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An Atypical DNA Polymerase Beta Overexpressed in Human Aml/Hl-60 Malignant Cells

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Abstract

Human acute myeloid leukemia cells overexpresses a beta–like DNA polymerase (EC 2.7.7.7) which is found to be a chromatin associated single subunit protein (66.5 kDa) purified by original extraction/gel filtration procedure allowing to gain the 122,000-fold purification degree as corrected to a total cell protein. The enzyme possesses some key DNA pol β-specific catalytic properties such as the processing of short (200_n-250_n) single strand DNA sequences, activation in the presence of 200 mM KCl, resistance to N-ethyl-melamide and Aphidicolin, lack of 3',5'-exonuclease activity, and low dTTP utilization rates (K_M=0.016 mM, K_{cat}=0.622 (μM dTTP/min)/mg protein). A possible significance of the unique enzyme studied as a target for its pharmaceutical inhibitors is under discussion. This work is a full–length version of a study presented as a Poster at the OMICS managed 2nd World Congress on Science Cancer and Therapy, Sept 10–12, 2012, San Antonio, TX.

Keywords: Acute myeloid leukemia; DNA polymerase beta; Target enzymes in cancer therapies

Introduction

DNA polymerases beta, EC 2.7.7.7 (DNA pol β), represent the special subpopulation of the rich-n-variable DNA polymerases superfamily. A remarkable peculiarity of DNA pol β relates to its participation in the DNA base–excision repair [1-4]. Being the chromatin-associated proteins [4,5], most DNA pol β species were found to be overexpressed in many malignant tumors [6-12]. It makes these enzymes the legitimate targets for inhibitors or, to be exact, for a chemotherapeutic attack provided by DNA pol β -recognizing high affinity suppressors playing a role of pharmaceutical agents [13-17].

The latter circumstance attracts an attention of not only enzymologists but of oncologists and pharmacologists as well [14,18,19]. However, a broad structural diversity of DNA pol β species isolated from normal and cancer cells dictates a necessity of detail structural and functional (catalytic) characterization of each one, usually tumor–specific, enzyme of this group.

Thus, most frequently, DNA pol β examples are Mg^{2+}-coordinating proteins having pI within 8.3–8.7 and a molecular mass within 35 kDa–55 kDa ranges, respectively [19-21]. Normally, these enzymes are too slowly produce the single strand DNA chain consisting of no more than 300 nucleotides (DNA repair requirement) showing a high resistance to such common DNA polymerase (alpha, gamma, epsilon, etc) specific inhibitors as Aphidicolin and N-ethyl-malemide [19,22]. A total lack of 3',5'-exonuclease activity is also a marking sign of DNA pol β [20-24].

On other hand, there are some remarkable instances of an exceptionally high, up to 260 kDa, molecular mass values estimated for several chromatin affiliated enzymes with the above specified catalytic activity (β-like DNA polymerases) [7,23,24]. Moreover, a DNA pol β molecular size itself might be a rather critical parameter in chromatin structural organization making an impact on genome expression control which seems to be particularly essential to the high molecular weight β-like enzymes [5,24].

Although the DNA pol β research counts nearly 20 years of its history, a list of tumors subjected to purification–characterization of their specific DNA pol β is still far of being completed. Noteworthy, one of the abundant human blood cancers, an acute myeloblast leukemia or AML, has not been engaged yet to isolate and investigate its specific DNA pol β . So this work is a first report to describe an original procedure of purification and characterization of such enzyme from chromatin of HL60 cells (AML).

Materials and Methods

Cell culture

The HL-60 human myeloid leukemia cell line has been purchased from the Hungarian Cell Bank, Pasteur Institute of Hungary, Szeged, NCBI Code C427. Cells were maintained in suspension culture at +37°C under 5% CO_2/air in RPMI 1640 (Gibco, UK) supplemented with 10% FCS and antibiotics: 100 U/mL Penicillin and 100 μg/mL Streptomycin. The cells were subcultured three times weekly, ATRA (Sigma, USA). This procedure has been originally adopted by Olins et al. [25] and then modified by Roy et al. [26].

Chromatin fractionation

The cells were precipitated by centrifugation at 12,000 rpm for 20 min (+4°C). The pellets were then suspended and homogenized in 5 volumes of 10 mM Tris-HCl (pH 7.80)/200 mM sucrose/1.5 mM EDTA/20 mM $MgCl_2$/0.5% (v/v) Triton X100 using the glass-teflon Potter-Elvehjem homogenizer with a snug-fitting pestle (1,800 rpm).

