



Supramolecular gel electrophoresis

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Abstract

Amphiphilic C_3 -symmetric tris-ureas self-assemble into supramolecular hydrogels in aqueous solution. These supramolecular hydrogels were used as matrices for the electrophoresis of biopolymers such as proteins and nucleic acids. A unique separation mode in comparison to that of typical sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was found during the electrophoresis of denatured proteins. Native proteins were separated on the basis of their isoelectric points and retained their activities. Affinity electrophoresis was realized by exploiting interactions between gelator glucosides and carbohydrate-binding proteins. Protein samples were efficiently recovered through an extremely simple operation, and up to 50% of the protein was extracted by centrifugation, which is a remarkable feature of electrophoresis using supramolecular hydrogels. Large DNA fragments that previously had been separated only by pulsed-field gel electrophoresis were separated using a supramolecular hydrogel matrix and a typical continuous-field electrophoresis apparatus. In this focus review, the author summarizes the electrophoresis of proteins and nucleic acids using our developed supramolecular hydrogel matrix.

Introduction

Gel electrophoresis is a very common experimental method for the separation and analysis of biopolymers such as proteins and nucleic acids and is extensively used in biological research [1, 2]. Polymer gels such as agarose and polyacrylamide are routinely used as electrophoretic matrices. This focus review outlines our contributions to the development of supramolecular gel electrophoresis, a novel electrophoresis method for the separation of proteins and DNA using supramolecular hydrogels formed by the self-assembly of low-molecular-weight compounds as matrices.

Tiselius reported the first protein electrophoresis of serum albumin in aqueous solution in the 1930s [3]. Later, in the 1950s, zone electrophoresis using gel matrices such as agarose and polyacrylamide was developed, and electrophoresis became a more popular method for the analysis of proteins [1]. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is now one of the most

widely used electrophoresis methods [4]. In SDS-PAGE, negatively charged denatured proteins migrate from the cathode to the anode. Proteins are separated mainly on the basis of molecular weight: proteins with smaller molecular weights are electrophoresed to the anode, while those with larger molecular weights remain closer to the cathode. Smaller proteins pass through the network structure of the polyacrylamide gel, a phenomenon known as the “molecular sieve effect”. Polyacrylamide gels, which are prepared by the copolymerization of acrylamide and *N,N*-methylenebisacrylamide (BIS), have been used as typical matrices for protein electrophoresis for over half a century; in addition to their ease of handling and low cost, their meshed structures are suitably sized for the separation of proteins, which is one of the most important characteristics of a polyacrylamide gel. Nevertheless, not all users are fully satisfied with polyacrylamide gels. For example, researchers occasionally find the recovery of protein samples from the polyacrylamide gel matrix following electrophoresis to be problematic. Although an excellent method for the specific detection of phosphorylated proteins exists, namely, Phos-tag SDS-PAGE [5], customizing affinity electrophoresis for specific proteins remains challenging. Radical technological innovation is essential in order to solve these problems. To that end, we felt that supramolecular gels were ideal candidates as novel matrix materials that would lead to key innovative electrophoresis technology.

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Supramolecular gels are physical gels formed by the self-assembly of small molecules referred to as “low-molecular-weight gelators” [6–9]. Supramolecular gels formed by gelation processes involving highly reversible noncovalent bonds are extremely flexible and are responsive to external stimuli. These flexible characteristics enable the application of supramolecular gels to a variety of smart materials [10, 11]. In particular, biocompatible supramolecular hydrogels have found uses in biological research, such as cell cultures, drug delivery, and sensors [12, 13]. Despite these research activities, to the best of our knowledge, there are no reports describing the use of supramolecular gels as electrophoretic matrices. The flexible and stimuli-responsive properties of supramolecular gels are advantageous for the recovery of proteins following electrophoresis. The ability to design low-molecular-weight gelators is desirable for the development of custom-made affinity electrophoresis methods for specific proteins. With this background in mind, we developed a protein electrophoresis method using supramolecular gel matrices and refer to this method as *Supramolecular Gel Electrophoresis (SUGE)*.

Supramolecular gel electrophoresis of denatured proteins (SDS-SUGE)[14]

A C_3 -symmetric tris-urea composed of three ureide groups and a benzene core was serendipitously discovered to be a low-molecular-weight organogelator[15]. Various supramolecular gels have been developed through the derivatization of the C_3 -symmetric tris-urea structure [16, 17]. A low-molecular-weight hydrogelator had been prepared from an amphiphilic C_3 -symmetric tris-urea by introducing hydrophilic groups into the outer shell of the hydrophobic C_3 -symmetric structure [18]. Another amphiphilic C_3 -symmetric tris-urea, **1**, based on a similar concept formed a supramolecular hydrogel with a Tris-glycine-SDS solution (TGS solution = 25 mM Tris, 195 mM glycine, 3.5 mM SDS; tris = tris(hydroxymethyl)aminomethane), a typical solution used in SDS-PAGE (Fig. 1) [14]. SDS-SUGE studies began using this supramolecular hydrogel as a matrix.

