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

Priyum K. Koonjul, Wolf F. Brandt, Jill M. Farrant[1] and George G. Lindsey*

Department of Biochemistry and [1]Department of Botany, University of Cape Town, Private Bag, Rondebosch 7701, South Africa

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 polyphenolic-rich resurrection plant *Myrothamnus flabellifolius* Welw. (Myrothamnaceae). RNA could only be isolated from this 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′-ATGGATC-CATGTCTGACGCAGGTAGAAA-3′) and (5′-GTTCTTACTTT-GAAAGCCTTAAGTAT-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

*To whom correspondence should be addressed. Tel: +27 21 650 2406; Fax: +27 21 685 5931; Email: lindsey@molbiol.uct.ac.za
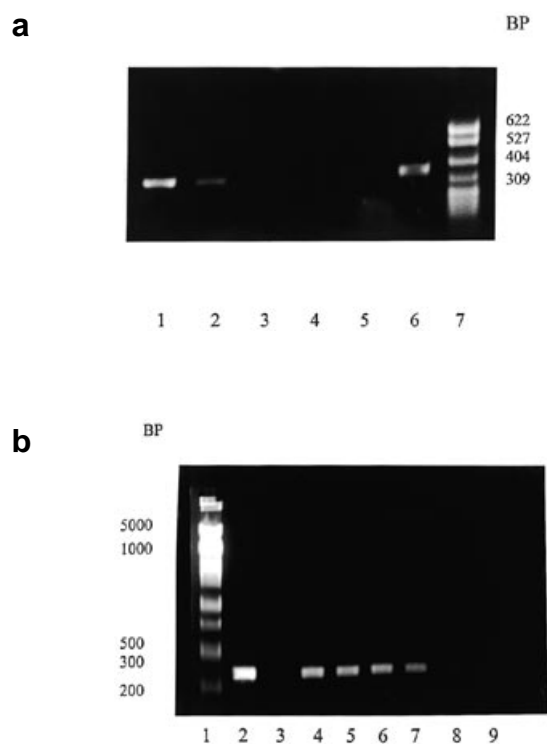
a

b

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 (*Hpa*II 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 (*Pst*I digest of bacteriophage λ); lane 2, positive control (no *M.flabellifolius* RNA added); 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%.

a

b

Figure 2. (**a**) Effect of green tea polyphenolic addition to PCR of the HSP 12 gene. Lane 1, molecular size marker (*Hpa*II 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 (*Hpa*II 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%.

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.

ACKNOWLEDGEMENTS

REFERENCES

1 Baxter,N.J., Lilley,T.H., Haslam,E. and Williamson,M.P. (1997) *Biochemistry*, **36**, 5566–5577.
2 Bruce,R.J. and West,C.A. (1989) *Plant Physiol.*, **91**, 889–897.
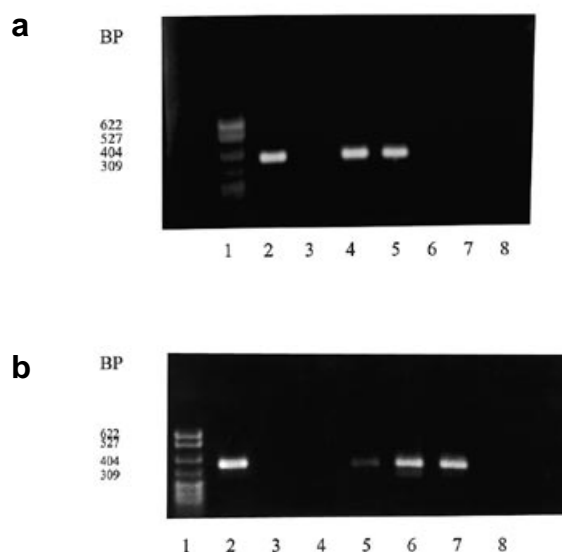3 Dry,I.B. and Robinson,S.P. (1994) *Plant Mol. Biol.*, **26**, 495–502.
4 Peterson,D.G., Boehm,K.S. and Stack,S.M. (1997) *Plant Mol. Biol. Rep.*, **15**, 148–153.
5 Ray,S.D., Guruparsad,K.N. and Laloraya,M.M. (1980) *J. Exp. Bot.*, **31**, 1651–1656.
6 Rice-Evans,C.A., Miller,N.J. and Paganga,G. (1996) *Free Rad. Biol. Med.*, **20**, 933–956.
7 Salunkhe,D.K., Jadhav,S.J., Kadam,S.S. and Chavan,J.K. (1982) *Crit. Rev. Food Sci. Nutr.*, **17**, 277–305.
8 Huh,N.W., Porter,N.A., McIntosh,T.J. and Simon,S.A. (1996) *Biophys. J.*, **71**, 3261–3277.
9 Su,X. and Gibor,A. (1988) *Anal. Biochem.*, **74**, 650–657.
10 Larson,R.A. (1988) *Phytochemistry*, **27**, 969–978.
11 Baker,S.S., Rugh,C.L. and Kamalay,J.C. (1990) *Biotechniques*, **9**, 268–272.
12 Katterman,F.R.H. and Shattuck,V.I. (1993) *Prep. Biochem.*, **13**, 347–359.
13 Mozer,T.J. (1980) *Plant Physiol.*, **65**, 834–837.
14 Soni,R. and Murray,J.A.H. (1994) *Anal. Biochem.*, **218**, 474–476.
15 Wan,C.Y. and Wilkins,T.A. (1994) *Anal. Biochem.*, **223**, 7–12.
16 Wilkins,T.A. and Smart,L.B. (1996) In Krieg,P.A. (ed.), *A Laboratory Guide to RNA Isolation, Analysis and Synthesis*. Wiley-Liss Inc., NY, pp. 21–41.
17 Loomis,W.D. (1974) *Methods Enzymol.*, **31**, 528–545.
18 Logemann,J., Schell,J. and Willmitzer,L. (1987) *Anal. Biochem.*, **163**, 16–20.
19 Staub,U., Polivka,H. and Gross,H.J. (1995) *J. Virol. Methods*, **52**, 209–218.
20 John,M.E. (1992) *Nucleic Acids Res.*, **20**, 2381.
21 Colpan,M., Schumacher,J., Brüggemann,W., Sanger,H.L. and Riesner,D. (1983) *Anal. Biochem.*, **131**, 257–265.
22 Dabo,S.M., Mitchell,E.D.,Jr and Melcher,U. (1993) *Anal. Biochem.*, **210**, 34–38.
23 Glisin,V., Crkvenjakov,R. and Byus,C. (1974) *Biochemistry*, **13**, 2633–2637.
24 Callahan,A., Morgens,P. and Walton,E. (1989) *Hort. Science*, **24**, 356–358.