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A new member of the dna polymerase beta superfamily in human myeloblast leukemia hl-60 cells

Dmitry A. Kuznetsov[2], Alexander A. Bukhvostov[1], Alexey P. Orlov[2] and Oleg A. Shatalov[3]
[1]I.M. Sechenov Moscow State Medical University, Russia
[2]N. I. Pirogov Russian National Research Medical University, Russia
[3]Moscow Regional Pedagogical University, Russia

HL-60 cell nuclear chromatin proteins were fractionated using an original procedure involving a consequent nuclease S / RNase A treatment, 0.5M NaCl/phenol – chloroform extraction, multiple acetone precipitation / pellet re-washing, nuclease S re-treatment, thermal – ultrasound incubation (80 KHz, 60^0 C, 30 min), and a scalar 30% - 70% ammonium sulfate saturation gradient application. The re-dissolved samples were subjected for gel filtration (TOYOPEARL HW 55F column, 1.6 x 70 cm). The DNA polymerase beta activity was found distributed within the 64 – 70 kDa peak; its SDS-PAGE analysis revealed a single band homogenous 66.5 kDa enzyme fraction. Once compared to a native SDS-free PAGE pattern, the monomer nature of enzyme has been confirmed. The resulting purification extent has been found equal to 122,000-fold, as corrected to a total cell protein. Being catalytically active monomer, which itself a very unique peculiarity for a beta family member, the enzyme purified was proven to be possessing such the beta – specific (EC 2.7.7.7) patterns as: (a) the marked capability to synthesize DNA sequences limited by size to 72 –260 nucleotides, (b) isoelectric point, 8.45, (c) over-activation in the presence of 200 mM KCl, (d) high resistance to N-ethyl-melamide (0.5 mM) and Aphidicolin (4-5 microgram/mL), (e) total lack of any 3',5'-exonuclease activity traces. Noteworthy, a uniqueness of the enzyme studied is also about its unusual molecular size (66.5 kDa) which has been found far larger as compared with the vast majority of species belonging to a beta super-family (30 – 45 kDa).

Biography

Dmitry A. Kuznetsov (born 1955), Ordinary Professor with the Department of Medicinal Nanobiotechnologies, Faculty of Biomedicine, N. I. Pirogov Russian National Research Medical University, Moscow, Russia. PhD in biochemistry (1981, M. I. Lomonosov Moscow State University, Moscow, USSR), DSc in biochemistry/pharmacology (1989, USSR Academy of Medical Sciences Institute for Nutrition Research, Moscow, Russia). Area of scientific interests covers chemical enzymology of metal-dependent phosphate transfer enzymes (nucleotidyl kinases, DNA polymerases, RNA polymerases) and their role in carcinogenesis including responses to the novel tumor specific enzyme cytostatic inhibitors.

kuznano@mail.ru