The following experimental SDS-SUGE procedure was established as a result of several investigations (Fig. 2). I) A glass capillary (inner diameter of 2 mm, length of 8 cm) was filled with a supramolecular hydrogel consisting of TGS solution containing both 2.0 wt% of **1** and agarose (TGS-solution gel). Agarose was used to physically reinforce the gel. The TGS-solution gel was sufficiently rigid for handling outside of the glass capillary even following electrophoresis, and the agarose gel itself had no separation ability for proteins under SDS-SUGE conditions. II) The protein sample was adsorbed onto one end of the supramolecular

hydrogel, and both ends of the capillary were filled with agarose gel. III) The capillary was immersed in the TGS solution in a submarine-type electrophoresis system and electrophoresed at varying voltages and times. IV) The electrophoresed gel was removed from the glass capillary and divided into eight equal parts (numbered 1 to 8 starting from the anode). V) Solutions extracted from the divided supramolecular hydrogel were analyzed using typical SDS-PAGE and stained with Coomassie brilliant blue (CBB). Proteins were extracted from the supramolecular hydrogel by simple centrifugation, resulting in the extraction of up to 50% of the protein. In comparison, only a few percent of the protein was obtained from a polyacrylamide gel after a similar operation. The efficient extraction of the protein from the gel during the SDS-SUGE procedure is attributable to the disintegration of the aggregated structure of the supramolecular hydrogel during centrifugation.

SDS-SUGE experiments were performed with samples containing proteins in the 6.5–116 kDa size range. A mixture of ovalbumin (45 kDa) and β -galactosidase (116 kDa) was subjected to SDS-SUGE at 100 V for 150 min. Smaller ovalbumin was detected in lanes 3 and 4 with the stronger band observed in lane 3. Larger β -galactosidase was detected in lanes 3 to 6 with the strongest band observed in lane 4. The smaller ovalbumin was electrophoresed closer to the anode than the larger β -galactosidase, as observed by SDS-PAGE; however, the separation was poor (Fig. 3a). SDS-SUGE of ovalbumin (45 kDa) and bovine serum albumin (66 kDa) showed similar separation behavior (vide infra). SDS-SUGE using proteins with molecular weights less than 45 kDa exhibited different separation behavior, as observed by SDS-PAGE; i.e., larger proteins showed

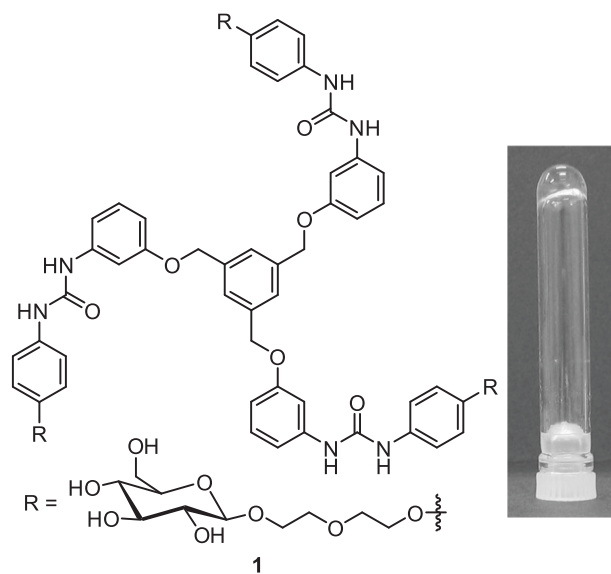


Fig. 1 Amphiphilic C_3 -symmetric tris-urea **1** and a photograph of its TGS-solution gel

