ANALYTICA CHIMICA ACTA 619 (2008) 101–109

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/aca

Evaluation of single-base substitution rate in DNA by affinity capillary electrophoresis

*Naoki Kanayama, Tohru Takarada**, *Hideaki Shibata, Ayumi Kimura, Mizuo Maeda**

Bioengineering Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

ARTICLE INFO

Article history:
Received 4 December 2007
Received in revised form
5 February 2008
Accepted 10 February 2008
Published on line 17 February 2008

Keywords:
DNA
Affinity capillary electrophoresis
Single-base mutation
Block copolymer
Hydrodynamic drag

ABSTRACT

Capillary electrophoretic separation of 60 mer single-stranded DNA (ssDNA) and a single-base-substituted ssDNA was demonstrated using a size- and composition-controlled poly(ethylene glycol)-oligodeoxyribonucleotide block copolymer (PEG-*b*-ODN) as an affinity ligand. Under appropriate conditions, PEG-*b*-ODN and ssDNA with a complementary sequence formed a reversible complex via hybridization during the electrophoresis, while the copolymer did not interact with the single-base-substituted ssDNA. The copolymer's PEG length determined the electrophoretic mobility of the ssDNA; upon formation of the complex, the electrically neutral PEG added hydrodynamic friction to ssDNA. Simultaneously using two types of PEG-*b*-ODN copolymers whose PEG segments were of different lengths, we achieved the complete separation of the 60 mer ssDNA, its single-base-substituted ssDNA, and impurities. This method was sensitive enough to quantify a slight amount (approximately 1%) of the single-base-substituted ssDNA. The present results suggest that our approach is applicable to quantitative detection of minor genotypes.

1. Introduction

One of the most important challenges in DNA analysis is the discrimination and quantification of a small number of minor sequences including single nucleotide polymorphisms (SNPs) in genomic samples. SNPs are genetic changes resulting from single nucleotide substitutions. These days, they are regarded as high-resolution genetic markers for mapping genes, identifying inherited diseases, and developing drug candidates [1,2]. Furthermore, they are now also utilized as powerful genetic markers in a variety of fields including agricultural science [3–6].

Based on the high specificity of nucleic acids in molecular recognition, SNP detection methods using allele-specific hybridization as well as primer extension and ligation have been proposed [7,8]. In these methods, the recognition of a target DNA sequence relies on hybridization with probe DNA. Since a single-base mismatch in the duplex makes only a small difference in the thermodynamic stability of a complementary duplex, these methods are intrinsically difficult to use to detect SNPs.

Thus far, we have developed an affinity capillary electrophoresis (ACE)-based DNA mutation assay that converts the mutation-induced small differences in the thermodynamic stability of duplexes to observable differences in their electrophoretic mobilities [9–12]. ACE is a powerful tool with which to examine affinity interaction between biomolecules, such as protein–ligand [13,14] and protein–DNA [15–17] interaction. Especially, gel-free ACE provides an excellent platform for studying affinity interactions under equilibrium conditions [18,19]. However, it is difficult to evaluate DNA–DNA affinity interaction in a gel-free CE system because DNA is a uniformly charged polyelectrolyte and its electrophoretic mobility is independent of its length and sequence. We recently reported

---

* *Corresponding authors.* Tel.: +81 48 467 9311; fax: +81 48 462 4658.
E-mail addresses: ttkrd@riken.go.jp (T. Takarada), mizuo@riken.go.jp (M. Maeda).

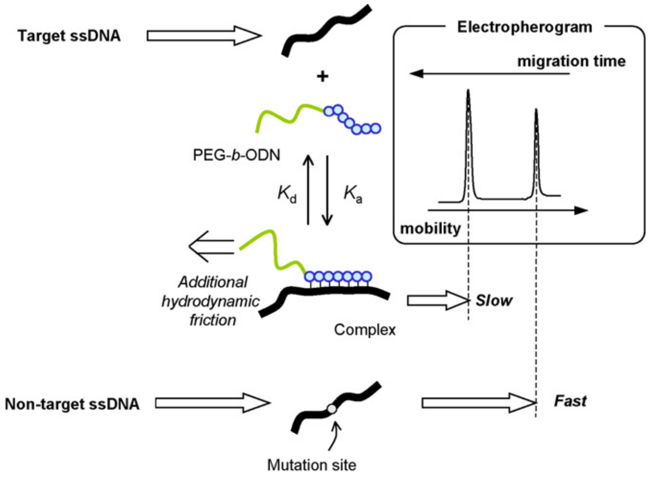
0003-2670/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2008.02.021

