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Separation of proteins using supramolecular gel electrophoresis†

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An amphiphilic low-molecular-weight hydrogelator 1 was synthesized. A tris-glycine-SDS solution gel of 1 was applied for electrophoresis to separate proteins. Centrifugation of a mixture of protein and a hydrogel of 1 enabled the recovery of protein. Various combinations of proteins were applied for supramolecular gel electrophoresis (SUGE), and remarkably poor mobility for small proteins (<45 kDa) was found.

Self-assembly of preorganized small molecules is a convenient approach to nanoscale architecture. A supramolecular gel composed of fibrous aggregates interlinked noncovalently is an attractive target in this area.[1] Most importantly, research on supramolecular hydrogels has been expanding in the last decade, because of their potential for biological applicability.[2] Great progress has been attained especially in the fields of tissue engineering or chemosensors.[3] In contrast, electrophoresis using supramolecular hydrogels remains as an undeveloped area of research. Gel electrophoresis is one of the most fundamental and frequently used techniques in the analysis of diverse biomolecules.[4] Polyacrylamide gel electrophoresis (PAGE), which was first reported in the middle of the last century, and sodium dodecyl sulfate PAGE (SDS-PAGE) play a central role in the analysis of proteins.[5] The application of supramolecular hydrogels to protein electrophoresis has potential advantages compared with polyacrylamide gels. One of them is the efficient recovery of protein samples. Polyacrylamide gels show strong affinity for proteins; thus, the recovery of proteins after PAGE is laborious.[6] The resolvable nature of supramolecular hydrogels is an advantage regarding the recovery of proteins, as the resolved gel would show lower affinity for proteins compared with the original gel. The other advantage is the potential for a novel separation manner depending on the structural nature of supramolecular hydrogels. A Phos-tag copolymerized polyacrylamide gel reported by Koike, Kinoshita, and co-workers exhibited high affinity for phosphorylated proteins, and attained unique electrophoresis.[7] This result indicates that the fine-tuning of gels enables the development of specialized electrophoresis. The structural divergence of supramolecular hydrogels favors the creation of electrophoretic techniques according to a specific purpose. In this manuscript, we report the synthesis of an amphiphilic low molecular weight (LMW) hydrogelator and its application to the electrophoresis of proteins. Efficient recovery of proteins and dramatically poor mobility of small proteins were observed using this supramolecular gel electrophoresis (SUGE) approach.

C_3-symmetric tris-urea LMW organogelators have been developed in our laboratory.[8] Peripheral modification of the organogelator with hydrophilic groups afforded an amphiphilic LMW hydrogelator.[9] The amphiphile **1** was designed and synthesized from commercial pentaacetyl α-D-glucoside using eight-step reactions (Fig. 1). A mixture of **1** and Tris-Glycine-SDS (TGS) solution (Tris: 25 mM; Glycine: 192 mM; SDS: 0.1%) yielded a hydrogel after brief heating; the minimum gelation concentration of 1 was estimated at 1.5 wt% (Fig. 2 inset). The hydrogel was stable at ambient temperature for a long period, and kept a high transparency, even at 5.0 wt%. The thermal stability (T_{gel})[10] of the hydrogel was increased by increasing the concentration of **1** as a typical characteristic of the supramolecular gel (T_{gel}; 46 °C for 1.5 wt%, 92 °C for 2.0 wt%, >100 °C for 3.0 wt%). Narrow fibers (average diameter = 70 nm) were observed in the scanning electron microscopy (SEM) images of the xerogel (Fig. 2).

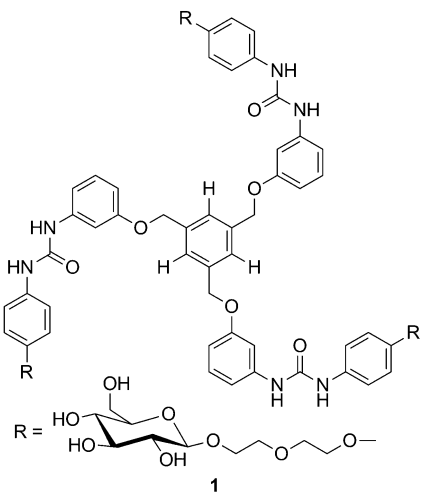

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Fig. 1 Chemical structure of LMW hydrogelator **1**.

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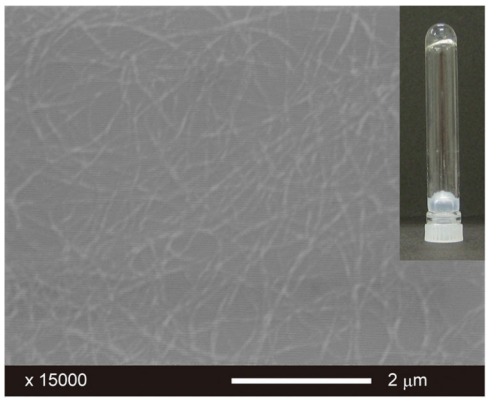

Fig. 2 Photograph and SEM image of TGS solution gel of **1**.

