Influence of Cd707 Ile→Leu substitution in N-terminal deletional variant of Taq DNA polymerase on stability and activity of the enzyme

Katarina Davalieva and Georgi D. Efremov

Research Centre for Genetic Engineering and Biotechnology, Macedonian Academy of Sciences and Arts,Krste Misirkov 2, Republic of Macedonia, E-mail: gde@manu.edu.mk

INTRODUCTION

The success, yield and specificity of the PCR, particularly for amplifications involving high cycle number, high CG content of the template and multiple primer pairs is affected by the DNA polymerase activity at room temperature. One way of preventing this activity is by genetic modifications of the DNA polymerase. For Taq DNA polymerase, mutations in the gene (Glu626Lys, Trp706Arg, Ile707Leu and Glu708Asp), were found to contribute to the significant decrease of the enzyme activity at room temperature, without compromising processivity at the optimal temperature (72°C) and thermostability at 95°C.

AIM OF THE STUDY

The aim of this study was to evaluate the usefulness of Ile707Leu cold-sensitive mutation in the N-terminal deletional variant of Taq DNA polymerase (Klentaq278) in PCR reaction and the influence of the mutation on overall stability of the enzyme.

MATERIALS AND METHODS

Construction and purification of Klentaq278 and Klentaq278(Ile707Leu

Klentaq278 is an N-terminal deletional variant of Taq polymerase, which lacks 278 amino acids from the N-terminus of the full-length wild-type enzyme. A fragment of 1668 bp containing the Klentaq278 gene was cloned into expression vector pGex-6P-1 (Amersham) and expressed as a fusion protein with glutathione S-transferase (GST). The nucleotide substitution ATT->CTT in codon 707 of the native Taq DNA polymerase gene was introduced in Klentaq278 gene by the method of overlap mutant primers (Figure 1). The fusion proteins were purified to homogeneity by affinity chromatography on Glutathione Sepharose 4 Fast Flow (Amersham). The thermostable polymerases had specific activity of 20,000 U/mg.

Polymerase activity at room temperature and thermostability test

Polymerase units were determined according to the standard definition of polymerase units (incorporation of 10 nmol of nucleotides in 30 min at 75oC). DNA polymerase activity at room temperature was assayed by standard DNA polymerase activity assay at 25°C and 75°C and was calculated as a fraction of the polymerase activity at 75°C. Thermostability of the polymerases were assayed for 1 hour at 95°C and the residual activity were determined by DNA polymerase activity assay.

PCR assay and incubation conditions

The PCR mixture (50 µl) contained PCR buffer (50 mM Tris-HCl, 2.5 mM MgCl2, 16 mM (NH4)2SO4, 0.1% Tween 20, pH 9.2), 3.5 mM $MgCl_2$, 200 µM dNTP, 20 pmol of forward and reverse primers and 100 ng human DNA. DNA polymerases (2 U) were added to the PCR mixture either by cold start during PCR set up or by hot start at 80°C. The influence of Na, K and Mg ions on the amplification capacities of the tested polymerases was assayed by PCR amplification of 631 bp fragment.

RESULTS

The Klentaq278(Ile707Leu) DNA polymerase showed reduced polymerase activity at room temperature for 12 times and approximately 88% of Klentaq278 thermostability. This substitution in the enzyme reduced twice the maximum tolerance of K and Mg ions, ten times the tolerance of Na ions and ten times the enzyme tolerance of blood and phenol as PCR inhibitors (Table 1). The major effect of the amino acid substitution was the reduction of the amplification capacity of the polymerase. When we tested the two DNA polymerases for the amplification of various length DNA fragments from the human β-globin gene (Figure 2), Klentaq278 DNA polymerase amplified all the fragment lengths while, Klentaq278 (Ile707Leu) DNA polymerase had limited performance, and could not amplify fragments of 1360 bp and 1677 bp, neither by cold or hot start.

