Tunable Molecular Sieving in Gel Electrophoresis Using a Poly(ethylene glycol)-Based Hydrogel

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Abstract
To achieve a tunable molecular sieving in gel electrophoresis, novel hydrogels consisted of a poly(ethylene glycol) based crosslinker and ionic monomers were developed. The gels showed specific shrinking regarding ionic solutions by suppressing an ionic repulsion in the gels as our previous report. Additionally, the intermolecular interactions such as hydrophobic interaction and π-π stacking among the adsorbed solutes also affected the specific shrinking of the gels. These gels were evaluated in gel electrophoresis using several glucans and DNA base pairs to examine the possibility of the molecular sieving. The gel prepared without ionic monomers showed the various separations depending on the differences of crosslinking degree. Also, the gel prepared with acrylic acid provided the various separation patterns of glucans based on the molecular sieving by simply changing pH of the buffer solutions for electrophoresis.

Keywords: Hydrogel; Molecular sieving; Gel electrophoresis; Shrinking

1. Introduction
For the separations of biomolecules, including proteins, sugars, and nucleic acids, liquid chromatography and electrophoresis are usually utilized. In particular, gel electrophoresis is commonly used owing to its simple procedure, possibility for large amount, and low cost. In most cases, the molecular sieving effect is employed for the electrophoresis separations of biomolecules in slab-gel [1], capillary gel [2,3], and microchip gels [4,5].

Polyacrylamide can be easily prepared with a high repeatability and controlled its polymer network by a simple change of monomer contents, so that polyacrylamide gel electrophoresis (PAGE), e.g., SDS-PAGE, is usually used for slab-gel electrophoresis [6]. Although the pore size of polyacrylamide can be controlled by the density of a crosslinker, N,N’-methylenebisacrylamide, and applicable for the effective separation of proteins, an appropriate gel-preparation is necessary for each range of the molecular weight and the toxicity of acrylamide monomer is of a problem. An alternative gel enabling tunable molecular sieving by a simple change of running solutions is attractive as a novel separation procedure with “single” slab-gel.

On the other hand, poly(ethylene glycol) (PEG) has a biodegradable property and high stability for pH, therefore PEG has been applied for an artificial bone and antibody [7,8]. Also, PEG has a high hydrophilicity, excluded volume effect, and high mobility, so that PEG based materials prohibit a non-specific adsorption of biomolecules [9,10]. According to these characteristics, a PEG-base gel is also expected to be utilized for gel electrophoresis. Recently, we reported novel hydrogels prepared with a PEG-based crosslinker and ionic monomers as a molecular-recognition responsive swelling/shrinking gel. In general, the swelling/shrinking of hydrogels is depending on the affinity between gels and solvents, the condition of charges in hydrogels, and the degree of crosslinking. Especially, the stimulus response for pH [11,12], temperature [13], ionic strength [14,15], pressure [16], and light [17] have been widely studied using PEG-containing hydrogels.

In order to develop a tunable molecular sieving in gel electrophoresis, we prepared novel PEG-based hydrogels with the reversible swelling/shrinking property by a simple change of running solutions. As a fundamental study, the swelling/shrinking behavior of the gels by intermolecular interactions was measured as a function of the crosslinking degree and the pH of running solutions.

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interaction among the adsorbed solutes to the gels was evaluated via batch adsorption as well as our previous study [18]. The separation of YOYO-1 labeled glucans and 8-aminonaphthalene-1,3,6-trisulfonic acid (APTS) labeled DNAs were carried out with the PEG-based gels in gel electrophoresis. The possibility of the molecular sieving effect was examined with the hydrogels prepared without any ionic monomers. The tunable molecular sieving by simple pH change of a running solution was also evaluated with an acrylic acid-containing PEG-based gel.