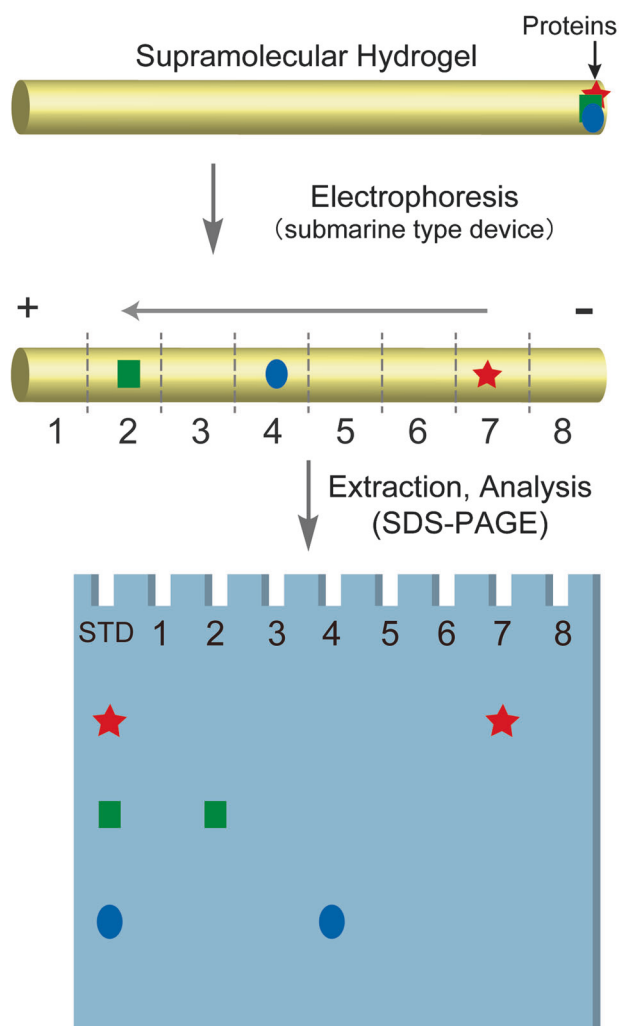


Fig. 2 Schematic representation of the SDS-SUGE procedure

greater mobility toward the anode. Electrophoresis of a mixture of aprotinin (6.5 kDa) and ovalbumin (45 kDa) was performed at 100 V for 150 min. SDS-PAGE analysis revealed that ovalbumin was distributed in lanes 3 to 5 and that the smaller aprotinin was present in lane 7 (Fig. 3b). A mixture of aprotinin (6.5 kDa) and lysozyme (14 kDa) was subjected to SDS-SUGE at 100 V for 180 min. SDS-PAGE analysis showed that lysozyme was mainly found in lane 5, while the smaller aprotinin was retained in lanes 6 and 7 closer to the cathode (Fig. 3c). We propose that two different separation mechanisms compete in the SDS-SUGE process. One is the molecular sieve effect, which is also operative in SDS-PAGE. String-like denatured proteins pass through the three-dimensionally intertwined fibrous network of **1**, and the smaller proteins exhibit greater mobilities. The other is the size-exclusion effect, which is common in gel filtration. Isolated spaces constructed by the supramolecular hydrogel of **1** may be suitable for retaining appropriately sized proteins against the electric current.

Therefore, protein samples are separated by mechanistic cooperativity. The molecular sieve effect is the dominant separation mode for proteins with molecular weights larger than 45 kDa, while the size-exclusion effect dominates for proteins with molecular weights less than 45 kDa.

Effect of SDS concentration on SDS-SUGE [19]

The addition of an ionic surfactant such as SDS was indispensable for the gelation of amphiphilic C_3 -symmetric tris-urea **1**. Furthermore, the concentration of the ionic surfactant influences the thickness of the fibrous aggregate that constitutes the gel [20]. The fibrous aggregates were thicker at lower SDS concentrations and became thinner as the SDS concentration increased, as evidenced by scanning electron microscopy (SEM). The xerogel prepared from the hydrogel formed using 9.0 mM **1** and 0.50 mM SDS exhibited intertwined fibrous aggregates with thicknesses from 100 to 600 nm (Fig. 4a), while the xerogel prepared from the hydrogel formed using 9.0 mM **1** and 4.0 mM SDS showed homogeneous fibrous aggregates with diameters in the 100–250 nm range (Fig. 4b). It is notable that a non-gelatinous dried solution of a mixture of 9.0 mM **1** and 10 mM SDS showed fibrous aggregates with large aspect ratios and diameters in the 50–80 nm range (Fig. 4c). Based on these results, we propose that the boundary between the dominant SDS-SUGE separation modes can be regulated by the SDS concentration.

TGS-solution gels were prepared using **1** (2.0 wt%), agarose (2.0 wt%), Tris (25 mM), glycine (195 mM), and SDS at concentrations in the 1.0–7.0 mM range [19]. As mentioned above, the dominant SDS-SUGE separation mode changed at 45 kDa when a TGS solution containing 3.5 mM SDS was used. The electrophoresis of ovalbumin (45 kDa) and bovine serum albumin (66 kDa) was performed using this TGS solution. The smaller ovalbumin migrated further toward the anode than the larger bovine serum albumin (Fig. 4d). The boundary between the separation modes appeared at approximately 66 kDa when SDS-SUGE was performed with a TGS solution containing 1.0 mM SDS, and the larger bovine serum albumin was observed to migrate further toward the anode than the smaller ovalbumin (Fig. 4e). The boundary between the separation modes appeared at approximately 14 kDa when SDS-SUGE was carried out using a TGS solution containing 7.0 mM SDS: the electrophoresis of a mixture of lysozyme (14 kDa) and carbonic anhydrase (29 kDa) revealed that the smaller lysozyme migrated further toward the anode than the larger carbonic anhydrase due to the dominance of the molecular sieve effect (Fig. 4f). However, the size-exclusion effect dominated during the electrophoresis of a

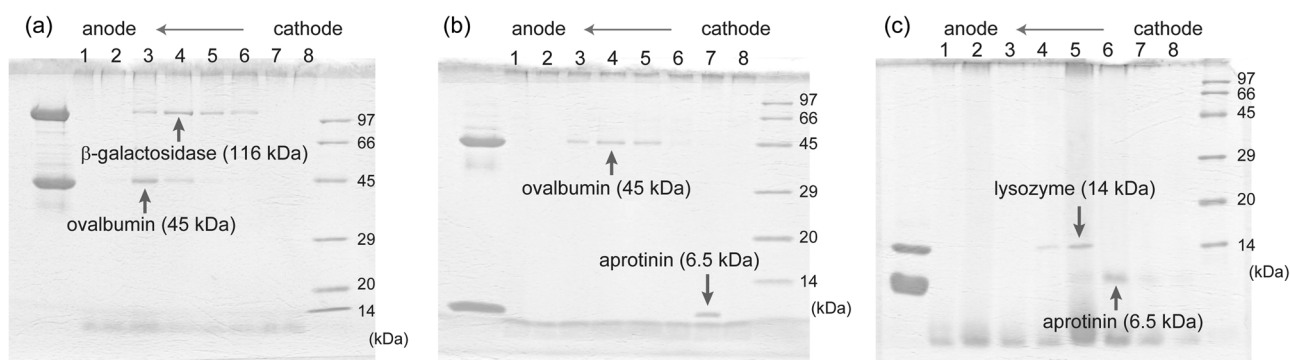


Fig. 3 SDS-PAGE analyses of SDS-SUGE-separated (a) ovalbumin (45 kDa) and β -galactosidase (116 kDa), (b) aprotinin (6.5 kDa) and ovalbumin (45 kDa), and (c) aprotinin (6.5 kDa) and lysozyme (14 kDa)