The aptitude of the TGS solution gel of **1** for electrophoresis aimed at separating proteins was tested. A capillary (ϕ = 1.7 mm) was filled with the TGS solution gel of **1**, and a color dye conjugated protein marker (DynaMarker® Protein Multi-Color III) was applied on one side. The capillary was applied to electrophoresis using a submarine electrophoresis system. The TGS solution gel of **1** held out against electrophoresis conditions. Six faded bands were observed after the electrophoresis (100 V, 40 min) using a 2.0 wt% TGS solution gel of **1**; however, a 12 wt% polyacrylamide gel filled capillary separated all eight proteins after submarine electrophoresis (135 V, 40 min) (Fig. S1, ESI†). The gel of **1** was too fragile to handle outside the capillary; therefore, it was difficult to analyze its exact separation capacity in detail. Accordingly, a mixed TGS solution gel consisting of **1** (2.0 wt%) and agarose (2.0 wt%) [**1**-AG gel] was used in subsequent electrophoretic experiments. The gel was sufficiently hard to handle outside the capillary, even after electrophoresis, and the agarose gel itself had no separation ability for proteins, at least in the range from 16 to 229 kDa.

Isolation of proteins from the **1**-AG gel was achieved using an extremely simple centrifugation step. The **1**-AG gel containing electrophoresed proteins was centrifuged (14 100 *g*). The supernatant was applied to SDS-PAGE, together with weighted references. Quantitative evaluation of extracted proteins was accomplished *via* analysis of a coomassie brilliant blue (CBB) stained polyacrylamide gel plate using the ImageJ software. Extraction of ovalbumin and lysozyme was attained with a yield of up to 27 and 43%, respectively. Other proteins were also recovered using this procedure, and their yields were about 20 to 40%. In contrast, this procedure was not effective for the polyacrylamide gel, and no proteins were detected in the extracts.

A typical procedure of SDS-SUGE using the **1**-AG gel is shown in Fig. 3. A capillary (ϕ = 2 mm, length = 120 mm) was filled with the **1**-AG gel (80 mm), a mixture of pre-denatured proteins was adsorbed onto an end of the gel, and both ends of the capillary were filled with an agarose gel (2.0 wt%). The capillary was sunk in TGS solution in the submarine electrophoresis system and electrophoresed using optional voltage and time. The electrophoresed gel was taken out of the capillary and divided into eight equal parts (numbered 1 to 8 from the anode side). Extracted solutions were analyzed using typical SDS-PAGE and CBB staining.

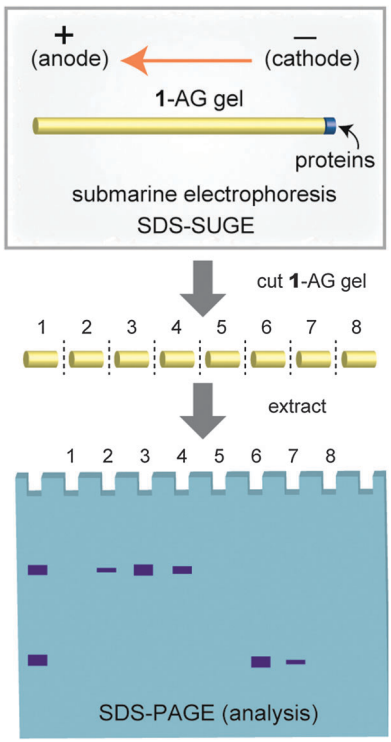

Fig. 3 Schematic representation of SDS-SUGE and following SDS-PAGE analysis.

A mixture of β-galactosidase (116 kDa) and ovalbumin (45 kDa) was employed for SDS-SUGE using the **1**-AG gel (100 V, 120 min.) (Fig. S2, ESI†). Smaller ovalbumin was detected in lanes 4 to 7, and a stronger band was found in lane 5. Larger β-galactosidase was detected in lanes 6 to 8, with the strongest band observed in lane 7. Ovalbumin was electrophoresed more to the anode side than β-galactosidase as well as in SDS-PAGE, however the separation was dull (Fig. 4a). Next, we chose a combination of ovalbumin and the much smaller lysozyme (14.4 kDa). SDS-SUGE was performed at 100 V for 170 min and the separation pattern was analyzed using SDS-PAGE followed by CBB staining (Fig. 4b and Fig. S2, ESI†). Ovalbumin was detected in lanes 3 and 4, whereas the smaller lysozyme was detected in lane 5. This result means that the smaller protein was retained closer to the cathode side compared with the larger one, *i.e.*, SDS-SUGE using the LMW hydrogelator **1** yielded a separation pattern that was different from that observed in typical SDS-PAGE. To identify the generality of this unusual separation pattern, aprotinin (6.5 kDa) was used in SDS-SUGE, instead of lysozyme. Electrophoresis of ovalbumin and aprotinin was performed at 100 V for 150 min (Fig. S2, ESI†). SDS-PAGE analysis showed that ovalbumin was distributed in lanes 3 to 5, and the smaller aprotinin was found in lane 7 (Fig. 4c). These results indicate that SDS-SUGE using the LMW hydrogelator **1** exhibits remarkably poor mobility for small proteins. Two different molecular sieve effects should be added to this electrophoretic technique. One is analogous to the separation mechanism of typical SDS-PAGE. String-like denatured proteins would pass through the three-dimensionally intertwining fibrous network of **1**, and smaller proteins would show large mobility. The other resembles the separation mechanism of gel filtration. Isolated spaces

