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Identification of DNA Polymerase δ in Eggs of a Teleost Fish (Loach)

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Abstract—DNA polymerase found in an extract from eggs of the teleost fish *Misgurnus fossilis* (loach) has been identified as an enzyme of the δ type. The enzyme was purified 4000- to 5000-fold from the extract by liquid chromatography. The DNA polymerase activity was sensitive to the inhibiting action of aphidicolin but resistant to N2-(*p*-*n*-butylphenyl)-2′-deoxyguanosine 5′-triphosphate (BuPdGTP). The enzyme activity correlates with the presence of a polypeptide with molecular mass of 120-130 kD that interacts specifically with polyclonal antibodies against calf thymus DNA polymerase δ as revealed by Western blotting and is presumably the catalytic subunit of the enzyme. The loach DNA polymerase possesses the 3′→5′-exonuclease activity specific to single-stranded DNA and catalyzes distributive elongation of primers in primer–template complexes.

Key words: replication, repair, DNA polymerases, DNA polymerase δ, oocytes, teleost fishes

DNA-dependent DNA polymerases are key enzymes of DNA metabolism; they play a prominent role in replication and repair of the cell genome. At least eight different DNA polymerases participate in DNA synthesis in eucaryotic cells. The structures and functions of these enzymes are studied intensively in a number of laboratories and are the theme of reviews appearing regularly in the literature [1, 2]. DNA polymerases α, δ, and ε are necessary for replication of nuclear DNA in yeast cells and probably in cells of higher eucaryotes. Primase, which is connected with DNA polymerase α, synthesizes short RNA primers in the region of initiation of replication and in the initial parts of Okazaki fragments that are elongated by DNA polymerase α. Then the processive elongation of the leading strand is catalyzed by DNA polymerase δ, and elongation of Okazaki fragments of the lagging strand occurs with the participation of DNA polymerase ε. DNA polymerase γ is involved in replication and repair of mtDNA. DNA polymerases ζ and η are able to overcome the structural barriers of the type of pyrimidine dimers in template DNA and are essential for replication of DNA in cells with damaged genomes [3, 4]. The function of DNA polymerase θ is still unknown [5]. The role of different DNA polymerases in the synthetic processes accompanying DNA repair has not been adequately studied. For an appreciable length of time, repair was believed to be associated only with the lowly processive DNA polymerase β. Recent investigations have shown that repair processes are dependent on PCNA (proliferating cell nuclear antigen); the data suggest the participation of DNA polymerase of type δ and probably ε in DNA repair [6-13]. Short gaps in DNA are thought to be filled by DNA polymerase β (short-patch repair), whereas DNA polymerase of type δ and probably ε are involved in filling long gaps (long-patch repair). For an appreciable length of time, DNA polymerase δ of vertebrates was thought to be a heterodimer containing, apart from the catalytic subunit (125-130 kD), a subunit with molecular mass of 48-55 kD that provides a connection of the enzyme with a processivity factor PCNA [14]. However, recently subunits with molecular masses of 66 kD [15, 16] and 12 kD [17] were identified in DNA polymerase δ. Both the enzymatic activities of the enzyme (DNA polymerase activity and 3′→5′-exonuclease activity) are connected with a high-molecular-weight polypeptide. Individual DNA polymerases in cells characterized by different rates of proliferation are substantially distinguished by their content and activity, and repair of damages may occur through alternative mechanisms with the participation of different DNA polymerases. The high rate of cell division for embryos of amphibians, echinoderms, and fishes early in their development suggests that an efficient system of repair of damages of cell genome functions exists in these cells. However, the

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mechanisms of repair processes in embryogenesis of animals are still poorly understood. In eggs and cells of early embryos of *Xenopus*, base excision repair is exerted by DNA polymerase β and/or δ [7]. The activity of DNA polymerase β in embryos of teleost fishes is very low [18], and repair potential is probably provided by other enzymes. To elucidate the mechanisms of regulation of DNA synthesis early in the development of animals, we are studying DNA polymerases stored in eggs of the teleost fish *Misgurnus fossilis* (loach). The goal of the present work was to identify DNA polymerase δ, which perhaps plays the role of the enzyme responsible for replication and repair of DNA in early embryos. A purified preparation of DNA polymerase δ was obtained. This enabled us to study the physicochemical and enzymatic properties of the enzyme. Later the interaction of loach DNA polymerase δ with DNA substrates simulating different repair intermediates and the ability of the enzyme to use duplexes with short gaps was studied [19].