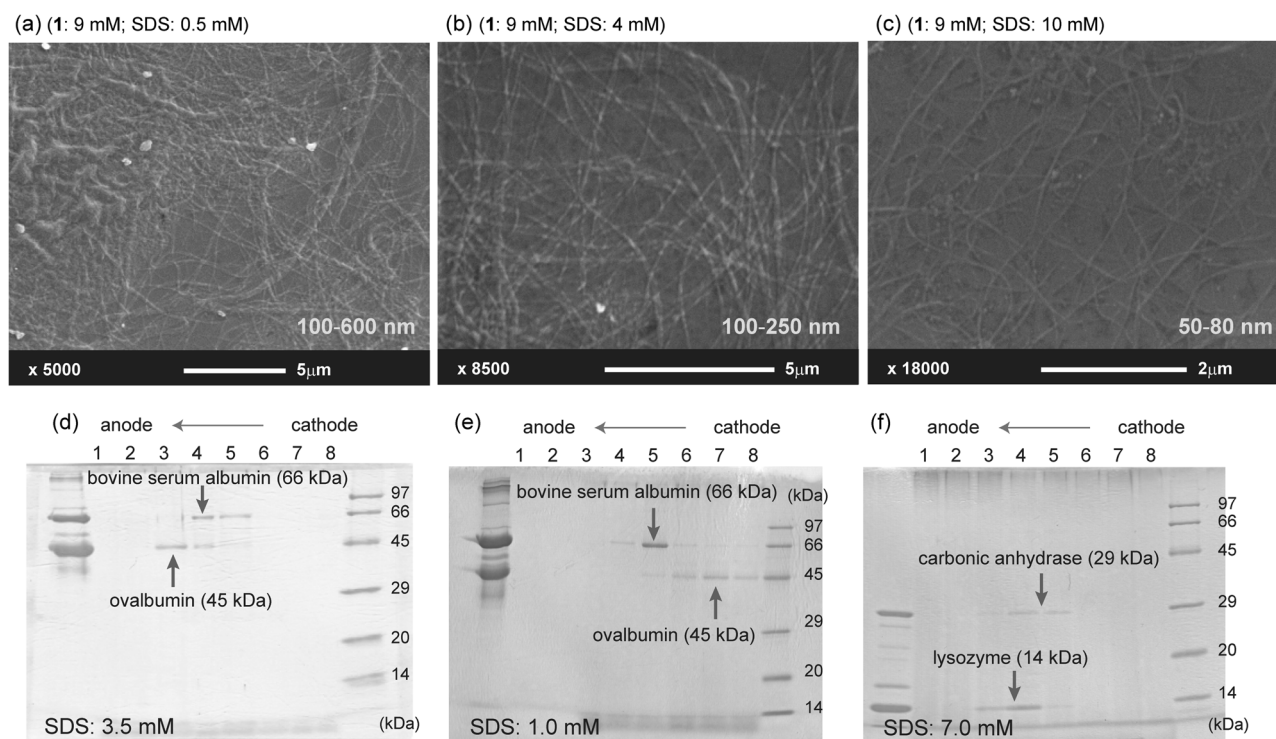


Fig. 4 SEM images of xerogels formed using (a) **1** (9.0 mM) and SDS (0.50 mM) and (b) **1** (9.0 mM) and SDS (4.0 mM). c SEM image of a non-gelatinous dried solution of **1** (9.0 mM) and SDS (10 mM). SDS-PAGE analyses of SDS-SUGE-separated (d) ovalbumin (45 kDa) and bovine serum albumin (66 kDa) using a TGS solution containing 25

mM tris, 192 mM glycine, and 3.5 mM SDS; (e) ovalbumin (45 kDa) and bovine serum albumin (66 kDa) using a TGS solution containing 25 mM tris, 192 mM glycine, and 1.0 mM SDS; and (f) carbonic anhydrase (29 kDa) and lysozyme (14.4 kDa) using a TGS solution containing 25 mM tris, 192 mM glycine, and 7.0 mM SDS

mixture of aprotinin (6.5 kDa) and lysozyme (14 kDa). The boundary between the separation modes can clearly be controlled by adjusting the SDS concentration.

