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Full Length Research Paper

Preparation of immunoaffinity column for rapid purification of human DNA polymerase delta and its subassemblies

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Mammalian deoxyribonucleic acid (DNA) polymerase delta (pol δ) is well characterized as a tightly associated heterotetrameric complex. It is thought to play a central role in chromosomal DNA replication and various DNA repair processes. However, the availability of highly purified active pol δ becomes one of the major barriers for its in-depth functional and structural analysis. In this work, a powerful immunoaffinity column was prepared. The human DNA pol δ and its subassemblies were reconstituted with a novel MultiBac system, over-expressed in insect cells, and followed by purification using immunoaffinity chromatography in combination with ion-exchange chromatography. Starting from 500 ml of infected Sf-9 cells, as much as 5 mg of recombinant pol δ with different subunit combinations was isolated near homogeneity with active forms that were conformed by the assays both on sparsely primed poly (dA)/oligo (dT) template-primer and on singly primed M13 DNA template. Thus, our home-made immunoaffinity column provides a significant advance in the isolation of pol δ, allowing its facile isolation from over-expressed insect cells or natural source in good yield and high purity.

Key words: DNA polymerase delta, immunoaffinity chromatography, reconstitution, MultiBac system, subassemblies.

INTRODUCTION

Among a family of eukaryotic deoxyribonucleic acid (DNA)-dependent DNA polymerases, three major replicative polymerases, α (pol α), δ (pol δ), and ε (pol ε), are viewed to be responsible for chromosomal DNA replication (Burgers, 1998; Waga and Stillman, 1998). The subunit composition of pol δ may vary among different eukaryotes. In fission yeast *Schizosaccharomyces pombe*, pol δ consists of four subunits, Pol3, Cdc1, Cdc27 and Cdm1 (Reynolds et al., 1998; Zuo et al., 2000; Bermudez et al., 2002). While, budding yeast *Saccharomyces cerevisiae* pol δ is a trimer of the first three subunits, Pol3p, Pol31p/Hys2, and Pol32p (Burgers and Gerik, 1998; Gerik et al., 1998). The smallest non-essential subunit Cdm1 has no apparent homologue in budding yeast (Reynolds et al., 1998). Human/mammalian pol δ was discovered as a new eukaryotic DNA polymerase that possesses both 5'-3' DNA polymerase and 3'-5' proofreading exonuclease activity in the laboratory of Dr. So (Byrnes et al., 1976; Byrnes et al., 1977). It contains four subunits, p125, p50, p68, and p12, as a counterpart of *S. pombe* pol δ (Hughes et al., 1999; Liu et al., 2000; Mo et al., 2000; Shikata et al., 2001).

In addition to its crucial role in DNA replication, pol δ is also a major participant in DNA repair processes and genetic recombination in which it is generally regarded as a primary enzyme to perform gap-filling (Sancar et al., 2004; Garg and Burgers, 2005; Branzei and Foiani, 2008). Extensive studies showed that pol δ itself may be a target of the DNA damage response. As replication stress or genotoxic agents triggered the degradation of the p12, pol δ was consequently converted from a heterotetramer to a trimer lacking the p12 subunit (Zhang et al., 2007).

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Also, it seems likely that loss of p12 modulated the rate of single-nucleotide (SN) and long patch (LP) in uracil-intiated base excision repair (BER) *in vitro* (Zhou et al., 2011). These findings raise a number of questions about the regulation of this enzyme and the integration of its functions, and how alterations in its function could contribute to the etiology of human cancer or other diseases that can result from loss of genomic stability. Therefore, a more in-depth investigation will be needed for its functional and structural analysis. However, one of the major obstacles is to obtain active pol δ complex and its subassemblies as an intact form in good yield and high purity. While we have developed efficient methods for the expression and purification of pol δ and its subassemblies, we have sought to improve both the quality and yields to facilitate our next study. In this work, we have made a great advance on: a) preparation of immunoaffinity column for its utilization for immunoaffinity chromatography by immobilizing a polyclonal antibody against p125 subunit onto the CarboLink coupling gel and b) improvement of over expression of pol δ subassemblies in Sf-9 cells with a novel baculovirus-based MultiBac system in which one or multiple subunits were constructed into one single recombinant virus with different combinations.

From 500 ml of infected Sf-9 cells, as much as 5 mg of different pol δ multi-subunit complexes could be obtained by purification using immunoaffinity chromatography in combination with ion-exchange chromatography. Thus, our work here offers a powerful approach to obviate those difficulties illustrated in the case of pol δ isolation from infected insect cells, from mammalian tissues or cultured mammalian cells (Lee et al., 1984; Jiang et al., 1995; Podust et al., 2002; Xie et al., 2002).

MATERIALS AND METHODS

All reagents and chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO), Ge Healthcare (Piscataway, Nj), Gibco-Brl, or Invitrogen, except as otherwise indicated.

