Detection of mutations in GC-rich DNA by bisulphite denaturing gradient gel electrophoresis

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ABSTRACT

Denaturing gradient gel electrophoresis (DGGE) in combination with PCR and ‘GC-clamping’ has proven highly efficient as a method for detection of DNA sequence differences. Due to strand dissociation phenomena, however, its use has been limited to the analysis of sequences with a relatively low content of GC pairs. This paper describes how treatment of template DNA with sodium bisulphite drastically lowers the melting temperature of very GC-rich sequences and renders them amenable to DGGE analysis. We demonstrate the use of bisulphite DGGE for rapid and efficient detection of mutations in the p16INK4/CDKN2 tumour suppressor gene.

Denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) separate DNA molecules on the basis of differences in thermal stability caused by differences in base sequence (1). The resolving power depends on the drop in electrophoretic mobility that occurs when part of a DNA molecule melts, resulting in the formation of a structure that is partly helical and partly random chain. PCR-mediated attachment of an artificial GC-rich sequence, a ‘GC-clamp’, provides a simple means for modulating the melting characteristics of most sequences into the two-domain profile that is considered optimal for detection of mutations (2).

Once DNA molecules have reached the retardation level in the denaturing gradient gel, the band of retarded molecules tends to disappear through complete strand dissociation if exposure to the electric field is prolonged. The rate of dissociation is determined by the equilibrium constant of strand dissociation at the temperature of migration arrest. The GC density of some sequences is so high that strand dissociation, despite extensive GC-clamping, is complete at the temperature where the partially melted structure is formed. This direct transition from the fully helical state to the fully single-stranded state prevents detection of sequence alterations and has been a major shortcoming of DGGE (1,3–5). The use of a psoralen derivative (‘ChemiClamp’) to covalently link one end of a DNA molecule has been shown useful as a means to prevent strand dissociation (6). However, the melting properties of a PCR product modified by ‘ChemiClamping’ cannot be predicted and may be suboptimal for resolution of mutations.

In this report we describe how very GC-rich DNA can easily be rendered amenable to GC-clamping and DGGE analysis by treatment with sodium bisulphite prior to PCR. Bisulphite converts unmethylated cytosine to uracil which is subsequently amplified as thymine (7–9). Figure 1 depicts the melt map (10) of a 250 bp genomic DNA region covering exon 1 of the p16INK4/CDKN2 tumour suppressor gene (11). This region has a GC content of 75% with a maximum Tm of 88°C. Treatment with sodium bisulphite and subsequent PCR would result in the formation of two distinct DNA molecules with GC contents of 50 and 26%, and predicted maximum Tm’s of 74 and 66°C, respectively (Fig. 1). Each of these two non-complementary strands can readily be subjected to mutation scanning by GC-clamping and DGGE. Because C→T and T→C transitions will remain undetected after bisulphite treatment of DNA, primer sets must be designed for both strands to ensure detection of all types of mutations.

Figure 2 illustrates the use of bisulphite DGGE for detection of mutations in the p16INK4/CDKN2 gene. Conventional DGGE analysis of a 150 bp region encompassing p16INK4/CDKN2 exon 1 resulted in lack of band focusing and loss of mutation resolution due to complete strand dissociation (data not shown). This observation is in agreement with computerized simulation of DNA melting (10), predicting that complete dissociation of the PCR product with a 40 bp GC-clamp attached becomes significant at a temperature ~0.7°C below the Tm of the lower melting domain. In contrast, a PCR product generated with primers specific for the lower strand of bisulphite-reacted DNA has a predicted low-stability domain Tm of 64°C, and strand dissociation with a 40 bp GC-clamp attached will not become significant below 72°C. Parallel DGGE analysis of PCR products of bisulphite-reacted DNA (Fig. 2) showed a single band at the expected position for all control samples, and an aberrant four-band pattern in a hereditary melanoma patient heterozygous

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for a C→T transition (Pro48Leu) in exon 1 of the p16INK4/CDKN2 gene (12).

The bisulphite DGGE procedure detailed herein may offer other advantages over conventional DGGE. First, because treatment of template DNA with sodium bisulphite drastically lowers the melting temperature, the use of the toxic substance formamide to generate a chemical gradient may be completely avoided (13). Second, the length of the GC-clamp required to prevent strand dissociation can be reduced (1). Third, because bisulphite converts only cytosine residues that are unmethylated (7), sequences that differ in methylation status will differ in nucleotide composition after treatment with sodium bisulphite and resolve at different positions in a denaturing gradient gel (unpublished results). This may provide an attractive alternative to other methods using bisulphite-converted DNA for detection and quantitation of DNA methylation, including methylation-specific PCR (14), methylation-sensitive single nucleotide primer extension (15) and procedures based on the use of restriction enzyme digestion (16,17).

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REFERENCES