Supramolecular gel electrophoresis of native proteins (native-SUGE)[21]

SDS-SUGE studies revealed that protein samples can be efficiently recovered from the supramolecular hydrogel

matrix by a simple procedure following electrophoresis. This observation encouraged us to investigate the electrophoresis of native proteins using the supramolecular hydrogel matrix (native-SUGE); the efficient recovery of the protein sample is an important criterion in native-protein electrophoresis. Unfortunately, amphiphilic C_3 -symmetric tris-urea **1** could not gel the TG solution (tris: 25 mM; glycine: 195 mM) typically used for the electrophoresis of native proteins (native-PAGE). Therefore, we first needed to develop a low-molecular-weight hydrogelator capable of

gelling the TG solution. We subsequently designed and synthesized amphiphilic C_3 -symmetric tris-urea **2** as a low-molecular-weight hydrogelator (Fig. 5) [22]; **2** not only gelled the TG solution and pure water but also a variety of aqueous solutions, including aqueous acid (8 M hydrochloric acid), aqueous base (7 M potassium hydroxide aqueous solution), and a concentrated salt solution (saturated saline). Furthermore, these supramolecular hydrogels exhibited thixotropic properties.

The electrophoresis of native acidic proteins was examined using the TG-solution gel of **2** [21]. The native-SUGE procedure was almost the same as that used for SDS-SUGE with the exception that the TG-solution gel of **2** was used and that agarose was not required. D-Lactate dehydrogenase (146 kDa, pI = 4.0), β -galactosidase (540 kDa, pI = 4.6), and ovalbumin (45 kDa, pI = 4.7) were used as sample

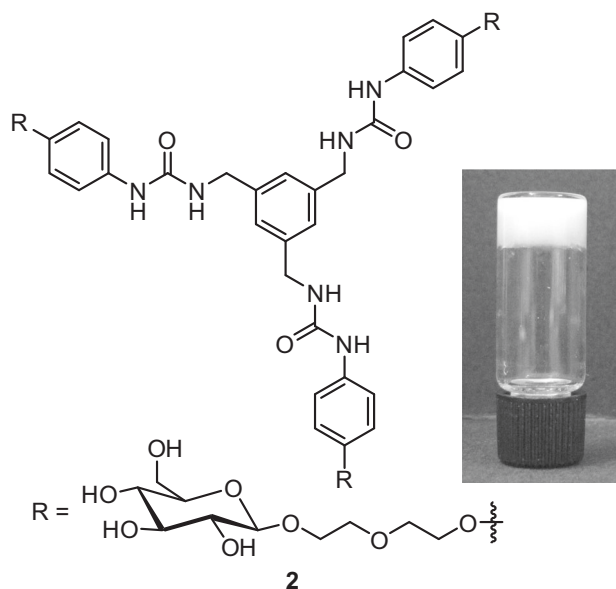


Fig. 5 Amphiphilic C_3 -symmetric tris-urea **2** and a photograph of its TG-solution gel

native acidic proteins. When a mixture of D-lactate dehydrogenase and β -galactosidase was subjected to native-SUGE, D-lactate dehydrogenase was detected in lanes 4 and 5, and β -galactosidase was detected in lanes 5 and 6 (Fig. 6a). The smaller and more acidic D-lactate dehydrogenase was more mobile and finished closer to the anode than the larger and less acidic β -galactosidase. When a mixture of D-lactate dehydrogenase and ovalbumin was subjected to native-SUGE, D-lactate dehydrogenase was detected in lanes 3 and 4, and ovalbumin was detected in lanes 5 and 6 (Fig. 6b). The larger and more acidic D-lactate dehydrogenase exhibited greater mobility toward the anode than the smaller and less acidic ovalbumin. Similarly, electrophoresis of a mixture of β -galactosidase and ovalbumin revealed the presence of β -galactosidase in lanes 4 and 5 and the presence of ovalbumin in lanes 5 and 6 (Fig. 6c). Again, the larger and more acidic β -galactosidase showed greater mobility toward the anode than the smaller and less acidic ovalbumin. These results reveal that these native acidic proteins were separated by native-SUGE mainly on the basis of their isoelectric points (pIs) using the TG-solution gel of **2**, while molecular weight had little influence.

A mixture of green fluorescent protein (GFP, 27 kDa, pI = 5.57) and red fluorescent protein (RFP, 27 kDa, pI = 5.65) was subjected to native-SUGE. Green and red fluorescent bands were observed upon irradiation of the supramolecular hydrogel with UV light during and after electrophoresis. The fluorescent bands observed in the supramolecular hydrogel of **2** corresponded to the GFP and RFP bands detected following SDS-PAGE analysis. The activity of D-lactate dehydrogenase was measured following native-SUGE in order to confirm that it had maintained its native form. D-Lactate dehydrogenase, which catalyzes the oxidation of lactate to pyruvate in the presence of nicotinamide adenine dinucleotide (NAD^+), retained 90% or more of its original activity following native-SUGE.