Homogenates were filtered through the 5-layer cotton graze and

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then the filtrates were centrifuged at 800 g for 30 min (+4°C). The pellets were collected and re-suspended in 10 volumes of 15 mM Tris-HCl (pH 8.0)/1.5 mM EDTA/10 mM $MgCl_2$/1.5 mM KCl/25 mM sucrose/Trypsin 20 µg/mL (w/v) and incubated at +37°C for 1 hr with a following centrifugation at 800 g, 40 min (+4°C). The resulted pellets were treated as a crude nuclei subcellular fraction, P0.8. To obtain nucleoplasm samples, separate portions of homogenate were utilized for isolation of P.08 fractions with their following Triton X100 treatment (2.0%, v/v)/15 mM Tris-HCl (pH 8.0) and a subsequent centrifugation at 150,000 g, 2 hrs (+4°C).

For isolation of chromatin, the crude nuclei fractions (P0.8) were subjected to procedure described by Voss et al. [27] and modified by Lerman et al. [28] with a subsequent phenol-chloroform extraction of total protein [28]. The resulting protein fractions were mixed with 10 volumes of an ice-cold acetone and kept at +4°C overnight. An acetone–insoluble material was precipitated at 20,000 rpm, 20 min, +4°C. The pellets were extensively re-washed with acetone using the same procedure and then dissolved in 5–6 volumes (w/v) of 25 mM potassium–phosphate (pH 6.30)/0.5% NaCl/1.5 mM EDTA/0.01% glutathione/0.05% heparin/1.0% 2-mercaptoethanol/80–100 U/mL nuclease S followed by 40 min incubation at +37°C. All post–incubation mixtures were treated by sonication at 80 KHz, 30 min, +60°C, under a non-stop extensive shaking. Then these mixtures were submitted to a scalar fractionation path reaching the 30%–70% ammonium sulfate saturation, consequently. The precipitates obtained were collected at 10,000 rpm, 20 min, and dissolved in 15 mM potassium phosphate buffer (pH 6.0)/0.2% NaCl (10 vols, w/v). The solutions were subjected to dialysis against 20 mM potassium phosphate buffer (pH 6.0) and lyophilized.

Gel filtration

The lyophilized powders were first dissolved in 15 mM potassium phosphate (pH 6.30)/5.0 mM $MgCl_2$/1.5 mM EDTA/0.0001% sodium azide and passed through the fiberglass filters with 0.3-0.4 µ pore diameter (Millipore 5R, Millipore, France). The transparent solutions were subjected to ultrafiltration on membranes with the molecular size exclusion limit of 5.0 kDa at 800 p.s.i. (Diaflo Y5.0 25 mm membranes, Amicon BV, The Netherlands). The membrane–retained material was then extracted with 10 mM Tris-HCl (pH 8.0)/1.0% 2-mercaptoethanol (v/v), 5.0 mL per a razor–disintegrated membrane, +30°C, 12 hrs, with a following concentration in a rotor evaporizer.

The 1.5-2.5 mL samples were then applied onto a 1.5 × 50 cm (V=98 mL) column packed with the TOYOPEARL HW 55F gel and equilibrated by the eluent buffer consisting of 15 mM potassium phosphate (pH 6.30)/5.0mM $MgCl_2$/ 0.0001% sodium azide. Elution rate: 0.8 mL/min (room temperature). In each one of the consequently eluted 1.5 mL fractions, the DNA polymerase activity has been measured according to Matsumoto and Kim [29] and modified by Piersen et al. [30]. To get the enzyme specific activity measure, the amounts of protein were estimated by the Bradford colometric method [31]. A column was calibrated with the protein markers set (Serva Heidelberg, Germany) to cover the following molecular mass values range: 12.5 kDa (cytochrome C)–24.0 kDa (trypsine)–45 kDa (ovalbumin)–70 kDa (HSA)–145.0 kDa (L-asparaginase).

Electrophoretic procedures

A slightly modified Laemmli method [32] has been employed to estimate both molecular mass and purification extent of the DNA polymerase isolated. The peculiar fractionation parameters: 10% PAAG separation system based on a standard Tris-glycine (pH 8.30)/0.5 % SDS, 0.1 × 100 × 100 mm slab gels, 180 V/gel (4.5 mA/cm). Calibration of gels was performed with a standard QR 460/5 protein marker kit covering the 12.5-120.0 kDa range (Miles Laboratories, USA).

A routine 2.0%-agarose gel electrophoretic technique has been employed [33]. For the nascent single strand DNA chain length estimation, the 150n–300n ranged poly (dT) markers were applied (Calbiochem-Novabiochem International, USA).

The descendent linear 10.0–3.0 pH gradient 1.0×80×80 mm PAAG slabs with a reported pI performance range of 9.6–3.5 (KomaBiotech, Korea) were used as originally described by Walker [34] and modified by Katoh [35]. Additionally, a cellophane–attached ultrathin 0.12 × 260 × 125 mm PAAG slabs with the 10.0–3.0 pH gradients (Clean Gel–IEF plates, GE HealthCare Europe GmbH, Germany) were used according to Görg et al. [36]. In a latter case, the processed and unfixed gels were also used to trace-or-exclude a 3´,5´-exonuclease activity in DNApolβ-fraction [37]. These IEF procedures were also employed to get the protein composition profiles of chromatin and nucleoplasm obtained from HL60 cells and from myelocytes of the adult human male donors [27,28].