2. Experimental

2.1. Chemicals and reagents

PEG 600 dimethacrylate (PEG-DMA) was kindly donated from Shin-Nakamura Chemical Co. Ltd. (Wakayama, Japan), and were utilized as received. Sodium p-styrenesulfate (SS) as functional monomers, 2,2′-azobisisobutyronitrile (VBTMAC) as a functional monomer, and other reagents were purchased from Wako Chemicals (Osaka, Japan). Acrylic acid (AA), vinylbenzyl trimethylammonium chloride (VBTMAC), and 9-aminopyrene-1,3,6-trisulfonic acid (APTS) were purchased from Sigma Aldrich Japan (Tokyo, Japan). Glucans, agarose, tris(hydroxymethyl) aminomethane, boric acid, EDTA, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Nacalai Tesque (Kyoto, Japan). DNA fragments (NoLimits DNA Fragment) were purchased from Thermo Fisher Scientific Inc. (Pittsburgh, PA). YOYO-1 was purchased from Life Technologies (Eugene, OR). PEG 600 dimethacrylate (PEG-DMA) was kindly donated from Shin-Nakamura Chemical Co. Ltd. (Wakayama, Japan), and were utilized as received. Sodium p-styrenesulfate (SS) as functional monomers, 2,2′-azobisisobutyronitrile (VBTMAC) as a functional monomer, and other reagents were purchased from Wako Chemicals (Osaka, Japan). Acrylic acid (AA), vinylbenzyl trimethylammonium chloride (VBTMAC), and 9-aminopyrene-1,3,6-trisulfonic acid (APTS) were purchased from Sigma Aldrich Japan (Tokyo, Japan). Glucans, agarose, tris(hydroxymethyl) aminomethane, boric acid, EDTA, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Nacalai Tesque (Kyoto, Japan). DNA fragments (NoLimits DNA Fragment) were purchased from Thermo Fisher Scientific Inc. (Pittsburgh, PA). YOYO-1 was purchased from Life Technologies (Eugene, OR).

2.2. Preparation of hydrogels

We synthesized various hydrogels by a simple copolymerization. PEG-DMA as a crosslinker, SS or AA or VBTMAC as a functional monomer, methanol aqueous solution as a porogenic solvent, and 1.0 or 5.0 wt% AIPD as a functional monomer, methanol aqueous solution as a porogenic solvent, and 1.0 or 5.0 wt% AIPD as a functional monomer, were mixed in 15 mL polypropylene tube (119 × 286 mm) and polymerized by UV irradiation (365 nm, 3 h) in a gel-tray, 130 mm × 59.5 mm × 13 mm (height).

2.3. Swelling/Shrinking behaviors of hydrogels in various solutions

The hydrogels were cut into a disk shape at about 5 mm thickness to measure their swelling/shrinking behaviors. The equilibrium swelling ratio was calculated from the following equation:[19,20]

\[ \text{Relative swelling ratio (RSR)} = \frac{V_s}{V_w} = \left(\frac{d_s}{d_w}\right)^3 \]

where \(d_s\) and \(d_w\) are the diameters of the immersed hydrogel in each solution and in water, respectively.

2.4. Gel electrophoresis

Gel electrophoresis was carried out with 6× loading buffer (Takara Bio, Tokyo, Japan) or TBE buffer for detection of DNA-based samples. Also, YOYO-1 labeled DNA [24] and APTS labeled glucans [25] were analyzed with the gels including the ionic monomers.

2.5. Instruments

Observation of swelling/shrinking of the hydrogels and gel electrophoresis were carried out with a digital microscope, Dinolite (Thanko, Tokyo, Japan) and Mupid-eXU (Advance, Tokyo, Japan), respectively.

3. Results and discussions

3.1. Fundamental shrinking behavior of the PEG-based gels

As previously reported, the PEG-based gels including ionic monomers showed the specific shrinkage in ionic solutions [18]. Interestingly, di-ionic solutes, such as 1,3-benzenedisulfonic acid or 4-(tributylammonium-methyl)benzyltributylammonium, performed as a pseudo-crosslinker in the cationic gel or the anionic gel, respectively. In consequence, the specific shrinkage of the gels was observed. Thus, to show the specific shrinkage in visually, astrazon orange R, which consists of two ammoniums and aromatic parts (Fig. S1.), was utilized as a pseudo-crosslinker to an anionic PEG-based gel including SS. As a result, the irreversible shrinkage of the gel was observed contrary to our expectation (Fig. S1.). We assumed that the irreversible shrinkage was caused by the other interactions including hydrophobic interaction and/or π-stacking among the adsorbed solutes to the gels.