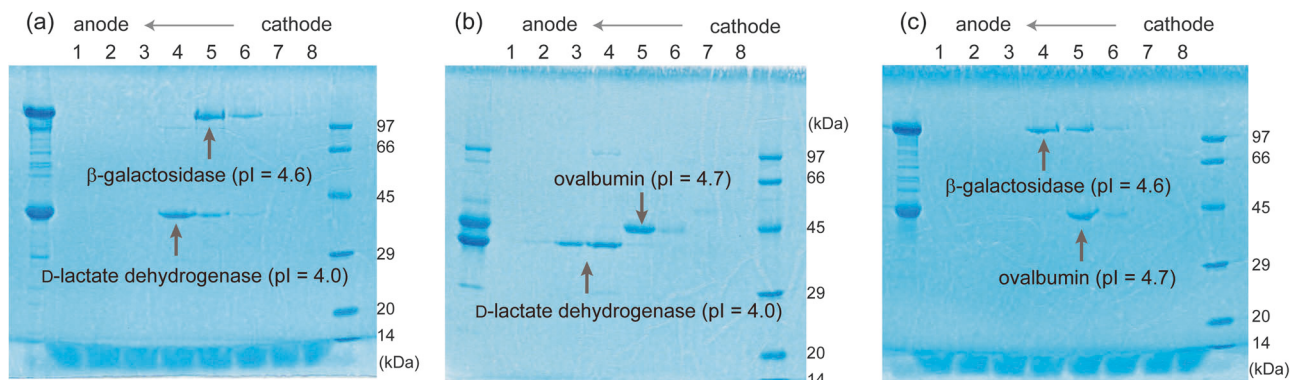


Fig. 6 SDS-PAGE analyses of native-SUGE-separated (a) D-lactate dehydrogenase (146 kDa, pI = 4.0) and β -galactosidase (540 kDa, pI = 4.6), (b) D-lactate dehydrogenase (146 kDa, pI = 4.0) and

ovalbumin (45 kDa, pI = 4.7), and (c) β -galactosidase (540 kDa, pI = 4.6) and ovalbumin (45 kDa, pI = 4.7)

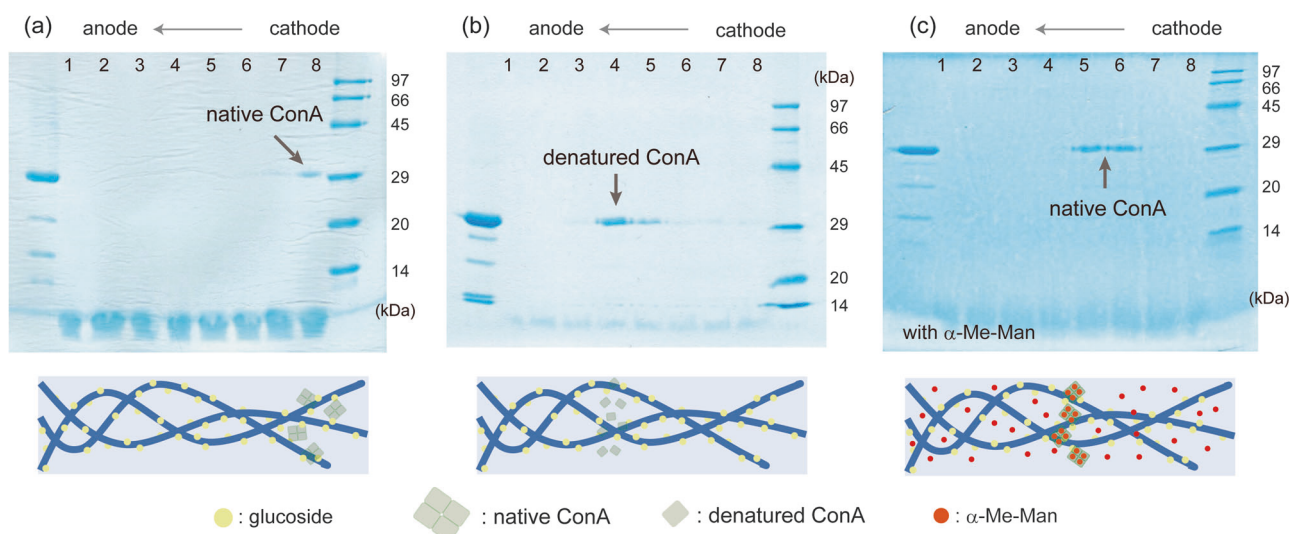


Fig. 7 SDS-PAGE analyses of ConA subjected to native-SUGE and the mechanisms proposed for each result. **a** Native-ConA (112 kDa (as a tetramer), pI = 4.4–5.5) in TG solution (25 mM Tris, 195 mM

glycine), **b** denatured-ConA in TG solution (25 mM Tris, 195 mM glycine), and **c** native-ConA in TG-Me α Man solution (25 mM Tris, 195 mM glycine, 51 mM Me α Man)

These results confirm that the native 3D structures and activities of proteins are retained during native-SUGE using the supramolecular hydrogel formed from **2**.

Affinity electrophoresis of lectin[21]

The surfaces of the nanofibers formed through the self-assembly of amphiphilic C_3 -symmetric tris-urea **2** are densely coated with glucosides introduced as hydrophilic groups [21]. The glucoside-coated nanofibers interact with appropriate carbohydrate-binding proteins (lectins) during electrophoresis, and the lectins involved in these interactions do not migrate as far in the supramolecular hydrogel as non-carbohydrate-binding proteins. The well-characterized lectin concanavalin A (ConA, tetramer = 112 kDa, pI = 4.4–5.5) was subjected to affinity electrophoresis using the TG-solution gel of **2**. Moderate association between **2** and ConA was expected on the basis of the known affinity between α -methyl-D-glucopyranoside and ConA ($K_a = 1.96 \times 10^3 \text{ M}^{-1}$). ConA was subjected to electrophoresis under typical native-SUGE conditions (100 V, 100 min). Most of the ConA remained at the starting cathode end (lane 8) (Fig. 7a). In contrast, the electrophoresis of denatured-ConA under the same conditions resulted in a considerably different outcome: the denatured-ConA migrated toward the anode and ended up in lanes 4 and 5 (Fig. 7b). These results indicate that interactions between native-ConA and the glucosides on the nanofiber surfaces inhibit its electrophoretic mobility. The low affinity of denatured-ConA toward glucosides results in moderate migration under the electrophoretic conditions depending on the isoelectric point. The addition of a saccharide with a

