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Abbreviations: MGC, minimum gelation concentration; SUGE, supramolecular gel electrophoresis; TBE, tris-boric acid-EDTA

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Abstract

Pulsed-field gel electrophoresis is a frequent technique used to separate exceptionally large DNA fragments. In a typical continuous field electrophoresis, it is challenging to separate DNA fragments larger than 20 kbp because they migrate at a comparable rate. To overcome this challenge, it is necessary to develop a novel matrix for the electrophoresis. Here, we de-scribe the electrophoresis of large DNA fragments up to 166 kbp using a supramolecular gel matrix and a typical continuous field electrophoresis system. C_3-symmetric tris-urea self-assembled into a supramolecular hydrogel in tris-boric acid-EDTA buffer, a typical buffer for DNA electrophoresis, and the supramolecular hydrogel was used as a matrix for electrophoresis to separate large DNA fragments. Three types of DNA marker, the λ-Hind III digest (2 to 23 kbp), Lambda DNA-Mono Cut Mix (10 to 49 kbp), and Marker 7 GT (10 to 165 kbp), were analyzed in this study. Large DNA fragments of greater than 100 kbp showed distinct mobility using a typical continuous field electrophoresis system.

Gel electrophoresis is a routine laboratory method in molecular biology for analyzing, separating, and purifying proteins or nucleic acids.[1,2] The first use of DNA electrophoresis was reported by Thorne in 1966, where polyoma virus DNA was separated using an agar gel.[3] To date, poly-acrylamide and agarose are frequently used as a gel matrix for DNA electrophoresis. Polyacrylamide gel, which has smaller pore size of 5–100 nm, is suitable to separate small DNA fragments below 500 base pair (bp). In contrast, agarose gel with pore size of 200–500 nm is typically used to resolve much larger DNA fragments (>100 bp). Larger DNA fragments generally migrate slower than smaller DNA fragments in these types of gel electrophoresis. In a typical continuous field electrophoresis, it is challenging to separate DNA fragments larger than 20 kbp because they migrate at a comparable rate.[2] Pulsed-field gel electrophoresis, which was first described by Schwartz and Cantor, has been a frequent technique to separate exceptionally large DNA fragments.[4,5] The pulsed-field gel electrophoresis technique is particularly useful for cloning large DNA fragments,[6] karyotype analysis of microorganisms,[7] and epidemiological analysis of infectious diseases;[8] however, it requires specialized equipment and long analysis time. Thus, the development of an alternative technique for the separation of large DNA fragments utilizing common equipment and a shorter analysis time will be valuable in the field.

Although the use of polyacrylamide and agarose as gel matrices has been established, the development of a novel gel matrix for electrophoresis has been challenging. Nevertheless, the availability of a novel gel matrix will promote advancements in electrophoresis technique. We have focused using supramolecular gels as novel matrix candidates for electrophoresis. Supramolecular gels are formed via self-assemblies of small molecules termed low-molecular-weight gelators.[9–12] Various applications of supramolecular gels especially in the biological and medical fields such as cell culture, drug delivery, and sensors, have been reported owing to their flexible and tunable characteristics.[13–16] Despite their wide applicability, the utility of supramolecular gel matrix for electrophoresis had never been reported until our report in 2011.[17] Since then, we have developed electrophoresis methods using supramolecular gel matrices constructed from self-assembled C_3-symmetric tris-urea gelators for denatured or native proteins.[17–19] The supramolecular gel has several advantages over the traditional polyacrylamide gel for protein electrophoresis, and protein can be efficiently recovered from the supramolecular gel matrix after electrophoresis. The unique separation mechanism is related to the structural feature of the gelator. During the investigation of acidic native proteins using supramolecular gel electrophoresis (SUGE), it was revealed that the amphiphilic tris-urea **1** formed coarse fibrous aggregates. Consequently, acidic

native proteins are separated based on their isoelectric points rather than their molecular weights.[19] This finding encouraged us to examine the utility of SUGE for the analysis of large DNA fragments. In this report, we describe the electrophoresis of large DNA fragments of 2 to 166 kbp using a supramolecular gel matrix constructed by the self-assembly of **1**.

