Substitution of Asn for Ser^{543} in the large fragment of *Taq* DNA polymerase increases the efficiency of synthesis of long DNA molecules

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Abstract Substitution of Asn for Ser^{543} in the large fragment of *Taq* DNA polymerase (*Klentaq*) increases several times the efficiency of synthesis of long (over 2 kbp) DNA molecules. The difference in the DNA synthesis efficiencies by the mutant and native enzymes increased with the increase in the DNA fragment length.

Key words: Polymerase chain reaction; *Taq* DNA polymerase; *Klentaq* DNA polymerase; *Tth* DNA polymerase

1. Introduction

DNA polymerases from *Thermus aquaticus* YT-1 (*Taq* DNA polymerase) and *Thermus thermophilus* HB8 (*Tth* DNA polymerase) belong to the group of *Escherichia coli* DNA polymerase I (Pol I)-like prokaryotic DNA polymerases. As thermostable enzymes, these polymerases are widely used in polymerase chain reaction (PCR). Despite a high similarity (identity of amino acid sequences 87%), *Taq* and *Tth* polymerases differ in their properties. Thus, *Tth* polymerase synthesizes long DNA molecules with a higher efficiency [1–3]. Earlier, we demonstrated that this property of *Tth* DNA polymerase is determined by the amino acid sequence of the 'thumb' subdomain of the enzyme [3], which is known to play an important role in the enzyme interaction with the primer–template duplex [4–8]. Comparison of amino acid sequences of the 'thumb' subdomains of *Taq* and *Tth* polymerases [3] and the X-ray data on the *Taq* DNA polymerase complex with DNA [8] revealed that of all non-identical residues, only Ser^{543} in *Taq* DNA polymerase is directly involved in the interactions with DNA. In *Tth* DNA polymerase, this residue is Asn^{545}. This allowed us to suggest that substitution of Asn for Ser^{543} may significantly increase the efficiency of synthesis of long DNA molecules by *Taq* DNA polymerase. This work was designed to study the effect of the S543N mutation on the capacity of the *Taq* DNA polymerase large fragment (*Klentaq* polymerase [9]) to synthesize DNA molecules of different lengths.

2. Materials and methods

2.1. General

A fragment of the *Taq* DNA polymerase gene encoding *Klentaq* polymerase [9] was cloned into pUC18 under the control of the *lac* promoter. The codon AGC for Ser^{543} was replaced by the codon AAC encoding Asn by means of PCR mutagenesis [10]. The nucleotide sequence of the mutant plasmids was verified by sequencing.

The genes for *Klentaq* polymerase and *KlentaqN* polymerase (containing the S543N mutation) were expressed in *E. coli* JM 109 cells. The enzymes were isolated and purified as described in [11].

2.2. Polymerase activity assay

The amount of enzyme that incorporated 10 nmol of total deoxyribonucleotides in the acid-insoluble fraction within 30 min under conditions described below was taken as one unit of activity.

The reaction mixture (50 μl) contained: 25 mM TAPS (pH 9.3), 50 mM KCl, 3.5 mM $MgCl_2$, 1 mM β-mercaptoethanol, 0.2 mM of each dNTP, 1 μCi [α-^{32}P]dATP, and 12.5 μg of activated salmon sperm DNA. Salmon sperm DNA (12.5 mg/ml) was activated in 10 mM Tris-HCl (pH 7.2) containing 5 mM $MgCl_2$ with pancreatic DNase I (0.03 U/ml) at 4°C for 1 h and then heated at 95°C for 5 min.

The polymerase activity was assayed at 73°C from the incorporation of the radioactively labeled nucleotide in the acid-insoluble fraction within 10 min.

2.3. PCR amplification

PCR was performed in a DNA Thermal Cycler 480 (Perkin-Elmer-Cetus). The reaction mixture (25 μl) contained 67 mM Tris-HCl (pH 8.8), 16.6 mM $(NH_4)_2SO_4$, 0.01% Tween 20, 0.2 mM of each dNTP, 3.5 mM $MgCl_2$, 10 pmol of each primer, 10 ng of λ DNA as a template, and 5 U of either *Klentaq* or *KlentaqN* DNA polymerase. The reaction included 25 cycles: 94°C for 30 s; 58°C for 30 s; 72°C for 100 s.

λ DNA fragments were amplified with the primers P1 (5′-GATGAGTTCGTGTCCGTACAACTGG) and P2 (5′- GGTTATCGAAATCAGCCACAGCGCC) (a 500-bp fragment); P1 and P3 (5′-TTCCCAGCCACACGCTGCATGACAT) (a 1270-bp fragment); P1 and P4 (5′-TAGCTGTCGTCATAGGACTCAGCG) (a 2000-bp fragment); and P1 and P5 (5′-TGTTGACCTTGCCTGCAGCAACGC) (a 2500-bp fragment).

