

Short communication

The influence of polyamines on polymerase chain reaction (PCR)*

Paweł Fiedorow and Zofia Szweykowska-Kulińska[✉]

Department of Gene Expression, Adam Mickiewicz University in Poznań, Międzychodzka 5, 60-371 Poznań, Poland

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Twelve different polyamines from three functional groups have been tested for their influence on polymerase chain reaction (PCR). Using crude total DNA from the liverwort *Pellia borealis* we have found that tri- and tetramines can strongly improve the efficiency of PCR.

Polymerase chain reaction became a very useful tool in many fields of molecular biology. One of the most difficult steps during PCR amplification of total DNA is to prepare a template of a quality good enough to obtain a specific PCR product. The isolation of the contaminant-free DNA is particularly difficult when one works with plant tissue material. The most common contaminants of plant DNA are polyphenols and polysaccharides which can decrease PCR specificity, efficiency or even completely inhibit the reaction. Problems with contaminants which inhibit PCR amplification were described in many publications [1-6]. Several DNA isolation methods have been developed, but the only method appropriate for obtaining contaminant-free DNA, is caesium chloride density gradient centrifugation. However, this method is rather expensive and time consuming. First information about the influence of spermidine (triamine) on PCR was published

by Wan & Wilkins in 1993 [2]. They found that spermidine at a proper concentration can suppress PCR inhibition or increase the quantity of PCR product amplified from crude, total cotton DNA purified by conventional methods (using RNase A and phenol/chloroform/isoamyl extraction). Polyamines bind tightly to negatively charged phosphate groups of RNA or DNA [7]. In mammalian cells, polyamines were shown to have a positive influence on initiation of DNA synthesis [8]. This effect is probably directly related to the affinity of polyamines to DNA. In order to study the influence of polyamines on PCR, we have used twelve different polyamines, which were added at three different concentrations to the PCR mixture.

Conventionally-purified total, crude DNA template from the liverwort *Pellia borealis* was used. PCR products could not be obtained from this template, probably because of the presence of polyphenol and terpenoid

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[✉]To whom correspondence should be addressed, Z. Szweykowska-Kulińska, Department of Gene Expression, A. Mickiewicz University in Poznań, Międzychodzka 5, 60-371 Poznań, tel./fax. (61) 61-55-96; E-mail: zofszwey@hum.amu.edu.pl.

contaminants. We have managed to overcome the PCR inhibition by addition of different polyamines. Our results show that polyamines work most probably universally on crude DNA preparations enabling PCR to proceed.

MATERIALS AND METHODS

Isolation of the liverwort *Pellia borealis* total DNA. DNA was extracted from 100 mg of haploid thallus of the liverwort *P. borealis* (grown *in vitro* in sterile conditions), according to Junghans & Metzclaff's procedure [9] with the following modifications: the DNA was resuspended in 200 μ l of H₂O containing 200 μ g/ml RNase A (TNE buffer was not used because it contains EDTA which can bind Mg²⁺ ions, important in PCR). Prior to the DNA precipitation by 96% ethanol, 0.1 volume of 3 M sodium acetate, pH 4.8, was added. After centrifugation DNA was resuspended in 80 μ l H₂O. Such a preparation gives usually about 10 ng/ μ l of *P. borealis* total DNA. The quality and size of the DNA were determined by 0.8% agarose gel electrophoresis, the quantity was measured spectrophotometrically.

PCR amplification of total DNA. PCR reaction mixture contained following components: 10 ng of total DNA per 10 μ l reaction [10], 0.6 μ M of each primer [11], 10 mM Tris/HCl (pH 8.8 at 25°C), 1.5 mM MgCl₂, 50 mM KCl, 0.1% (v/v) Triton X-100, 200 μ M of each dNTP [11], and 0.012 units of Prime Zyme DNA polymerase (Polygen Wrocław, Poland) or Boehringer Mannheim Taq DNA polymerase per microliter of reaction mixture. Two synthetic primers specific for plant tRNA^{Leu} gene, were used [12]. Both primers were 32 nucleotide-long and contained sequences generating *Bam*HI and *Eco*RI restriction sites on 5' end and 3' end of PCR product respectively:

(5'-CGGGATCCGTCAG-
GATGGCCGAGTGGTCTAAG-3')

(5'-CAGAATTCTGTCAGAAGTGGG-
ATTTGAACCCA-3')

We used also two RAPD (Random Amplified Polymorphic DNA) primers:

(5'-GTCCCGACGA-3')

(5'-AACGTAGGCC-3')

PCR was carried out in an Eppendorf cycler. For tRNA^{Leu} primers a two-step amplification was used: the initial denaturation at 95°C for 5 min was followed by 3 cycles of: 1 min denaturation at 94°C, 1 min hybridization at 37°C and 1 min elongation at 72°C, and 30 cycles of: 1 min at 94°C, 1 min at 52°C and 1 min at 72°C. The PCR was terminated by an elongation cycle of 5 min at 72°C. PCR with RAPD primers was carried out as follows: the initial denaturation at 95°C for 5 min was followed by 50 cycles of: 1 min denaturation at 92°C, 2 min hybridization at 35°C and 2 min elongation at 72°C. The PCR was terminated by an elongation cycle of 5 min at 72°C. PCR reactions with tRNA^{Leu} primers were performed in the presence of different polyamines at concentrations of: 0.3, 1.0 and 3.0 mM. We have used twelve different polyamines belonging to the following three groups:

