Available online at www.sciencedirect.com
ScienceDirect
Reactive & Functional Polymers 67 (2007) 1373–1380
REACTIVE & FUNCTIONAL POLYMERS
www.elsevier.com/locate/react

Poly(ethylene glycol)-oligodeoxyribonucleotide block copolymers for affinity capillary electrophoretic separation of single-stranded DNAs with a single-base difference

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

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

Received 1 June 2007; received in revised form 17 July 2007; accepted 18 July 2007
Available online 28 July 2007

Dedicated to Professor Teiji Tsuruta on the occasion of his 88th birthday (Beiju).

Abstract

An affinity capillary electrophoretic method was developed to detect a single-base difference of single-stranded DNA (ssDNA). Poly(ethylene glycol)-oligodeoxyribonucleotide block copolymers (PEG-*b*-ODN) were prepared for use as a novel affinity ligand. We introduced a running buffer solution of PEG-*b*-ODN into a capillary tube, and electrophoretically separated a mixture of chemically synthesized 20 mer ssDNA (normal ssDNA) and a single-base-substituted 20 mer ssDNA (mutant ssDNA). When the base sequence of PEG-*b*-ODN was designed to be complementary to part of the normal ssDNA, the migration rate of the normal ssDNA was significantly decreased by reversible hybridization with PEG-*b*-ODN, depending on the base number of PEG-*b*-ODN, the salt concentration of the running buffer, and the capillary temperature. In contrast, the mobility of mutant ssDNA did not change because the interaction with PEG-*b*-ODN was negligible. Optimization of the analytical conditions gave two distinct peaks, one for normal and the other for mutant ssDNA, on the electropherogram, allowing for facile discrimination of the single-base difference. The results indicate that PEG-*b*-ODN is a promising affinity ligand for the capillary electrophoretic separation of normal and single-base mutated ssDNA.

Keywords: Affinity capillary electrophoresis; Poly(ethylene glycol); DNA; SNPs; Duplex formation

1. Introduction

Single nucleotide polymorphisms (SNPs) are genetic changes resulting from single nucleotide substitutions. They are utilized as high-resolution genetic markers for mapping genes, identifying inherited diseases, and discovering new drugs [1]. Though originally used in genetics and medicine,

* 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).

1381-5148/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.reactfunctpolym.2007.07.036

SNP markers have recently been considered a powerful tool in agricultural [2] and environmental sciences [3]. Consequently, development of a method to detect SNPs has been one of the most important issues in analytical science. A number of detection methods have been developed, including DNA microarrays [4], fluorescence resonance energy transfer-based assays [5], and polymerase chain reaction-based methods [6]. However, these established methods are relatively labor-intensive and time-consuming.

Capillary electrophoresis has several advantages, such as simple procedures, short running time, high-resolution, and small amount of sample [7]. To discriminate a single-base difference of ssDNA, two electrophoresis-based methodologies have already been developed: the single-strand conformation polymorphism (SSCP) assay [8] and affinity capillary electrophoresis [9,10]. SSCP is based on the difference in folded structures between normal and mutant ssDNA. The SSCP assay is a very simple method, but it is applicable only when each folded ssDNA can show a detectably different electrophoretic mobility.

In contrast, affinity electrophoresis is a general method of separating biological molecules through the use of specific interactions between antigens and antibodies, enzymes and substrates, ligands and receptors, and so on [11]. To separate normal and mutant ssDNA by affinity capillary electrophoresis, we have utilized ODN-*grafted* polyacrylamide (PAAm-*g*-ODN) to serve as a pseudo-immobilized affinity ligand [9,10]. Since the sequence of ODN (6–12 mer) was designed to be complementary to the relatively small area around the mutation site of normal ssDNA, the duplex formation between sample ssDNA and affinity ligand ODN was reversible and in the dynamic equilibrium state. This weak interaction with ODN allowed mutant ssDNA to migrate without binding too strongly with PAAm-*g*-ODN pseudo-immobilized in a capillary tube. As a result, the migration of normal ssDNA was slower than that of mutant ssDNA, which could not form a duplex with ODN due to a single-base mismatch. Using capillary tubes filled with the running buffer solution of PAAm-*g*-ODN, we previously achieved the single-step separation of normal and mutant ssDNA without any gradient control over temperature or denaturants [9]. Since the separation efficiency was closely associated with the melting temperature (T_m) of the duplex between sample ssDNA and affinity ligand ODN, the appropriate sequence of ODN can be inferred from the T_m value under analytical conditions [10].

In the present work, we prepared poly(ethylene glycol)-ODN block copolymers (PEG-*b*-ODN) for a novel affinity ligand. Thus far, some research groups have reported the application of PEG-oligonucleotide block copolymers to deliver antisense DNA and siRNA [12]. PEG is water-soluble, electrically neutral, and free from nonspecific interaction with biological molecules, including DNA. We considered that all these features were well suited for an affinity ligand. Various end-functionalized PEG derivatives, which have highly controlled polymerization degrees and narrow molecular weight distributions, are commercially available. Block-type conjugation of the PEG derivative to ODN will give a size- and composition-controlled affinity ligand. Here, we demonstrate the electrophoretic separation of normal and mutant ssDNA by using PEG-*b*-ODN as an affinity ligand.

2. Experimental

2.1. Materials

All reagents were commercially available and used without further purification unless otherwise noted. Maleimide-terminated PEG (mPEG-MAL, $M_n = 5000$) was purchased from Nektar Therapeutics. 5′-FITC-labeled ssDNA and 5′-thiol-terminated ODN (ODN-SH) were purchased from Tsukuba Oligo Service. The ODN-SH was purified through a NAP-5 column (GE Healthcare) before use. The DNA concentration was determined by measuring the absorbance at 260 nm.

2.2. Synthesis of PEG-b-ODN

The ODN-SH (7 mer: 5′-GCACCCC-3′, 110 nmol) in 750 μl of 10 mM Tris–HCl buffer (pH 7.4) was deoxidized by bubbling with Ar gas for 5 min. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP: 110 nmol) and mPEG-MAL (1.6 mg, 330 nmol) were added to the buffer solution of ODN-SH. The mixture was allowed to react overnight at room temperature and was then loaded on a gel filtration column packed with Sephadex G-100 gel (GE Healthcare) to remove unreacted ODN-SH and TCEP. Further purification was carried out using anion exchange chromatography (Q-Sepharose Fast Flow, GE Healthcare) to remove excess mPEG-MAL. The gradient (40 min) was 0–100% (0–15 min,