Chinese Chemical Letters Vol. 11, No. 11, pp. 1015–1018, 2000

A Novel Sieving Medium for Separation of DNA Fragments-Poly(acrylamide-dimethylacrylamide)

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Abstract: A short chain poly (acrylamide-dimethylacrylamide) was synthesized in water phase using isopropanol as a chain transfer agent. This copolymer can form a stable dynamic coating on the inner surface of the capillary, thereby suppressing the electroosmotic flow and DNA-capillary wall interaction. The high efficient separation of DNA fragments and SSCP analysis were obtained in bare capillaries using this copolymer as a sieving medium.

Keywords: Poly (acrylamide-dimethylacrylamide), DNA, capillary electrophoresis.

Introduction

Capillary electrophoresis (CE) in polymer solution has become an attractive alternative to slab gel electrophoresis for DNA analysis[1, 2]. This technique has been successfully applied for mutation detection, genotyping, DNA sequencing and gene expression[3-7]. In order to perform DNA analysis, the inner surface of the capillary must be modified, usually by covalent bonding of hydrophilic polymers. However, the covalent coating will increase the cost and often causes problems related to capillary deterioration and coating inhomogeneity.

In the present work we first synthesized a novel copolymer-poly (acrylamidedimethyla crylamide), which can form a stable dynamic coating on the inner surface of the capillary and then demonstrated that the separation of DNA fragments and SSCP analysis were successfully obtained in bare capillaries using this copolymer as sieving medium.

Experimental

Materials

Acrylamide, pUC 18 DNA fragments (12 fragments) and N-tris (hydroxymethyl) methyl -3-aminopropanesulfonic acid (TAPS) were obtained from Sigma Chemical Co. (St. Louis, MO). N,N'-Dimethylacrylamide was from Aldrich-Chemie (Germany). N,N,N',N'- tetramethylenediamine and ammonium peroxydisulfate, were purchased

from Bio-Rad Laboratories (Hercules, CA). The pH of TAPS buffer was adjusted with 1 mol/L sodium hydroxide. SYBR Green I (concentration not given) in dimethyl sulfoxide was purchased from Molecular Probes. QIAamp blood kits were products of QIAGEN Co. (Hilden, Germany). Fused silica capillaries with 75 μm internal diameter (i.d.) and 365 μm outer diameter (o.d.) were from J&W Scientific (Folsom, CA) and Yongnian Optical Fiber Factory (Yongnian, Hebei, China). 5-Fluorescein-labeled primers were synthesized by Eurogentec (Seraining, Belgium). Water double-distilled was used for preparation of all aqueous solutions.

Synthesis of short-chain linear poly (acrylamide-dimethylacrylamide) (PADMA)

PADMA was synthesized in water using isopropanol as a chain transfer agent to control the molecular weight of the copolymer. Briefly, 110 mL water and 3.8 ml isopropanol were added to a vessel containing a given of dimethylacrylamide and acrylamide. The mixture was degassed with helium for 30 min and heated at 50 ^{o}C in water bath 20 min. Then, 0.625 ml 10% (v/v) N,N,N',N'-tetramethylenediamine and 0.625 ml 10% (w/v) ammonium peroxydisulfate were added, and the polymerization took place for 1.5 h at 50 ^{o}C.

The reaction product was extensively dialyzed against water for two days, using a 12,000 molecular weight cutoff dialysis membrane tubing (Thomas Scientific, Philadephia, PA), lyophilized. The sieving medium was prepared by dissolved the copolymer powder in separation buffer.

DNA extraction and PCR reactions

DNA was extracted from whole blood using a QIAamp blood kit according to the instruction from the manufacture. The PCR mixture contained 10 mmol/L Tris-HCl , pH 9.0, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 0.01% (w/v) gelatin, 0.1% Triton X-100, 125 μmol/L of each dNTP, 0.2 μmol/L of each primer, 1 U Taq polymerase (Super Taq, HT Biotechnology Ltd., UK) and approximately 100 ng template DNA in final volume of 100 μL. PCR was performed on a DNA Thermocycler (Perkin-Elmer, USA) using a thermocycling profile with 36 cycles of 94 ^{o}C for 20 s, 58 ^{o}C for 30 s and 72 ^{o}C for 20 s. The primers used were 5'-fluorescein-CTTTGGGGAGCTGAAGGACTACTAC (for-ward) and 5'-fluorescence-CACTTTGTGACCATTCCGGTTTG (reverse). The Primers define the PCR product of 163 bp in length including a position 1298. The PCR products were precipitated with ethanol according to the reference[3] and then dissolved in 50 μL diluted buffer (0.1 xTBE). The samples were stored at –20 ^{o}C until analysis.

