The Accessory Subunit of Mammalian DNA Polymerase γ Is a Functional Homodimer

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Introduction: Mitochondrial DNA is replicated and repaired by a nuclear-encoded DNA polymerase, Polγ, distinct from the polymerases that replicate and repair nuclear DNA. Polγ is composed of two subunits, a catalytic subunit of 125-140 kDa and an accessory subunit of 35-51 kDa. The small subunit, PolγB, has been characterized as a processivity factor for the polymerase [1-4]. Upon interaction with the catalytic subunit, PolγB increases the affinity of the polymerase for DNA and promotes tighter nucleotide binding, increasing the polymerization rate.

Methods and Materials: The crystal structure was solved at a resolution of 1.95 Å using multiple anomalous diffraction (MAD) of a crystal of selenomethionine PolγB in combination with data of a native crystal to 2.25 Å resolution and non-crystallographic symmetry averaging. Crystals were analyzed at the National Synchrotron Light Source at the Brookhaven National Laboratory, beam lines X26c and X25. Mouse SeMet PolγB crystals belong to space group P2₁2₁2₁ with unit cell dimensions a=96.62 Å, b=133.42 Å, c=135.04 Å and α=β=γ=90°. Crystals were measured at three wavelengths close to the Se absorption edge chosen to maximize f'' and delta f' of Se. Data collection for the isomorphous native crystals was carried out at a wavelength of 1.1 Å.

Deletions mutants of human PolγB lacking solvent exposed loops and hairpins were designed based on the structure, expressed and purified. They were characterized by native gel electrophoresis in the presence of different DNA constructs and/or PolγA and by glycerol gradient sedimentation.

Results: The 1.95 Å crystal structure of mouse PolγB shows high similarity to the glycyl-tRNA synthetase fold. However, residues thought to be required for tRNA synthetase activity are not conserved in PolγB. PolγB forms a homodimer stabilized by a unique intermolecular four-helix bundle. A human PolγB mutant lacking the four-helix bundle failed to dimerize in solution, lost its PolγA-stimulating activity but retained the ability to bind with PolγA to a primer-template construct, indicating that the functional holoenzyme contains two molecules of PolγB. Two mutants lacking surface β-hairpin motifs retained activity as a processivity factor, but lost the ability to bind folded ssDNA.

Conclusions: We report the first evidence that the accessory subunit of mitochondrial DNA polymerase, PolγB, is functional as a homodimer and associates with one copy of the catalytic subunit in a heterotrimeric holoenzyme. The evolutionary relationship of PolγB to aminoacyl tRNA synthetases is reflected in conservation of nucleic acid binding properties, since surface loops involved in tRNA recognition by aaRS appear to be important for the interaction of PolγB with folded ssDNA. As a processivity factor, PolγB exhibits unique properties. It is clearly distinct from the sliding clamps like PCNA and has little structural similarity to thioredoxin. The ability of PolγB to bind DNA suggests a parallel to the herpes virus DNA pol processivity factor, UL42, although the detailed DNA binding properties and structures differ considerably.

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References: