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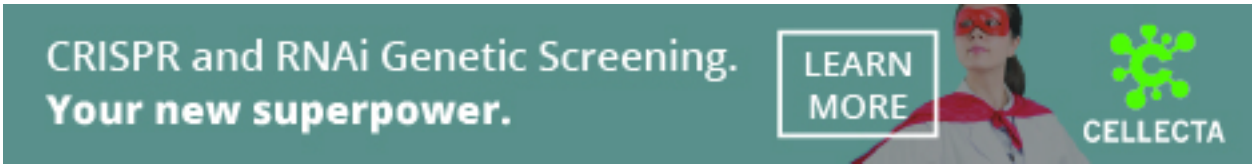

Interference of PCR Amplification by the Polyamines, Spermine and Spermidine

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In a recent article describing the effects of various reaction conditions, Blanchard et al.[1] mentioned that spermine and spermidine at concentrations between 0.5 and 3 mM had no effect on PCR amplification[2,3] with their sequence-specific primers. Because we have observed with arbitrary primers and other DNA templates the concentration-dependent effects of spermine and spermidine either alone or together in PCR, we would like to report our experiences here. The present experiments were run in 1991 and may therefore carry outdated features.

The observation that barley (*Hordeum vulgare*) DNA samples isolated by two different methods, that of Wienand and Feix[4] and the other of Wagner et al.,[5] yielded different results when used as PCR templates gave an impetus to the present study. We deduced that a component was carried along with DNA from the solutions used in the latter method, which keeps the nucleic acids precipitated in the early stages of the procedure with PEG in the presence of polyamines. It appeared that the polyamines, spermine and spermidine, either alone or together in the PCR mix, explained the observed patterns of amplification described below. Hence, some polyamines were supposed to be carried with the DNA over the subsequent steps of isolation. Polyamines have been used in a few assay buffers of nucleic acids affecting enzymes, but their effects were not found to be beneficial in all cases, for example, with restriction endonucleases.[6,7]

MATERIALS AND METHODS

DNA Isolation

Seedlings of barley cv. *Adorra* and its near isogenic experimental lines, 86-HA2-64 and 86-HA2-65, were used at the two-leaf stage. They were frozen with liquid nitrogen and ground (while frozen) with a mortar and pestle. The DNA was prepared according to the method of Wienand and Feix[4] with modifications, that is, ending the procedure after ethanol precipitation by washing twice with 70% ethanol first containing ammonium acetate (10 mM, molecular biology

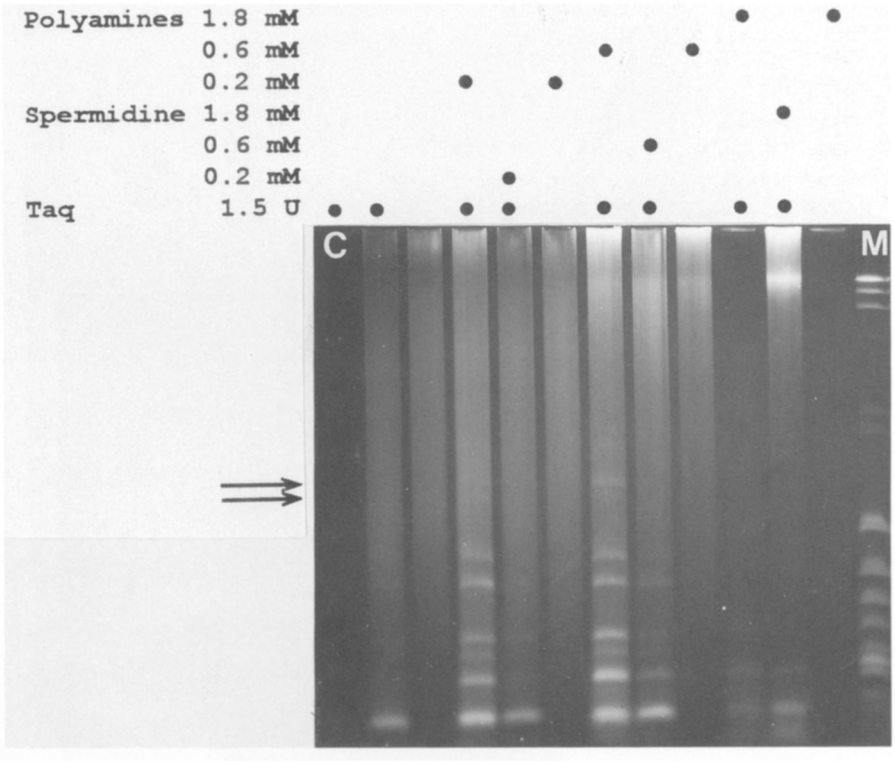

FIGURE 1 *Taq* amplification of barley cv. 86-HA2-64 DNA in the presence of the polyamines, spermine and spermidine at a molar ratio of 1 : 2, or spermidine alone at different concentrations. (C) Control without template DNA; (M) molecular size marker. Black dots indicate the presence of various compounds in the reaction mixtures resolved in the electrophoretic tracks below. The upper arrow indicates the 1400-bp zone; the lower arrow, the 1200-bp zone.