Production of polyclonal antibody

For antigen preparation, the His-tagged p125 was generated by the polymerase chain reaction (PCR) using a full-length *POLD 1* cDNA as a template. The generated PCR fragments were digested with *Bam*HI and *Eco*RI, subcloned in-frame into the *Bam*HI-*Eco*RI sites of pFastBac HTc expression vector, and sequenced. The primer pairs used here were as follows: forward, 5'-AGCTACGGATCCGGATGGATGGCAAGCGGCGGCC-3'; backward, 5'-AGCTACGAATTCTCACCAGGCCTCAGGTCCAGGG-3' (*Bam*HI and *Eco*RI sides are underlined). Growth and maintenance of Sf-9 insect cells, preparation of recombinant baculoviruses and infection of the cells were performed according to manufacture's instruction (Invitrogen).

His-tagged p125 was expressed in one liter of Sf-9 cells in suspension culture, purified by nickel-nitrilotriacetic acid agarose (Qiagen), and followed by further purification using fast protein liquid chromatography (FPLC) on mono Q 5/50 Glitch (GL) column (Ge Healthcare). The highly purified protein was analyzed by mass spectrometry to further confirm amino acid sequence before immunization. The polyclonal antibody against human pol δ catalytic subunit was produced by immunizing rabbits with highly purified His-tagged p125 protein. The collected antiserums were precipitated at 40 to 50% saturation of $(NH4)_2SO_4$ and followed by the low-salt purification on a column packed with 5 ml pierce protein A/G plus agarose as described (Harlow and Lane, 1988).

Mass spectrometry analysis

The specific bands corresponding to human pol δ catalytic subunit p125 protein were excised manually from the polyacrylamide gel with a sterile scalpel and digested with trypsin according to Li et al. (2006b) method. The digested samples were analyzed by an ultraflex matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF–TOF) instrument (Bruker, Germany). Peptide mass fingerprinting (PMF) was performed by comparing the masses of identified peptides to NCBI protein database using the MASCOT search engine (http://www.matrixscience.com).

Preparation of immunoaffinity column

The preparation of immunoaffinity column was performed using CarboLink™ immobilization kit (Pierce) according to the manufacture's instruction. The support was the CarboLink coupling gel (6% cross-linked beaded agarose) which is ideal for immobilizing polyclonal antibodies *via* its abundant carbohydrates located on the Fc portion of the molecules. Briefly, the highly purified antibody (about 200 mg) in a total volume of 30 ml of coupling buffer was oxidized by adding 100 mg of sodium meta-periodate (resulting in 460 mM $NaIO_4$). The mixture was incubated at room temperature for 30 min and then desalted by desalting column. CarboLink resin (30 ml) was washed with 5 gel-bed volumes of coupling buffer and mixed with the desalted oxidized antibody. The column contents were mixed in suspension by end-over-end rotating at room temperature for 6 h and then at 4°C overnight. The coupled resins were washed with three gel-bed volumes of coupling buffer subsequently with phosphate buffered saline (PBS) and 10 gel-bed volumes of wash solution (1 M NaCl) and equilibrated with TGEE buffer (50 mM Tris-HCl, 0.1 mM EGTA, 0.5 mM EDTA, 10% glycerol, pH 7.8).

Construction, expression and purification of recombinant human DNA pol δ assemblies

The construction of recombinant human DNA pol δ and its subassemblies was performed with a novel MultiBac system as described (Zhou et al., 2011), according to manufacture's instruction provided by Dr. T. J. Richmond (Berger et al., 2004). Four subunit fragments of p125, p50, p68 and p12 containing *Bam*HI and *Xba*I restriction sites in each fragment were generated by PCR using the templates kindly provided by Dr. Ellen H. Fanning. The generated PCR products were digested with *Bam*HI and *Xba*I, subcloned in-frame into the *Bam*HI- *Xba*I sites of MCS1 of transfer vector pFBDM with different subunit combinations and sequenced. The preparation of recombinant baculoviruses harboring different subunit combinations was performed according to manufacture's instruction (Invitrogen). For the expression of the pol δ four-subunit enzyme and its subassemblies, 500 ml of Sf-9 cells at 2×10^6 cells/ml in suspension culture was infected with generated corresponding baculoviruses at a multiplicity of infection (MOI) of two. The cells were collected 72 h post-infection, and the cell pellets were either treated directly with lysis buffer or stored at -80°C.

A highly standardized protocol for rapid isolation of recombinant pol δ heterotetramer and its subassemblies was developed using newly made immunoaffinity column in combination with FPLC chromatography on mono Q column essentially as described for the isolation from a *Bombyx mori* bioreactor generated pol δ (Zhou et al., 2011).

