The Effects of Plant Polysaccharides and Buffer Additives on PCR

ABSTRACT

A survey of the inhibitory effects of various plant polysaccharides on PCR amplification of a 974-bp section of rbcL *in spinach revealed that most of the polysaccharides tested (arabinogalactan, carrageenan, dextran, gum guar, gum karaya, gum locust bean, inulin, mannan, pectin, starch and xylan) were not inhibitory. In contrast, two of the acidic polysaccharides (dextran sulfate and gum ghatti) were inhibitory. The addition of 0.5% Tween 20 reversed the inhibitory effects of gum ghatti (polysaccharide:DNA ratio of 500:1). The inhibitory effect of dextran sulfate (50:1) could be reversed by the addition of Tween 20 (0.25% or 0.5%), DMSO (5%) or polyethylene glycol 400 (5%), but none of these three additives were effective at a 100:1 ratio of dextran sulfate/DNA.*

INTRODUCTION

PCR is widely being used to amplify DNA (4). Polysaccharides are common contaminants in DNA extracted from plant tissues (2). Most conventional plant DNA purification methods can remove proteins, but may not be effective for removing polysaccharides (see Reference 2 for discussion). In a previous report (2), acidic plant polysaccharides were shown to be inhibitory for *Hin*dIII restrictions of lambda DNA. Neutral plant polysaccharides were found to be non-inhibitory. The effect of polysaccharides on PCR amplification of genomic plant DNA is not known. The objectives of this study were to examine the effects of neutral and acidic plant polysaccharides on PCR amplification and investigate various buffer additives for reversing PCR inhibition.

MATERIALS AND METHODS

Polysaccharides were obtained from Sigma Chemical (St. Louis, MO) and mixtures prepared as previously outlined (2), except spinach DNA was used instead of lambda DNA. DNA was extracted from locally obtained fresh spinach (*Spinacia oleracea* L.) leaves using the hot CTAB protocol (3). PCR was performed in a volume of 25 µl containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 1.5 mM $MgCl_2$, 0.01% gelatin and 0.1% Triton® X-100, 0.2 mM of each deoxynucleoside triphosphates (dNTPs), 0.4 µM primers, 3 ng of spinach DNA and 2 units of Promega *Taq* DNA polymerase (Madison, WI). Initially, a ratio of polysaccharide to DNA of 500:1 (w/w) was used. Lower ratios of polysaccharide/DNA were investigated for polysaccharides that showed inhibition at the 500:1 ratio. The *rbc*L primers used for PCR were BU1: 5′–3′ (TCA ACT GGT ACA TGG AC) and BU2: 5′–3′ (GC AGG CAT ATG CCA AAC). These primers amplify a 974-bp section of *rbc*L in spinach DNA and are based on conserved consensus sequences applicable to higher plants (6). Amplification was performed in an MJ Research Programmable Thermal Cycler (Watertown, MA). The thermal cycle used was 94°C (1.5 min) for initial strand separation, then 25 cycles at 37°C (2 min), 72°C (3 min) and 94°C (1 min). Two additional steps were used as follows: 37°C (2 min) and 72°C (10 min) for final extension. Amplification products were analyzed by electrophoresis in 1.5% agarose gels and detected by staining with ethidium bromide. DMSO (5%), formamide (5%), glycerol (5%, 9%, 13%), polyethylene glycol (PEG) 400 (5%), bovine serum albumin (BSA) (2 µg/ml) and Tween 20 (0.25%, 0.5%, 1.0%, 2.0%) were added to the PCR buffer to attempt reversal of the inhibition of PCR (1,5,7).

RESULTS AND DISCUSSION

The neutral polysaccharides (arabinogalactan, dextran, gum guar, gum locust bean, inulin, mannan and starch) did not inhibit PCR amplification of spinach DNA (Table 1). However, two of the acidic polysaccharides, dextran sulfate and gum ghatti, inhibited PCR amplification. Previously, these polysaccharides have been shown to inhibit restriction of lambda DNA by *Hin*dIII (2). One should note that dextran sulfate is not a natural plant product; however, gum ghatti, which contains about 9.5% D-glucuronic acid (8), is a natural product in the legume family. Although we (2) have previously shown that carrageenan, gum karaya, pectin and xylan inhibited restriction of lambda DNA by *Hin*dIII, these acidic polysaccharides do not appear to

