Biochemical and functional characterization of the DNA polymerase encoded by bacteriophage vB_EfaS-271

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Most DNA polymerases lose catalytic efficiency as temperature drops below 20°C, creating a fundamental barrier for field-deployable isothermal diagnostics that must operate without active heating. This work began with a gene found in the genome of bacteriophage vB_EfaS-271, isolated from Gdańsk sewage, encoding a previously uncharacterized B-family DNA polymerase designated Efa. The enzyme proved remarkably cold-active: primer extension assays showed robust DNA synthesis from -5°C to 50°C. Direct comparisons at -10°C revealed that Efa generates a full-length product, whereas T4, T7, and E. coli Pol I remain essentially inactive. This raised a compelling mechanistic question: what molecular adaptations allow Efa to catalyze DNA synthesis when most polymerases cease to function?

Sequence analysis and structural prediction provided the first clue. Efa possesses a canonical B-family polymerase core (palm, fingers, thumb, and 3' to 5' exonuclease domains) but carries an unexpected N-terminal extension (residues 27-124) that folds into an oligonucleotide-binding (OB) fold, a motif normally found in single-stranded DNA-binding proteins such as bacterial SSB or eukaryotic RPA. To probe its function, we generated a truncated variant, Efa □N, lacking this domain. At 10°C, wild-type Efa incorporated nucleotides under single-turnover conditions with an observed rate constant of 27.3 s-1, whereas Efa □N was more than 500 times slower (0.05 s-1). On structured hairpin templates, wild-type Efa extended through secondary structure to produce full-length product, while the truncated mutant accumulated exclusively at the stall site.

X-ray crystallography revealed the structural basis of this cold adaptation. The apoenzyme structure at 2.3-Å resolution showed the N-terminal OB-fold perched above the polymerase core. Ternary complexes with primer-template DNA captured two distinct conformational states: an engaged state, in which the OB-fold clamps a 6-nucleotide 5-prime overhang through contacts involving K53, Y74, R79, and F105, and a loose state (with a 4-nucleotide overhang), in which the domain swings away by 4 Ångströms and rotates by 22 degrees. This structural switching translates directly into kinetic control. On long overhangs, wild-type Efa displayed rapid single-nucleotide incorporation. Shortening the overhang to 4 nucleotides reduced kobs by approximately 130-fold, collapsing its behavior to that of the truncated mutant. Arrhenius analysis showed that N-terminal engagement was associated with a different apparent temperature dependence of the observed reaction under the conditions tested.

Together, these data support a model in which Efa is, to our knowledge, the first structurally characterized cold-active B-family polymerase from a bacteriophage and reveal that its N-terminal OB-fold functions as an integrated SSB module that stabilizes single-stranded DNA and accelerates nucleotide addition at low temperature. This modular architecture, a conventional polymerase core coupled to an accessory DNA-binding domain that dynamically engages the template depending on overhang length, provides a natural blueprint for engineering multi-domain polymerases with tailored temperature profiles. Efa's unique properties position it as a promising platform for developing low-temperature isothermal amplification technologies that could operate at ambient temperature without specialized heating equipment, expanding access to molecular diagnostics in resource-limited settings.

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