The amphiphilic tris-urea **1** was synthesized from commercial pentaacetyl α-D-glucoside using eight-step reactions as we reported previously.[20] A mixture of **1** and tris-boric acid-EDTA (TBE) buffer (45 mM tris/boric acid, 1.0 mM EDTA), a typical buffer for DNA electrophoresis, formed a supramolecular hydrogel after heat treatment (Figure 1). The minimum gelation concentration (MGC) of **1** to TBE buffer was 0.25 wt%. Mixtures of **1** and TBE buffer below the MGC appeared as viscous solutions; as the concentration of **1** was increased, the resulting hydrogel became opaque. The utility of SUGE for DNA analysis using a supramolecular hydrogel of **1** was examined using the following procedure, as illustrated in Figure 1. 1) A mixture of **1** and TBE buffer in a glass tube was heated on a hot plate. The obtained solution was drawn into a glass capillary (ϕ of 2 mm, length of 120 mm). The solution in the glass capillary was left at an ambient temperature for 1 day, and the gelatinated matrix was used for electrophoresis. The length of the supramolecular hydrogel was adjusted to 80 mm. 2) Three types of

DNA marker, the λ-Hind III digest (2 to 23 kbp), Lambda DNA-Mono Cut Mix (10 to 49 kbp), and Marker 7 GT (10 to 165 kbp), were analyzed in this study. The DNA marker was placed at one end of the hydrogel and the glass capillary was left to stand vertically to allow for DNA marker absorption. Both ends of the glass capillary were then filled with agarose gel. 3) The glass capillary was submerged in TBE buffer in a submarine electrophoresis system, and electrophoresis was performed at 25 °C. DNA samples were electrophoresed from the cathode to the anode end. 4) The electrophoresed gel was removed from the glass capillary and divided into 8 or 20 equal parts (numbered lane 1 to 8 or 20, from the anode end). 5) Each hydrogel slice was placed into a micro-tube and frozen at −20 °C for 3 h. Subsequently, 20 μL of TBE buffer was added into the microtube, then the mixture was centrifuged at 14,500 g for 10 min. 6) The supernatant was freeze-dried and 15 μL of loading buffer (0.5% bromophenol blue/xylene cyanol, 6% glycerol, 1.0 mM EDTA) was subsequently added. The solution was analyzed using a typical DNA electrophoresis procedure using agarose H, followed by staining with ethidium bromide.