Diamines

ethylenediamine [en]	H ₂ N(CH ₂) ₂ NH ₂
1,2-diaminopropane	CH ₃ CH(NH ₂)CH ₂ NH ₂
1,3-diaminopropane [tn]	H ₂ N(CH ₂) ₃ NH ₂
1,4-diaminobutane [putrescine]	H ₂ N(CH ₂) ₄ NH ₂
1,5-diaminopentane [cadaverine]	H ₂ N(CH ₂) ₅ NH ₂
1,6-hexanediamine	H ₂ N(CH ₂) ₆ NH ₂

Triamines

diethylenetriamine [dien or 2,2-tri]	H ₂ N(CH ₂) ₂ NH(CH ₂) ₂ NH ₂
<i>N</i> -(2-aminoethyl)-1,3-propanediamine [2,3-tri]	H ₂ N(CH ₂) ₃ NH(CH ₂) ₂ NH ₂
<i>N</i> -(3-aminopropyl)-1,3-propanediamine [3,3-tri]	H ₂ N(CH ₂) ₃ NH(CH ₂) ₃ NH ₂
spermidine	H ₂ N(CH ₂) ₄ NH(CH ₂) ₃ NH ₂

Tetramines

<i>N,N'</i> -bis(3-aminopropyl)-1,3-propanediamine [3,3,3-tet]	$\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_3\text{NH}_2$
spermine	$\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}(\text{CH}_2)_3\text{NH}_2$

PCR products were analyzed on 4% agarose (Sea Plaque GTG produced by FMC Bio Products) or 2% agarose (Promega) gels.

RESULTS AND DISCUSSION

Crude (non purified on caesium chloride gradient) total DNA from the liverwort *P. borealis* completely inhibited amplification by PCR (Fig. 1, lane 13, and Fig. 2, lane 13). The inhibitory effect was probably due to the presence of polyphenol and terpenoid compounds. We found that addition of crude total liverwort DNA to the PCR reaction mixture which contained tRNA^{Leu} gene tem-

hornwort (*A. crispulus*) and liverwort (*P. borealis*) DNA template has a similar or even the same origin. Many publications indicate that polyphenols, polysaccharides and terpenoids are the main factors that inhibit PCR carried out with plant total DNA template [1, 3, 4, 6]. In order to overcome this problem we tested the effect of twelve polyamines at different concentrations on the crude template from *P. borealis*. We have found that 0.3 mM concentration of tri- or tetramines (2,3-tri, 3,3-tri, spermidine, 3,3,3-tet and spermine) suppressed the PCR inhibitory effect (Fig. 2, lanes 2–6), the only exception being the triamine 2,2-tri (Fig. 2, lane 1). In PCR samples where tri- or tetramines (except 2,2-tri) were added we obtained a product of the expected size of about 90-bp, which indicates amplification of liverwort tRNA^{Leu} genes. The same experiment was carried out with 1 mM polyamine concentration. In this case the efficiency of PCR was even higher. We have obtained the product of expected size



Figure 1. Electrophoresis on 4% agarose gel of PCR products using crude *P. borealis* DNA as a template and tRNA^{Leu} primers.

M, relative molecular mass standard: pUC19 digested with *Hae*III (80, 102, 174, 257/267, 298, 434/458, 587 bp fragments). All polyamines were used at 1 mM concentration. Diamines — 1, 2, 3, 4, 5, 6 (en, 1,2-diaminopropane, tn, putrescine, cadaverine, 1,6-hexanediamine, respectively); triamines — 7, 8, 9, 10 (2,2-tri, 2,3-tri, 3,3-tri, spermidine, respectively); tetramines — 11, 12 (3,3,3-tet and spermine, respectively); 13, sample with total DNA template without any polyamine, shows suppression of PCR caused by total DNA contaminants.

plate cloned into a plasmid, completely inhibited PCR. Without liverwort DNA, PCR on a plasmid template worked perfectly (not shown). A very similar PCR inhibitory effect, caused by impurities of total DNA template preparations, was found in the hornwort *Anthoceros crispulus* [13]. Hornworts form a small class of bryophytes with an unclear but close relationship to liverworts and mosses. It is possible that PCR inhibition using the

(90-bp) in every PCR sample to which tri- or tetramines (2,2-tri, 2,3-tri, 3,3-tri, spermidine, 3,3,3-tet, spermine) were added (Fig. 1 lanes 7–12). Using 1 mM concentration we have obtained the PCR product even with 2,2-tri (triamine), which was not able to suppress PCR inhibition at 0.3 mM concentration (Fig. 1, lane 7). PCR samples containing 1 mM diamines did not give any product (Fig. 1 lanes 1–6). At 3 mM concentration of the

polyamines in the PCR mixture the expected product was obtained only with spermidine (triamine) (Fig. 2, lane 10). In addition we have found that the presence of diamines at both 0.3 or 3 mM concentration did not induce PCR amplification (not shown). The

also increase the specificity of amplification; 1 mM spermidine in the PCR reaction mixture suppressed the amplification of unspecific PCR products of apolipoprotein B-100 gene, and led to a single product of the expected size (R.D. Plewa, personal communi-



Figure 2. Electrophoresis on 4% agarose gel of PCR products using crude *P. borealis* DNA as a template and tRNA^{Leu} primers.