the use of poly(ethylene glycol)-oligodeoxyribonucleotide block copolymers (PEG-*b*-ODNs) with well-controlled size and composition in gel-free CE to facilitate the ACE-based DNA mutation assay [20,21]. ODNs 7–9 mer in length were employed as affinity probes. Their sequences were designed to be complementary to part of target single-stranded DNA (ssDNA). The center of this part should contain the single-base mutation site. Conjugation of the electrophoretically neutral PEG segment to the 5′-terminus of the affinity probe ODN significantly retarded electrophoretic migration in the electric field. When PEG-*b*-ODN was added to the running buffer, target ssDNA formed a complex with PEG-*b*-ODN via duplex formation between the complementary region of target ssDNA and the affinity probe ODN segment of PEG-*b*-ODN, and they were in equilibrium during electrophoresis, as follows.

$$\text{target ssDNA} + \text{PEG-}b\text{-ODN} \underset{K_d}{\overset{K_a}{\rightleftarrows}} \text{complex} \qquad (1)$$

The PEG segment could give hydrodynamic friction to target ssDNA by complex formation; accordingly, the electrophoretic mobility of target ssDNA was significantly retarded compared with non-target ssDNA (Scheme 1). We have already proven that the electrophoretic mobility of target ssDNA depends on the affinity strength between the affinity probe ODN and the target ssDNA [21]. Under appropriate conditions, a mixture of 20 mer ssDNA and its single-base-substituted one was separated into two distinct peaks on the electropherogram, allowing for the facile detection of single-base substitution within the 20 mer ssDNA sequence.

In this study, we extended the analytical target to a longer ssDNA (60 mer) with polymerase chain reaction (PCR)-amplifiable fragment length. A mixture of a 5′-FITC-labeled 60 mer ssDNA (normal ssDNA) and its single-base-substituted 60 mer ssDNA (mutant ssDNA) was employed as an analytical sample (sample ssDNA, Table 1). The sequence of normal ssDNA and mutant ssDNA corresponds to the mitochondrial cytochrome *b* codon 133–153 of cucumber powdery mildew and its single-base mutant at codon 143 (GGT to GCT), respectively. It has been reported that this single-base mutation (G143A mutation) in the cytochrome *b* gene was closely related to the development of strobilurin-related fungicide resistance in several fungal pathogens, including cucumber powdery mildew [22,23]. A facile and quantitative method for detecting SNPs will be useful for monitoring the frequency of the strobilurin-resistant cytochrome *b* allele in field populations of fungal pathogens.



Scheme 1 – Schematic illustration of affinity electrophoretic ssDNA separation by using PEG-*b*-ODN.

Here, we described the electrophoretic separation behavior of 60 mer ssDNA and its single-base-substituted one using PEG-*b*-ODN as an affinity ligand. Moreover, we designed a novel ACE system that enabled reliable and sensitive detection of mutant sequence in samples, regardless of the presence of unexpected impurities. This *dual-conjugate* system relies on the simultaneous use of two PEG-*b*-ODNs having different PEG molecular weights and different ODN sequences; one is complementary to the normal ssDNA, while the other is complementary to the mutant ssDNA. The new system was applied to quantify the contents of mutant sequences in the

Table 1 – Sequences of sample ssDNA and affinity probe ODN used in this study

Name	Sequence
Sample ssDNA[a]	
Normal ssDNA	5′-TT TTA CCT TGG GGA CAA ATG AGT TTT TGG G**G**T GCA ACT GTT ATT ACT AAT TTA TTT TCT G-3′
Mutant ssDNA	5′-TT TTA CCT TGG GGA CAA ATG AGT TTT TGG G**C**T GCA ACT GTT ATT ACT AAT TTA TTT TCT G-3′
Affinity probe ODN	
ODN(7N)	3′-CC CCA CG-5′
ODN(8N)	3′-ACC CCA CG-5′
ODN(9N)	3′-ACC CCA CGT-5′
ODN(10N)	3′-A ACC CCA CGT-5′
ODN(8M)	3′-ACC CGA CG-5′

[a] The 5′-terminus of sample ssDNA was labeled with FITC.