Table 1. Effect of different concentrations of Na+, K+ and Mg2+ ions on the amplification capacities* of Klentaq278 and Klentaq278(Ile707Leu) thermostabile DNA polymerases

	mM	*Klentaq278*	*Klentaq278 (Ile707Leu)*
NaCl	0	+	+
	5	+	-
	10	+	-
	20	+	-
	40	-	-
	60	-	-
	80	-	-
	100	-	-
KCl	0	+	+
	5	+	+
	10	+	+/-
	20	+	-
	40	-	-
	60	-	-
	80	-	-
	100	-	-
$MgCl_2$	3,5	+	+
	4,5	+	+
	5,5	+	+
	8,5	+	+/-
	13,5	+/-	-
	23,5	-	-
	33,5	-	-

*PCR amplification of 631 bp fragment of human β-globin gene. The results were scored as follows: (+) strong intensity of PCR product on agarose gel; (+) weak intensity of PCR product on agarose gel; (-) no visible PCR product on agarose gel.

Cd (Taq gen)	701	702	703	704	705	706	**707**	708	709	710	711	712	713	
M707-F				5'-	GCC	TGG	**CTT**	GAG	AAG	ACC	CTG	-3'		
Taq gen 5'-	GGG	AAG	GTG	CGG	GCC	TGG	**ATT**	GAG	AAG	ACC	CTG	GAG	GAG	-3'
M707-R		3'-	CAC	GCC	CGG	ACC	**GAA**	CTC	TTC	-5'				

Figure 1. Design of overlap mutant primers for ATT->CTT mutation in Cd 707 of Taq DNA polimerase gene

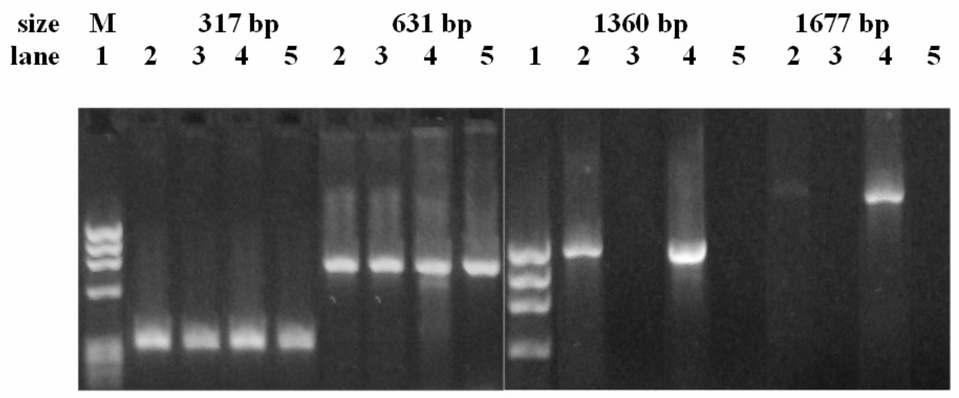

Figure 2. PCR amplifications of various human β-globin gene fragments with Klentaq278 and Klentaq278(Ile707Leu) polymerases. Lane 1-marker IX (Amersham); lane 2- Klentaq278 polymerase with cold start; lane 3- Klentaq278 (Ile707Leu) polymerase with cold start; lane 4- Klentaq278 polymerase with manual hot start; lane 5- Klentaq278 (Ile707Leu) polymerase with manual hot start.

CONCLUSION

The mutation Ile707Leu in Klentaq278 DNA polymerase reduces the overall processivity and stability of the enzyme and limits the application of this DNA polymerase in PCR only to small fragments. Klentaq278 (Ile707Leu) DNA polymerase is suitable for amplification of single or multiple fragments without hot start but only up to 1 kb length.

ACKNOWLEDGMENTS

This work was supported by the funds for science of the Macedonian Academy of Sciences and Arts (No.09-40/12), Skopje, Republic of Macedonia.