DNA polymerase (ec 2.7.7.7) activity measurements

The enzyme catalytic activity was measured by method [22] adopted for 0.15 mL incubation mixtures consisting of 50 mM Tris-HCl (pH 8.0)/8.0 mM dithiothreitol/15 mM $MgCl_2$/15% glycerol (v/v)/27 µg act DNA, calf thymus/50 µg each of dATP, dCTP, dTTP, dGTP/0.25 µmole [Methyl-1,2-^{3}H]dTTP (90–120 Ci/mmol, NET520A, NEN)/150 mM NaCl. The tritium-labeled nucleotide was purchased from New England Nuclear, USA. These compound concentration values were first pre-optimized within both pH 6.0–9.0 and 5.0 mM–50.0 mM $MgCl_2$ ranges. These mixture samples were first pre-incubated at +37°C for 60 min. Then 5.0–7.5 µg of pure enzyme was added to each one of these running samples and they were incubated at +37°C for 60 min longer. The ice cold incubation samples (0°C, 60°C after pre-incubation) as well as the trypsin treated samples (20 µg/mL trypsin, Merck GmbH, Germany, +37°C, 60°C) were taken for controls.

The post-incubation mixtures were subjected to a quantitative extraction of the DNA ultramicro–amounts using an AccuPrep Genomic DNA Extraction Kit (Bioneer Corp., Korea) as described by Mikami et al. [38] and modified by Haratian et al. [39]. The extracted DNA aliquots were used for electrophoretic determination of the DNApolβ-processed DNA chain sizes [33] and for [^{3}H]-radioactivity measurements in Wallac 2200LX LS Counter (Wallac OY, Finland). The DNA pol β -specific catalytic activity values were expressed in [^{3}H] cpmDNA/mg enzyme. The protein ultramicro amounts were estimated according to Fukami et al. [40]. The DNA ultramicro amounts measurements were performed in diluted water solutions as described by Müller et al. [41].

The kinetic constants, K_M (mM) and K_{cat} ([µM dTTP/min]/mg enzyme), were estimated by the free dTTP pool depletion rates [42] measured using the HPLC analysis of acetone–soluble fractions of pre- and post-incubation mixtures: Altex 1800E (18×220 mm) column/ODS-S5CN stationary phase/mobile phase, 10-60% linear pyridine gradient based on 10% water-methanol/2,000 p.s.i./+22°C / Waters DL600 HPLC Analytical System [42,43].

Spectrometry

The UV-spectrophotometry (Lambda 1050 Scanning

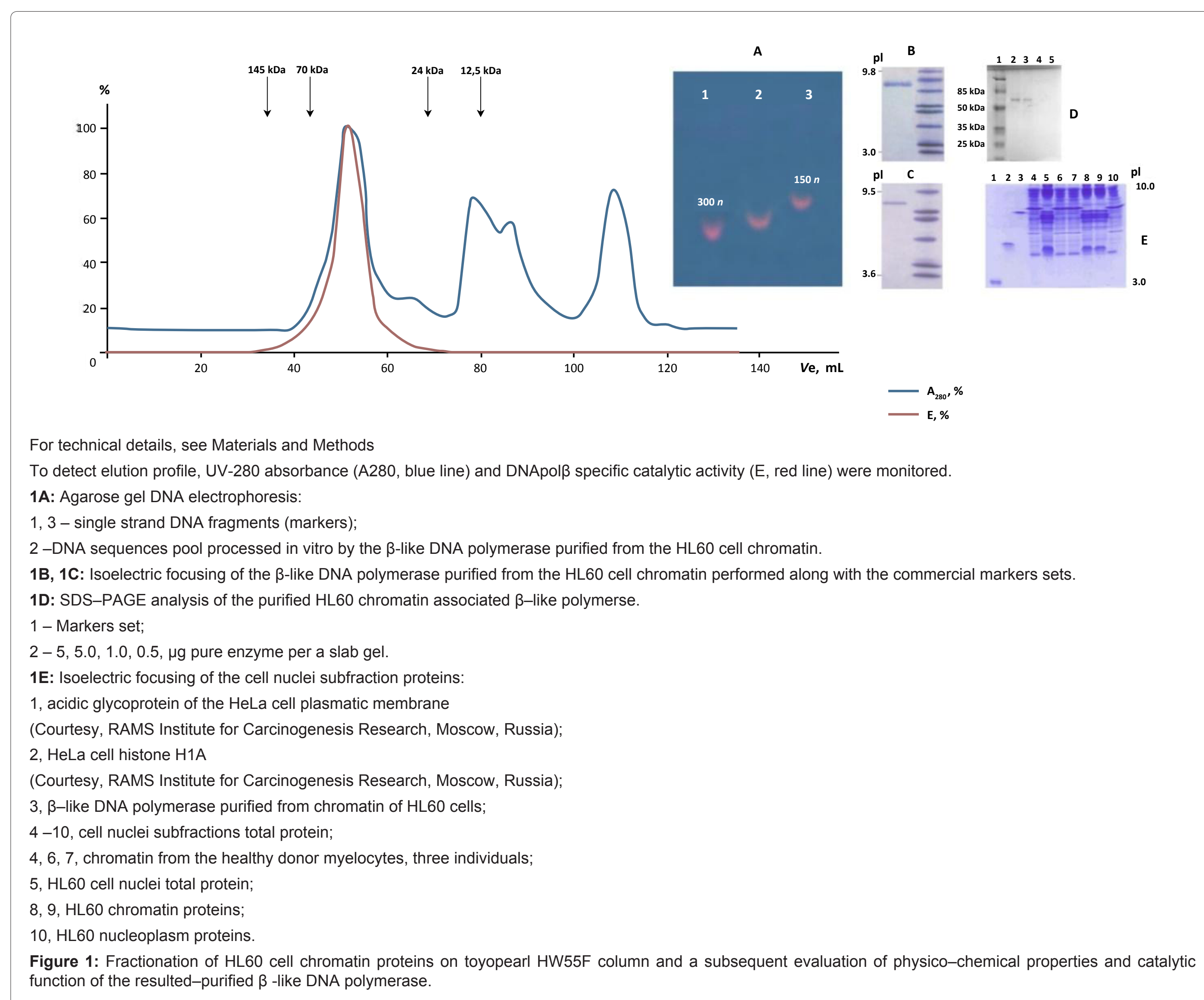
Spectrophotometer, Perkin Elmer, Inc., USA) and a circular dichroism spectrometry (J815 CD Spectrometer, JASCO, Inc., USA) were employed using the Spectra Manager II cross-platform software (JASCO) for automated data treatment. To dissolve the lyophilized enzyme samples, 10 mM potassium phosphate adjusted to pH 5.20/pH 6.20/pH 8.30 (UV absorption tested in 190 nm–400 nm range) and to pH 8.00 (CD spectrometry, 190 nm-255 nm) has been employed.