Purification of pol δ complex from Hela cell extracts

The purification of pol δ four-subunit complex from Hela cells was performed essentially as described for its isolation from HEK 293T or Hela cells (Zhang et al., 2007; Meng et al., 2009).

Preparation of recombinant human proliferating cell nuclear antigen (PCNA)

The recombinant human PCNA expressed in *Escherichia coli* was purified to near homogeneity by a protocol as previously described (Zhang et al., 1995; Zhou et al., 2011).

DNA polymerase activity assay

The standard assay for pol δ activity was performed using sparsely primed poly (dA)/oligo (dT) as the template as described (Zhang et al., 2007; Meng et al., 2009; Zhou et al., 2011). Assay using singly primed M13 DNA as the template was performed as previously described (Zhang et al., 2007; Zhou et al., 2011).

Western blotting analysis

Western blotting was performed as described (Zhou et al., 2011). The antibodies used here were mouse polyclonal antibody against p50 (ZJM5002), rabbit polyclonal antibodies against p68 (ZJR6803), p12 (ZJR1204) previously prepared in our laboratory, and newly made rabbit polyclonal antibody against p125 (ZJR12501) prepared in this work. The second antibodies were alkaline phosphatase (AP)-conjugated goat anti-mouse or anti-rabbit IgG. Perfect protein western blot kit (Novagen) was used for signal generation.

Protein concentration determination

Protein concentrations were determined by the Bradford method with bovine serum albumin as a standard, or by “in-gel” determination of the catalytic subunit p125 concentration using catalase as a protein standard (Zhou et al., 2011).

RESULTS

Production of polyclonal antibody against human pol δ catalytic subunit p125

We chose baculovirus expression system to produce His-tagged p125 fusion protein by inserting full-length *POLD1* fragment into the pFastBacHTc plasmid between the *Bam*HI and *Eco*RI sites. Restriction analysis of selected colonies showed that a 3332 bp fragment was inserted into pFastBacHTc (Figure 1A). DNA sequencing confirmed that the *POLD1* was correctly fused to N-terminal His-tag with correct DNA sequence. The obtained recombinant pFastBacHTc-POLD1 plasmid was transformed into DH10Bac competent cells. After transposition, the recombinant bacmids harboring *POLD1* were isolated from the selected white phenotypes. PCR analysis confirmed that *POLD1* gene was successfully transposed to the bacmid with an expected band of -4 kb of the PCR products with the p125 forward amplification primer and RV-M reverse amplification primer (Figure 1B). The recombinant baculoviruses were then generated by the transfection of recombinant bacmid DNAs into Sf-9 cells and verified by western blotting with the anti-His antibody. The bands of His-p125 protein were detected as expected molecular mass (Figure 1C).

About 2 mg of highly purified soluble His-p125 protein from one liter of infected Sf-9 cells was obtained (Figure 1D). The amino acid sequence of obtained protein was further verified by mass spectrometry (data not shown). After immunizing two New Zealand rabbits using highly purified His-p125 protein as immunogen, about 200 mg of polyclonal antibody was purified in high purity from 120 ml of collected antiserum (final termination bleeds) by the precipitation of $(NH_4)_2SO_4$ and on a protein A/G plus agarose packed column (Figure 2A). The efficiency of obtained antibody was tested by western blotting with Hela cell extracts. Optimized concentration of purified antibody at 0.05 to 0.1 µg/ml could result in a high sensitivity for detecting the interaction between antibody and endogenous p125 subunit of pol δ in good specificity (Figure 2B, lanes 4 and 5).

Preparation of immunoaffinity column

To develop immunoaffinity column, about 200 mg, a combination of four preparations of highly purified polyclonal antibody, was used for its immobilization. After periodate oxidation of the carboxyl groups in the Fc portion of the antibodies, -140 mg of antibody was coupled to 30 ml of hydrazide-activated CarboLink™ gel, that is, about 4.7 mg of antibody was coupled per milliliter of resin. The coupling efficiency was also judged by comparing starting material (before coupling, Bc) with that after coupling (Ac) reaction on 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) stained with Coomassie blue (Figure 2C).

Expression and purification of recombinant human pol δ and its subassemblies

A set of recombinant baculoviruses for heterotetramer, two trimers lacking p12 or p68, and two-subunit enzyme (p125/p50) were generated with a novel MultiBac system by inserting the cDNAs for the pol δ subunits into a single transfer vector pFBDM in any one of combinations, each in an individual expression cassette. About 500 ml of Sf-9 cells in suspension culture was infected with generated recombinant viruses in individuals. The infected cell