MATERIALS AND METHODS

Mature loach eggs were obtained as described by Neifakh [20].

Enzymes and reagents. Calf thymus DNA polymerase δ and rabbit polyclonal antibodies against calf thymus DNA polymerase δ were provided by D. Mozzherin (State University of New York at Stony Brook). Antibodies against rabbit IgG conjugated with peroxidase were purchased from Sigma (USA). Polynucleotide kinase of phage T4 was from Boehringer (Germany).

Fractionation of DNA polymerases. *Buffers.* Buffer A: 50 mM potassium phosphate buffer, pH 7.5, containing 10% glycerol, 5 mM 2-mercaptoethanol, 1 mM EDTA, 10 mM $Na_2S_2O_5$, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 μM leupeptin, pepstatin (1 μg/ml), and aprotinin (1 μg/ml). Buffer B: 10 mM potassium phosphate buffer, pH 7.5, containing 10% glycerol, 5 mM 2-mercaptoethanol, 1 mM EDTA, 10 mM $Na_2S_2O_5$, 0.2 mM PMSF, 1 μM leupeptin, pepstatin (1 μg/ml), and aprotinin (1 μg/ml). Buffer C is identical to buffer B except that concentration of glycerol is increased to 20%. Buffer D: 10 mM potassium phosphate buffer, pH 7.5, containing 50% glycerol, 5 mM 2-mercaptoethanol, 1 mM EDTA, 10 mM $Na_2S_2O_5$, 0.2 mM PMSF, 1 μM leupeptin, pepstatin (1 μg/ml), and aprotinin (1 μg/ml).

Chromatography of extract from eggs on a DEAE-Toyopearl column. All procedures were carried out at 0-4°C. The extract was obtained by centrifugation of eggs using an SW-28 rotor (Beckman, USA) at 20,000 rpm for 30 min as described by Minin et al. [21]. The extract (4.5 ml) was suspended in buffer A in at 1 : 3 ratio and centrifuged at 105,000*g* for 1 h. Buffer B was added to supernatant to potassium phosphate concentration of 20 mM. The solution was placed on the column with DEAE-Toyopearl (1.6 × 10 cm) equilibrated with buffer B. The rate of elution was 20 ml/h. The column was washed with 40 ml of buffer C and eluted with buffer C in which the concentration of potassium phosphate increased linearly to 0.4 M over a period of 10 h. The rate of elution was 20 ml/h. In fractions obtained, we determined the activity of DNA polymerases and the content of immunoreactive proteins by Western blotting using polyclonal antibodies against calf thymus DNA polymerase δ.