Results

As seen from the data presented in Figure 1, the procedure we proposed allows to isolate the perfectly purified 66.5 kDa monomer protein (Figure 1d and Figure 1 gel filtration profile) with a marked DNA polymerase activity limited to produce DNA chains within 200_n–250_n size range (Figure 1a). The enzyme purified is found to possess the following properties: pI=8.45 (Figures 1B, C and E); pH 8.0/15 mM $MgCl_2$ optimal incubation parameters (Materials and methods); kinetic constants estimated by dTTP utilization mode, K_M=0.016 mM and K_{cat}=0.622 (μM dTTP/min)/mg protein.

The enzyme is overexpressed in HL60 cells compared to normal myelocytes being affiliated with chromatin not nucleoplasm (Figure 1e). In the same IEF tests, a total lack of 3',5'-exonuclease activity was found for purified enzyme employing the technique described by Görg et al. and Rule [36,37]–see Materials and methods. UV absorption spectra of the enzyme isolated shows not only a lack of impurities such as poly- and oligonucleotide contaminants but a nativity of the protein as well [44] (Figure 2). A high nativity extent along with a predominantly alpha-helix contribution to a secondary structure of enzyme [44] has been detected by CD-spectrometry (Figure 3).

The enzyme is sensitive to ddTTP inhibitor being resistant to Aphidicolin and N-ethyl-melamide, a high concentration of KCl (200 mM) leads to a sharp increase of catalytic activity (Table 1). A progressive increase of the enzyme beta-specific catalytic activity occurred in the consequent steps of purification procedure is seen clearly from the data listed in Table 2. As corrected to a total cell protein, the final yield (purification extent) of the enzyme isolated was found equal to 1:122,000 (w/w). To make sure that the DNA polymerase activity measured (Table 2) was indeed related to a beta-type enzyme, it has always been determined in the presence of Aphidicolin, 5.0 μg/mL [19,22,24].



For technical details, see Materials and Methods

To detect elution profile, UV-280 absorbance (A280, blue line) and DNApolβ specific catalytic activity (E, red line) were monitored.

1A: Agarose gel DNA electrophoresis:

1, 3 – single strand DNA fragments (markers);

2 –DNA sequences pool processed in vitro by the β-like DNA polymerase purified from the HL60 cell chromatin.

1B, 1C: Isoelectric focusing of the β-like DNA polymerase purified from the HL60 cell chromatin performed along with the commercial markers sets.

1D: SDS–PAGE analysis of the purified HL60 chromatin associated β–like polymerse.

1 – Markers set;

2 – 5, 5.0, 1.0, 0.5, µg pure enzyme per a slab gel.