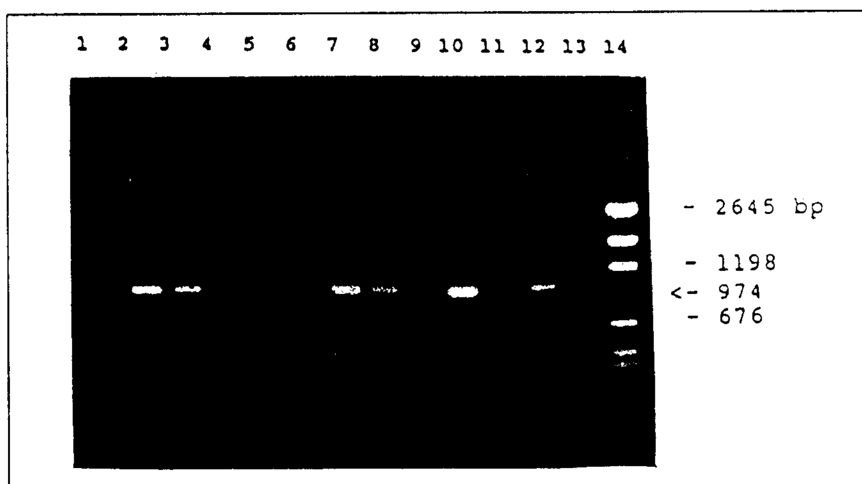

Figure 1. Effect of SDS, dextran sulfate and gum ghatti on PCR amplification of a 974-bp portion of *rbc*L gene in spinach. Lane 1 = dextran sulfate (100:1) + 0.5% Tween 20; lane 2 = dextran sulfate (50:1) + 0.5% Tween 20; lane 3 = dextran sulfate (5:1); lane 4 = dextran sulfate (50:1); lane 5 = dextran sulfate (100:1); lane 6 = dextran sulfate (500:1); lane 7 = gum ghatti (500:1) + 0.5% Tween 20; lane 8 = gum ghatti (100:1); lane 9 = gum ghatti (500:1); lane 10 = + 0.01% SDS + 0.5% Tween 20; lane 11 = + 0.01% SDS; lane 12 = positive control (DNA); lane 13 = negative control (no DNA); lane 14 = pGEM marker.

Table 1. Comparison of the Effects of Various Plant Polysaccharides on the PCR Amplification of a 974-bp Portion of the *rbc*L Gene in Spinach Using 500:1 (Polysaccharide:DNA, w/w)

Neutral Polysaccharides	PCR Amplification	Acidic Polysaccharides	PCR Amplification
Arabinogalactan	+	Carrageenan	+
Dextran	+	Dextran sulfate	-
Gum guar	+	Gum ghatti	-
Gum locust bean	+	Gum karaya	+
Inulin	+	Pectin	+
Mannan	+	Xylan	+
Starch	+		

+ = Amplification
- = No amplification

inhibit PCR amplification. In view of the inhibitory nature of dextran sulfate, it is somewhat surprising that carrageenan (which contains approximately 80% kappa with one sulfate per disaccharide and 20% lambda with three sulfates per disaccharide) was not inhibitory. Pectin is quite variable in nature. We found apple pectin (pH 4.1, ca. 76% galacturonic acid, 7% methoxy groups) to not be inhibitory, but other plant pectins might have different effects.

Lower ratios of polysaccharides to DNA (100:1, 50:1, 5:1) were further investigated for dextran sulfate and gum ghatti. Gum ghatti did not inhibit PCR at 100:1, 50:1 or 5:1 ratios. The inhibitory effect of gum ghatti at 500:1 was reversed by the addition of 0.5% Tween 20 (Figure 1). Dextran sulfate inhibited PCR amplification at 500:1, 100:1 and 50:1, but not at 5:1 ratio. The addition of Tween 20 (0.25% or 0.5%, v/v) reversed the inhibitory effect of dextran sulfate at the 50:1 ratio (Figure 1). The addition of Tween 20 (0.5%) has previously been reported to enhance *Taq* polymerase activity (5). However, the addition of Tween 20 in amounts of up to 2% (v/v) did not reverse the inhibitory effects of dextran sulfate at 500:1 and 100:1 ratios (data not shown). The addition of either DMSO (5%) or PEG 400 (5%) reversed the inhibitory effect of dextran sulfate (50:1). Neither was effective in reversing the effect of dextran sulfate at a 100:1 ratio. The addition of BSA (2 µg/ml), formamide (5%) or glycerol (5%, 9%, 13%) did not reverse the inhibitory effect of dextran sulfate even for the 50:1 dextran sulfate/DNA ratio.

The inhibitory nature of some polysaccharides with free acidic groups is further demonstrated by contrasting dextran and dextran sulfate. Dextran (neutral) had no interfering effects at 500:1 ratio, whereas dextran sulfate was very inhibitory (Table 1). A common sulfate detergent used in DNA extraction (sodium dodecyl sulfate [SDS]) has been reported to inhibit PCR (5). We confirmed (5) that SDS (0.01%) does inhibit PCR amplification, and the addition of Tween 20 (0.5%) reverses the SDS inhibition (Figure 1).

It appears that plant polysaccharides may not present as large a problem for PCR as we have previously found for restrictions (2). The addition of Tween 20, DMSO or PEG might be used to aid in the amplification of DNA from recalcitrant plant species and is under further investigation.

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