reaction mixtures (100 μl) contained: (1) (3′-[^{3}H]dC$_1$)dT$_{100}$ annealed to poly(dA) (0.025:0.05 optical units), (2) (3′-[^{3}H]dT$_1$)dT$_{100}$ annealed to poly(dA) (0.025:0.05 optical units) or (3) (5′-[^{32}P])dT$_{100}$ annealed to poly(dA) (0.025:0.05 optical units), and other components (see Section 2). Reactions were started by addition of pol δ (0.07 units) and carried out at 30°C. At time points 0, 30, 60, and 120 min, 20-μl portions were removed and processed to determine the acid-insoluble radioactivity. The initial radioactivity of the probes corresponding respectively to 2580 cpm (1), 6010 cpm (2), and 2070 cpm (3) was taken as 100%.

A strong binding of loach pol δ to DNA probes containing unpaired 3′-tails suggests the involvement of the enzyme 3′→5′ exonuclease domain in this interaction. In agreement with this prediction, the duplexes with mismatched bases at the 3′-ends served as much more efficient substrates for 3′→5′ exonuclease of pol δ, as compared to the duplexes with properly matched bases (Fig. 5). The loach pol δ removed more than 60% labeled residues dC from 3′-ends of dT$_{100}$ in duplexes dT$_{100}$:poly(dA) at conditions when digestion of 3′-terminal residues dT in similar duplexes was negligible (compare lines 1 and 2). Thus, the preferential hydrolysis of unpaired 3′-termini in duplexes might be determined by a high binding affinity of pol δ for DNA duplexes with 3′-tails.

4. Discussion

The distributive mode of DNA synthesis on single-stranded templates is usual for eukaryotic DNA polymerases δ functioning in the absence of the processivity factors PCNA, RF-C, and ATP [22–24]. However, the loach pol δ was capable of filling small gaps in partial DNA duplexes by processive synthesis in the absence of any accessory factors (Fig. 1). This means that an oligonucleotide properly positioned downstream of the template site stabilizes the productive complex of pol δ with the DNA substrate. The interaction by the enzyme with the 5′-terminus of the downstream fragment presumably permits it to polymerize nucleotides without dissociation from DNA after each catalytic event. In agreement with this model, the loach pol δ showed a much higher binding affinity for partial duplexes containing appropriate single-stranded gaps than for the standard primer–template (Fig. 2).

Eukaryotic pol β catalyzes the distributive synthesis on single-stranded DNA but the processive synthesis on small gaps in duplexes [8]. The stabilization effect of gapped DNA is caused by the interaction of 5′-deoxyribose phosphate lyase (dRP lyase or AP lyase) domain of pol β with the 5′-terminus of the downstream fragment [9–11]. A slow dRP lyase activity was detected recently in the family A DNA polymerases including *Escherichia coli* DNA polymerase I and eukaryotic DNA polymerase γ [28]. The dRP lyase domain presumably permits the formation of stable complexes of *Xenopus* pol γ with 5′-ends in partial duplexes (Mikhailov, Pinz, Bogenhagen, unpublished observations). However, the dRP lyase activity was never observed in DNA polymerases of the δ-type. The 5′→3′ exonuclease domain presented in some DNA polymerases such as *E. coli* DNA polymerase I may also participate in the interaction with 5′-terminal groups in DNA [29]. But we have not detected the respective activity in the loach pol δ (Fig. 5, line 3), and the reason for the stabilization effect of gapped DNA on the polymerization activity of pol δ remains unknown.

The primary function of 3′→5′ exonuclease associated with pol δ is proofreading of nascent DNA, i.e. a rapid hydrolysis of the mismatched 3′-terminal residue of primer in the course of synthesis. However, the loach pol δ binds efficiently to DNA duplexes containing several unpaired residues at 3′-ends (Fig. 4) and is able to hydrolyze a single-stranded

DNA lacking secondary structure (data not shown). Thus, the branched molecules with 3′-tails may serve as substrates for pol δ. Participation of pol δ in DNA repair was usually related to a PCNA-dependent pathway that has been well documented in a variety of systems [14–21]. The data described here suggest that the direct action of pol δ on gapped and tailed molecules that present common intermediates in the repair process may occur allowing pol δ to participate particularly in the long-patch excision base repair of nuclear DNA.

Acknowledgements: The authors thank Dmitry Mozzherin for kindly providing some oligonucleotides, and Sergei Vassetzky and George Rohrmann for critical reading of the manuscript and helpful discussion. This work was supported, in part, by the Russian Foundation for Basic Research (Project no. 00-04-49183).