1E: Isoelectric focusing of the cell nuclei subfraction proteins:

1, acidic glycoprotein of the HeLa cell plasmatic membrane

(Courtesy, RAMS Institute for Carcinogenesis Research, Moscow, Russia);

2, HeLa cell histone H1A

(Courtesy, RAMS Institute for Carcinogenesis Research, Moscow, Russia);

3, β–like DNA polymerase purified from chromatin of HL60 cells;

4 –10, cell nuclei subfractions total protein;

4, 6, 7, chromatin from the healthy donor myelocytes, three individuals;

5, HL60 cell nuclei total protein;

8, 9, HL60 chromatin proteins;

10, HL60 nucleoplasm proteins.

Figure 1: Fractionation of HL60 cell chromatin proteins on toyopearl HW55F column and a subsequent evaluation of physico–chemical properties and catalytic function of the resulted–purified β -like DNA polymerase.

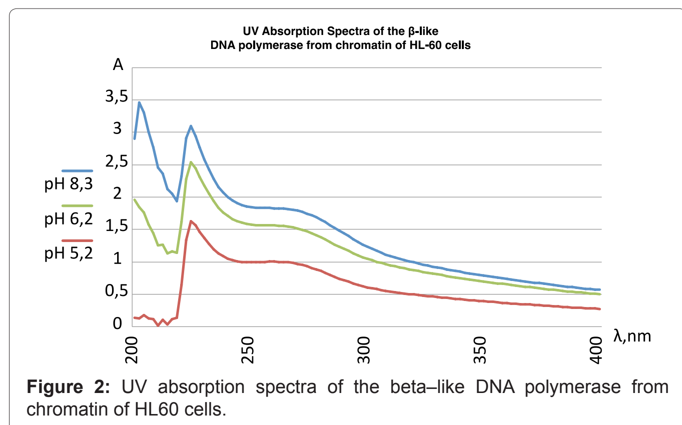


Figure 2: UV absorption spectra of the beta–like DNA polymerase from chromatin of HL60 cells.

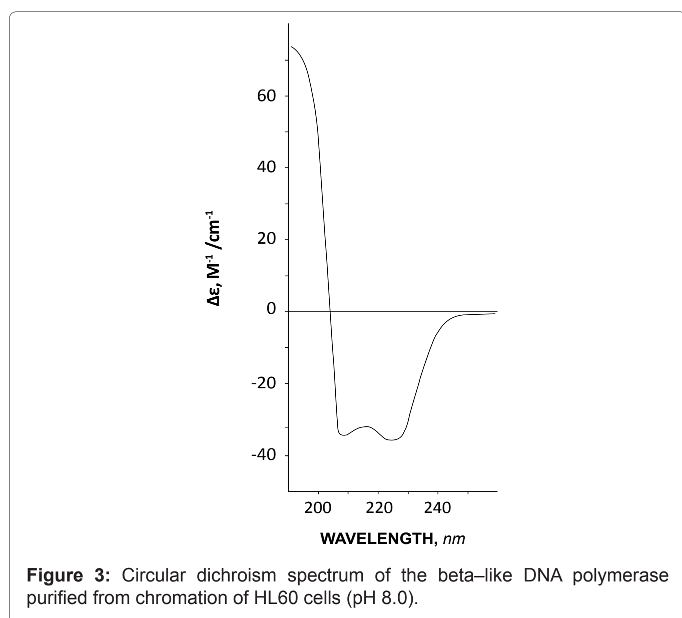


Figure 3: Circular dichroism spectrum of the beta–like DNA polymerase purified from chromation of HL60 cells (pH 8.0).

Discussion

The reason why the enzyme studied considered a β -like DNA polymerase not just a DNA pol β is a relatively large molecular size (66.5 kDa) (Figure 1) of this single-subunit protein. This alone makes this enzyme a sort of "non canonical" to fit a conventional definition [1,2,19–21] of DNA pol beta. For such rare and peculiar cases, a β-like DNA polymerase notion has been deliberately proposed [45-47]. A comparison between the molecular size determination data obtained by

(a) gel filtration column chromatography (native conditions allowing enzyme to work perfectly), and by