Purification of DNA polymerase δ. DNA polymerase δ was isolated from 12 ml of extract of eggs. The extract was obtained as described above. The supernatant was placed on a column with phosphocellulose P-11 (2.5 × 15 cm) equilibrated with buffer B at the rate of 50 ml/h. The column was washed with 350 ml of buffer B containing 0.23 M KCl and eluted with 0.5 M KCl in buffer B. The active fractions were combined and dialyzed against buffer C. Then the preparation was placed on a column with DEAE-Toyopearl (1.6 × 8.5 cm) equilibrated with buffer C at the rate of 20 ml/h. The column was washed with 120 ml of buffer C containing 20 mM potassium phosphate and eluted with buffer C in which the concentration of potassium phosphate increased linearly from 20 to 90 mM over a period of 6 h. The rate of elution was 20 ml/h. Fractions possessing DNA polymerase activity sensitive to aphidicolin but resistant to N2-(*p*-*n*-butylphenyl)-2′-deoxyguanosine 5′-triphosphate (BuPdGTP) were combined. Dry ammonium sulfate was added to the preparation by small portions with stirring until they completely dissolved. The final concentration of $(NH_4)_2SO_4$ was 0.5 M. Then the preparation was placed on a column with phenyl-Sepharose (1 × 3.5 cm) equilibrated with buffer C containing 0.5 M $(NH_4)_2SO_4$ at the rate of 10 ml/h. The column was washed with 6 ml of buffer C containing 0.5 M $(NH_4)_2SO_4$ and then eluted at the rate of 8 ml/h it two stages (each over a period of 2 h): first by 0.5-0 M gradient of $(NH_4)_2SO_4$ in buffer C and then by 0-1% gradient of Triton X-100 in buffer C. DNA polymerase δ was eluted in two fractions: the first fraction (δ1) corresponded to 0.37-0.10 M interval of $(NH_4)_2SO_4$ and the second fraction (δ2) was obtained in 0.6-0.9% interval of Triton X-100. The fractions were dialyzed separately against buffer C and placed on identical columns with single-stranded DNA-cellulose (0.8 × 1.7 cm) equilibrated with buffer C with the rate of 5 ml/h. The columns were washed with 2.5 ml of buffer C and eluted with a 0-0.8 M gradient of KCl in buffer C over a period of 4 h with the rate of 5 ml/h. The active fractions were dialyzed against buffer D and stored at –20°C.

Immunoblotting. The proteins were separated by electrophoresis in 8%-polyacrylamide gel with SDS. Then the semi-dry transfer of the proteins from the gel to nitrocellulose membrane was carried out. The membrane was incubated at room temperature for 1 h in buffer TNT (10 mM Tris-HCl buffer, pH 7.5, 150 mM NaCl and

0.1% Tween 20) containing 20% serum of cattle and polyclonal antibodies against calf thymus DNA polymerase δ in dilution 1 : 1000. Then the membrane was washed several times by buffer TNT and incubated for 1 h in buffer TNT containing 20% serum of cattle and antibodies against rabbit IgG conjugated with peroxidase in dilution 1 : 5000. After washing in buffer TNT the membrane was placed in 50 mM Tris-HCl buffer, pH 7.5, containing diaminobenzidine (0.1 mg/ml) and 30% H_2O_2 until the coloring appears.

DNA substrates. To prepare the primer template, we used 21-mer (AAGCGGAGTGTATGTGCAGTG) as primer and 51-mer (CTTCTATGATCCCGGCACTCTGTGTGGCTACACTGCACATACACTCCGCTT) as template. The primer was labeled at the 5′-end using [γ-^{32}P]ATP and polynucleotide kinase of phage T4 and hybridized with template in molar relationship 1 : 1.2.

Elongation of primers using DNA polymerase δ. Processivity of DNA polymerase δ was determined in samples (20 μl) containing 0.42 pmol of [5′-^{32}P]21:51-mer, 30 mM Tris-HCl, pH 7.5, 6 mM $MgCl_2$, BSA (100 μg/ml), 2 mM dithiothreitol, and dATP, dGTP, dCTP, and TTP (50 μM each). The reaction was initiated by the addition of 0.05 U of the DNA polymerase at 30°C. Aliquots (5 μl) were withdrawn at intervals (1, 2, and 4 min) and mixed with 7 μl of terminating solution containing formamide and dyes (bromphenol blue and xylene cyanol). The preparations were analyzed by electrophoresis in 10%-polyacrylamide gel in the presence of 8 M urea.

