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Polymerase-chain reaction: analysis of DNA/DNA hybridization by capillary electrophoresis

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Capillary electrophoresis (CE) has been recently described as a very useful technology to analyse double-stranded DNA (dsDNA) as well as single stranded DNA (ssDNA) (1, 2). This technology is fast, reproducible and very efficient, especially if the detection is performed (a) with on-column sample stacking, together with addition in the buffer system of agents such as ethidium bromide (2), or (b) with laser-induced fluorescence of DNA fragments using compounds such as thiazole orange (3).

Analysis by CE of DNA restriction fragments and polymerase chain reaction (PCR) products toward detection of the AIDS (HIV-1) virus in blood has been recently reported (4, 5). Although this approach could be extremely interesting, detection of DNA/DNA hybridization by CE has not been reported (5, 6).

In this communication, we demonstrate that CE can be used to detect hybridization between a 299 nucleotides long ssDNA and a complementary 28-mer.

ssDNA was prepared as follows: SK145 and SK39 HIV-1 primers (their location is shown in Figure 1A) (5) were used to amplify a *gag* portion (5 ng/reaction) of the HIV-1 genome. PCR was performed in 25 μl of 100 mM KCl, 100 mM Tris–HCl pH 8.3, 2 mM $MgCl_2$ by using 2 U/reaction of *Taq*I polymerase (Perkin-Elmer). The PCR cycles were 1 min denaturation at 92°C, 1 min annealing at 55°C and 1 min elongation at 72°C. Unbalanced PCR was performed with a SK145:SK39 ratio of 10:1 (250 ng of SK145, 25 ng of SK39). Figure 1B shows the agarose gel electrophoresis of SK145/SK39 PCR products, together with the analysis of unbalanced SK145/SK39 PCR. ssDNA was purified by standard methods (7) and analysed by agarose gel (Figure 1B, lane d). Electrophoresis was carried out in 3% agarose (agarose:NuSieve = 3:1).

CE was performed on a P/ACE System 2050 (Beckman Instruments, Palo Alto, CA, USA) in the reversed polarity mode, i.e., a negative potential at the injection end of the capillary. The temperature of the capillary was set at 20°C. Ultraviolet absorbance was monitored at 254 nm. Postrun analysis of data was performed by using the Beckman Gold Software (version V711). A fused deactivated (100 μg internal diameter, 40 cm length to detector) silica capillary was used (Beckman Instruments, Palo Alto, CA, USA). Before each separation step a 4 min buffer rinse was performed under high pressure. Samples were introduced into the capillary at a negative polarity of 4 kV for 40 s. Separations within the coated capillary were performed at negative polarity under constant voltage of 12 kV (about 10 μA). The CE buffer system consisted of 100 mM Tris base, 100 mM boric acid, 0.5% propyl-methyl-cellulose to pH 8.3. The buffer was filtered to remove particulates. In our CE protocol, ethidium bromide (10 μg/ml) was present in the buffer system during voltage separation but not in the sample to be analysed. All the DNA samples were ethanol-precipitated and resuspended in water before CE analysis.

Three DNA samples were analysed by CE: (a) SK145/SK39 PCR product; (b) ssSK145/SK39 PCR product and (c) ssSK145/SK39 PCR products pre-hybridized with the complementary SK150 probe. Hybridization was performed in 100 mM KCl, 100 mM Tris–HCl pH 7.4, 2 mM $MgCl_2$ for 15 min at 55°C. The results obtained are shown in Figure 2 and demonstrate that hybridization of ssSK145/SK39 PCR product to the SK150 oligonucleotide (750 ng) causes a shift in the mobility of HIV-1 ssPCR product (Figure 2E). This effect appears to be strictly sequence-specific since unrelated