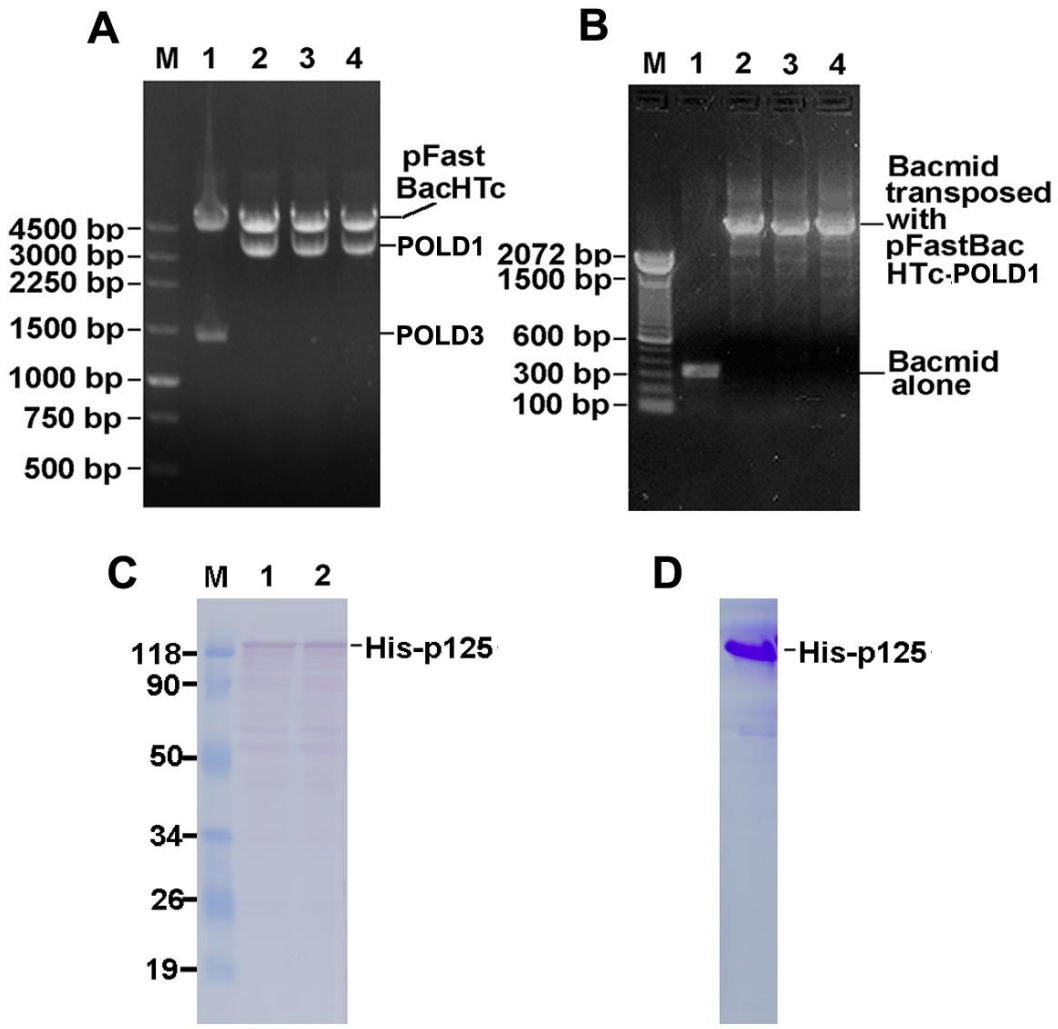

Figure 1. Analysis for the preparation of the immunogen used in the production of polyclonal antibody against human DNA pol δ catalytic subunit p125. A, Agarose gel electrophoresis of recombinant plasmid pFastBacHTc-POLD1 digested with *Bam*HI and *Eco*RI. M, DNA marker; lane 1, pFastBacHTc-POLD3 as a control; lanes 2 to 4, pFastBacHTc-POLD1 selected from three colonies. B, Agarose gel electrophoresis of PCR analysis for confirmation of the transposition of *POLD1* into bacmids through Tn7. M, DNA marker; lane 1, PCR product amplified from bacmid alone (as a control) with M13-47 forward and RV-M reverse primers; lanes 2 to 4, PCR product amplified from the bacmids transposed with pFastBacHTc-POLD1 isolated from the selected white phenotypes with the p125 forward and RV-M reverse primers. C: Western blotting analysis for the generation of recombinant viruses for His-tagged p125 with anti-His antibody. M, protein maker in kDa; lane 1 and 2, two preparations of recombinant viruses. D, A peak fraction of highly purified His-p125 protein on Mono Q column analyzed on 12% SDS-PAGE gel stained with Coomassie blue.

extract was directly applied onto a 20 ml immunoaffinity column. Of each assembly, the eluted peak fractions containing all expected subunits were combined, dialyzed, loaded on a 1 ml mono Q 5/50 GL column, and eluted with 20-bed volumes of a linear gradient of NaCl from 0.1 to1 M in TGEED buffer (50 mM Tris–HCl, pH 7.8, 10% glycerol, 0.5 M EDTA, 0.1 mM EGTA and 1 mM dithiothreitol), respectively. The peak activities were eluted between 350 and 400 mM NaCl in TGEED buffer (data not shown). Eluted fractions were judged by 12% SDS-PAGE gel and stained with Coomassie blue (Figure 3).