3′→5′-Exonuclease assay. The activity of 3′→5′-exonuclease was estimated by the reduction in 3′-[^{3}H]poly(dT) radioactivity after incubation with the enzyme. The reaction mixture (50 μl) contained 3′-[^{3}H]poly(dT) (0.02 relative unit/ml, 7000 cpm), 20 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, nuclease-free BSA (100 μg/ml), 10% glycerol, 1 mM dithiothreitol, and 5 μl of the enzyme-containing fraction. Double-stranded DNA substrates and substrates suitable for determination of the activity of 5′→3′-exonucleases were obtained as described previously [22]. After 30-min incubation at 37°C, the reaction was stopped and the radioactivity of acid-insoluble fraction was determined as described previously [23]. The amount of the enzyme catalyzing the liberation of 1 nmole of dNMP from DNA over a period of 60 min was taken to be one unit of the 3′→5′-exonuclease activity.

Binding of DNA polymerase δ with DNA substrates. DNA polymerases δ (0.02-0.07 U) were incubated in the presence of 0.01 pmol of [5′-^{32}P]21:51-mer in 10 μl of 10 mM Tris-HCl buffer, pH 8.0, containing BSA (100 μg/ml) and 2 mM dithiothreitol at 22-23°C for 15 min. Then the samples were analyzed by electrophoresis in 6%-polyacrylamide gel (acrylamide/bisacrylamide, 60 : 1) (60 × 100 × 0.75 mm) prepared with 20 mM Hepes buffer, pH 8.0, containing 0.1 mM EDTA. Electrophoresis was carried out at 80 V in 20 mM Hepes buffer, pH 8.0, containing 0.1 mM EDTA and 2 mM thioglycolic acid at room temperature. The gel content was transferred to DE81 paper and dried under vacuum.

Other methods. The enzymatic activity of DNA polymerases was measured by incorporation of [^{3}H]dNMP into acid-insoluble product as described previously [24]. The amount of the enzyme catalyzing inclusion of 1 nmole of dNMP in DNA over a period of 60 min was taken to be the unit of the DNA polymerase activity. Protein concentration was determined spectrophotometrically. Salt concentration in fractions obtained in the chromatographic experiments was measured using a conductometer.

RESULTS

Identification of DNA polymerase δ in extracts of loach eggs. Extracts of loach eggs were fractionated using column chromatography on ion-exchanger DEAE-Toyopearl. Proteins were eluted with a 0-0.4 M gradient of potassium phosphate buffer. The activity of DNA polymerases in the fractions was determined using activated DNA as a template primer in the absence and in the presence of BuPdGTP, a selective inhibitor of DNA polymerase α (Fig. 1). The main DNA polymerase activity in extracts corresponded to DNA polymerase α. This enzyme was eluted from the ion exchanger at concentration of potassium phosphate higher than 100 mM (fractions 16-24) and was sensitive to the inhibiting action of aphidicolin and BuPdGTP. DNA polymerase α of loach was isolated and characterized by us previously [25, 26]. In fractions eluted in the interval from 30 to 80 mM (frac-

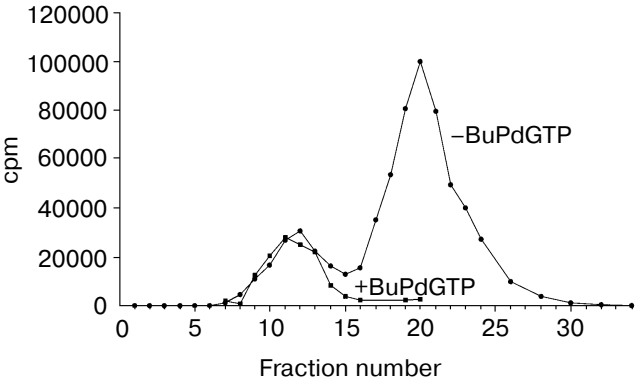

Fig. 1. Fractionation of DNA polymerases of loach by chromatography on a DEAE-Toyopearl column. The extract of eggs was obtained as described in "Materials and Methods". The enzymes were eluted from the column with a 10-400 mM gradient of potassium phosphate buffer. The activity of DNA polymerases was measured in the absence of BuPdGTP (–BuPdGTP) or in the presence of 0.7 μM BuPdGTP (+BuPdGTP).

