Journal of Chromatography A, xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Tunable separations based on a molecular size effect for biomolecules by poly(ethylene glycol) gel-based capillary electrophoresis

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ARTICLE INFO

Article history:
Received 15 March 2017
Received in revised form 13 June 2017
Accepted 15 June 2017
Available online xxx

Keywords:
Capillary gel electrophoresis
Poly(ethylene glycol)
Molecular size
Crosslinking density
Biomolecules

ABSTRACT

We report novel capillary gel electrophoresis (CGE) with poly(ethylene glycol) (PEG)-based hydrogels for the effective separations of biomolecules containing sugars and DNAs based on a molecular size effect. The gel capillaries were prepared in a fused silica capillary modified with 3-(trimethoxysilyl)propylmethacrylate using a variety of the PEG-based hydrogels. After the fundamental evaluations in CGE regarding the separation based on the molecular size effect depending on the crosslinking density, the optimized capillary provided the efficient separation of glucose ladder (G1 to G20). In addition, another capillary showed the successful separation of DNA ladder in the range of 10–1100 base pair, which is superior to an authentic acrylamide-based gel capillary. For both glucose and DNA ladders, the separation ranges against the molecular size were simply controllable by alteration of the concentration and/or units of ethylene oxide in the PEG-based crosslinker. Finally, we demonstrated the separations of real samples, which included sugars carved out from monoclonal antibodies, mAbs, and then the efficient separations based on the molecular size effect were achieved.

1. Introduction

Gel electrophoresis (GE) is one of powerful tools for the efficient separation of biomolecules, such as polysaccharides, nucleic acids, and proteins. Therefore, a variety of GE methods have been widely employed for the separations in the field of biochemistry, medical science, pharmacology, and food science [1–12]. In most cases, a simple molecular sieving effect is employed for these separations, so that we have a number of possibilities for using separation media corresponding to the targeting compounds. In fact, a great number of applications using GE have been reported for the separations of polysaccharides [13–15] and proteins [16–21], especially the applications to DNAs separations have been widely examined [22–27]. Furthermore, the formats of electrophoresis are not only slab gels and capillary gel electrophoresis (CGE) but microchips in recent researches [28–31].

In general, agarose gels and polyacrylamide (PAA) gels are usually utilized for GE [32,33]. The agarose gel can be easily prepared and allows the effective separation of DNAs in the range of 0.1–60 k base pair (bp) by controlling the concentration of agarose. However, the agarose gel is not suitable for the separation of small size differences because of its larger pores. On the other hand, the pore sizes of a PAA gel are controllable, so that the smaller DNAs can be separated. Meanwhile, the range of the suitable molecular size is limited in the PAA gels. Additionally, the toxicity of the acrylamide monomer and the non-specific interactions by amide groups toward biomolecules are also problematic in using PAA [34]. Instead of these gels, poly(ethylene glycol) (PEG) has attracted attention as another separation medium in GE. As well known, PEG shows several advantages, including higher biocompatibility, lower non-specific interaction, and low toxicity of the monomers. In addition, a variety of PEG derivatives having several ethylene oxide (EO) units are commercially available. Therefore, we expected that the PEG-based hydrogels can be useful for the separation medium in GE [35,36]. As previous studies regarding the PEG-based separation in GE, X. Dou et al. reported the separation of RNA fragments ranged from 100 to 10,000 nt in PEG and polyethylene oxide (PEO) solutions with different molecular weight and different concentration in capillary electrophoresis [37]. Furthermore, T. Sakai et al. reported unique PEG-based separations by CGE using tetra-PEG, and reported the physical and chemical properties of the PEG gels related to the separation behavior based on the molecular sieving

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http://dx.doi.org/10.1016/j.chroma.2017.06.043
0021-9673/

effect [38–42]. Similar to these interesting results, we also reported the PEG-based hydrogels using PEG dimethacrylate (PEGDMA) as a crosslinker, and applications to the responsible swelling/shrinking gel [43], the protein imprinting [44,45], and the tunable molecular separations in GE [46]. Despite of these studies regarding PEG-based media in GE, the fundamental evaluations concerning the relations between the concentrations of the monomers contributing the crude density of the polymer network and the molecular sieving effect had never been discussed.

In this study, we aim to develop universal media for the efficient separation based on the molecular size in CGE: a variety of PEG-based hydrogels were prepared with PEGDMA by changing the concentration and EO unit in a capillary to control the polymer network. The separable ranges of the molecular weight for glucose and DNA ladders were evaluated with the prepared capillaries by CGE. Additionally, the separation of sugars carved out from monoclonal antibodies (mAbs) was demonstrated as a practical application in the CGE analysis.

