APPLICATION OF THE CAPILLARY DNA CHROMATOGRAPHY IN THE PATERNITY TESTING USING APOB AMPLIFIED ALLELES

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Allele resolution and separation of "in vitro" amplified DNA is currently performed by gel electrophoresis followed by stain or probe detection (1,2). However, these are time-consuming procedures and require extensive manual operations.

Capillary electrophoresis (CE) has been successfully used as an alternative to standard gel electrophoresis for analysing DNA restriction fragments and PCR products of single viral and human sequences (3,4). The advantage of CE over other separation techniques is that only nanolitres of samples are required for each procedure, and rapid, efficient, sensitive and automated analyses can be achieved (5).

We have investigated the separation of amplified polymorphic alleles from the human APOB locus using CE with a coated fused silica column.

MATERIALS AND METHODS

Human genomic DNA was extracted by standard techniques and amplified "in vitro" using APOB specific amplimers (6). Amplified fragments were subjected to a phenol-chloroform (3:1 v/v) extraction and precipitated with cold ethanol and lyophilized. Samples were reconstituted in 10 μl of distillated water. CE was performed on the P/ACE™ system 2000 (Beckman, USA) in the reversed polarity mode. The temperature was set at 25°C and UV absorbance was monitored at 260 nm. A surface-modified fused silica capillary (100μm i.d.) was used for all analyses. Aliquots (1:100 diluted) of purified PCR products were run for 22 min at 12 KV.

Peaks were identified as specific alleles by calibration curve of migration time vs fragment sizes using ϕX174 Hae III digested DNA.

RESULTS AND DISCUSSION

Complete and reproducible resolution of the APOB alleles in the range of 560 to 900 molecular weight was obtained without ambiguity. The electropherogram pattern was comparable to the profile obtained by agarose gel electrophoresis (Fig.1).

We have applied this technique to analyze retrospectively 10 cases of disputed paternity, previously shown to be fully informative for the APOB segregation. Paternity attribution was confirmed in 4 cases and excluded in 6. In three experiments in which DNA samples from two persons were co-injected, four distinct migration peaks were detected. Thus, the presence of two APOB genotypes, heterozygous for non-sharing alleles, was unambigously resolved. This result proves that non-paternity is readily and accurately detected using the present protocol.

Personal experience demonstrates high efficiency in separating the APOB amplified alleles using CE, which is proposed as a method for automatizing forensic DNA analysys.

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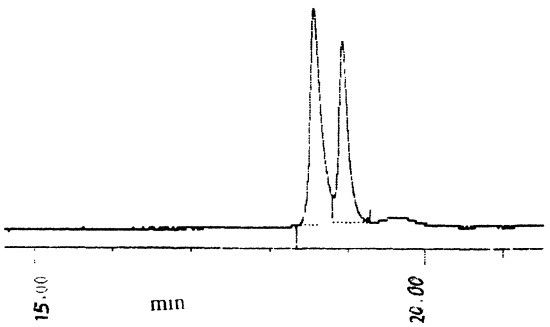

Fig.1. CE separation of APOB amplified fragments from an individual with alleles 35 and 39. Detection was by absorbance at 260 nm (0.05 full scale). Migration times (min) are indicated on the x-axis.

References

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