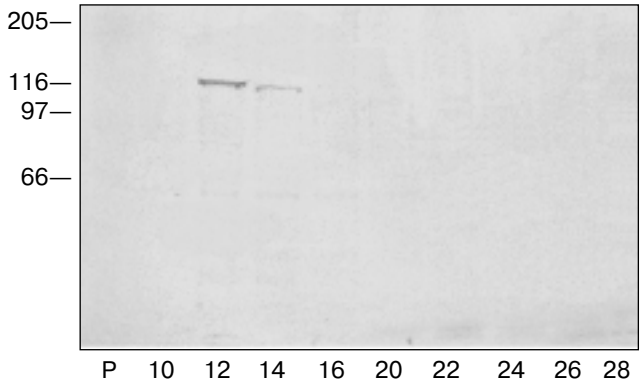

Fig. 2. Western blotting of proteins from fractions of extract of loach eggs. Fractions were obtained by chromatography on a column with DEAE-Toyopearl (Fig. 1). Proteins were separated by electrophoresis in 8%-polyacrylamide gel in the presence of SDS. Polyclonal antibodies against calf thymus DNA polymerase δ were used as the primary antibodies. Numbers of fraction used in the experiments are shown in the horizontal axis (P are the proteins that were not bound with the ion exchanger).

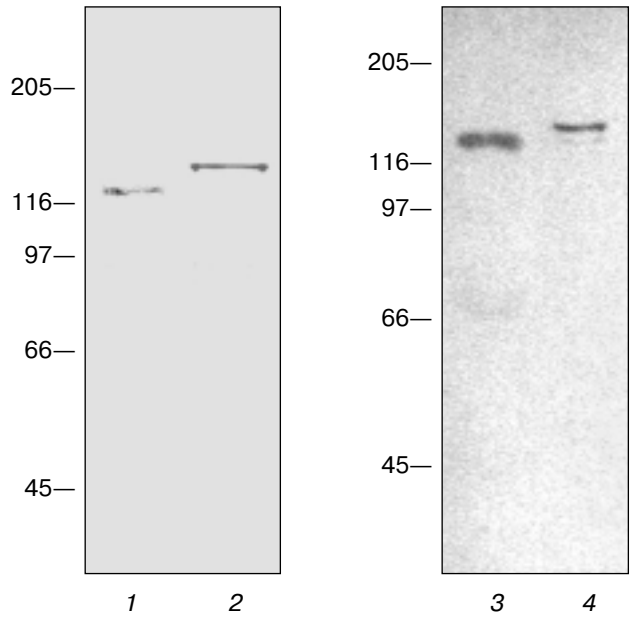

Fig. 3. Western blotting of the preparations of loach DNA polymerase δ obtained by chromatography on columns with single-stranded DNA-cellulose. Proteins were separated by electrophoresis in 8%-polyacrylamide gel in the presence of SDS. Polyclonal antibodies against calf thymus DNA polymerase δ were used as the primary antibodies. The following preparations of DNA polymerases were placed on the columns: *1*) δ1 (0.15 U); *2*) δ2 (0.5 U); *3*) δ1 (2.5 U); *4*) calf thymus DNA polymerase δ (0.08 U).

tions 9-14), minor DNA polymerase activity sensitive to aphidicolin but resistant to BuPdGTP was found. This activity may correspond to DNA polymerases δ and ε and probably other unidentified enzymes. To identify DNA polymerase δ in fractions obtained with a gradient, the corresponding proteins were analyzed by SDS-PAGE followed by Western blotting with the use of polyclonal antibodies against calf thymus DNA polymerase δ (Fig. 2). Fractions 12 and 14 showed an immunoreactive polypeptide with molecular mass of 120-130 kD. There were no other immunoreactive polypeptides in these fractions. (Special experiments showed that fractions 1-4 and 17-19 lacked immunoreactive polypeptides.) The molecular mass of 120-130 kD corresponds to the molecular mass of the catalytic subunit of DNA polymerases δ from various sources [2, 14]. Thus, the immunoreactive polypeptide with molecular mass of 120-130 kD is probably the catalytic subunit of DNA polymerase δ of loach. We use the fact that chromatography on DEAE-Toyopearl results in separation of DNA polymerases δ and α for elaboration of a preparative method of isolation of DNA polymerase δ from loach spawn.

