Semi-wet peptide/protein array using supramolecular hydrogel

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The protein microarray is a crucial biomaterial for the rapid and high-throughput assay of many biological events where proteins are involved. In contrast to the DNA microarray, it has not been sufficiently established because of protein instability under the conventional dry conditions. Here we report a novel semi-wet peptide/protein microarray using a supramolecular hydrogel composed of glycosylated amino acetate. The spontaneous gel-formation and amphiphilic properties of this supramolecular hydrogel have been applied to a new type of peptide/protein gel array that is compatible with enzyme assays. Aqueous cavities created in the gel matrix are a suitable semi-wet reaction medium for enzymes, whereas the hydrophobic domains of the fibre are useful as a unique site for monitoring the reaction. This array system overcomes several drawbacks of conventional protein chips, and thus can have potential applications in pharmaceutical research and diagnosis.

Microarrays of biomolecules have facilitated the high-throughput analysis of complicated biological events, and have great potential not only in basic research, but also in diagnostics and drug discovery as demonstrated by the great success of the DNA microarray[1–3]. The protein array is now being actively developed to evaluate the activities of various proteins in a high-throughput manner, because proteins encoded by DNA are direct indicators in many biological systems[4–6]. However, compared with the DNA array, the preparation of a useful protein array is generally difficult because of protein denaturation under the conventional dry conditions. When one considers the natural location of proteins (high water content of 70% net weight) in living systems, wet conditions are appropriate for a protein in its array. In contrast to dry devices, unfortunately, a wet array or chip has to date not been well developed[7]. Gels can be regarded as an intermediate between the dry and wet systems, because gels contain a large amount of immobilized solvent. For the past two decades, macromolecular hydrogels consisting of chemically or physically crosslinked polymers have been actively studied as a unique soft material[8–11]. In addition to such a conventional polymer gel, low-molecular-weight organo-[12–15] or hydrogelators[16–26] have been developed, which self-assemble into polymer-like fibres to immobilize solvents, forming the so-called supramolecular gels. In particular, supramolecular hydrogels seem very promising in many biomaterial applications due to their compatibility with the rational design of nanostructures, and because of their spontaneous gelation and rapid degradation.

We now report a supramolecular structure of the low-molecular-weight hydrogelator **1**, which consists of a glycosylated amino acid scaffold (Fig. 1a). The deduced structural features indicate that well-ordered interactions such as hydrogen bonding, van der Waals packing, and hydrophobic interactions are accumulated in a one-dimensional mode parallel to the fibre axis. Using the unique amphiphilicity, spontaneous gel-formation, and the low critical concentration (that is, the lowest concentration needed to form a hydrogel) of hydrogelator **1**, we subsequently constructed a new semi-wet peptide/protein gel array in which peptides or proteins are entrapped in the active form. This supramolecular protein/peptide chip is envisioned to be applicable to protein/enzyme analyses based on high-throughput activity.

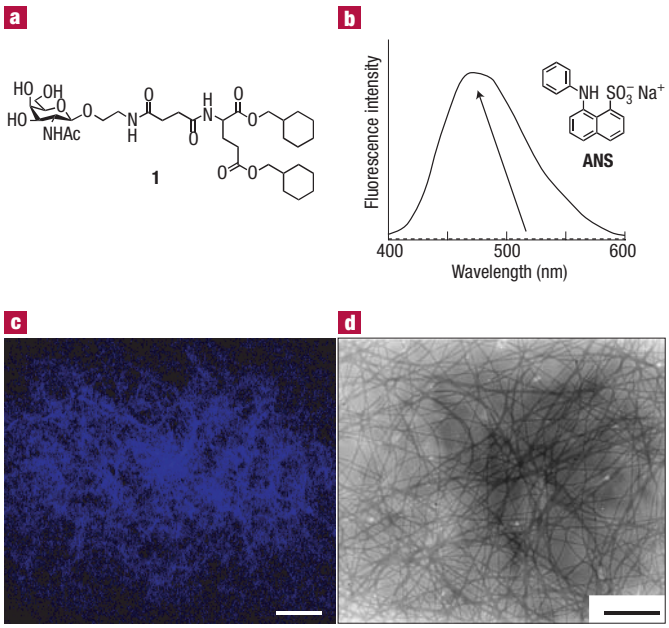

Figure 1 Structural and physicochemical analyses of the supramolecular hydrogel. a, Molecular structure of **1**. **b**, Fluorescence spectra of hydrogel and solution containing environmental fluorescent probes (solid lines: hydrogel; dotted line: solution). [fluorophore] = 10 μM in 25 mM phosphate buffer (pH 8.0), [gelator] = 0.25 wt%, excitation wavelength = 380 nm. The very weak emission of ANS in aqueous solution (almost the bottom line) changes to the enhanced peak in hydrogel along the arrow. **c**, A confocal laser scanning micrograph of a hydrogel containing ANS. [ANS] = 20 μM in H_2O, [gelator] = 0.13 wt%. To obtain a clear contrast, we used 0.13 wt% of the hydrogel in this case. Scale bar: 10 μm. **d**, A TEM photograph of a hydrogel without staining. [gelator] = 0.25 wt%. Scale bar: 500 nm.