M, molecular weight standard: pUC19 digested with *Hae*III (80, 102, 174, 257/267, 298, 434/458, 587 bp fragments). Concentration of polyamines: 0.3 mM, triamines — 1, 2, 3, 4 (2,2-tri, 2,3-tri, 3,3-tri, spermidine, respectively); tetramines — 5, 6 (3,3,3-tet, spermine respectively); concentration of polyamines: 3 mM, triamines — 7, 8, 9, 10 (2,2-tri, 2,3-tri, 3,3-tri and spermidine, respectively); tetramines — 11, 12 (3,3,3-tet and spermine, respectively); 13, sample with total DNA template without any polyamine.

effect of polyamines on PCR was tested with other pair of primers. For this purpose RAPD primers were used (see Materials and Methods) and the same effect was found of spermidine at 1 mM concentration on amplification of *P. borealis* crude total DNA template (Fig. 3). The obtained PCR products were of

cation). The apolipoprotein B-100 gene was amplified from crude total human DNA. Thus it seems that spermidine can improve the PCR carried out with animal DNA as a template. The mechanism which enables polyamines to improve the specificity and efficiency of PCR is still unknown. Many

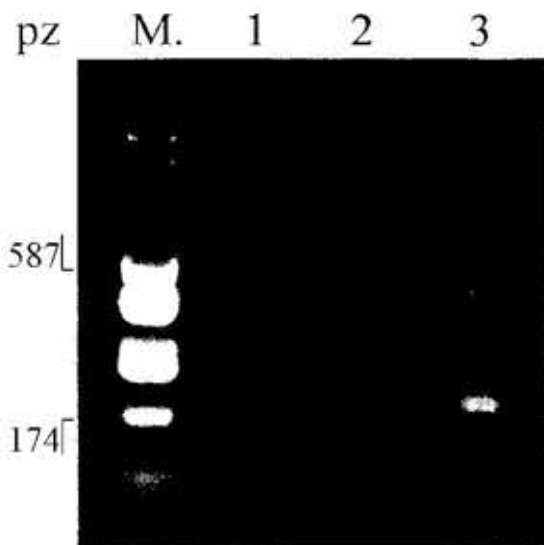


Figure 3. Electrophoresis on 2% agarose gel of PCR products using crude *P. borealis* DNA as a template and RAPD oligonucleotides as primers.

M, relative molecular mass standard: pUC19 digested with *Hae*III (80, 102, 174, 257/267, 298, 434/458, 587 bp fragments). 1, PCR reaction control. In the PCR reaction water was used instead of *P. borealis* crude DNA template, in the presence of RAPD primers; 2, PCR reaction containing *P. borealis* crude DNA template and RAPD primers. 3, PCR reaction containing *P. borealis* crude DNA template, RAPD primers and 1 mM spermidine.

200–600 bp in length. Some observations indicate that spermidine can, in some cases, not only suppress the inhibition of PCR but

observations indicate that polyamines affect the enzymatic reactions in which nucleic acids are involved (also PCR), directly by their

ability to bind nucleic acids, without having any effect on enzyme itself [14]. The interaction of nucleic acids with polyamines can change the conformation of DNA or RNA [15, 16]. Polyamines can induce B-DNA to Z-DNA conformational transition [17,18], and stabilize the conformation of tRNA [19, 20]. Probably because of this influence on the DNA conformation, polyamines can stimulate many enzymatic reactions associated with nucleic acids (including PCR). Polyamines can be very helpful in our efforts to increase the efficiency of PCR, but it is important to point to a danger that is connected with the use of this method. We have found that in the case of primers which show a tendency to create primer-dimer artifacts, polyamines (spermine in particular), can increase the primer-dimer amplification (as it was observed when primers for tRNA^{Tyr} gene were used; data not shown). This effect is probably linked to conformational changes in primers caused by polyamine-DNA affinity.

We conclude that 1 mM concentration of tri- and tetramines (particularly spermidine) can suppress the PCR inhibition and strongly improve the PCR efficiency. This PCR inhibition is suppressed regardless to the source of thermostable DNA polymerase used in the reaction. We tested two different enzymes: Taq DNA polymerase (not shown) and Prime Zyme DNA polymerase. The possibility of using tri- or tetramines as a tool for enhancing the quality and quantity of PCR amplification products, can be a great help for everyone who uses PCR.

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