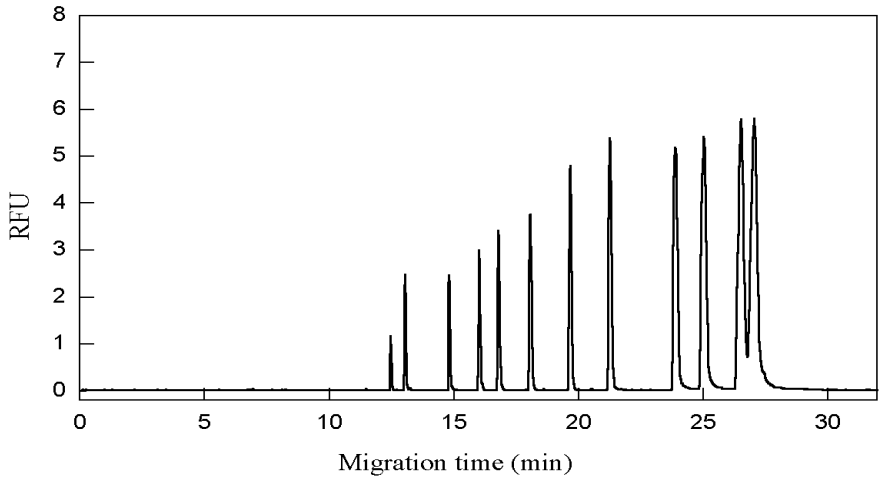
Capillary electrophoresis procedure

The Beckman P/ACE System 5500 (Beckman Instruments, Fullerton, CA) was used in this study. It was equipped with a LIF detector. A new capillary was first rinsed with 1mol/L HCl solution for 10 min, then with water for 5 min and finally filled with copolymer sieving medium. For double-strand DNA analysis, SYBR Green I was added

to the sieving medium at a dilution of 1:5000. Samples were introduced by electrokinetic injection at –5 kV for 10 s, and the electrophoresis was performed at reverse polarity mode. Between each run, the capillary was rinsed with the separation buffer for 4 min and then filled with the fresh sieving medium.

Results and Discussion

Figure 1. Separation of pUC 18 Msp I digests (26, 34, 67, 89, 110, 147, 190, 242, 353, 404, 489, 501 bp) in a bare capillary using PADMA as sieving medium.



Electrophoretical conditions: capillary, 37 cm total length, 30 cm effective length, 75 μm i.d., 365 μm o.d.; sieving medium, 5% PADMA (dimethylacrylamide to acrylamide ratio, 1:9); buffer, 100 mmol/L TAPS, pH 7.8; Temperature, 25 ^{o}C; the applied voltage, 8 kV; RFU, relative fluorescence units.

Figure 1 showed the separation of pUC 18 DNA fragments in a bare capillary using PADMA (the dimethylacrylamide to acrylamide ratio, 1: 9) as sieving medium. The system was characterized by high separation efficiency and the pUC 18 DNA fragments were well resolved under the conditions. The similar resolutions for pUC18 DNA fragments were obtained using a series of PADMAs with different dimethylacrylamide to acrylamide ratio. The copolymers were also successfully applied as sieving media for SSCP analysis of methylenetetrahydrofolate reductase gene. Three genotypes of A1298A, A1298C and C1298C were clearly distinguished by CE with PADMA medium (data not shown).

Our preliminary results demonstrated that the PADMA was an attractive alternative to other non-cross linked polymers commonly used in CE.

Acknowledgments

This work was supported by The National Natural Science Foundation of China (Grant No. 29975008).

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Received 8 May 2000