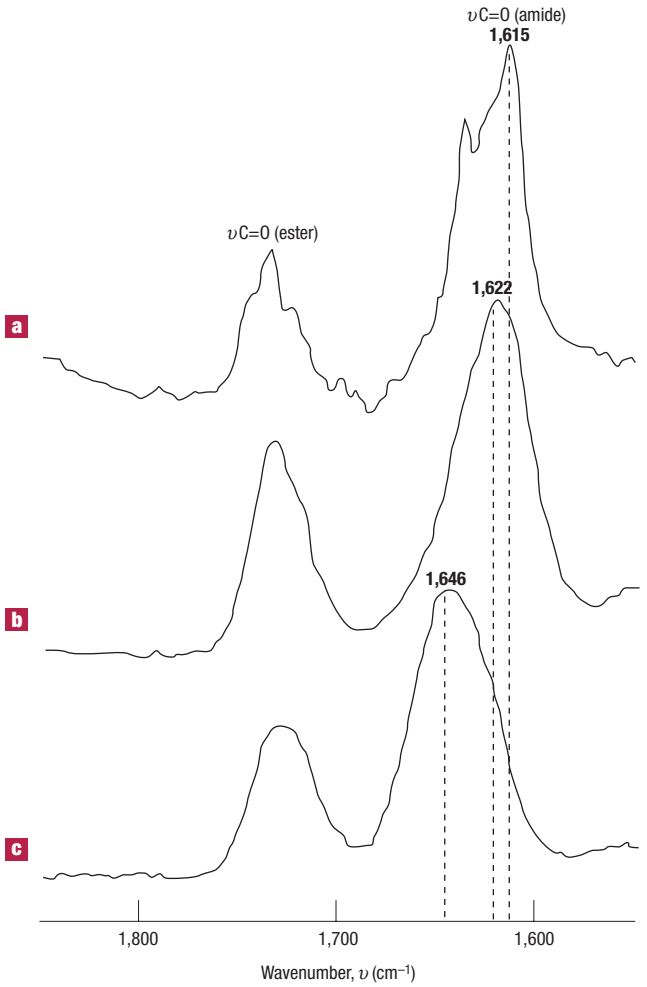

Figure 2 Infrared spectral shift of the amide carbonyl region of 1 depending on its aggregation state. a, Hydrogel state. **b**, Amorphous solid state. **c**, Homogeneous solution of **1** dissolved in $CDCl_3/CD_3OD$ (3/1 (v/v)).

From a small library of saccharide-appended amino acetates prepared by combinatorial solid-phase lipid synthesis[15,24,25,27,28], we discovered several excellent low-molecular-weight hydrogelators, especially the optimized structure **1**, which gelates water at very low concentrations (critical gel concentration = 0.1 wt%)[25]. Based on the weight percent of the hydrogelators, it is calculated that approximately 38,000 molecules of water are immobilized by one molecule of the gelator **1** in the supramolecular hydrogel.

We noticed the stronger emission of an environmentally sensitive fluorescent probe mixed with the hydrogel **1**, compared with that of the probe in aqueous solution[29]. Figure 1b shows the fluorescence spectra of these probes dissolved in a homogeneous aqueous solution and in the hydrogel. In the case of the hydrophobic probes ANS (1-anilinonaphthalene-8-sulphonic acid) and DANSen (5-dimethylaminonaphthalene-1-(*N*-2-aminoethyl)sulphonamide), a remarkable blue shift in the emission maximum was observed relative to that in aqueous solution, along with the intensified emission. In contrast, the relatively hydrophilic probe EDANS (5-((2-aminoethyl)amino)naphthalene-1-sulphonic acid) did not show such a strong emission (See Supplementary Information, Fig. S1). These results suggest that a hydrophobic domain is created in the present hydrogel, which can be stained with fluorescent probes. A confocal laser scanning microscopy image (Fig. 1c) of the hydrogel containing ANS clearly showed the entangled microfibrils with a diameter of several micrometres in the wet gel state. A very important fact is that these fibrils emit a blue fluorescence due to ANS, indicating that each fibre contains the well-developed hydrophobic core that is able to bind hydrophobic molecules. The fibre structure was observed at higher resolution by transmission electron microscopy (TEM) of the gel (Fig. 1d). The diameter of the fibres ranges from 20 nm to 100 nm. This value is roughly tenfold smaller than that observed using confocal laser scanning microscopy, suggesting that thin fibres are assembled to form thick fibrils in the hydrogel state.