(b) SDS–PAGE analysis (tough denaturing conditions) show no difference in molecular mass of the enzyme purified (66.5 kDa, both gel filtration and SDS–PAGE) (Figure 1). This proves a monomer, i. e. single-subunit, nature of the enzyme which itself is an unusual, attention catching, DNA pol β-characterizing pattern. On other hand, a numerous known DNA pol β-specific marker signs ("taxonomic criteria") like a unique capability to get overactivated in the presence of 200 mM KCl [22], high resistance to Aphidicolin and N-ethyl-melamide [19,22,24], short (smaller than 300 n) single strand DNA chains processed [1,3,4,24], a total lack of 3',5'- exonuclease activity [2,5,20,45], slow rate of the nascent DNA chain growth (low processivity) indicates that the enzyme belongs namely to a DNA pol β group. All these criteria listed were experimentally found and measured for the enzyme studied (Figure1 and Table 1, K_M and K_{cat}-see Results). So we're indeed dealing with a special type of DNA pol β or, better to say, β -like DNA polymerase. Both kinetic constants measured (Results) shows a rather slow reaction processing rate which is in a favor to precise and accurate, error-free, DNA repair known for most of the DNA pol β species [1,10,12,18,47]. As per the pI value estimated (8.45), Mg^{2+} dependence and sensitivity to ddTTP (Figure 1 and Table 1, Materials and methods), these patterns are common for all kinds of DNA polymerase subpopulations including a DNA pol β group as well [13,19,22]. A mere fact that the chromatin associated enzyme we purified is overexpressed in malignant cells compared to the normal ones (Figure 1 e) is in a good accordance with the variable data claiming a critical role of DNA pol β in carcinogenesis [6-17]. These data are about to offer a concept of cytostatic effect caused by a selective turning off the DNA pol β function in tumor as a part of the enzyme targeted cancer therapies [14,17-19]. Considering this point, a detail study on pure DNA pol β species isolated from cancer cells (Results) seems practically the same task as a study on the tumor specific molecular target for new drugs or pharmacophores [13,16,17].

Noteworthy, our purification procedure allows not only to separate some non-specific chromatin structural "ballast" proteins but to remove the DNA pol β peptide inhibitors [6,13,29] as well (Figure 1 and Table 2).

A known DNA polymerase beta - specific resistance to Aphidicolin [19,22,24] helps to trace an increase of its catalytic activity in crude biological extracts and subcellular fractions in a course of the multi-step enzyme purification procedure (Results, Table 2). This is an advantage of the inhibitor chase sufficient to turn off all DNA polymerases except for their beta subpopulation [22,24,29]. The UV-absorption spectra show some peculiarities (Figure 2). Thus, there is no marked absorption in a region close to 210 nm (peptide bond) at pH 5.20 while the maximum absorption was registered over there at pH 8.30. Since pH-optimum (8.0–see Materials and methods) and pI 8.45 (Figures 1b,1c and 1e) makes enzyme active, this protein is expected to show a high level of

Agent tested	DNA pol β activity, [^{3}H]DNA cpm/mg protein (M ± SEM, n=6)
Aphidicolin, 5.0 μg/mL	30,789 ± 398
N-ethyl-melamide, 0.5 mM	27,632 ± 437
ddTTP, 2.5 μM	1,370 ± 186
Trypsin, 20 μg/mL	207 ± 16
KCl, 200 mM	74,613 ± 441
No effectors added (optimized incubation mixture)	29,838 ± 322

Table 1: Catalytic activity of the beta-like DNA polymerase purified from HL60 chromatin: effects of inhibitors and potassium chloride.

Enzyme purification step	DNA pol β–specific catalytic activity, [^{3}H]DNA c.p.m./mg protein (M ± SEM, *n*=6)
Total cell homogenate	588 ± 63
Crude nuclei (P0.8)	1,864 ± 87
Crude chromatin	2,476 ± 118
Phenol-chloroform extraction	6,081 ± 202
Ammonium sulfate 30%-70% saturation gradient	10,112 ± 233
Diaflo Y5.0 ultrafiltration	19,423 ± 270
TOYOPEARL HW 55F Gel Chromatography	32,687 ± 331

Table 2: An increase of DNA pol β activity in a multi–step purification procedure.

nativity in UV-absorption spectra registered at pH 8.30. So the lack of 210 nm–absorbance at pH 5.20 reflects a mild misfolding occurred under acidic conditions [44]. Having a moderate-low UV-absorption peak nearby 280 nm (aromatic amino acids residues), we should pay attention to 225 nm–high absorption revealed at all pH tested (Figure 2) which is, most likely, relates to tryptophan contribution typical for some alpha helix–rich chromatin proteins [44]. UV-spectrophotometry as well as the CD-spectrometry data (Figure 3) reveals a nativity of enzyme at pH close to 8.0 and a high degree of the protein purity.

The ellipticity of the CD spectrum obtained is typical for the "far" UV (190–240 nm) and show the native structure of the alpha-protein with the secondary structure comprising insignificant quantity of the beta-fold elements [44,48]. So the enzyme studied is a predominantly alpha-helical monomer with a common for chromatin proteins alkaline pI point (Figures 1b,1c,1e,2 and 3). A superficial, UV-accessible, location of the Trp residues usually means a rigid globular structure enriched with the alpha helical elements [44,49] which corresponds to our results (Figure 2).

Conclusions

Human acute myeloblast leukemia cells, HL60, are found to be overexpressing a chromatin associated DNA polymerase beta–like enzyme that manifests the EC 2.7.7.7–specific activity being different from a vast majority of DNA pol β species by molecular mass (66.5 kDa) of the catalytic autonomous monomer.

For a complete purification of this enzyme, an original multi-extraction/gel chromatography procedure has been proposed.