In order to confirm the shrinking behavior of the PEG-based gels, the effect of the crosslinking density in regard to swelling/shrinking of the gels was examined since the evaluation was not completed in our previous study. A number of PEG-based gels with PEG-DMA (0.9 mmol), SS (0.6 M to solvents), and AIZP (1.0 wt% to monomers) were prepared as changing the amount of the solvent (20 mM...
HEPES buffer, pH 7.5). In this case, the gels should be swelled in pure water because of the ionic repulsion between ionic groups in the gels. The equilibrium swelling ratio (ESR) of each gel is summarized in Fig. 1. As expected, the ESR value became larger as increasing the volume of the solvent. When we used a HEPES buffer more than 16 mL in this condition, the gelation was not completed because of the lack of the crosslinking density. Thus, we fixed the total volume of solvents at 14 mL for the gel preparation in following evaluations.

3.2. Gel shrinkage in ionic solutions

The aim of this study is to achieve a tunable molecular sieving using single gel by a simple change of running solutions in gel electrophoresis. To understand the shrinking behavior of the gels, we examined the effect of a variety of solutes against the gel shrinkage in a consideration of intermolecular interactions among adsorbed solutes to the gels. Fig. 2 shows the relative swelling ratio (RSR) of the cationic gel prepared with VBTMAC or the anionic gel prepared with SS in various sulfonic solutions. A certain degree of shrinks were observed in all the solution because of the moderation of the ionic repulsion in each gel. In evaluations using alkyl sulfate with the cationic gel (a), the RSR value was gradually decreased as the alkyl chain became longer, although the value was almost the same in the anionic gel. Herein, the hydrophobic interaction was worked among adsorbed solutes resulting a gradable gel shrinking. Furthermore, when sodium 2-naphthalenesulfate (Naph-SO₃) was used as a solute, the drastic shrinkage was observed in the cationic gel. In a consideration of the relationship of the logarithm of the octanol-water partition coefficient log \( P_{\text{o/w}} \) [26,27], the values of \( n \)-pentane, \( n \)-hexane, and naphthalene are 3.62, 4.11, and 3.35, respectively. As shown in Fig. 2, although the hydrophobicity of a naphthalene moiety is lower than that of pentyland hexyl-, the RSR value of the cationic gel was much lower in Naph-SO₃ solution. We assumed that the \( \pi \)-stacking as well as the hydrophobic interaction among the adsorbed Naph-SO₃ was worked, and then the drastic shrinkage of the gel was occurred. In case of using astrazon orange R described above, further pseudo-crosslinking by two ammoniums as well as the \( \pi \)-stacking and hydrophobicity were worked all together, resulting the irreversible shrinkage of the gel.

As mentioned above, the cationic gel using VBTMAC was shrunk in 10 mM Naph-SO₃ solution to less than 10% volume toward the stable condition in water. To examine its swelling/shrinking reversibility, a progressive soaking into water, Naph-SO₃ solution, NaCl solution, and water was carried out, and then the variation of the RSR was measured. The RSR values and the photo images are summarized in Fig. 3 and Fig. S2, respectively. According to Fig. 3, as well as

![Equilibrium swelling ratio of the PEG-based gel. The gel was prepared with 0.9 mmol PEG-DMA, 0.6 M SS in 20 mM HEPES buffer (pH 7.5), and 1.0 wt% AIZP toward the monomers.](image1)