Isolation of DNA polymerase δ. To obtain the preparation of DNA polymerase δ of loach in the preparative quantities, column chromatography of extract of unfertilized eggs was carried out sequentially on phosphocellulose, DEAE-Toyopearl, phenyl-Sepharose, and single-stranded DNA-cellulose as described in "Materials and Methods". In some experiments additional purification of the enzyme preparations was performed by centrifugation in glycerol gradients. Chromatography on phosphocellulose yielded a combined preparation of DNA polymerases. DNA polymerase α was separated on a column with DEAE-Toyopearl as shown in Fig. 1. DNA polymerase β was not bound to phenyl-Sepharose and was separated when the preparation was placed on this sorbent. Chromatography on phenyl-Sepharose yielded additional fractionation of the preparation of DNA polymerase δ. Part of the DNA polymerase activity was eluted from phenyl-Sepharose when the concentration of $(NH_4)_2SO_4$ decreased from 0.5 to 0 M (fraction δ1), whereas the main activity was eluted only in the presence of nonionic surfactant, 1% Triton X-100 (fraction δ2). Then chromatography of both fractions was carried out on single-stranded DNA-cellulose. Western blotting with the use of antibodies against calf thymus DNA polymerase δ showed that fractions obtained by chromatography on DNA-cellulose and possessing the DNA polymerase activity contained immunoreactive polypeptides with molecular mass of 120 or 130 kD (Fig. 3). The apparent molecular mass of the immunoreactive polypeptide in fraction δ1 was ~120 kD (lanes *1* and *3*). The position of this polypeptide in the gel is coincident with that for a minor fraction in the preparation of calf thymus DNA polymerase δ that is probably a product of proteolysis of the catalytic subunit of the enzyme with molecular

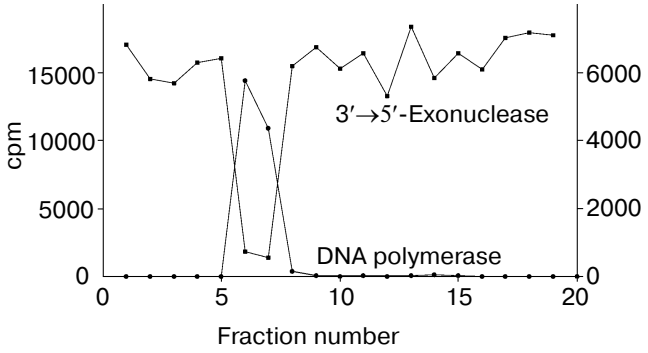

Fig. 4. Chromatography of a preparation of DNA polymerase δ1 that was obtained by fractionation on phenyl-Sepharose on a column with single-stranded DNA-cellulose. The preparation of DNA polymerase δ1 was obtained as described in "Materials and Methods". Proteins were eluted with a 0-0.8 M gradient of KCl. The DNA polymerase activity in fractions obtained was measured by the inclusion of label into activated DNA (left ordinate). The right ordinate corresponds to the 3′→5′-exonuclease activity measured by hydrolysis of 3′-[^{3}H]poly(dT).

mass of ~125 kD (lane *4*). On centrifugation of the preparation δ1 obtained in the gradient of glycerol and chromatography on phenyl-Sepharose, the amount of the polypeptide with molecular mass 120 kD in fractions determined by silver staining correlated with the DNA polymerase activity (data not presented). These results suggest that the immunoreactive polypeptide with molecular mass of 120 kD found in the preparation δ1 is the catalytic subunit of DNA polymerase δ. The apparent molecular mass of the immunoreactive polypeptide in fraction δ2 exceeds that for fraction δ1 and was found to be 130 kD (Fig. 3, lane *2*). Thus, fraction δ1 may be formed by limited hydrolysis of fraction δ2. The specific DNA polymerase activity of the purified preparations with respect to activated DNA was 1000 U per mg of protein for fraction δ1 and 400 U per mg of protein for fraction δ2. The degree of purification in relation to the homogenate of spawn was 4000-5000-fold.