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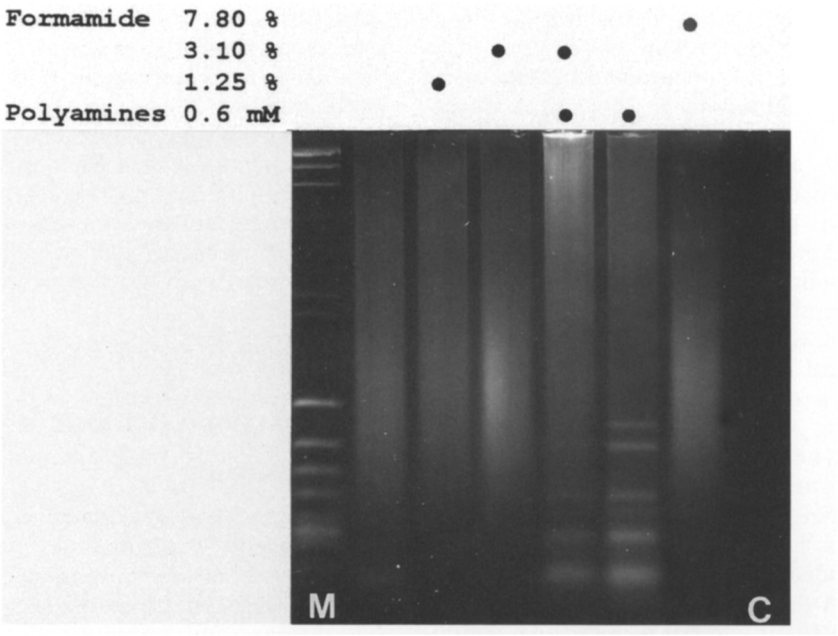

FIGURE 2 *Taq* amplification of barley cv. 86-HA2-64 DNA in the presence of various concentrations of formamide with or without polyamines (spermine and spermidine, molar ratio 1 : 2). (M) Molecular size marker; (C) control without template DNA.

grade from Sigma) and then without ammonium acetate. RNA was removed with DNase-free RNase I "A" (Pharmacia). High levels of RNA may be suppressive to PCR amplification.[8] DNA was dissolved in TE buffer (10 : 1, pH 7.4). DNA concentrations were determined with a DNA fluorometer (Hoefer) using calf thymus DNA as the standard. Most of the experiments were done with cv. 86-HA2-64 DNA; some were repeated with DNAs of other genotypes.