![Relative swelling ratio of the gels in sulfate solutions. The gels were prepared with 0.9 mmol PEG-DMA, 14 mL methanol/water = 9/5 (v/v), 1.0 wt% AIZP toward monomers, and 2.1 mmol (a) VBTMAC or (b) SS. Solutions: 25 mM sodium butylsulfate (Butyl-SO₃), sodium \( n \)-pentylsulfate (Pentyl-SO₃), sodium \( n \)-hexylsulfate (Hexyl-SO₃), sodium 2-naphthalenesulfate (Naph-SO₃), and NaCl.](image2)

![Swelling and shrinking reversibility of the gel. Gel: same as Fig. 2-(1). Solutions: (a) water (before) → (b) 25 mM Naph-SO₃ aq. (24 h) → (c) 50 mM NaCl (24 h) → (d) water (after) (24 h).](image3)
as above evaluation, the volume of the cationic gel was shrunk at about 8% when 25 mM Naph-SO$_3$ solution was employed. In a NaCl solution, most of Naph-SO$_3$ molecules were removed from the gel by the strong ionic strength, and then the volume of the gel was completely recovered to the original condition in water. Furthermore, similar results were obtained in the anionic gels. Consequently, these results clearly showed the swelling/shrinking reversibility of the PEG-based gels containing ionic monomers.

3.3. Tunable gel shrinking by pH changing of the solutions

Tunable molecular sieving in gel electrophoresis by a simple control of a buffer solution, e.g., change of pH, is attractive ideally and practically. Although the reversible swelling/shrinking of the PEG-based gels was observed by changing solutions in above discussions, these gels were prepared with specific monomers including the strong ionic groups. Therefore, it was expected that a simple pH change should not be worked for the gel shrinking. Therefore, another gel was prepared with AA, of which $pK_a$ is 4.25, and the swelling/shrinking behavior of the gels was evaluated. The RSR value of the gel in each pH is shown in Fig. 4 (a). As expected, the gel showed the specific swelling toward the pH change. When a lower pH solution was employed, most of carboxy groups should be protonated, so that the gel kept smaller volume. In contrast, a higher pH solution provided the deprotonation of carboxy groups, resulting the swelling of the gel by the ionic repulsion. Furthermore, in the middle pH condition around $pK_a$ of AA, the volume of the gel was also reflected since a part of carboxy groups were deprotonated. Additionally, the reversibility of the swelling/shrinking of the gel by the pH change was evaluated. As shown in Fig. 4 (b), the gel volume was completely recovered by a simple pH change in repeated operations ($n = 3$).

Consequently, we confirmed the possibility of a tunable molecular sieving with the PEG-based gel by a simple pH change of the running solutions.

3.4. Gel electrophoresis using the PEG-based hydrogels

To confirm the ability for a molecular sieving in the gels, the PEG-based gel prepared without any ionic monomers was employed for the separation of three kinds of YOYO-1 labeled DNA base pair in gel electrophoresis. The results of gel electrophoresis using the gels, which was prepared with a different crosslinker content, are shown in Fig. 5. In general, the polymer network is thickly as increasing crosslinking points, so that it is expected that the migration of smaller molecules becomes slower by using a higher-crosslinked gel in gel electrophoresis. As our expectation, each DNA base pair was separated in the gel using 0.9 mmol crosslinker, whereas all the DNA base pairs appeared on closed area in the gel using 2.7 mmol crosslinker. These results suggested that the PEG-based gels can be utilized for molecular sieving in gel electrophoresis.

Thereupon, we evaluated the gels including ionic monomers for a tunable molecular sieving by a simple change of the running solutions. Firstly, the gels prepared with strong ionic monomers such as SS or VBTMAC were evaluated for the separation of DNA base pairs or glucans. Unfortunately, these gels indicated cracks in a few minutes after the voltage application. Although we controlled the strength of the applied voltage and the running solutions, the issue was not overcome (Fig. S3). Secondly, the gels prepared with AA, in which the reversible swelling/shrinking was confirmed by a simple pH change of the immersed solutions, were evaluated in gel electrophoresis. To confirm the shrinkage of the gel, a NaCl solution and TBE buffer were employed for immersed solutions of the gel in advance. As a result, the gel was more shrinking in the TBE buffer. Then, the separations of APTS derivative glucans [25] were carried out using the same solutions for the migration. As shown in Fig. 6, the separation patterns of glucans we re different in each other due to the polymer network of the gel, in fact, the separation of glucans was better in the TBE buffer. Also, the cracks were not observed in the evaluation using the gel even after 60 min at 100 V.