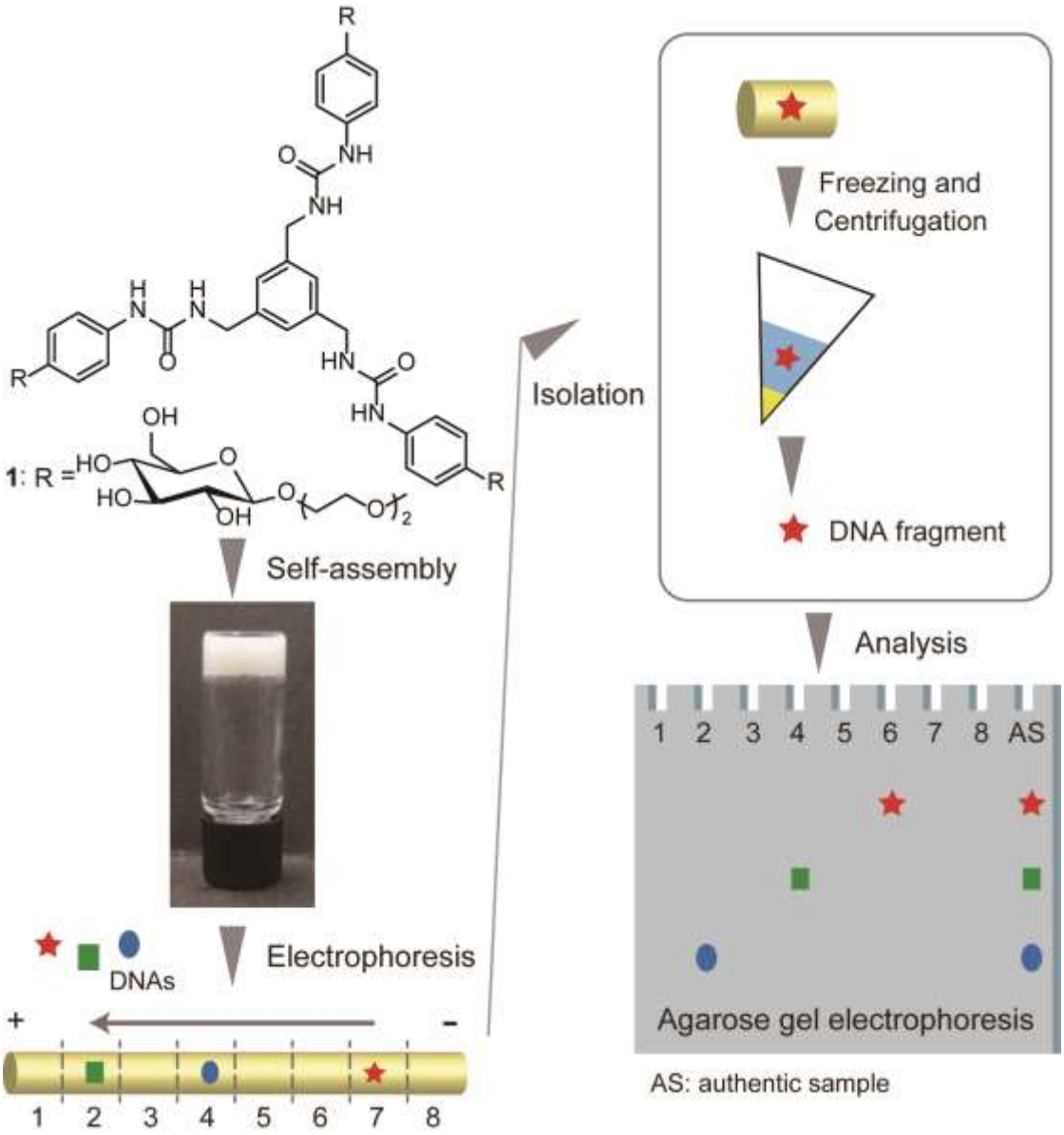

Figure 1. Outline of the DNA-SUGE procedure.

We first examined the utility of DNA-SUGE to analyze λ-Hind III digest containing 2.0, 2.3, 4.4, 6.6, 9.4, and 23.1 kbp DNA fragments. In the first experiment, the electrophoresis was performed using 2.0 wt% supramolecular hydrogel of **1** at 150 V for 90 min (Figure 2A). The supramolecular hydrogel was divided into 8 equal parts after

electrophoresis. The DNA fragments were separated according to their length, with shorter fragments displaying higher mobility than longer fragments (Figure 2A). The 2.0 kbp DNA fragment was found in the most anodic lane 2, while the 2.3, 4.4, and 6.6 kbp DNA fragments were found in lanes 3, 4, and 5, respectively. Larger DNA fragments of 9.4 and 23.1 kbp appeared mainly in lane 6, and a thin band corresponding to the 23.1 kbp DNA fragment was observed in lane 7. Under this condition, the six DNA fragments were finely separated. It is remarkable that DNA fragments with similar length, i.e., the 2.0 and 2.3 kbp fragments, were fully separated.

A subsequent electrophoresis was performed using 2.0 wt% supramolecular hydrogel of **1** at 100 V for 150 min. (Figure 2B). In this experiment, the supramolecular hydrogel was divided into 20 equal parts after electrophoresis (Figure 2B). The 2.0 and 2.3 kbp DNA fragments were found in lanes 5 to 7, but the resolution was inferior to that obtained using the former condition. The 4.4 and 6.6 kbp DNA fragments were observed in lanes 11 and 13, respectively, while the 9.4 kbp fragment was found in lanes 14 and 15, with lane 14 containing the strongest band. The 23.1 kbp DNA fragment was found in lanes 15 and 16. Nevertheless, the results indicated that a low voltage was more effective to separate large DNA fragments, and separation of the 9.4 and 23.1 kbp fragments was clearer at 100 V than that obtained at 150 V.