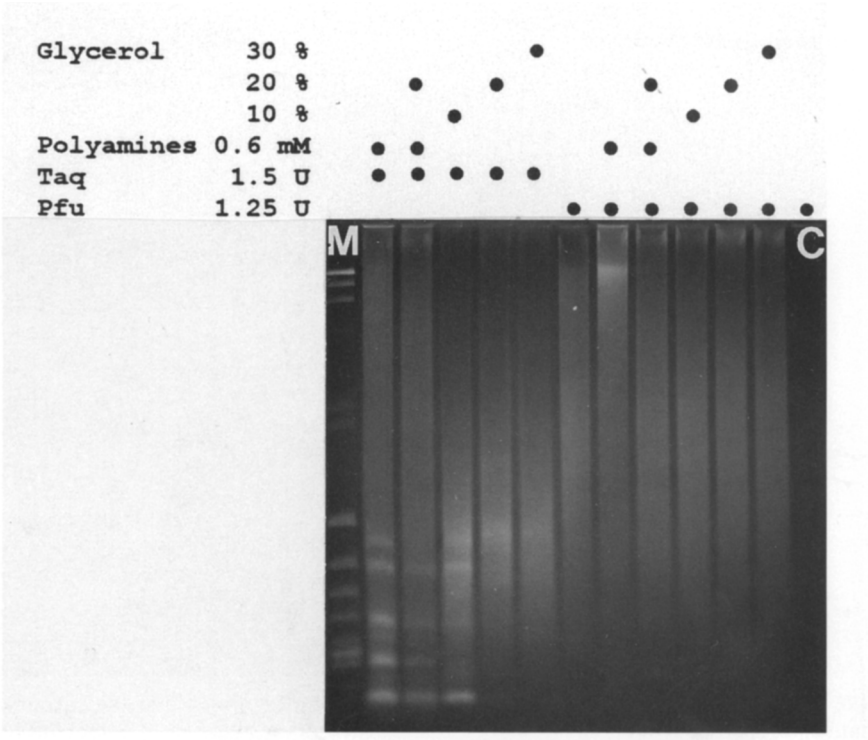

FIGURE 3 *Taq* and *Pfu* amplification of barley cv. 86-HA2-64 DNA as the template with various concentrations of glycerol with or without polyamines (spermine and spermidine, molar ratio 1:2). (M) Molecular size marker; (C) *Pfu* control without template DNA.

Primers and PCR Conditions

The primers were prepared with an ABI DNA/RNA 392 synthesizer using Small-Scale Wide-Pore T CPG columns (according to the instructions of the manufacturer) with ABI chemicals. The primers were used without purification, or they were purified further with Millipore Sep-Pak C_{18} columns.[9–12] The arbitrary primer sequences were TTACCTCCACTTGCACT and CAGCCACACGAGGACCT. Each of them was applied at ~1.5 μM concentration. The amount of the template DNA was ~0.5 μg in a final volume of 50 μl covered with 40 μl of mineral oil (Sigma) in 0.5-ml tubes. Spermine tetrahydrochloride and spermidine (molecular biology grade) were from Sigma, analytical grade glycerol from Merck, and analytical grade formamide from Fluka. Before use, formamide was deionized with Bio-Rad AG508-X8 beads.[9] The thermotolerant DNA polymerases used or tested were as follows: AmpliTaq from Perkin-Elmer Cetus (1.5 unit per reaction), *Taq* from Promega (1.5 unit per reaction), Vent from New England Biolabs (1 unit per reaction), and *Pfu* from Stratagene (1.25 unit per reaction). The 10 × buffer supplied by each manufacturer was used in the master mix containing the dNTPs (Promega or Perkin-Elmer Cetus), each at a concentration of 300 μM. Spermine and spermidine, in the molar ratio of 1 : 2, were added from stock solutions to make a final pooled concentration of 0.05, 0.2, 0.6, or 1.8 mM. The PCR (Techne PHC-2) running parameters were 10 cycles of 97°C, 55°C, and 72°C (each for 1 min), and 30 cycles of 94°C, 55°C (both for 1 min), and 72°C (for 2 min). The run was terminated at 72°C for 4 min. The amplified DNA was resolved with horizontal electrophoresis in 0.8% SeaPlaque agarose (FMC) gels using 1× TBE buffer. Samples of 30 μl of the PCR products were mixed with 15 μl of sample buffer[13] containing ethidium bromide.[14] The gels were poststained with ethidium bromide if necessary. The molecular size marker was a λ DNA *Hin*dIII/ΦX-174 RF DNA *Hin*cII digest (Pharmacia), with the following visible zones after electrophoresis (from top to bottom, see Figs. 1–4): 23130, 9416, 6557, 4361, 2322, 2027, 1057, 770, 609, 495, 392, 345, 341, 335, 297, and 291 bp. The buffer pH was measured using a Knick 761 Calimatic pH meter with an Ingold