strong affinity for ConA to the electrophoresis buffer solution might improve the electrophoretic migration of native-ConA during native-SUGE. α -Methyl-D-mannopyranoside (Me α Man) was selected for this task because of its stronger affinity for ConA ($K_a = 0.82 \times 10^4 \text{ M}^{-1}$) than that of α -methyl-D-glucopyranoside. A TG-Me α Man solution (25 mM Tris, 192 mM glycine, 51 mM Me α Man) was used for the electrophoresis of native-ConA. SDS-PAGE analysis showed that ConA was present in lanes 5 and 6 (Fig. 7c). Native-ConA migrated much further toward the anode in the presence of Me α Man than it did under typical electrophoretic conditions.

Supramolecular gel electrophoresis of DNA (DNA-SUGE)[23]

Polyacrylamide and agarose are frequently used as gel matrices for DNA electrophoresis[2]. Polyacrylamide gel, which has small pore sizes in the 5–100 nm range, is suitable for the separation of small DNA fragments below 500 base pairs (bp). In contrast, agarose gel, with pore sizes of 200–500 nm, is typically used to resolve much larger DNA fragments (> 100 bp). Larger DNA fragments generally migrate more slowly than smaller DNA fragments in these types of gel electrophoresis. The separation of DNA fragments larger than 20 kbp by typical continuous-field electrophoresis is challenging because the fragments migrate at comparable rates. Pulsed-field gel electrophoresis, which was first reported by Schwartz and Cantor, was developed to separate very large DNA fragments [24, 25]. Pulsed-field gel electrophoresis is useful for the cloning of large DNA fragments, karyotype analyses of microorganisms, and

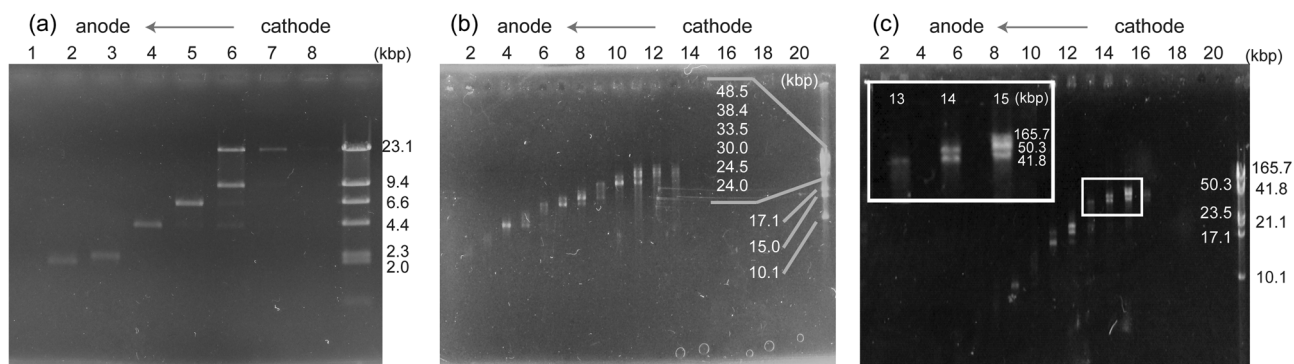


Fig. 8 Agarose H gel electrophoresis following DNA-SUGE-separated (a) λ -Hind III digest (2.0, 2.3, 4.4, 6.6, 9.4, and 23.1 kbp DNA fragments), (b) Lambda DNA-Mono Cut Mix (10.1, 15.0, 17.1, 24.0, 24.5,

30.0, 33.5, 38.4, and 48.5 kbp DNA fragments), and (c) Marker 7 GT (10.1, 17.7, 21.1, 23.5, 41.8, 50.3, and 165.7 kbp DNA fragments)

epidemiological analyses of infectious disease, but this technique requires special equipment and long analysis times. The ability to separate and analyze large DNA fragments using a typical continuous-field electrophoresis apparatus provides a valuable alternative technique to pulsed-field gel electrophoresis. The results of the native-SUGE experiments suggest that the network structure of the supramolecular hydrogel formed from **2** is coarse and/or flexible, allowing large biopolymers to pass through. Consequently, native acidic proteins are separated on the basis of their isoelectric points rather than their molecular weights. This finding encouraged us to examine SUGE for the analysis of large DNA fragments.

A supramolecular hydrogel formed from amphiphilic C_3 -symmetric tris-urea **2** and TBE solution (45 mM Tris, 45 mM boric acid, 1.0 mM EDTA) was used for the electrophoresis of large DNA fragments (DNA-SUGE)[23]. DNA-SUGE was performed using almost identical procedures as those for SDS-SUGE and native-SUGE. Three DNA markers, the λ -Hind III digest (2–23 kbp DNA fragments), Lambda DNA-Mono Cut Mix (10–49 kbp DNA fragments), and Marker 7 GT (10–165 kbp DNA fragments), were analyzed in this study. Following electrophoresis, the supramolecular hydrogel was divided into eight or 20 equal parts. Their extracts were analyzed by typical DNA electrophoresis using the agarose H gel, followed by staining with ethidium bromide.