Finally, we demonstrated the separation of glucans using the gel prepared with AA by a simple pH change of the running solutions. The solutions including pH 10.8 and 2.5 were employed for migrations since the obvious differences in swelling and shrinking were observed as above discussion.

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**Fig. 4.** Swelling and shrinking of the gel prepared with AA. Gel: 0.9 mmol PEG-DMA, 14 mL water, 1.0 wt% AIZP toward monomers, and 2.1 mmol AA. (a) RSR values in various pH, (b) swelling and shrinking reversibility of the gel in 20 mM phosphate buffers or a carbonate buffer for 4 h.
Fig. 5. Separation of DNA base pairs by gel electrophoresis. Gel: 14 mL 1x TBE buffer, 5.0 wt% AIZP toward PEG-DMA, and (a) 0.9 mmol, (b) 1.8 mmol, (c) 2.7 mmol PEG-DMA, under UV irradiation (365 nm) for 3 h. Larger sized gels were prepared at same mole ratio. Electrophoresis: running buffer, 1x TEB buffer; applied voltage, 135 V; migration time, (a) 20 min (b) 25 min (c) 25 min; sample, YOYO-1 labeled 22.7 ng/μL DNA base pair.

Fig. 6. Gel electrophoresis of glucans using the gel prepared with AA. Gel: 0.9 mmol PEG-DMA, 2.1 mmol AA, 14 mL water, 5 wt% AIZP toward monomers. Electrophoresis: running solution, (a) 3 mM NaCl (b) 1x TBE buffer; applied voltage, 100 V; migration time, 40 min, sample, APTS derivative glucans.

Fig. 7. Gel electrophoresis of glucans in different pH conditions using the PEG-based gel prepared AA or an agarose gel. Gel: (a) and (a’) 0.9 mmol PEG-DMA, 2.1 mmol AA, 14 mL water, 5 wt% AIZP toward monomers; (b) and (b’) 1.0 wt% agarose gel. Electrophoresis: running solution, (a) and (b) 20 mM carbonate buffer (pH 10.8), (a’) and (b’) 20 mM phosphate buffer (pH 2.5); applied voltage, 100 V; migration time, (a) and (a’) 40 min, (b) and (b’) 10 min; sample, APTS derivative glucans.
The results are shown in Fig. 7. Here, as a comparison result, the separation of glucans by a commercially used agarose gel is also showed. As shown in these results of the gel prepared with AA, glucans were completely separated in higher pH condition, whereas glucans appeared similar position in lower pH condition. We assumed that swelling condition by the deprotonation in higher pH made suitable polymer network for the separation of glucans using in this evaluation. In addition, when an agarose gel was employed for the separation, the separation patterns were almost the same regardless of pH conditions. These results clearly revealed that the PEG-based gels have the possibility of a tunable molecular sieving in gel electrophoresis, and several applications using this concept will be possible in the future by further examinations.

4. Conclusions
The hydrogels prepared with the PEG-based crosslinker and ionic monomers showed the reversible swelling/shrinking properties by simply changing the immersed solutions. These gels were specially shrunk by the intermolecular interactions including the hydrogen bond and π-stacking among the adsorbed solutes onto the gels. Additionally, the gels prepared with AA provided a variation of separation patterns for glucans due to the structural change of the polymer network by a simple pH change of the running solutions in gel electrophoresis. The results suggested the possibility of a tunable molecular sieving with single gel by just changing the running solution in gel electrophoresis. We strongly expect that the results shown in this study will lead to the other applications such as the separations of biomolecules in gel or capillary electrophoresis in the future.

Supporting information
The procedures of an agarose gel preparation and the derivatization of samples, the photo images of gels are available via the WEB at http://chromsoc.jp/Journal.html.

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