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Betaine improves the PCR amplification of GC-rich DNA sequences

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ABSTRACT

Betaine improves the co-amplification of the two alternatively spliced variants of the prostate-specific membrane antigen mRNA as well as the amplification of the coding cDNA region of *c-jun*. It is suggested that betaine improves the amplification of these genes by reducing the formation of secondary structure caused by GC-rich regions and, therefore, may be generally applicable to ameliorate the amplification of GC-rich DNA sequences.

Polymerase chain reaction (PCR) is a widely applied approach in molecular biology. GC-rich DNA sequences often require laborious work to optimize the amplification assay. Additives, DMSO (1) and glycerine (2), nucleotide analogs as 7-deaza dGTP (3) and dITP (4) or DNA template denaturation by NaOH (5) were introduced to optimize the amplification.

Co-amplifying both the prostate-specific membrane antigen (PSM) mRNA and the alternatively spliced variant (PSM′), differing by a deletion of 266 bases (6), by reverse transcription (RT) and PCR with one primer set located upstream (P1: 5′-AAACACTGCTGTGGTGGA) or downstream (P2: 5′-TAG-CTCAACAGAATCCAGGC) from the deletion, we found that the ratio of PSM and PSM′ mRNA expressed in the prostatic cancer cell line LNCaP was <1 (Fig. 1). This result is in contrast to data obtained by a RNase protection assay (6). Since the deletion contains GC-rich sequences (66% GC) the RT–PCR was optimized by both the involvement of 10% DMSO or 10% glycerine (Fig. 1) and the application of PCR assays developed for fidelity and yield by selection of special Taq DNA polymerases. Unfortunately, these experimental approaches do not ameliorate the depressed amplification of PSM mRNA (Fig. 2). However, the inclusion of betaine, purchased as monohydrate (Sigma), improves the amplification of PSM mRNA fundamentally (Fig. 1). Betaine is effective with conventional Taq DNA polymerases (Promega; Pharmacia Biotech) as well as with PCR assays designed for hot start PCR (AmpliTaq Gold™, Perkin Elmer) or for long and accurate (LA) PCR (Expand™ High Fidelity Taq, Boehringer Mannheim; TaKaRa LA Taq, Boehringer Ingelheim Bioproducts) using, according to Barnes (7), a mixture of a minor proofreading enzyme and Taq DNA polymerase (Fig. 2). Increasing betaine concentrations increase the signal intensity

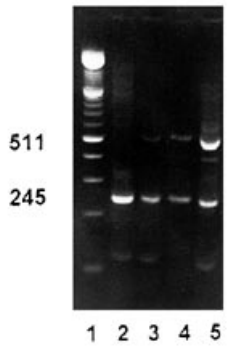

Figure 1. Effect of additives on the amplification of alternatively spliced variants of PSM mRNA. cDNA synthesis; with M-MLV reverse transcriptase (Promega), 1 µg RNA, isolated with TRIzol™ Reagent (GIBCO BRL) and primer P2 at 50°C for 30 min. The amplification was performed with the 2.5 U Taq DNA polymerase (Promega) per 50 µl PCR assay, 1.7 mM $MgCl_2$, 0.2 µM primer P1 and primer P2, hot-start and 25 cycles (annealing at 55°C, elongation at 72°C, denaturation at 95°C, each for 1 min) were used. PSM 511 bp, PSM′ 245 bp: lane 1, DNA marker XIV (Boehringer Mannheim); lane 2, without additives; lane 3, 10% DMSO, 5 U Taq DNA polymerase were used since DMSO inhibits the enzyme (1); lane 4, 10% glycerine; lane 5, 1 M betaine.

of the PSM amplicon and decrease that of the PSM′ amplicon. This relationship has similarities with the effect of increasing concentrations of an internal standard on the amplification efficiency of the target observed in the competitive PCR (8). Obviously, betaine makes PSM cDNA accessible for Taq DNA polymerase and favours, in this way, competition with the amplification of PSM′. The optimal betaine concentration for the amplification of the alternatively spliced PSM variants amounts to ~1 M (Fig. 3).

A further example of the effectiveness of betaine for the PCR of GC-rich sequences, is the improvement of the amplification of *c-jun* prevented by a basic region containing 72% GC (1). For the amplification of the coding cDNA inserted in pBluescript ~2.5 M betaine is optimal (Fig. 3). Thus, the optimal betaine concentration seems to be dependent on the composition of the amplified DNA sequence.

Rees *et al.* (9) demonstrate that betaine reduces or even eliminates the base pair composition-dependent DNA thermal

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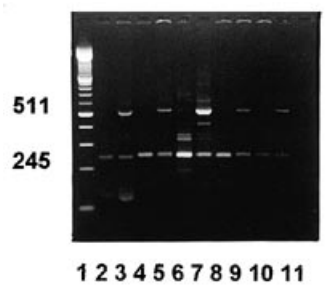

Figure 2. Improvement of PCR assays obtained from several suppliers by betaine. The PCR assays were performed as described by the manufacturers including the modifications outlined in Figure 1 and without or with 1 M betaine (lanes 3, 5, 7, 9, 11). Lane 1, DNA marker XIV (Boehringer Mannheim); lanes 2 and 3, Taq DNA polymerase (Promega); lanes 4 and 5, AmpliTaq Gold™ (Perkin Elmer); lanes 6 and 7, TaKaRa LA Taq (Boehringer Ingelheim Bioproducts); lanes 8 and 9, Taq DNA polymerase (Pharmacia Biotech); lanes 10 and 11, Expand™ High Fidelity Taq (Boehringer Mannheim).

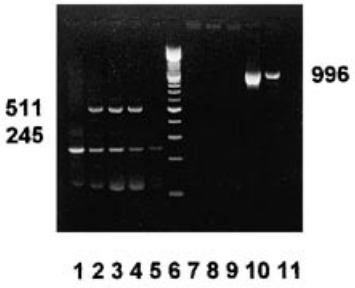

Figure 3. Effect of betaine on the amplification of both the alternatively spliced PSM variants and the *c-jun* coding cDNA fragment. PCR assay as described in Figure 1 including the following betaine concentrations: 0, 0.5, 1, 2.5 and 5 M (lanes 1–5 and 7–11). Lanes 1–5, PSM′ and PSM; lanes 7–11, *c-jun* coding cDNA fragment inserted in pBluescript (50 ng), each 1 µM primer J1 (5′-ATGACTGCAAAGATGGAAACG) and primer J2 (5′-TCAAAATGAA-AGCAACTGCTGCG), 30 cycles (annealing at 53°C, elongation at 72°C, denaturation at 95°C, each for 1 min); lane 6, DNA marker XIV (Boehringer Mannheim).

melting transition. Mytelka *et al*. (10), studying a novel class of DNA sequences that cause DNA polymerase to pause, observed that T7 DNA polymerase often recognizes pause sites near putative hairpin–loop structures. The addition of betaine relieves these pauses.

In summary, the application of betaine in the PCR assay improves the amplification of both the PSM and *c-jun* cDNA and may be generally applicable to ameliorate the amplification of GC-rich DNA sequences.

Note

While this paper was under review, we became aware of an independent study of *Baskaran et al*. (11) in which betaine is applied in combination with DMSO for the uniform amplification of a mixture of DNA with varying GC content. Hengen (12), referring to an information sheet for LA–PCR provided by Wayne Barnes, pointed out that 1.3 M betaine and 1.3% DMSO added to LA–PCR mixtures improves processivity.

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