Single base substitutions are detected by double strand conformation analysis

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Double strand DNA in aqueous solution is thought to have an intrinsic curvature which is dependent on its nucleotide sequence. According to the ‘wedge’ model, the neighboring base-pair stacks are considered as geometrically independent and the DNA axis deflections are described by 16 wedge angles (1). This sequence-dependent curvature may be directly related to the friction encountered by a DNA segment during electrophoresis in porous gels. Thus, a base substitution in a critical position of a double-stranded DNA sequence, corresponding to one of the above mentioned dinucleotide stacks, should induce a modification of the curvature and, consequently, of the electrophoretic mobility. Here, we report on the detection of single base substitutions in PCR-amplified DNA segments by Double-Strand-Conformation-Analysis (DSCA).

Originally, electrophoretic mobility shifts were observed in 8% non-denaturing polyacrylamide gels (PAA) in three different PCR amplification products of the dystrophin gene of subjects affected with Duchenne muscular dystrophy (2). After sequencing, they turned out to correspond to base substitutions and not, as expected, to numerical base changes. Therefore, a series of experiments was set up in order to explore the conditions for detecting base substitutions by high-resolution polyacrylamide electrophoresis under non-denaturing conditions.

Four different mutations, previously identified by chemical cleavage in exons 53, 55, 60 and 70, respectively of the human dystrophin gene (3), were used to validate the method. Mobility shifts were observed in all but one of the exon-specific PCR products on 15% or 20% PAA gels (data not shown). The same technique was then adopted to screen the dystrophin exons 3, 6, 19, 41 and 48 in a cohort of 20 DNAs from patients affected with Duchenne or Becker muscular dystrophies.

PCR primer sequences were taken from the literature (4,5). Amplification conditions were as described previously (2,6). Samples were loaded onto vertical PAA gels (29:1 acrylamide: bisacrylamide, 22×20×0.1 cm) of different concentrations (6.5% to 20%). Electrophoresis was carried out at room temperature in TBE buffer (1×TBE: 89 mM Tris, 89 mM boric acid, 2 mM EDTA) at 10 V/cm for varying length of time (10 to 44 hrs). Gels were silver stained and dried for documentation.

The method proved able to detect band shifts in five patients (Figure 1a) which were subsequently confirmed by direct sequencing as the base substitutions C386T, C711A, A2525G, C6107T and 7121-113A—T. Furthermore, it was also possible to detect the band shift caused by mutation C6107T in a female heterozygote (Figure 1b). In order to rule out that the double band seen in the heterozygote may be due to the formation of heteroduplex DNA molecules in the later stages of the PCR reaction, a PCR product of similar size obtained from a heterozygote for a known 5 bp deletion (6) was run on the same gel (Figure 1b, lane B). The resulting band pattern clearly ruled

Figure 1. Electrophoretic separation under non-denaturing conditions (8% PAA gel) of various exons of the dystrophin gene. a) Lanes: A and G: controls; B: C711A (in exon 6); C: C6107T (in exon 41); D: C386T (in exon 3); E: A2525G (in exon 19); F: 7121-113A—T (in exon 48). b) Lanes A, C, D, F: controls; B: female heterozygote for the mutation 6643del5 (ref. 6); E: female heterozygote for the mutation C6107T.
The analysis of the five novel mutations identified so far shows that different base substitutions (C—T, C—A, A—T and A—G) are equally capable to induce a modification of the double-strand conformation as predicted by the wedge-model theory. A curvature map was obtained for each mutant amplicon by the computer program CURVATURE (7), kindly provided by Dr Shpigelman. In all cases, a remarkable modification of the curvature profile was observed as the consequence of the base substitution (Figure 2).

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