Thus far, a pol δ heterotetramer and its subassemblies, purified to near homogeneity, were obtained. We routinely obtained as much as 4 to 5 mg of pol δ complexes from 500 ml Sf-9 cell culture. A summary for the specific activities and yield of preparations for purification of recombinant pol δ heterotetramer is shown in Table 1. Besides its excellent behavior in purification of recombinant pol δ complexes, our newly made

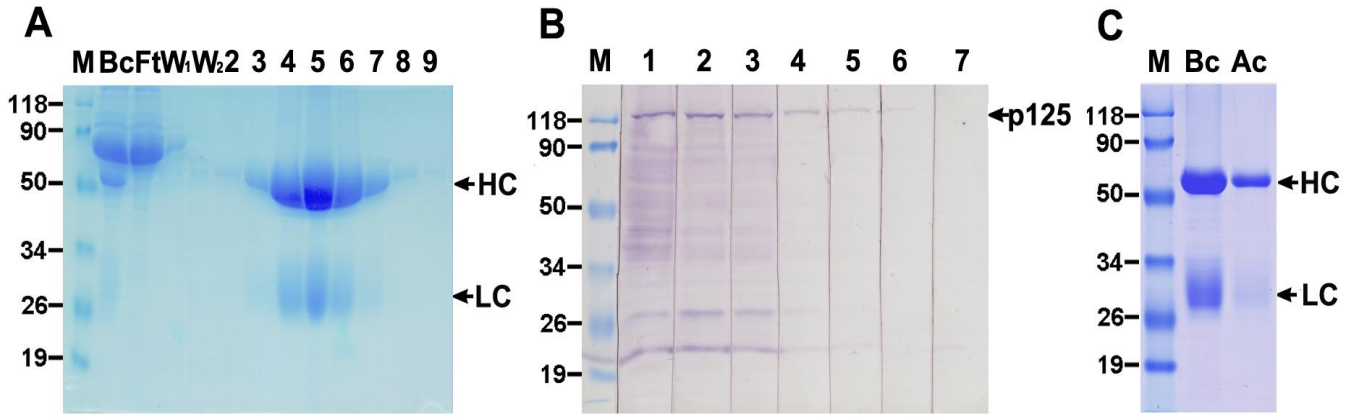

Figure 2. Analysis for the production of polyclonal antibody and preparation of immunoaffinity column. A, Coomassie blue stained SDS-PAGE analysis for the purified polyclonal antibody against p125 after affinity chromatography by a 5 ml protein A/G plus column. The dialyzed sample in PBS after ammonium sulfate precipitation (Bc), flow-through (Ft), wash (W_1 and W_2), and eluted fractions were analyzed on 12% SDS-PAGE followed by Coomassie blue staining. Protein marker in kDa is indicated by M. The heavy and light chains are marked by arrows. B, Measurement of sensitivity and specificity of purified antibody by western blotting with Hela cell extracts. The seven lanes showing decreasing concentrations of antibody contained 0.8, 0.4, 0.2, 0.1, 0.05, 0.01 and 0 µg/ml of antibody/slice membrane, respectively. The detected endogenous p125 is marked by an arrow. C, Judgment of coupling efficiency of immunoaffinity column on 12% SDS-PAGE stained with Coomassie blue. Bc, starting material before coupling reaction; Ac, the material after coupling reaction; M, protein marker in kDa.