References

[1] Wood, R.D. and Shivji, M.K. (1997) Carcinogenesis 18, 605–610.
[2] Budd, M.E. and Campbell, J.L. (1997) Mutation Res. DNA Repair 384, 157–167.
[3] Sankar, A. (1996) Annu. Rev. Biochem. 65, 43–81.
[4] Sobol, R.W., Horton, J.K., Kuhn, R., Gu, H., Singhal, R.K., Prasad, R., Rajewsky, K. and Wilson, S.H. (1996) Nature 379, 183–186.
[5] Chagovetz, A.M., Sweasy, J.B. and Preston, B.D. (1997) J. Biol. Chem. 272, 27501–27504.
[6] Ahn, J.W., Kraynov, V.S., Zhong, X.J., Werneburg, B.G. and Tsai, M.D. (1998) Biochem. J. 331, 79–87.
[7] Wilson, D.M. and Thompson, L.H. (1997) Proc. Natl. Acad. Sci. USA 94, 12754–12757.
[8] Prasad, R., Beard, W.A. and Wilson, S.H. (1994) J. Biol. Chem. 269, 18096–18101.
[9] Matsumoto, Y. and Kim, K. (1995) Science 269, 699–702.
[10] Piersen, C.E., Prasad, R., Wilson, S.H. and Lloyd, R.S. (1996) J. Biol. Chem. 271, 17811–17815.
[11] Matsumoto, Y., Kim, K., Katz, D.S. and Feng, J.A. (1998) Biochemistry 37, 6456–6464.
[12] Horton, J.K., Prasad, R., Hou, E. and Wilson, S.H. (2000) J. Biol. Chem. 275, 2211–2218.
[13] Prasad, R., Dianov, G.L., Bohr, V.A. and Wilson, S.H. (2000) J. Biol. Chem. 275, 4460–4466.
[14] Shivji, M.K.K., Kenny, M.K. and Wood, R.D. (1992) Cell 69, 367–374.
[15] Matsumoto, Y., Kim, K. and Bogenhagen, D. (1994) Mol. Cell. Biol. 14, 6187–6197.
[16] Aboussekhra, A., Biggerstaff, M., Shivji, M.K.K., Vilpo, J.A., Moncollin, V., Podust, V.N., Protic, M., Hubscher, U., Egly, J.M. and Wood, R.D. (1995) Cell 80, 859–868.
[17] Budd, M.E. and Campbell, J.L. (1995) Mol. Cell. Biol. 15, 2173–2179.
[18] Klungland, A. and Lindahl, T. (1997) EMBO J. 16, 3341–3348.
[19] Zeng, X.R., Jiang, Y., Zhang, S.J., Hao, H. and Lee, M.Y.W.T. (1994) J. Biol. Chem. 269, 13748–13751.
[20] Matsumoto, Y., Kim, K., Hurwitz, J., Gary, R., Levin, D.S., Tomkinson, A.E. and Park, M.S. (1999) J. Biol. Chem. 274, 33703–33708.
[21] Araujo, S.J., Tirode, F., Coin, F., Pospiech, H., Syvaoja, J.E., Stucki, M., Hubscher, U., Egly, J.M. and Wood, R.D. (2000) Genes Dev. 14, 349–359.
[22] Hindges, R. and Hubscher, U. (1997) J. Biol. Chem. 378, 345–362.
[23] Waga, S. and Stillman, B. (1998) Annu. Rev. Biochem. 67, 721–751.
[24] Mikhailov, V.S. (1999) Mol. Biol. 33, 498–510.
[25] Sharova, N.P., Dimitrova D.D., Abramova, E.B., Dmitrieva, C.B. and Mikhailov, V.S. (2001) Biokhimiya 66, in press.
[26] Mikhailov, V.S. and Gulyamov, D.B. (1983) Eur. J. Biochem. 135, 303–306.
[27] Mikhailov, V.S., Marlyev, K.A., Ataeva, J.O., Kullyev, P.K. and Atrazhev, A.M. (1986) Nucleic Acids Res. 14, 3841–3857.
[28] Pinz, K.G. and Bogenhagen, D.F. (2000) J. Biol. Chem. 275, 12509–12514.
[29] Xu, Y., Grindley, N.D. and Joyce, C.M. (2000) J. Biol. Chem. 275, 20949–20955.