Powder X-ray diffraction of the freeze-dried hydrogel (xerogel) provided two clear diffraction peaks (See Supplementary Information, Fig. S2). The peak corresponding to the long spacing of 38 Å is slightly smaller than twice the molecular length of **1**. The other peak in the wider-angle region corresponded to 4 Å, which fits well with the molecular thickness of the cyclohexyl ring, suggesting the tight packing of the hydrophobic tail in the gel fibre of **1**. Fourier transform infrared spectroscopy (FT-IR) of the hydrogel displayed a unique peak assigned to the amide carbonyl stretching at 1,615 cm^{-1} (Fig. 2). Compared with the conventional amide carbonyl (1,646 cm^{-1}), which appeared in **1** homogeneously dissolved in $CDCl_3/CD_3OD$ mixture, the peak was shifted to a lower energy and comparable to that observed for the well-developed hydrogen-bonding β-sheet networks in natural proteins[30]. Interestingly, such a large shift did not occur in the amorphous solid (1,622 cm^{-1}). Thus, it

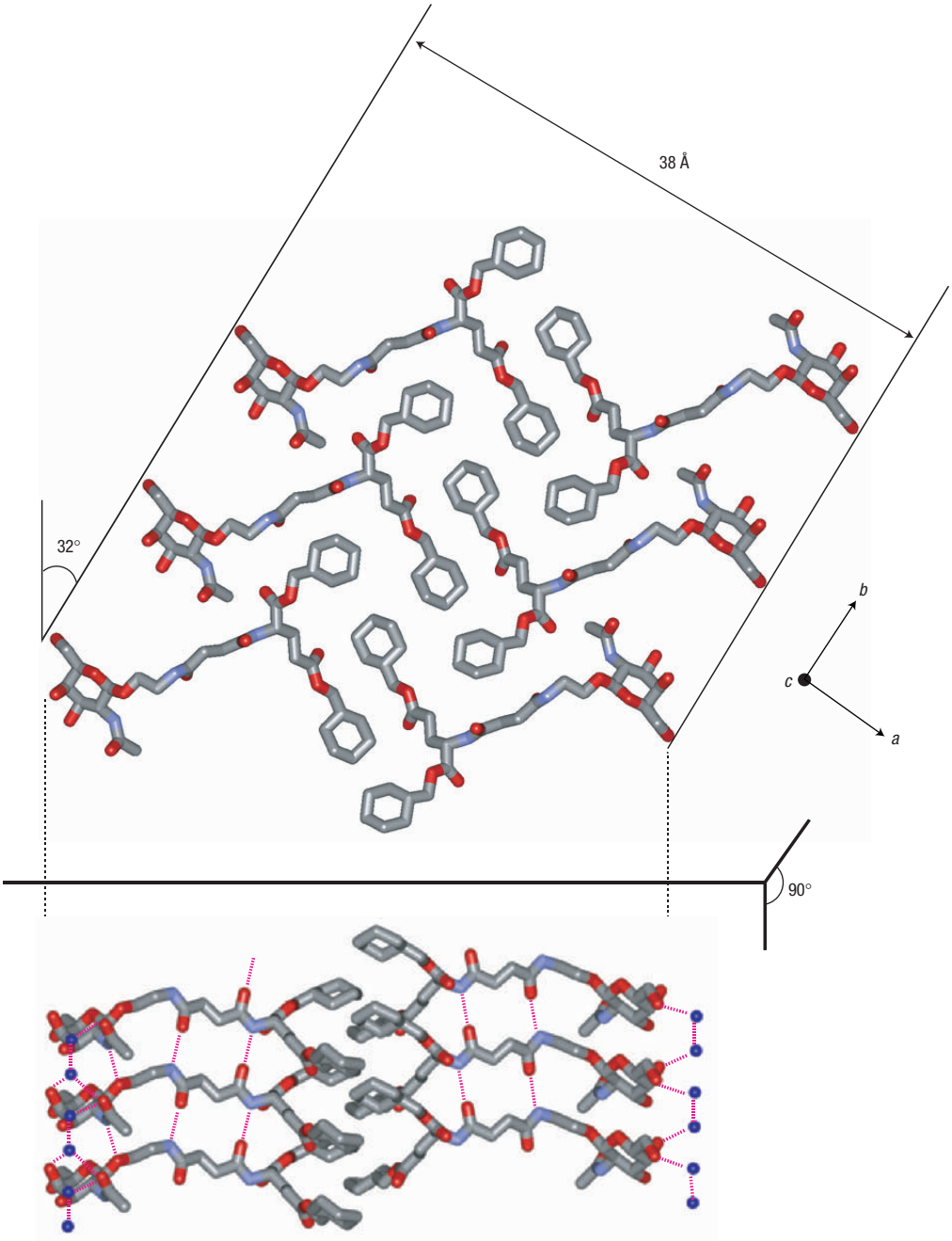

Figure 3 Crystal structure analysis of the molecular packing of 1 prepared from a hydrogel. A top and side view of crystal lattices shows the interdigitated bimolecular structure. Grey: carbon; red: oxygen; light blue: nitrogen; dark blue: oxygen of water molecules. The red dashed lines represent hydrogen-bonding networks.

is concluded that the hydrogen-bonding network is significantly well ordered in the hydrogel fibres.

At a higher gelator concentration (2 wt%), the hydrogel turned into thin platelet crystals within several days. An obtained single crystal was successfully analysed by X-ray crystallography (Fig. 3). The amphiphilic molecular structure provides a bimolecular layer, in which it is apparent that the hydrophobic moieties face each other, and is thus a fundamental structure permitting a continuous hydrophobic domain to be formed. This agrees well with the structure postulated on the basis of the spectroscopic data. In the hydrophobic domain, one methyl-cyclohexyl unit in 1 is interdigitated into the facing molecule in a well-packed manner. The other methyl-cyclohexyl is slightly tilted and forms face-to-face packing with the nearest methyl-cyclohexyl ring in the