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Journal of Chromatography A, 1109 (2006) 127–131
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Affinity capillary electrophoresis with a DNA-nanoparticle conjugate as a new tool for genotyping

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Available online 30 January 2006

Abstract

We have developed a novel method for genotyping based on free solution affinity capillary electrophoresis. We prepared DNA-nanoparticle conjugates by mixing biotin-modified DNA and NeutrAvidin-modified polystyrene nanoparticles; this mixture was then injected into a capillary. Subsequently, we injected the fluorescent-labeled sample DNAs into the capillary, applied the voltage, increased its temperature after 7 min, and detected the fluorescence at its anodic end. This novel method was applied for genotyping human c-K-*ras*, and the three genotypes were definitely distinguishable with high reproducibility. This method can be easily automated, and it is useful for high-throughput gene mutation analysis.

Keywords: Affinity capillary electrophoresis; Free solution affinity capillary electrophoresis; Gene point mutation; Genotyping; Nanoparticle

1. Introduction

Recent developments in the field of molecular biology have revealed that small mutations of certain genes are the definitive origin of cancers and genetic disease. These findings have highlighted the importance of gene mutation assays (genotyping) that are based on differences in DNA base sequences in the field of diagnostics and medicine [1].

A variety of methods have been proposed for genotyping. The currently proposed methods can be broadly classified into two categories: enzyme-based methods and hybridization-based methods [2,3]. The enzyme-based methods use the inherent catalytic specificity of enzymes for their substrates. Among these, the single base extension (SBE)-based methods have proved to be particularly attractive due to their robustness, and adaptability to various detection formats, including gel and capillary electrophoresis [4,5], solution-phase homogeneous assay [6,7], MALDI-TOF mass-based spectrometry [8], and solid-phase microtiter plates [9] or microarrays [10,11]. However, the SBE-based methods usually require complicated experimental procedures, including size separation of the product or removal of surplus substrates, which limit the potential for automation. The hybridization-based methods depend on single base differences in hybridization stability between a short oligonucleotide probe and the target DNA. The hybridization-based methods are attractive because of the simple procedures involved. Several rapid genotyping methods have been developed, such as TaqMan or 5′ nuclease assay [12], Molecular Beacon [13,14], iFRET [15], and Hybridization Probe Assay [16]. These methods are not cost effective, because they require fluorescently labeled oligonucleotide probes that are tailor-made and expensive. Every method has its own advantages and disadvantages, and novel ideas for further improving genotyping are required.

An affinity capillary electrophoresis (ACE) method, which is based on hybridization, is considered to be one of the promising methods for genotyping. ACE is a technique developed by combining the selectivity of bioaffinity recognition with the high separation power of capillary electrophoresis. In this method, an affinity ligand DNA that possesses a complementary sequence to the mutation site of the sample DNA is fixed in a capillary either by binding it to the inner surface of the capillary [17,18], or in a polymer matrix filled in the capillary [19–23]. During electrophoretic migration, the target DNA is trapped by the ligand, or it moves slowly due to its interaction with the ligand. On the other hand, the non-target DNA moves fast in the absence of any interaction. Thus, the target and non-target DNAs are separated

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doi:10.1016/j.chroma.2005.12.110

based on the difference in their affinities for the ligand DNA. ACE can distinguish any mutation and is currently being used for genotyping [18,21–23]. The drawback of this method is that the immobilization of the ligand DNA onto the inner surface of the capillary or in the polymer matrix is time consuming and labor intensive. In addition, when the polymer matrix is once filled in the capillary, it is not exchangeable, and the repeated use of the capillary causes undesired effects (memory effects) due to the accumulation of various components contained in the analytical samples.

Another ACE method for genotyping has been reported; this method uses fluidized DNA-polyacrylamide conjugates (free solution ACE) [24]. In this method, two kinds of conjugates that contain different ligand DNAs are used. These conjugates also migrate in the capillary; however, the mobility of the two conjugates is different because the DNA content of the conjugates is different, and the separation of DNA is performed successfully. The advantage of this method is that there are no memory effects because the conjugates are washed out from the capillary every time and not used repeatedly. The drawback of this method is that the preparation of the conjugates is time consuming and labor intensive.

Recently, nanosize particles have been used for the separation and detection of biomolecules. Nanoparticles have a high reactive surface to volume ratio, and hence, a large amount of ligand DNA can be immobilized on their surface. It has recently been reported that the use of NeutrAvidin-modified polystyrene nanoparticles simplified the preparation of a DNA-nanoparticle conjugate [25].