References

1. Dianov GL, Parsons JL (2007) Coordination of DNA single strand break repair. DNA Repair 6: 454–460.
2. Parsons JL, Dianova II, Khoronenkova SV, Edelmann MJ, Kessler BM, et al. (2011) USP47 is a deubiquitylating enzyme that regulates base excision repair by controlling steady-state levels of DNA polymerase Î². Mol Cell 41: 609-615.
3. Gieseking S, Bergen K, Di Pasquale F, Diederichs K, Welte W, et al. (2011) Human DNA polymerase beta mutations allowing efficient abasic site bypass. J Biol Chem 286: 4011-4020.
4. Kidane D, Jonason AS, Gorton TS, Mihaylov I, Pan J, et al. (2010) DNA polymerase beta is critical for mouse meiotic synapsis. EMBO J 29: 410-423.
5. Aves SJ, Liu Y, Richards TA (2012) Evolutionary diversification of eukaryotic DNA replication machinery. Subcell Biochem 62: 19-35.
6. Falcieri E, Cataldi A, di Baldassarre A, Robuffo I, Miscia S (1996) Morphological patterns and DNA polymerase regulation in apoptotic HL60 cells. Cell Struct Funct 21: 213-220.
7. Bergoglio V, Pillaire MJ, Lacroix-Triki M, Raynaud-Messina B, Canitrot Y, et al. (2002) Deregulated DNA polymerase beta induces chromosome instability and tumorigenesis. Cancer Res 62: 3511-3514.
8. Albertella MR, Lau A, O'Connor MJ (2005) The overexpression of specialized DNA polymerases in cancer. DNA Repair (Amst) 4: 583-593.
9. Lang T, Dalal S, Chikova A, DiMaio D, Sweasy JB (2007) The E295K DNA polymerase beta gastric cancer-associated variant interferes with base excision repair and induces cellular transformation. Mol Cell Biol 27: 5587-5596.
10. Dalal S, Chikova A, Jaeger J, Sweasy JB (2008) The Leu22Pro tumor-associated variant of DNA polymerase beta is dRP lyase deficient. Nucleic Acids Res 36: 411-422.
11. Yang J, Parsons J, Nicolay NH, Caporali S, Harrington CF, et al. (2010) Cells deficient in the base excision repair protein, DNA polymerase beta, are hypersensitive to oxaliplatin chemotherapy. Oncogene 29: 463-468.
12. Ljungman M (2010) The DNA damage response--repair or despair? Environ Mol Mutagen 51: 879-889.
13. Kodvanj L, Aranyi J, Fesus N, Udvardi S (2004) Chromatin associated DNA polymerases and their inhibitors. Protocols in Mammalian Genome Research. Miskolc University Press: Miskolc – Sztged – Budapest.
14. Mizushina Y (2009) Specific inhibitors of mammalian DNA polymerase species. Biosci Biotechnol Biochem 73: 1239-1251.
15. Ljungmann M (2009) Targeting in the DNA damage response in cancer. Chem. Rev 109: 2929-2950.
16. Martin SA, McCabe N, Mullarkey M, Cummins R, Burgess DJ, et al. (2010) DNA polymerases as potential therapeutic targets for cancers deficient in the DNA mismatch repair proteins MSH2 or MLH1. Cancer Cell 17: 235-248.
17. Jaiswal AS, Banerjee S, Aneja R, Sarkar FH, Ostrov DA, et al. (2011) DNA polymerase Î² as a novel target for chemotherapeutic intervention of colorectal cancer. PLoS One 6: e16691.
18. Rechkunova NI, Lavrik OI (2010) Nucleotide excision repair in higher eukaryotes: mechanism of primary damage recognition in global genome repair. Subcell Biochem 50: 251-277.
19. Roettger MP, Bakhtina M, Kumar S, Tsai MD (2010) Catalytic mechanism of DNA polymerases. Comprehensive Natural Products. Part 2. Elsevier: Amsterdam.
20. Sobol RW, Horton JK, Kühn R, Gu H, Singhal RK, et al. (1996) Requirement of mammalian DNA polymerase-beta in base-excision repair. Nature 379: 183-186.
21. Podlutsky AJ, Dianova II, Podust VN, Bohr VA, Dianov GL (2001) Human DNA polymerase beta initiates DNA synthesis during long-patch repair of reduced AP sites in DNA. EMBO J 20: 1477-1482.
22. Sakaguchi K, Boyd JB (1985) Purification and characterization of a DNA polymerase beta from Drosophila. J Biol Chem 260: 10406-10411.
23. Cazillis M, De Recondo AM, Frayssinet C (1975) High molecular weight deoxyribonucleic acid polymerase of LF hepatoma. Purification and properties. Biochim Biophys Acta 407: 133-146.
24. Beard WA, Wilson SH (2006) Structure and mechanism of DNA polymerase Beta. Chem Rev 106: 361-382.
25. Olins AL, Herrmann H, Lichter P, Olins DE (2000) Retinoic acid differentiation of HL-60 cells promotes cytoskeletal polarization. Exp Cell Res 254: 130-142.
26. Roy MK, Thalang VN, Trakoontivakorn G, Nakahara K (2004) Mechanism of mahanine-induced apoptosis in human leukemia cells (HL-60). Biochem Pharmacol 67: 41-51.
27. Voss DO, Plaut GWE, Hagihara H, Clendenin JS (1967) Fractionation of chromatin compounds isolated from the Mammalian neoplastic cell nuclei. Meth Enzymol 10: 326-341.
28. Lerman MI, Abakumova EV, Podobed OV, Zlatopolsky AD (1976) Isolation and properties of the DNP- and RNP-particles from the Ehrlich ascite carcinoma cells. Advanced Methods in Biochemistry. Meditsina Publ. Moscow.
29. Matsumoto Y, Kim K (1995) The nuclear DNA polymerases beta: activity shifts and the DNA gaps beta–elimination control, Science 269: 699-702.
30. Piersen CE, Prasad R, Wilson SH, Lloyd RS (1996) on the 5',3'-deoxynucleotidyl transferase catalytic activity expressed by the nuclear DNA polymerase beta in Mammalian cells. J Biol Chem 271: 1781-1785.
31. Bradford MM (1976) An improved colorimetric technique for protein measurement. Analyt Biochem 72: 348-354.
32. Laemmli UL (1970) An efficient polyacrylamide gel electrophoresis system for proteins separation. Nature 227: 690-695.
33. Reichman ME, Rice SA, Thomask CA, Doty P (2002) Further examination of the molecular weight and size of deoxyribonucleic acid. J Amer Chem Soc 76: 3047- 3053.
34. Walker JM (1994) Isoelectric focusing of proteins in polyacrylamide gels. Methods in Molecular Biology. Series B. Springer Berlin Heidelberg.
35. Katoh R (2011) Analytical Techniques in Biochemistry and Molecular Biology. Springer: Berlin – Dortrecht – Heidelberg.
36. Görg A, Postel W, Westermeier R (1978) Ultrathin-layer isoelectric focusing in polyacrylamide gels on cellophane. Anal Biochem 89: 60-70.
37. Rule GS (1984) Quantitative assay of deoxyribonuclease activity after