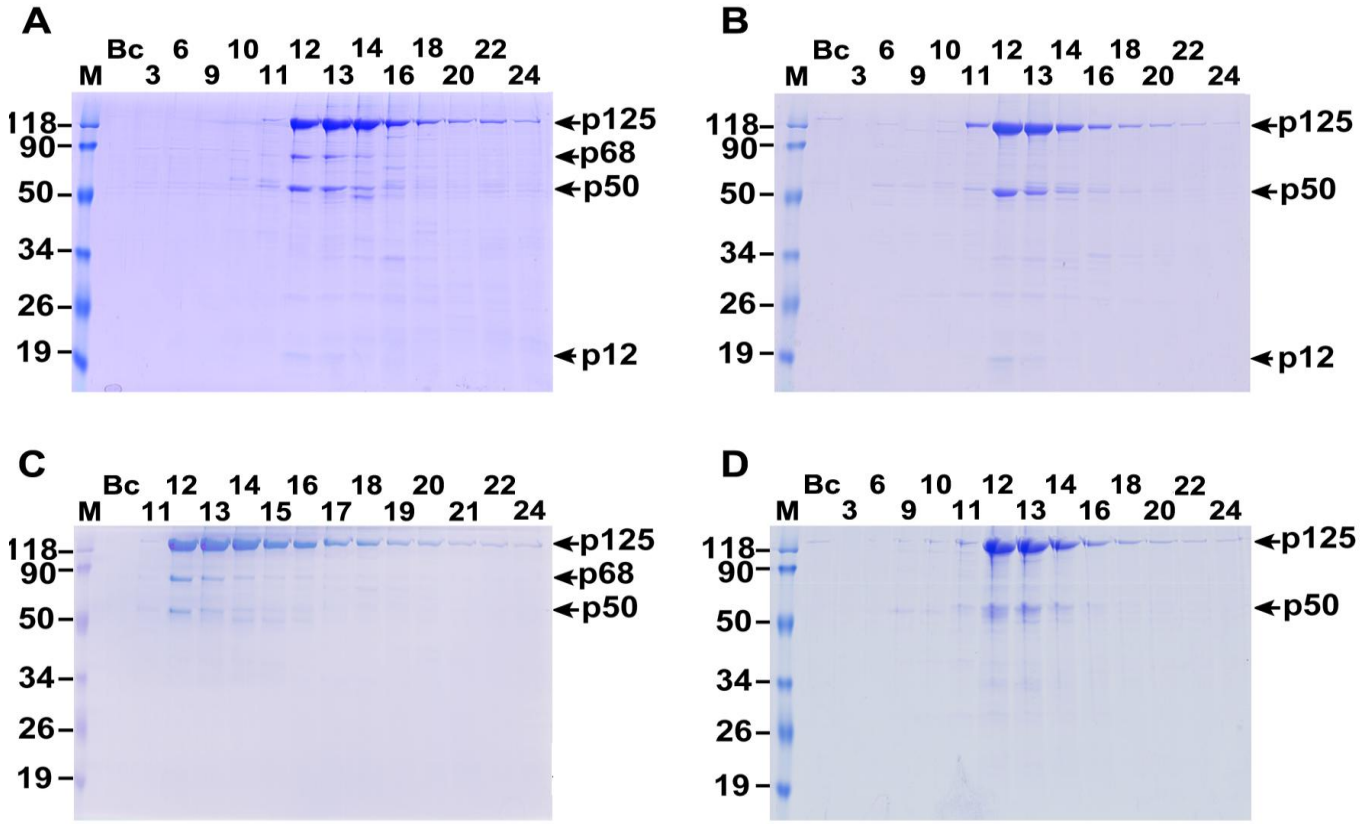

Figure 3. Coomassie blue stained SDS-PAGE analysis for the preparations of pol δ assemblies after their further purification on Mono column. The fractions across peak from the immunoaffinity chromatography step were combined, dialyzed, and passed through a Mono Q column. The starting materials after immunoaffinity chromatography (Bc) and eluted fractions were analyzed by 12% SDS-PAGE followed by Coomassie blue staining. Pol δ four subunits are marked by arrows. Panels A, B, C, and D show the preparations of pol δ4, trimer lacking p68 (pol δ3-p68), trimer lacking p12 (pol δ3-p12), and dimer p125/p50, respectively. Protein marker in kDa is indicated by M.

Table 1. Purification of recombinant DNA pol δ four-subunit complex from 500 ml of infected Sf-9 cells[a].

Step	Total volume (ml)	Protein (mg)	Activity (unit)	Specific activity (unit/mg)
Cell extract	200	801.5	ND[b]	-
Immunoaffinity	120	12.21	148962	12200
Mono Q	3.0	5.01	131262	26200

[a]The purification procedure was carried out as described under materials and methods. DNA polymerase activity was assayed on poly(dA)/oligo(dT) template-primer. [b]Activity in supernatant of cell extracts cannot be accurately measured.