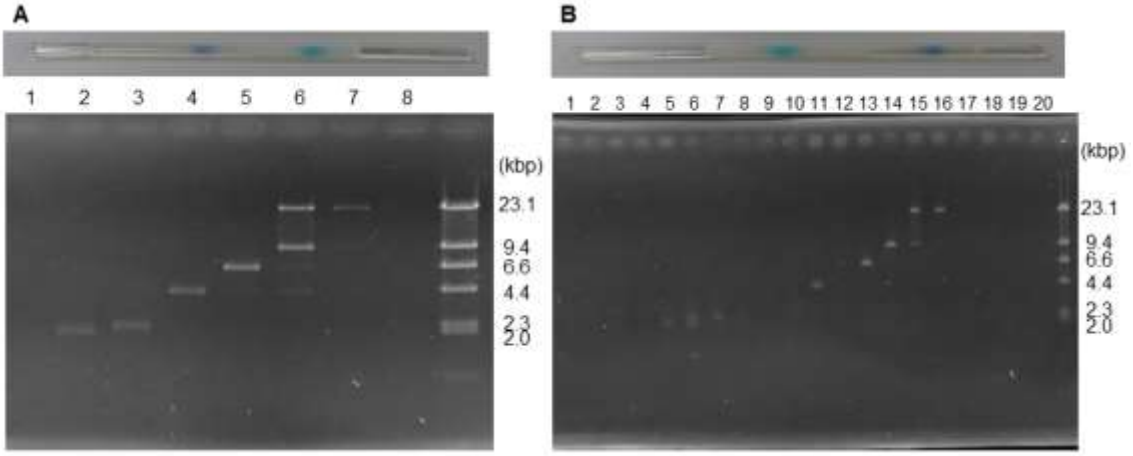

Figure 2. Photographs of glass capillary after DNA-SUGE (top) and following agarose H gel electrophoresis (bottom) of λ-Hind III digest (2.0, 2.3, 4.4, 6.6, 9.4, and 23.1 kbp DNA fragments) using 2.0 wt% hydrogel of **1** (A) at 150 V for 90 min, and (B) at 100 V for 150 min.

The Lambda DNA-Mono Cut Mix, which contains nine DNA fragments of 10.1, 15.0, 17.1, 24.0, 24.5, 30.0, 33.5, 38.4, and 48.5 kbp in length, was also analyzed using the DNA-SUGE method. The Lambda DNA-Mono Cut Mix contains larger DNA fragments than the λ-Hind III digest described above. However, the DNA-SUGE conditions that were effective for separating the λ-Hind III digest were not optimal for the analysis of Lambda DNA-Mono Cut Mix. Optimization experiments revealed that a low concentration of supramolecular hydrogel of **1** and low voltage were more effective to separate multiple DNA fragments, although a high concentration of supramolecular

hydrogel of **1** showed fine resolution. Thus, the electrophoresis for Lambda DNA-Mono Cut Mix was performed using 1.0 wt% supramolecular hydrogel of **1** at 50 V for 6 h, and the hydrogel was divided into 20 equal parts after electrophoresis (Figure 3). Using this condition, the 10.1 kbp DNA fragment appeared primarily in lane 4. It is notable that this method could separate the 15.0 and 17.1 kbp DNA fragments, which were mainly found in lanes 7 and 8, respectively. 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 resolution of the agarose H gel electrophoresis.

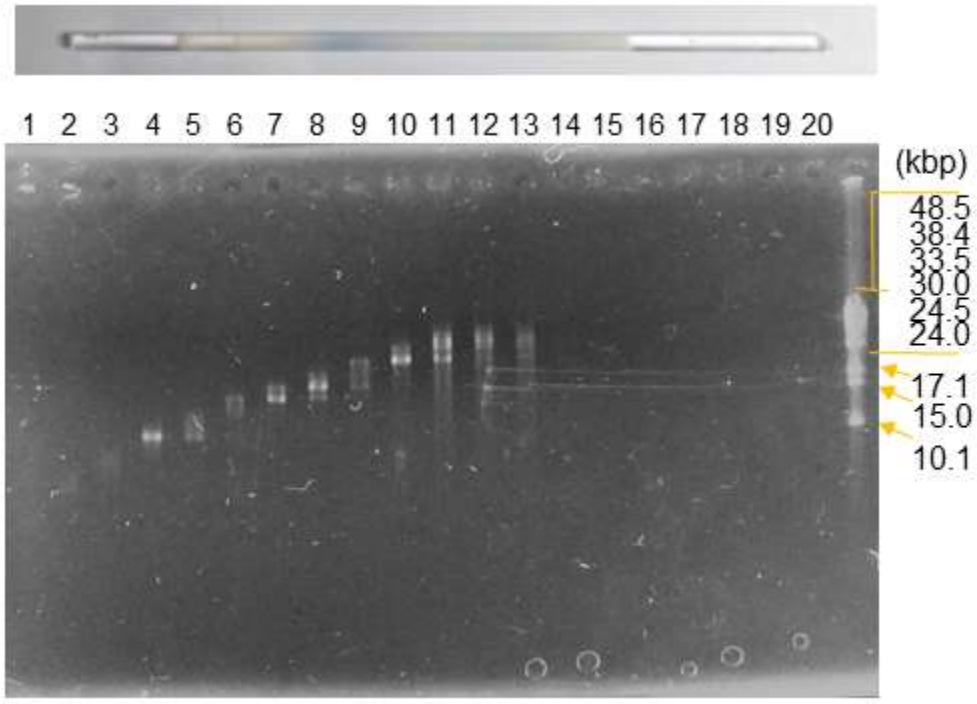

Figure 3. Photographs of glass capillary after DNA-SUGE (top) and following agarose H gel electrophoresis (bottom) of Lambda DNA-Mono Cut Mix (10.1, 15.0, 17.1, 24.0,

24.5, 30.0, 33.5, 38.4, and 48.5 kbp DNA fragments) using 1.0 wt% hydrogel of **1** at 50 V for 6 h.