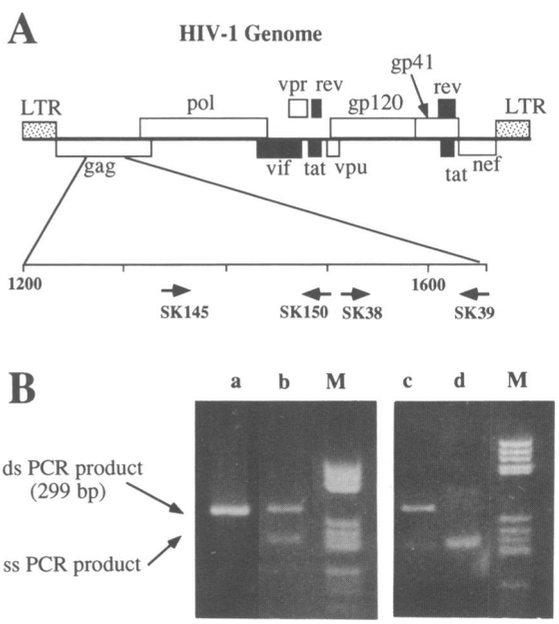

Figure 1. (A) Location of the SK145, SK150, SK38 and SK39 primers in the *gag* portion of HIV-1 genome. (B) Agarose gel electrophoresis of SK145/SK39 PCR products. a: SK145:SK39 = 1:1; b: SK145:SK39 = 10:1; c, d: analysis of purified dsPCR (c) and ssPCR (d) products. Molecular weight markers are indicated (M, HaeIII restricted pBR322 plasmid DNA).

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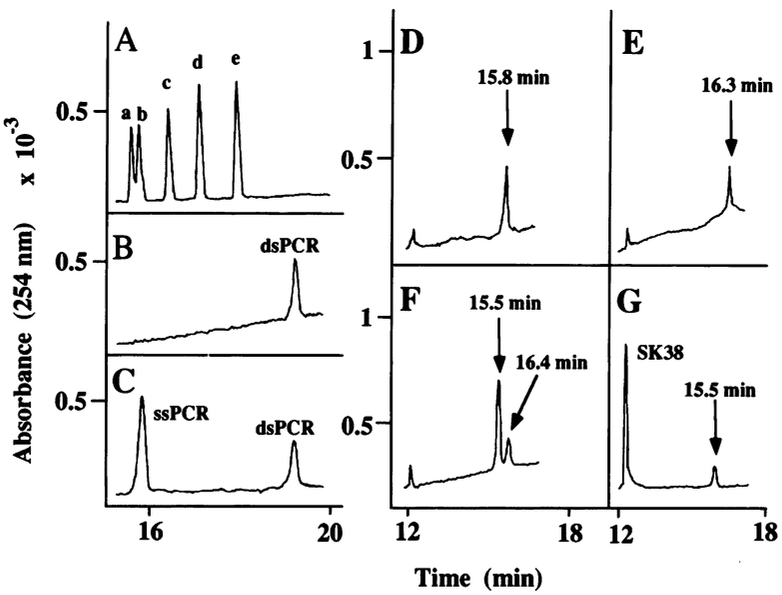

Figure 2. CE electropherograms of (**A**) molecular weight markers (the size of these dsDNAs are a = 184, b = 192, c = 213, d = 234, e = 267); (**B**) PCR products obtained with a ratio 1:1 of SK145 and SK39 primers; (**C**) unbalanced PCR products obtained with a ratio 10:1 of SK145 and SK39 primers; (**D**) purified ss(SK145/SK39) PCR; (**E**) purified ss(SK145/SK39) PCR products hybridized with 750 ng of SK150 primer as described in text; (**F**) mixture of (a) purified ss(SK145/SK39) PCR and (b) purified ss(SK145/SK39) PCR products hybridized with SK150; (**G**) purified ss(SK145/SK39) PCR products hybridized with 750 ng of SK38 primer as described in text. Migration time of ss(SK145/SK39) PCR products (15.5–15.8 min) is different from the migration time of purified ss(SK145/SK39) PCR products hybridized with SK150 (16.3–16.4 min).

oligonucleotides do not cause any shift in the electropherogram (Figure 2G and data not shown).

These data suggest that CE technique can be used as a one-step diagnostic approach (a) to identify HIV-1 PCR products and (b) to demonstrate the specificity of the amplification on the basis of a shift in the electrokinetic mobility of HIV-1 ssPCR product caused by a complementary synthetic oligonucleotide.

The picomole–femtomole (10^{-12}–10^{-15} mol) and the attomole–zeptomole (10^{-18}–10^{-21} mol) sensitivity ranges of UV–CE and laser-equipped CE (1–3, 8), respectively cannot be approached by standard electrophoresis techniques, unless hybridization is performed. A further advantages of CE over standard electrophoretic techniques is that CE discriminates between DNA fragments of very similar molecular weight (1–3).

We are using CE analysis of unbalanced PCR products, hybridized to internal oligonucleotides, to detect HIV-1 genomic sequences. Our results suggest that CE could be an integral part of automated diagnostic systems based on the use of laboratory workstations for DNA isolation, preparation of PCR reactions and analysis of PCR products.

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