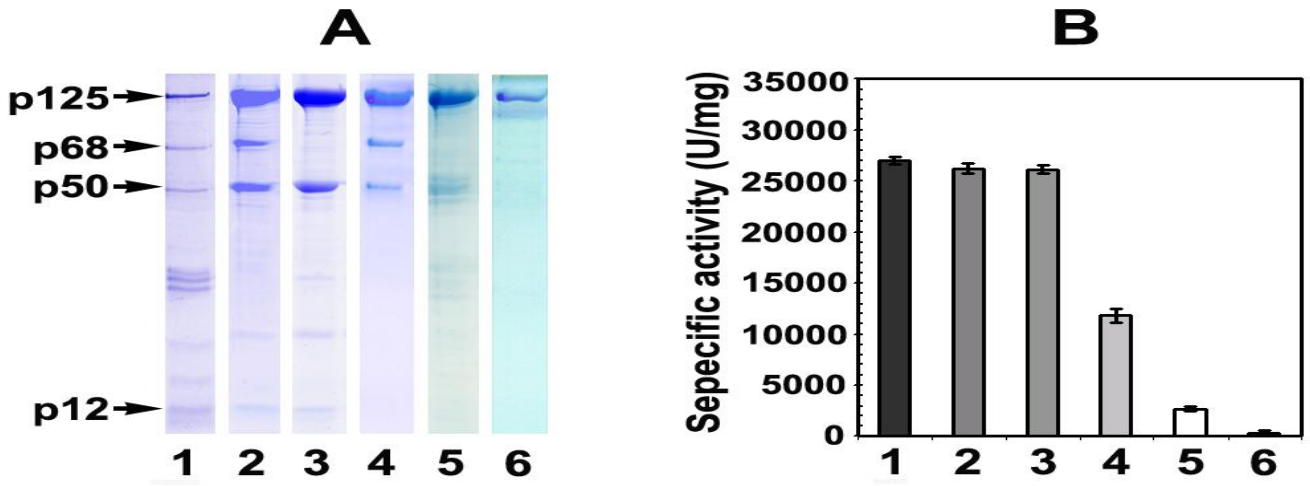

Figure 4. Comparison of the activities of pol δ and its subassemblies on poly (dA)/oligo(dT) primer-template. A, The proteins used in polymerase activity assays judged on Coomassie blue stained SDS-PAGE gel. Lane 1, A peak fraction of native pol δ enzyme isolated from cultured Hela cells by immunoaffinity chromatography used as a control; lanes 2 to 5, the peak fractions of pol δ4, pol δ3-p68, pol δ3-p12, and dimer p125/p50 purified to near-homogeneity after Mono Q column step, respectively; lane 6, a His-tagged p125 protein after purification by nickel-nitrilotriacetic acid agarose and Mono Q column. B, Pol δ activity assay. Six lanes show the same preparations of pol δ assemblies as indicated in panel A. 200 fmoles of each assembly were used in each assay in the presence of PCNA. The vertical axis indicates the specific activity (in triplicate) in units/mg and the horizontal axis shows the different enzyme assemblies as shown in panel A. PCNA, proliferating cell nuclear antigen.

immunoaffinity column was also suitable for the isolation of native pol δ complexes from natural source. We tested the column by the isolation of pol δ from 150 plates of cultured Hela cells (about 3×10^8 cells), resulting in an intact four-subunit complex near homogeneity with expected molecular masses judged by Coomassie blue stained 12% SDS-PAGE gel from which a peak fraction on the gel was shown (Figure 4A, lane 1).

Comparison of the activities of highly purified pol δ complexes

The specific activities of highly purified recombinant pol δ complexes in the eluted peak fraction 12 after FPLC chromatography on mono Q (Figure 4A, lane 2 to 6) were compared with that of the native pol δ holoenzyme isolated from cultured Hela cell extract after immunoaffinity chromatography (Figure 4A, lane 1) on sparsely primed poly (dA)/oligo(dT) template-primer in the presence of PCNA. As shown in Figure 4B, the specific activity of recombinant pol δ4 was calculated to be 26,200 units/mg, almost the same as the 27,000 units/mg of native pol δ holoenzyme isolated from Hela cells. The trimer lacking p68 was as active (26,130 units/mg) as pol δ4, which is consistent with that previously reported (Podust et al., 2002; Li et el., 2006a). Surprisely, the trimer lacking p12 exhibited a 45% activity over its progenitor; while, the dimer of p125/p50 was PCNA responsive and also exhibited an unexpected 10% activity over pol δ4.

The DNA elongation activities of these purified pol δ complexes were also compared on singly primed M13 DNA. As shown in Figure 5, the pol δ4 exhibited a perfect ability to elongate primer DNA on M13 template. The synthesized products are around 7 kb, but, the trimer lacking p68 (pol δ3-p68) showed lowered activities and the presence of shorter products around 3 kb. The trimer lacking p12 (pol δ3-p12) possessed almost the same activity as pol δ4 at higher enzyme concentrations. Interestingly, the dimer of p125/p50 also appeared lower activities at high enzyme concentrations which is