We first used DNA-SUGE to analyze the λ -Hind III digest DNA marker, containing 2.0, 2.3, 4.4, 6.6, 9.4, and 23.1 kbp DNA fragments. The electrophoresis was performed using 2.0 wt% of the supramolecular hydrogel formed from **2** at 150 V for 90 min (Fig. 8a). The DNA fragments were separated according to their lengths, with shorter fragments displaying higher mobilities than longer fragments. The 2.0 kbp DNA fragment was found in lane 2, the most anodic lane, while the 2.3, 4.4, and 6.6 kbp DNA fragments were found in lanes 3, 4, and 5, respectively. The larger 9.4 and 23.1 kbp DNA fragments appeared mainly in

lane 6, and a thin band corresponding to the 23.1 kbp DNA fragment was observed in lane 7. Under these conditions, the six DNA fragments were finely separated. It is remarkable that DNA fragments with similar lengths, i.e., the 2.0 and 2.3 kbp fragments, were fully separated.

Subsequently, the separation of the Lambda DNA-Mono Cut Mix, containing 10.1, 15.0, 17.1, 24.0, 24.5, 30.0, 33.5, 38.4, and 48.5 kbp DNA fragments, was examined. The electrophoretic conditions that separated the λ -Hind III digest were unsuitable for the separation of the Lambda DNA-Mono Cut Mix. Several investigations revealed that a low concentration of the supramolecular hydrogel of **2** and a low voltage were more effective for the separation of multiple DNA fragments, although high concentrations of the supramolecular hydrogel of **2** resulted in fine resolution. As a result, the Lambda DNA-Mono Cut Mix was subjected to DNA-SUGE using 1.0 wt% of the supramolecular hydrogel formed from **2** at 50 V for 6 h, and the supramolecular hydrogel was divided into 20 equal parts following electrophoresis (Fig. 8b). The 10.1 kbp DNA fragment was mainly detected in lane 4, while the 15.0 kbp fragment was mainly detected in lane 7, and the 17.1 kbp DNA fragment was mainly in lane 8, confirming clear separation. The 24.0 to 48.5 kbp fragments were observed in lanes 10 to 13; although these DNA fragments were loosely separated, their identification was problematic owing to the low resolving power of agarose H gel electrophoresis.

The separation of Marker 7 GT, containing 10.1, 17.7, 21.1, 23.5, 41.8, 50.3, and 165.7 kbp DNA fragments was examined. DNA-SUGE using 2.0 wt% of the supramolecular hydrogel formed from **2** at 40 V for 9 h resulted in suitable separation (Fig. 8c): the 10.1 and 17.7 kbp DNA fragments were detected only in lanes 9 and lane 11, respectively, while the 21.1 and 23.5 kbp DNA fragments were detected in lane 12. The 41.8 kbp DNA fragment appeared in lanes 13 to 15, while the 50.3 kbp DNA fragment was found in lanes 14 and 15. The largest DNA fragment (165.7 kbp) was found only in lane 15. It is worth

mentioning that the 50.3 and 165.7 kbp DNA fragments showed different mobilities following DNA-SUGE.

Summary and outlook

Electrophoresis is an important and indispensable experimental method in modern life science research because of its ability to separate and analyze biopolymers. Electrophoresis using polyacrylamide- or agarose-gel matrices is a well-established and mature method for the separation and analysis of proteins and nucleic acids. However, researchers still require novel and innovative electrophoresis methods that overcome some of the problems associated with existing technology, including the abilities to recover proteins from the matrix following electrophoresis and to customize affinity electrophoresis for specific proteins. The development of electrophoresis methods that sufficiently satisfy these requirements using current matrices is difficult. Technological evolution will be triggered by the development of new electrophoresis matrices. The authors have focused on high-flexibility supramolecular hydrogels as novel biopolymer-electrophoresis gel matrices and have studied electrophoresis methods for denatured proteins (SDS-SUGE) and the electrical properties of native proteins, which resulted in the development of native-SUGE and a novel DNA electrophoresis method (DNA-SUGE). SDS-SUGE exhibited unique separation characteristics due to the coexistence of two different separation modes based on the molecular sieve and size-exclusion effects. We showed that the dominant separation mode depends on the molecular weight of the sample protein. In native-SUGE, native proteins were separated mainly on the basis of their isoelectric points and retained their activities following electrophoresis. Affinity electrophoresis was achieved by exploiting interactions between the glucoside moieties of the gelator and lectin. Protein samples were efficiently recovered by an extremely simple operation in these SUGE methods. Large DNA fragments that were difficult to separate without pulsed-field gel electrophoresis could be separated by DNA-SUGE using a typical continuous-field electrophoresis apparatus. A variety of SUGE methods for the separation of biopolymers have been developed. On the other hand, some problems remain to be solved. For instance, the fragility of the supramolecular hydrogel makes SUGE operation difficult, and the multistep synthesis of the low-molecular-weight hydrogelator restricts the ability to use large quantities of the matrix. However, supramolecular hydrogels have enormous potential as novel matrices for the electrophoresis of biopolymers. We believe that SUGE is an innovative experimental technique that will be routinely used in the future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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