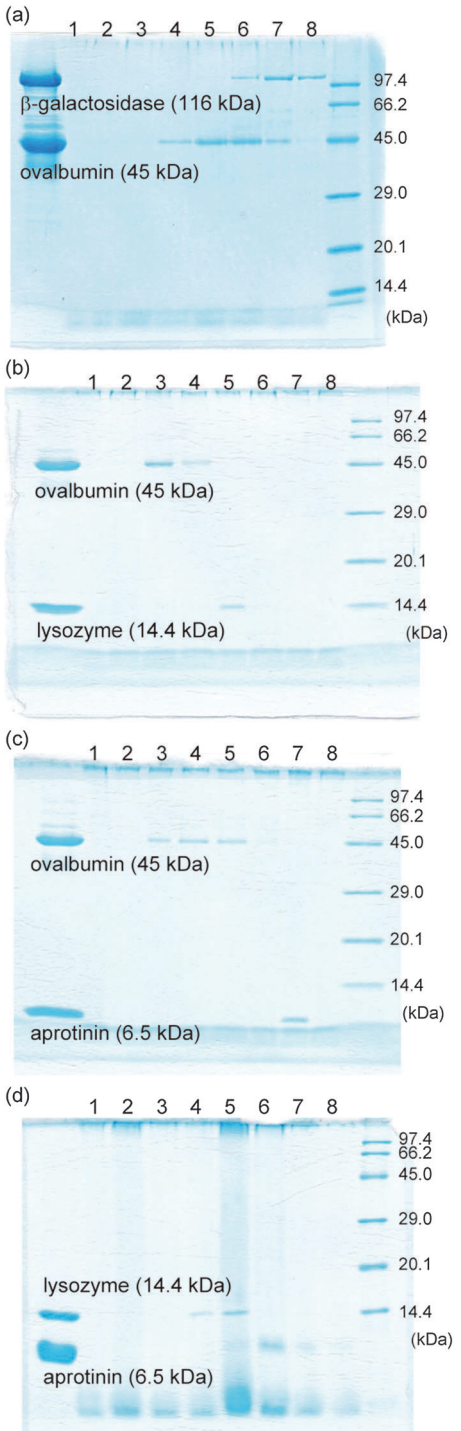

Fig. 4 SDS-PAGE analyses of SDS-SUGE (**1**-AG gel) separation of (a) β-galactosidase (116 kDa) and ovalbumin (45 kDa) (12% polyacrylamide gel); (b) ovalbumin and lysozyme (14.4 kDa) (15% polyacrylamide gel); (c) ovalbumin and aprotinin (6.5 kDa) (15% polyacrylamide gel); (d) lysozyme and aprotinin (15% polyacrylamide gel).

constructed from the self-assembly of **1** may be suitable for retaining the proper size of proteins against an electric current. Larger proteins were chiefly influenced by the former mechanism. SDS-SUGE of ovalbumin and carbonic anhydrase (29 kDa) was performed at 100 V for 170 min. SDS-PAGE analysis showed that ovalbumin was identified mainly in lanes 4 and 5, and the smaller carbonic anhydrase was detected in lane 6 (Fig. S3, ESI†). The relatively scanty separation of ovalbumin and carbonic anhydrase seems to support the above-mentioned mechanism. SDS-SUGE allowed the separation of small-size proteins. A mixture of lysozyme (14.4 kDa) and aprotinin (6.5 kDa) was used for SDS-SUGE at 100 V for 180 min (Fig. S2, ESI†). SDS-PAGE analysis showed that lysozyme was mainly found in lane 5, and smaller aprotinin was retained at the more cathodic lanes 6 and 7 (Fig. 4d). Electrophoresis using the TGS solution gel of **1** (1.5 or 3.0 wt%) and agarose (2.0 wt%) yielded similar separation tendencies; however, the relative mobility changed slightly (Fig. S4, ESI†). This suggests that the unique separation originates in the properties of the pseudopolymer of **1** itself.

In conclusion, we have demonstrated the potential of supramolecular hydrogels as a basis for electrophoresis. Some advantages, such as a simple extraction procedure and unique separation pattern, may render this technique superior to the classical agarose or polyacrylamide gel electrophoreses. The development of a practical procedure of supramolecular hydrogel electrophoresis is currently being investigated in our laboratory.

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