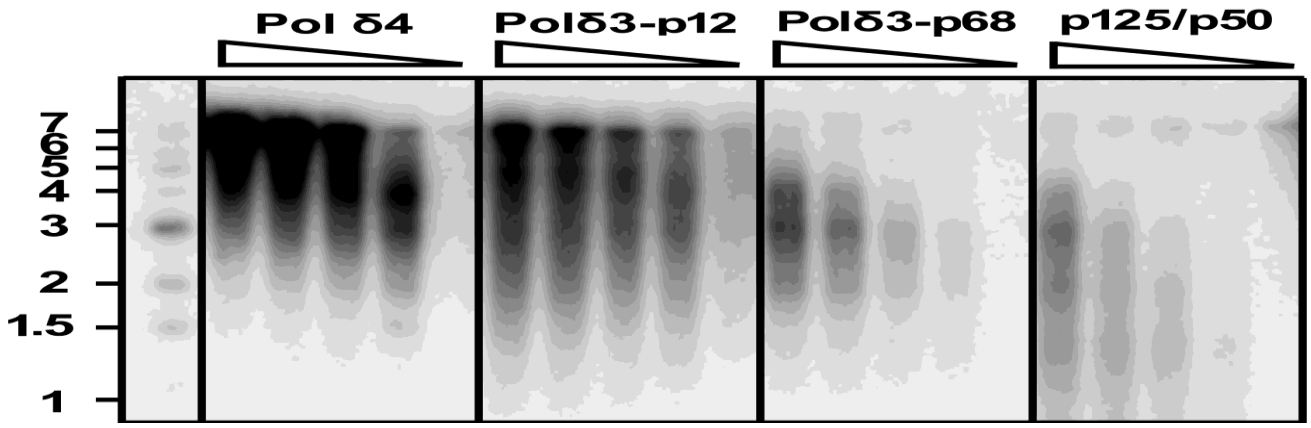

Figure 5. Analysis of DNA elongation activities on singly primed M13 DNA template. Horizontal bar on the left indicates the positions in Kbs of DNA markers labeled with [γ-^{32}P] ATP. The panels from left to right show the behaviors of the enzymes in M13 assays as pol δ4, pol δ3-p12, pol δ3-p68, and dimmer p125/p50. The lanes in each panel from left to right show decreasing amounts of enzyme as 200, 100, 50, 25, 0 fmoles.

consistent with that in poly (dA)/oligo(dT) assays.

DISCUSSION

The study of the enzymology of the mammalian DNA polymerases has been extremely difficult due to the small amounts of enzyme that could be recovered (Lee et al., 1984; Jiang et al., 1995; Hughes et al., 1999). Although the procedure was improved by the introduction of immunoaffinity chromatography in which a column was developed by immobilizing a monoclonal antibody against catalytic subunit onto AvidChrom hydrazide gel, only small amount, that is, -0.3 mg of enzyme could be obtained from 0.75 kg of calf thymus in which four columns were involved (Jiang et al., 1995). While we sought to develop an efficient strategy for the overproduction and isolation of pol δ complexes, we focused mainly on two aspects, the improvement of expression system and the development of a powerful immunoaffinity column for rapid isolation of enzymes in both good purity and high yields.

MultiBac is a simple and versatile system for generating recombinant baculovirus DNA to express protein complexes comprising many subunits. With MultiBac system, we have greatly simplified our constructions of pol δ multiple subunits assemblies in any one of subunit-combinations, that is, heterotetramer pol δ4, two trimers of pol δ3-p68 and pol δ3-p12, as well as a dimer of p125/p50. CarboLink™ coupling gel is ideal for immobilizing polyclonal antibodies through Fc portion of the molecule. Such antibodies are properly oriented with their antigen-binding sites unobstructed, offering greater purification capability. With our newly made powerful column by immobilizing a polyclonal antibody against catalytic subunit onto the CarboLink coupling gel, we developed a highly standardized protocol for rapid isolation of recombinant pol δ complexes in milligrams by two columns only. The activities of highly purified pol δ and its subassemblies using this column were analyzed both on sparsely primed poly (dA)/oligo (dT) template-primer and on singly primed M13 DNA template.

The specific activity of high purified recombinant pol δ4, as much as 5 mg protein from 500 ml Sf-9 infection, exhibited a high value up to 26200 units/mg (Table 1) which is comparable with those of pol δ preparations isolated from Hela cells (27,000 units/mg in this work), calf thymus (26,400 units/mg by four columns) (Lee et al., 1984; Jiang et al., 1995), and baculoviral co-infection-based reconstitution (20830 units/mg by three columns) (Xie et al., 2002). The trimer lacking p12 exhibited a 45% activity over its progenitor (Figure 4B), which differs from those (extremely low levels of activity, generally 5 to 7% of that of pol δ4) previously reported by us and others (Podust et al., 2002; Li et al., 2006a). It could be partially explained by the fact that these isolated enzymes might be highly unstable. Surprisely, the dimer of p125/p50 was PCNA responsive and exhibited an unexpected 10% activity over pol δ4. We now for the first time have a recombinant dimer that behaves like the native source pol δ dimer isolated from calf thymus and human tissues (Lee et al., 1984, 1991).

Similar behaviors of these purified enzymes were observed on singly primed M13 DNA template except the pol δ3-p68 which was defective in M13 assay while it was active in poly (dA)/oligo(dT) assay. These different behaviors on our observation need to be further investigated. Thus, the availability of highly purified pol δ complexes in milligrams with reproducible purity and specific activities, as well as the facilitated production of pol δ subassemblies by the use of our immunoaffinity column in combination with ion-exchange chromategraphy, will have and has already had a major impact on our research.

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