Marker 7 GT, which contains 10.1, 17.7, 21.1, 23.5, 41.8, 50.3, and 165.7 kbp DNA fragments, was also analyzed in this study. Marker 7 GT contains large DNA fragments of more than 100 kbp. The electrophoresis of Marker 7 GT was performed using 1.0 wt% supramolecular hydrogel of **1** at 50 V for 9 h, and the supramolecular hydrogel was divided into 20 equal parts after electrophoresis (Figures 4A). Smaller DNA fragments migrated closer to the anode side than larger fragments. The 10.1 and 17.7 kbp DNA fragments were clearly separated, and the 10.1 kbp fragment appeared in lane 2 and the 17.7 kbp fragments appeared in lanes 4 to 7. Unfortunately, separations of the 17.7 to 23.5 kbp and 41.8 to 165.7 kbp DNA fragments were not clear using this condition. Following optimization, a condition using 2.0 wt% supramolecular hydrogel of **1** at 40 V for 9 h was found to be more suitable for the analysis. The supramolecular hydrogel was divided into 20 equal parts after the electrophoresis (Figures 4B). Using this condition, the 10.1 kbp DNA fragment appeared in lane 9. Although the 17.7 kbp DNA fragment appeared broadly using the former condition, it was exclusively found in lane 11 using the new condition. Lane 12 contained the 21.1 and 23.5 kbp DNA fragments. 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 of 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 mobility following DNA-SUGE.

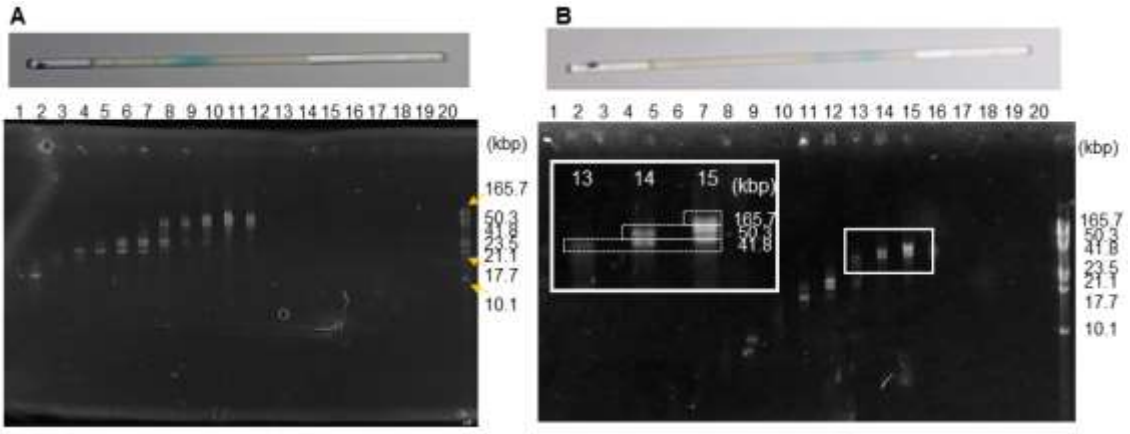

Figure 4. Photographs of glass capillary after DNA-SUGE (top) and following agarose H gel electrophoresis (bottom) of Marker 7 GT (10.1, 17.7, 21.1, 23.5, 41.8, 50.3, and 165.7 kbp DNA fragments) (A) using 1.0 wt% hydrogel of **1** at 50 V for 9 h, and (B) using 2.0 wt% hydrogel of **1** at 40 V for 9 h.

In summary, we have developed an electrophoresis method for separating large DNA fragments using a supramolecular hydrogel of **1** as the matrix. This is the first example of DNA separation using a SUGE technique. The DNA-SUGE method allowed the separation of large DNA fragments using a typical continuous field electrophoresis; previously, this separation required the use of pulsed-field gel electrophoresis. The present procedure is not enough practical yet in respect of running cost, operability, etc.

However, this study showed that supramolecular hydrogel can be a candidate for next-generation DNA electrophoresis. We believe further investigations will open an innovative DNA electrophoresis technique utilizing characteristics of supramolecular hydrogel.

Acknowledgements

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