Characteristic of DNA polymerase δ. Apart from the DNA polymerase activity, the preparations of DNA polymerase δ possessed 3′→5′-exonuclease activity specific to single-stranded DNA. This activity was readily revealed at the terminal stages of purification after the separation of admixed nucleases. The ratio of the exonuclease activity to the DNA polymerase activity was rather high, ~0.2. When carrying out chromatography of the preparations of δ1 and δ2 on single-stranded DNA-cellulose and sedimentation in gradients of glycerol, the 3′→5′-exonuclease activity in fractions obtained correlated with the DNA polymerase activity. Figure 4 shows results obtained for fractionation of δ1 on the column with single-stranded DNA-cellulose. Both the enzymatic activities (polymerase and exonuclease activities) were eluted from the column at 0.2 M KCl as a single homogeneous peak. The preparations of loach DNA polymerase δ did not reveal 5′→3′-exonuclease activity with respect to single- or double-stranded DNA. When incubated with double-stranded DNA, 3′→5′-exonuclease in the DNA polymerase δ preparations removed efficiently the unpaired 3′-end nucleotide residues but poorly hydrolyzed the paired 3′-end nucleotide residues. Thus, the 3′→5′-exonuclease activity observed is evidently connected with the proofreading function of DNA polymerase δ [27].

In the course of the nucleotidyltransferase reaction, loach DNA polymerase δ exerted distributive elongation of primers on single-stranded templates. The main product of the reaction catalyzed by fraction δ1 with excess of the primer–template complex *21:51-mer was primers extended by 1-2 nucleotides (Fig. 5). Products of exonuclease hydrolysis of primers from 3′-ends were also detected. DNA polymerase δ1 elongated primers dT_{10} on template poly(dA) through the distributive mechanism. In the case of fraction δ2, the elongation was analogous (data not presented).

The rate of migration of the primer–template *21:51-mer complex with DNA polymerase δ isolated

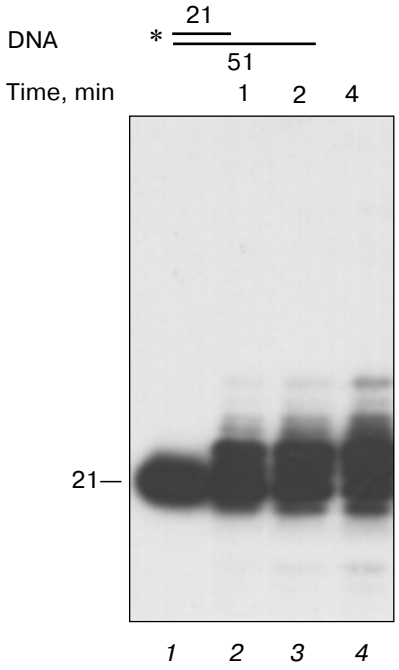

Fig. 5. Elongation of primers [5′-^{32}P]21-mer by DNA polymerase δ1 in primer–template complex *21:51-mer. The reaction was carried out for 1, 2, and 4 min (*2-4*). The products of the reaction were analyzed by electrophoresis in 10%-polyacrylamide gel in the presence of 8 M urea. Lane *1* shows the migration of the original primer [5′-^{32}P]21-mer in the gel.