In this study, we developed a novel, free solution ACE method using one DNA-nanoparticle conjugate instead of the two DNA-polyacrylamide conjugates described above. The immobilization of biotinylated probe DNA onto the surface of NeutrAvidin-modified polystyrene nanoparticles was achieved by simple mixing of the DNA and the nanoparticles. We applied this novel ACE method for genotyping c-K-*ras* with a single-base difference.

2. Principle of genotyping by the novel ACE method using a DNA-nanoparticle conjugate

A schematic representation of the principle of the novel ACE system is shown in Fig. 1. In this system, the electroosmotic flow is suppressed by using a coated capillary. First, a solution of biotin-modified single-stranded DNA (ssDNA), which contains the complementary sequence to the target DNA, is mixed with NeutrAvidin-modified polystyrene nanoparticles to obtain DNA-nanoparticle conjugates. Next, this mixture is introduced into the capillary from the cathodic end (Fig. 1a); subsequently, a solution of a sample containing fluorescently-modified ssDNAs is injected (Fig. 1b). A high electric voltage is then applied (Fig. 1c). The conjugates and the ssDNAs migrate electrophoretically toward the anode. During the migration, the mobility of the conjugates is lower than that of the ssDNAs, and hence, the ssDNAs catch up with the conjugates. The non-target DNA continues to migrate through the capillary, while the target DNA hybridizes with the ligand DNA on the conjugates and moves with the conjugates (Fig. 1d). Thus, the target DNA is separated from the non-target DNA. Following this, the hybridized DNA is released by increasing the temperature of the capillary. The DNAs are detected with a laser induced fluorescence detector at the anodic end (Fig. 1e). Genotypes are distinguished by calculating the ratios of the trapped DNA peak area to the total peak area in the electropherograms.

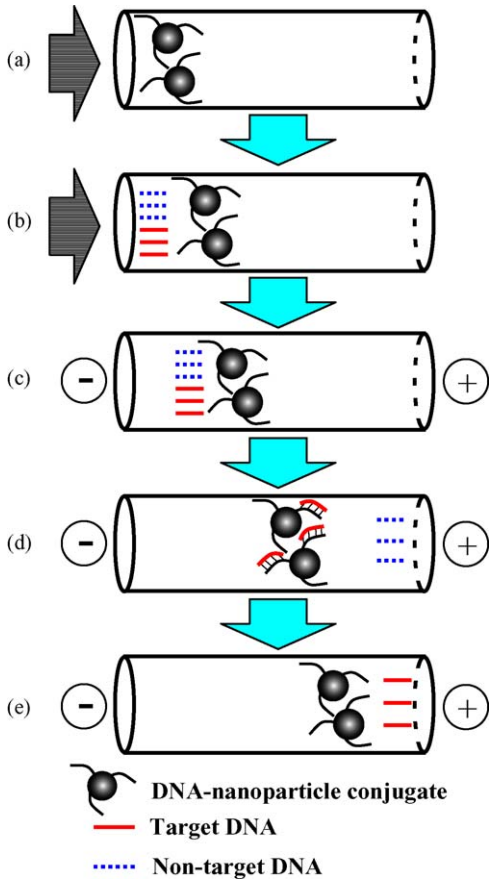

Fig. 1. Schematic principle of ACE using DNA-nanoparticle conjugate. (a) Inject the conjugate using pressure, (b) inject the sample using pressure, (c) applied the voltage, (d) trap the target DNA onto the conjugate, and (e) release the trapped DNA by raising the capillary temperature.

3. Experimental

3.1. Chemical and reagents

Taq DNA polymerase Hot Start version, 10× PCR buffer, dNTPs, and *ras* Mutant Set c-Ki-*ras* codon 12 that contained normal and mutant c-K-*ras* of the human genome were purchased from TaKaRa Bio (Shiga, Japan). The oligonucleotides used as the forward (5′-(Cy5)GACTGAATATAA ACTTGTGG-3′) and reverse (5′-(biotin)ATCGTCAAGGCACTCTTGCC-3′) primers for the preparation of 60-bp DNA fragments from human c-K-*ras*, ligand DNA (5′-GCCACTAGCTCC-3′) that was labeled at the 5′ end with NHS-LC-biotin II, and 12-mer normal (5′-GGAGCT**G**GTGGC-3′) and mutant (5′-GGAGCT**A**GTGGC-3′) c-K-*ras* were chemically synthesized (high-performance liquid chromatography-purified grade) by TaKaRa Bio. The underlined letter indicates the mutation point.