2. Experimental

2.1. Materials

Methanol of the HPLC grade, acetic acid, sodium cyanoborohydride ($NaBH_3CN$), tetrahydrofuran (THF), tris(hydroxymethyl)aminomethane (Tris), boric acid, sodium hydroxide (NaOH), hydrochloric acid (HCl), acrylamide, 2,2′-azobis-[2-(2- imidazolin-2-yl)propane] (AIZP), *N*,*N*'-methylenebisacrylamide (MBAC), and ethylenediaminetetraacetic acid (EDTA) were purchased from Nacalai Tesque (Kyoto, Japan), 3-(trimethoxysilyl)propylmethacrylate (γ-MAPS) from Tokyo Chemical Industry (Tokyo, Japan), 2,2-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (AIYP), ammonium peroxodisulfate (APS), *N*,*N*,*N*',*N*'-tetramethylethylenediamine (TEMED), D(+)-glucose, maltose monohydrate, and maltoheptaose from Wako Pure Chemical Industries (Osaka, Japan), PEGDMA (9G, 14G, and 23G; M_W = 536, 736, and 1136, respectively) and glucose oligomer from Shin-Nakamura Chemical (Wakayama, Japan), YOYO-1 and DNA ladder from Thermo Fisher Scientific K. K. (Yokohama, Japan), 9-aminopyrene-1,4,6-trisulfonic acid (APTS) from Sigma-Aldrich Japan (Tokyo, Japan). Deionized water was obtained from a Milli-Q Direct-Q 3UV system (Merck Millipore, Tokyo, Japan). A fused-silica capillary was purchased from Polymicro Technologies (Phoenix, AZ, USA).

2.2. Instruments

CE analyses were carried out by a P/ACE MDQ (Beckman Coulter, Fullerton, CA, USA) with a laser-induced fluorescence (LIF) 488 nm Laser Module. Measurements of pH of all the solutions were carried out by an F–52 pH meter (Horiba, Kyoto, Japan). IX71 (Olympus, Tokyo, Japan) and Eppendorf Thermomixer (Eppendorf AG, Hamburg, Germany) were used as a fluorescence microscope and a mixer, respectively.

2.3. Preparation of gel capillaries

A fused silica capillary (50 cm × 50 μm i.d.) was flushed with 1.0 M aqueous NaOH for 1 h, water for 5 min, 1.0 M aqueous HCl for 2 h, and methanol for 15 min by a syringe pump followed by N_2 gas. Then, the capillary was reacted with 50 vol% γ-MAPS in methanol at 40 °C for 16 h by flushing with a syringe pump. Finally, the reacted capillary was washed with methanol and dried, and then a vinyl-modified capillary was obtained.

To prepare the gel capillaries with PEGDMA or PAA, solutions for diluting monomers were prepared. An 89 mM aqueous Tris-boric acid solution was prepared (1 × TB, pH 8.64). EDTA was diluted with 1 × TB to 2 mM (1 × TBE, pH 8.31). A PAA solution was prepared with acrylamide/MBAC at 40%T (total monomer concentration) and 5%C (weight percentage of crosslinker). Each pre-polymerization solution shown in Table 1 or Table 2 was filled into the vinyl-modified capillary and sealed tightly with Teflon tape in the end of the capillary. The capillaries were left for 16 h at 65 °C in water bath with AIZP or at room temperature with APS/TEMED. Then, both the end of the capillary were cut for 5 cm length to remove the void and fix to the CE instrument.

Table 1
Composition of PEGDMA-based hydrogels.

Crosslinker (9G, 14G, or 23G) (mg)	Initiator	Solvents[a] (1×TB or 1×TBE) (mL)	Ratio of crosslinker (volume%)
57	10% APS aq., 57 μL	2.95	1.7
68	TEMED, 11.4 μL	2.94	2.0
79	or	2.93	2.4
159	AIYP, 5.7 mg	2.86	4.7
238		2.78	7.1
318		2.71	9.4
398		2.64	11.8
477		2.57	14.2
557		2.49	16.5
637		2.42	18.9

[a] TB buffer and TBE buffer were utilized for the capillaries to be analyzed glucose ladder and DNA, respectively.

Table 2
Composition of PAA-based hydrogels.

	PAA solution (5%C) (μL)	Initiator	Solvents[a] (1 × TB or 1 × TBE) (mL)
3%T	225	10% APS aq., 15 μL	2.78
5%T	375	TEMED, 3 μL	2.63
10%T	750		2.25
15%T	1125		1.88
20%T	1500		1.50

[a] TB buffer and TBE buffer were utilized for the capillaries to be analyzed glucose ladder and DNA, respectively.

2.4. Sample preparations

APTS labeled glucose ladder (G1 ~ G20) was prepared as follows: 2.5% aqueous APTS 3.0 μL, 100 mM aqueous glucose ladder 3.0 μL, acetic acid 2.25 μL, and water 6.75 μL were mixed in a polypropylene tube and stirred. After adding 1.0 M $NaBH_3CN$ in THF 5.0 μL, the mixture was reacted at 55 °C in water bath for 2 h. Then, the mixture was diluted with a 1 × TB buffer to 100 μL. By the same procedures, glucose, maltose, maltopentaose, and maltoheptaose were labeled by APTS.

YOYO-1 labeled DNA ladder (100 ~ 1500, 2072 bp, 1 μg/μL) was prepared as follows: DNA ladder 10 μL and 100 μM YOYO-1 20 μL were mixed in a polypropylene tube and left at room temperature for 1 h. Then, the mixture was diluted for 100 times with a 1 × TBE buffer. Each DNA containing 100, 500, 1000, and 1500 bp was labeled by the same procedures.

For real sample analyses, sugars carved out from mAbs were prepared. The APTS labeled sugars from mAbs-A and -B were separated by CGE with a PEGDMA-based gel capillary. The detailed procedures for the sample preparation is described in Supplementary Information.

Please cite this article in press as: T. Kubo, et al., Tunable separations based on a molecular size effect for biomolecules by poly(ethylene glycol) gel-based capillary electrophoresis, J. Chromatogr. A (2017), http://dx.doi.org/10.1016/j.chroma.2017.06.043