Inclusion of polyvinylpyrrolidone in the polymerase chain reaction reverses the inhibitory effects of polyphenolic contamination of RNA

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ABSTRACT

Polysaccharides, secondary metabolites and polyphenolics are known to co-isolate with nucleic acids from plant tissues resulting in inhibition of molecular manipulations. RNA isolated from the polyphenolic-rich resurrection plant, Myrothamnus flabellifolius, was demonstrated to inhibit a standard polymerase chain reaction used as an assay despite the inclusion of the polyphenolic-binding compound poly(1-vinylpyrrolidone-2) (PVP) into the RNA isolation medium. This inhibition was, however, reversed by the addition of PVP into the PCR mixture itself. Confirmation of the inhibitory effect of polyphenolics on PCR was obtained by addition of green tea polyphenolics to the standard PCR assay. This inhibition was also reversed by the simultaneous inclusion of PVP.

Polyphenolics occur at different concentrations in the leaves, bark and fruit of most higher plants (1–7). An important characteristic of many polyphenolics is their propensity to form complexes with nucleic acids (7–16). Hence a variety of protocols (11–23) have been developed to avoid inhibition of molecular biological reactions.

We are interested in genes induced by desiccation in the species in the presence of poly(1-vinylpyrrolidone-2) (PVP) and when chaotropic salt was excluded from the isolation medium (24). However, although this RNA appeared to be of high quality (A\(_{260}\)/A\(_{280}\) in the range 1.9–2.0 with distinct 18S and 28S rRNA bands) and could be reverse transcribed, any subsequent amplification of the cDNA by PCR was unsuccessful. It has been reported that even traces of contamination of an RNA sample with polyphenolics and/or polysaccharides inhibit further manipulations, for example in vitro translation (9) and RT–PCR (19). To investigate the effect of possible contaminants in the M. flabellifolius RNA extract on the PCR, a standard PCR assay was set up using chromosomal DNA extracted from Saccharomyces cerevisiae as the template. The primers used (5′-ATGGATCCAGTGCAGCAGGTAGAAA-3′) and (5′-GTCTCTATCTTTGAAAGCCCTTAACTGAT-3′) recognised the HSP 12 gene sequence yielding the expected product of 355 bp after amplification. A secondary minor product of 310 bp was also observed at high loading. PCR was performed using 130 ng of template, 20 pmol of each primer, 0.1 mM dNTPs, 1 mM MgCl\(_2\), 0.005% BSA, 10 mM Tris–HCl, pH 8.4, 50 mM KCl, 0.001% gelatin. These conditions were found to result in the optimum yield of product. The yeast DNA was amplified by hot start PCR after which the Taq polymerase was added. The amplification cycles used were as follows: five cycles of 93°C for 45 s, 50°C for 30 s, 72°C for 60 s, 25 cycles of 93°C for 30 s, 65°C for 30 s, 72°C for 60 s.

Addition of 2 ng RNA extracted from M. flabellifolius to the PCR mixture resulted in an amplification of the target DNA equivalent to that of the control reaction with the production of a fragment of the anticipated size (Fig. 1a). The yield of product declined substantially, however, when the amount of input M. flabellifolius RNA was increased to 20 ng; no product was observed when this input was further increased to 200 ng. Since PVP has been reported to bind polyphenolics (9,20), we investigated whether the addition of PVP to the PCR mixture might reverse the inhibitory effects of contaminants in the M. flabellifolius leaf RNA preparation. The standard PCR assay, therefore, was carried out in the presence of different final concentrations of PVP of molecular weight 25 000–30 000 (Merck) together with 200 ng of M. flabellifolius leaf RNA. Product formation was observed in the presence of 0.5–2% PVP (Fig. 1b). The product yield, however, was less than that observed with the control PCR. Increasing the PVP concentration above 2% resulted in a markedly reduced product yield.

To confirm our assumption that PCR inhibition was caused by polyphenolic contamination of the M. flabellifolius leaf RNA preparation, the standard PCR assay was repeated in the presence of a commercial extract of polyphenolics from green tea (Sigma). Although product inhibition was not observed when up to 50 ng polyphenolics was added to the reaction, no product was formed in the presence of 500 ng polyphenolics (Fig. 2a). To investigate whether the inhibitory effects of polyphenolic addition could be reversed by the simultaneous addition of PVP, PCR was carried out in the presence of 500 ng polyphenolics together with different concentrations of PVP. It was found (Fig. 2b) that the inhibitory effects of polyphenolic addition could indeed be

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PVP and the to be inhibitory to PCR, possibly due to interaction between the PVP used be optimised since an increase in concentration proved of PVP. We would recommend that the actual concentration of RNA from polyphenolic-rich plants be performed in the presence between 1 and 2% PVP.

reversed and that product formation occurred in the presence of between 1 and 2% PVP.

We suggest, therefore, that any molecular manipulation using RNA from polyphenolic-rich plants be performed in the presence of PVP. We would recommend that the actual concentration of PVP used be optimised since an increase in concentration proved to be inhibitory to PCR, possibly due to interaction between the PVP and the Taq polymerase, although the presence of inhibitory contaminants in the PVP itself cannot be ruled out.

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REFERENCES


Figure 1. (a) PCR of the HSP 12 gene from yeast chromosomal DNA in the presence of RNA isolated from M. flabellifolius. Lane 1, 2 ng M. flabellifolius RNA; lane 2, 20 ng; lane 3, 200 ng; lane 4, 2 µg; lane 5, negative control (no template DNA); lane 6, positive control (no M. flabellifolius RNA added); lane 7, molecular size marker (HpaII digest of pBR 322). (b) Effect of PVP addition to PCR of the HSP 12 gene (1 U of Taq polymerase, 0.005% bovine serum albumin) in the presence of 200 ng of M. flabellifolius RNA. Lane 1, molecular size marker (PstI digest of bacteriophage λ); lane 2, positive control (no polyphenolic addition); lane 3, negative control (no template DNA); lane 4, 0.5% final concentration PVP added; lane 5, 1%; lane 6, 1.5%; lane 7, 2%; lane 8, 3%; lane 9, 5%.

Figure 2. (a) Effect of green tea polyphenolic addition to PCR of the HSP 12 gene. Lane 1, molecular size marker (HpaII digest of pBR 322); lane 2, positive control (no polyphenolic addition); lane 3, negative control (no template DNA); lane 4, 5 ng polyphenolics added; lane 5, 50 ng; lane 6, 500 ng; lane 7, 1 µg; lane 8, 2 µg. (b) Effect of PVP addition to PCR of the HSP 12 in the presence of 500 ng of green tea polyphenolics. Lane 1, molecular size marker (HpaII digest of pBR 322); lane 2, positive control (no polyphenolic addition); lane 3, negative control (no template DNA); lane 4, 0.5% final concentration PVP added; lane 5, 1%; lane 6, 1.5%; lane 7, 2%; lane 8, 3%.