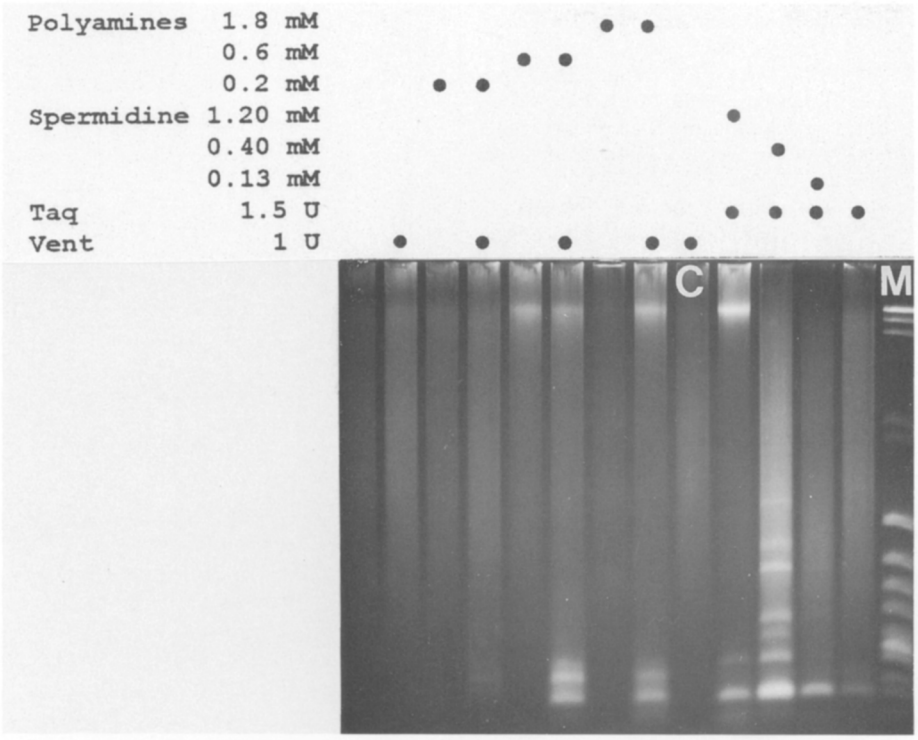

FIGURE 4 Vent amplification of barley cv. 86-HA2-64 DNA in the presence of various concentrations of polyamines (spermine and spermidine, molar ratio 1 : 2). *Taq* amplification with spermidine. (C) Vent control without template DNA; (M) molecular size marker.

combined electrode and a temperature sensor.

RESULTS AND DISCUSSION

If not specified otherwise, the results describe amplifications with AmpliTaq polymerase. The optimum concentration of the pooled polyamines was found to be 0.6 mM (Fig. 1). Without added polyamines, PCR normally resulted in zero or one zone, or up to six zones of short fragments. A polyamine concentration of 0.2 mM usually resulted in 6 fragments, and that of 0.6 mM in up to 10 fragments. The largest fragments were ~1800 bp (weakly visible), followed by one of 1400 bp, and one of 1200 bp. These large fragments were observed in most runs at the optimum concentration of the polyamines. When tested separately, spermidine was more efficient than spermine (result not shown) in promoting the amplification. The optimum concentrations of spermine and spermidine ranged from ~0.4 M to 0.6 mM. However, the maximal promotion was obtained with the mixture (Fig. 1), the ratio of which was obtained by the referred method.[5] The highest polyamine concentrations were sometimes observed to cause precipitation in the samples.

Formamide was shown to promote the specificity of amplification.[15] In the present material, with formamide alone at the concentrations of 1.25%, 3.1%, or 7.8%, no amplification was observed. Formamide (3.1%) in combination with polyamines (0.6 mM) partially inhibited the promoting effect of polyamines (Fig. 2). A barley GC-rich sequence was reported to be successfully amplified in the presence of glycerol.[16] In the present material, glycerol was tested at final concentrations of 10%, 20%, and 30%, and the 20% concentration was also amplified in a reaction mix containing 0.6 mM polyamines. Glycerol partly inhibited the effect of polyamines but, curiously, at 10% concentration, slighty promoted amplification of fragments of similar sizes as did polyamines at a concentration of 0.6 mM (Fig. 3).

Of the different polymerases suitable for PCR, *Pfu* did not produce any visible amplification either with or without polyamines or glycerol (Fig. 3). Vent polymerase amplification was promoted with the polyamine mix at an optimum concentration of 0.6 mM. However, Vent amplified the three or four shortest fragments common to those of *Taq*, but their amplification intensities were different (Fig. 4).

The effect of polyamines cannot simply be explained as a pH effect because no coherent rise of pH was seen in the *Taq* buffer at these concentrations. At concentrations of 0, 0.2, 0.6, and 1.8 mM of the polyamines, the pH readings of the 1× *Taq* buffer varied from 8.32 to 8.45 at 19.4°C, and from 7.27 to 7.58 at 72°C. Raising the assay buffer pH has been shown to promote amplification but to decrease the stringency of the PCR amplification.[1]

The radiolabeled fragments of 1200 and 1400 bp hybridize to restricted barley DNA. The present observations imply also that the DNA isolation method may be a source of variation in PCR amplification. A number of other variables, too, have been shown to affect the results.[1,8,17–19] It is possible that the binding of polyamines on DNA affects the apparent availability of the DNA template. The ratio of primer to template is one of the efficiency determinants in PCR.[19] A preliminary study using another arbitrary primer pair and barley DNA as the template supports the observed promotion of PCR amplification by polyamines (results not shown). The use of a DNA isolation method with polyamines in the solution involves the risk of carryover of the polyamines with the DNA at a concentration affecting their template properties in PCR.

ACKNOWLEDGMENTS

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