3. Results

The capacity of the mutant polymerase (*KlentaqN*) containing Asn at position 543 to synthesize λ DNA fragments of different lengths was compared to that of *Klentaq* polymerase. The efficiency of synthesis was determined from the amount of the PCR-amplified DNA molecules. The data on the amplification of λ DNA fragments of 500, 1270, 2000, and 2500 bp are shown in Fig. 1. It was found that for both polymerases, the amounts of the synthesized 500- and 1270-bp fragments were the same, whereas the amounts of the 2000- and 2500-bp fragments were higher in the reactions with the mutant enzyme. Note that the maximum difference in the product yields was observed for the 2500-bp fragment, which suggests that the difference in the efficiency of DNA synthesis by *Klentaq* polymerase and its mutant form would increase with the increase in the amplified fragment length.

The efficiency of 2500-bp λ DNA fragment synthesis was assayed. Different amounts of *Klentaq* or *KlentaqN* DNA polymerases were added to the reaction mixture, and the yields of

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Abbreviations: S543N, mutation Ser^{543} to Asn; PCR, polymerase chain reaction

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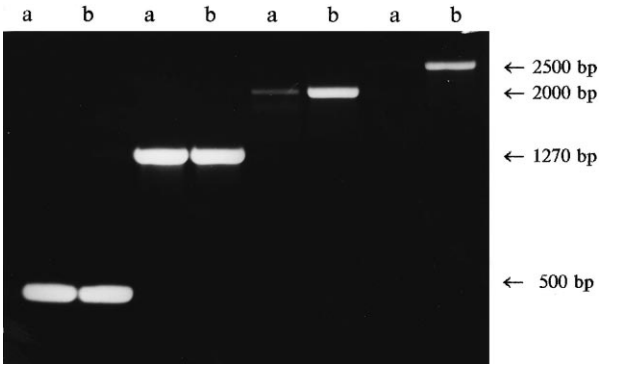

Fig. 1. Electrophoretic analysis of PCR amplification products of phage λ DNA fragments (500–2500 bp) in 1.2% agarose gel. The fragments were amplified with (lanes a) *Klentaq* and (lanes b) *KlentaqN* DNA polymerases.

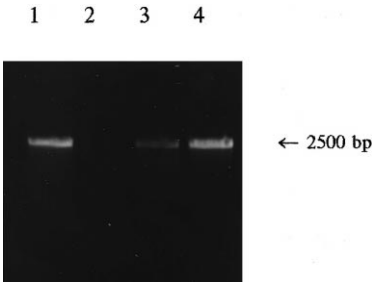

Fig. 2. Electrophoretic analysis of PCR amplification products of phage λ DNA fragment (2500 bp) in 1% agarose gel: (lane 1) 4 U of *KlentaqN* DNA polymerase; (lanes 2–4) 4, 7, and 10 U of *Klentaq* DNA polymerase, respectively.

the synthesized DNA fragment were compared. Fig. 2 shows that similar yields of the amplified fragment were obtained when 10 U of *Klentaq* polymerase or only 4 U of the mutant enzyme were used.

4. Discussion

In this work, we studied the effect of the Ser^{543}Asn substitution on the capacity of the large fragment of *Taq* DNA polymerase to synthesize long DNA molecules.

The fact that Ser^{543} is a highly conserved residue suggests its essential role in DNA synthesis. Comparison of amino acid sequences of 27 DNA-dependent DNA polymerases belonging to different groups [4] revealed that only six of them contain no serine at this position in the 'thumb' subdomain.

The significance of this residue for the polymerase interaction with DNA was demonstrated in studies of the conserved fragment of the phage ϕ29 DNA polymerase 'thumb' subdomain [12]. Thus, substitution of Gly for Ser^{388} (which corresponds to Ser^{543} in *Taq* DNA polymerase [4]) resulted in a 10-fold decrease in the efficiency of the phage ϕ29 DNA replication, although the polymerase activity with short DNA templates remained high [12]. It appeared that substitution of Ser deteriorates the interactions between the polymerase and the primer–template duplex, and consequently decreases the efficiency of synthesis of long DNA molecules.

Earlier, we demonstrated that the higher efficiency of *Tth* DNA polymerase in the synthesis of long DNA molecules as compared to that of *Taq* DNA polymerase is determined to a considerable extent by the difference in the amino acid sequences of the 'thumb' subdomains of these enzymes [3]. In particular, *Tth* DNA polymerase contains Asn^{545} at the position corresponding to Ser^{543} in *Taq* DNA polymerase. Considering the high extent of homology between these polymerases and the fact that Ser at the corresponding position is a highly conserved residue, we suggest that particularly Asn^{545} (instead of Ser) determines the higher efficiency of *Tth* DNA polymerase in the synthesis of long DNA molecules. Actually, we found that substitution of Asn for Ser^{543} considerably increased the efficiency of synthesis of long DNA molecules by *Klentaq* DNA polymerase.

X-ray data on the *Taq* DNA polymerase complex with DNA demonstrated the direct involvement of Ser^{543} in the enzyme binding to the primer–template duplex [8]. The substitution of Asn for Ser^{543} probably alters the length and geometry of hydrogen bonds formed by this residue and phosphate groups of the template and affects the capacity of the enzyme to synthesize long DNA molecules.

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