isoelectric focusing in polyacrylamide gels: pH control and effects of enzyme diffusion. Anal Biochem 138: 99-106.

38. Mikami T, Satoh N, Hatayama I, Nakane A (2004) Buthionine sulfoximine inhibits cytopathic effect and apoptosis induced by infection with human echovirus 9. Arch Virol 149: 1117-1128.
39. Haratian K, Shamsi Shahrabadi M, Sardari S (2007) Buthionine sulfoximine inhibits cytopathic effects and apoptosis induced by infection with AIK-HDC strain of measles virus. Iran Biomed J 11: 229-235.
40. Fukami T, Uchiyama K, Yoshimura Y, Watanabe T, Nakazawa H (1996) Ultramicro-analysis by use of light-scanning photoacoustic densitometry for electrophoresed protein in human hair. Anal Biochem 238: 60-64.
41. Müller WE, Obermeier J, Totsuka A, Zahn RK (1974) Influence of template inactivators on the binding of DNA polymerase to DNA. Nucleic Acids Res 1: 63-74.
42. Varfolomeyev SD (2005) Enzymatic catalysis: kinetics, catalytic site structures, bioinformatics. Chemical and Biological Kinetics. Part 2: Biological Kinetics Chemistry Publ. Moscow.
43. Kuznetsov DA, Govorkov AV, Zavijalov NV, Sibileva TM, Richter V, et al. (1986) Fast estimation of ATP/ADP ratio as a special step in pharmacological and toxicological studies using the cell-free translation systems. J Biochem Biophys Methods 13: 53-56.
44. Finkelstein AV, Ptitsyn OV (2005) Physics of Proteins. University Publishing House (UPB): Moscow.
45. Kornberg A, Baker TA (2005) DNA Replication, (2ndedn.), Nucleic Acids Research Science Books Publ. New York.
46. Nunthawarasilp P, Petmitr S, Chavalitshewinkoon-Petmitr P (2007) Partial purification and characterization of DNA polymerase beta-like enzyme from Plasmodium falciparum. Mol Biochem Parasitol 154: 141-147.
47. Sungchul J (2012) Molecular Theory of a Living Cell. Springer. New York.
48. Dolgikh DA, Abaturov LV, Bolotina IA, Brazhnikov EV, Bychkova VE, et al. (1985) Compact state of a protein molecule with pronounced small–scale mobility: bovine alpha-lactalbumin. Eur Biophys J 13: 109-121.
49. Dolgikh DA, Lebedev YO, Tiktopulo EI, Ptitsyn OV (2000) Spectroscopy methods in protein structure studies, In: Advanced Techniques in Molecular Biology and Biophysics . Naukova Dumka.