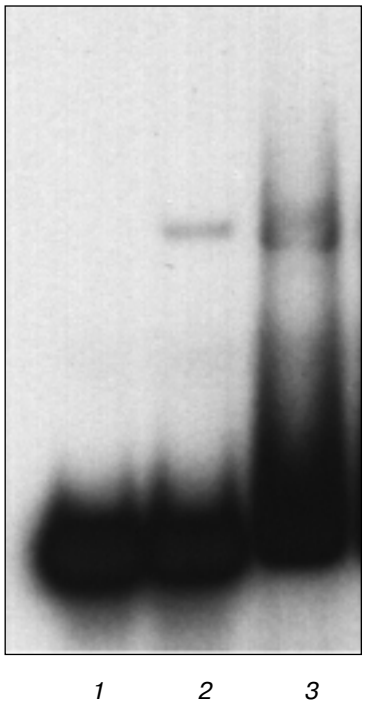

Fig. 6. Comparison of the electrophoretic mobility of complexes between DNA substrates and DNA polymerases δ of loach and calf thymus. The reaction mixtures (10 μl) contained 0.01 pmol of [5′-^{32}P]21:51-mer and the preparations of loach DNA polymerase δ1 (0.02 U) (*2*) or calf thymus DNA polymerases δ (0.07 U) (*3*). The reaction mixture corresponding to lane *1* lacked the enzyme. After 15-min incubation at room temperature, the products of the reaction were analyzed by electrophoresis in 6% polyacrylamide gel.

from loach spawn in gel (Fig. 6, lane *2*) was found to be close to that for the complexes of primer–template with calf thymus DNA polymerase δ (lane *3*). We have presented formal evidence for the presence of functional DNA polymerase in the electrophoretically detected complexes of DNA substrates with the protein [19]. We used calf thymus DNA polymerase δ that was characterized in detail in [14, 16, 17, 28] as a control. PAGE of calf thymus DNA polymerase δ yielded two zones with similar electrophoretic mobilities, suggesting that the enzyme preparation contained the products of limited proteolysis (Fig. 3, lane *4*). Thus, DNA polymerases δ of loach and calf thymus can form complexes with DNA substrates that are similar in structure and properties.

DISCUSSION

We have characterized DNA polymerase δ of loach eggs using inhibitor analysis as well as on the basis of physicochemical, enzymatic, and immunochemical properties:

1) the DNA polymerase activity of the enzyme is sensitive to the inhibiting action of aphidicolin but resistant to BuPdGTP;

2) the DNA polymerase activity correlates with the presence of a polypeptide with molecular mass of 120-130 kD that interacts with polyclonal antibodies against calf thymus DNA polymerase δ;

3) apart from DNA polymerase activity, the enzyme possesses 3′→5′-exonuclease activity specific to single-stranded DNA;

4) the enzyme catalyzes distributive synthesis of DNA in the absence of auxiliary factors (PCNA, replication factor C, and ATP).

This set of properties distinguished between DNA polymerase δ, on one hand, and DNA polymerases α, β, and γ isolated earlier from loach cells [18, 22, 25, 26] as well as DNA polymerase ε and recently discovered DNA polymerases ζ, η, and θ [3-5], on the other hand. The similarity of the complexes formed by primer–template 21:51-mer and DNA polymerases δ of calf thymus and loach eggs (Fig. 6) supports the validity of identification of this enzyme in loach cells and is additional evidence for structural resemblance of DNA polymerases δ of teleost fishes and mammals.

When purifying DNA polymerase δ by liquid chromatography, heterogeneity was observed, the latter resulting in the separation of the enzyme into fractions δ1 and δ2 with phenyl-Sepharose. The different molecular masses of the immunoreactive polypeptides, namely 120 kD in fraction δ1 and 130 kD in fraction δ2 (Fig. 3), suggest that the reason for the appearance of fraction δ1 may be limited proteolysis of the original form of the enzyme. However, a question is raised as to whether limited proteolysis accompanies the procedure of purification of the enzyme or proteolysis is connected with physiological processing of the enzyme in eggs. It should be noted that eggs contain a considerable store of the enzymes and structural proteins providing a rapid increase in growth of the embryo. The form in which DNA polymerase δ is stored remains unknown.

Identification of DNA polymerase δ in loach eggs and the preparation of partially purified enzyme allowed us to study the possible role of DNA polymerase δ in DNA repair in experiments with model DNA substrates simulating repair intermediates [19].

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