same layer. In addition, two sets of one-dimensional hydrogen-bonding networks (dashed lines in Fig. 3) are found in the spacer region. The direction of each hydrogen-bonding network is opposite, which is similar to the anti-parallel β-sheet in natural proteins. Such a well-oriented β-sheet-like structure found in the spacer unit is consistent with the data for the significantly low-energy-shifted carbonyl stretching in FT-IR. On the other hand, the saccharide units segregate from the hydrophobic core to form a well-oriented hydrophilic layer in the crystal structure. Two water molecules are found to link the sugar

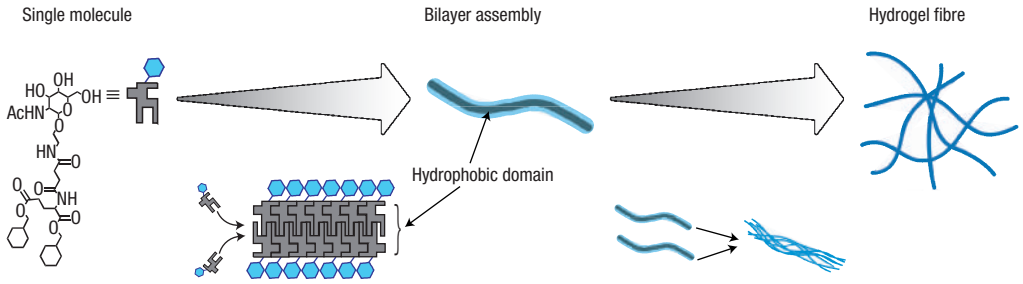

Figure 4 Schematic representation of the hierarchal molecular assembly of 1 to form a supramolecular hydrogel.

moieties to give complicated hydrogen-bond networks. In the acetamide moiety of GalNAc (*N*-acetyl-D-galactosamine, the glycosyl part of hydrogelator **1**), the carbonyl group forms two hydrogen bonds with two distinct water molecules, and the NH group is connected by hydrogen bonding with the acetal oxygen of the neighbouring GalNAc group. These hydrogen bonds align along the same direction as other interactions, such as the hydrogen bonding of spacer amides and van der Waals packing of cyclohexyl groups. These cooperative interactions play an important role in stabilizing the one-directional fibre structure.

Thus, the hierarchal assembly of the present supramolecular hydrogel is proposed as summarized in Fig. 4. As a fundamental nanoscale structure, the bimolecular layer of the gelators is stabilized through both the tight packing of the hydrophobic tails and a well-developed network of multiple hydrogen-bonding amide units. This assembly forms fibres that contain extensive hydrophobic domains in their core, and an oriented saccharide interface. The thin fibres are entangled to give thick fibrils. The thick fibrils are entangled again to form many microcavities in which water molecules are efficiently immobilized, so that a macroscopic hydrogel consisting of self-assembled small molecules is spontaneously prepared.

The observed low critical hydrogel concentration is compatible with entrapping active, native-state enzymes in the supramolecular

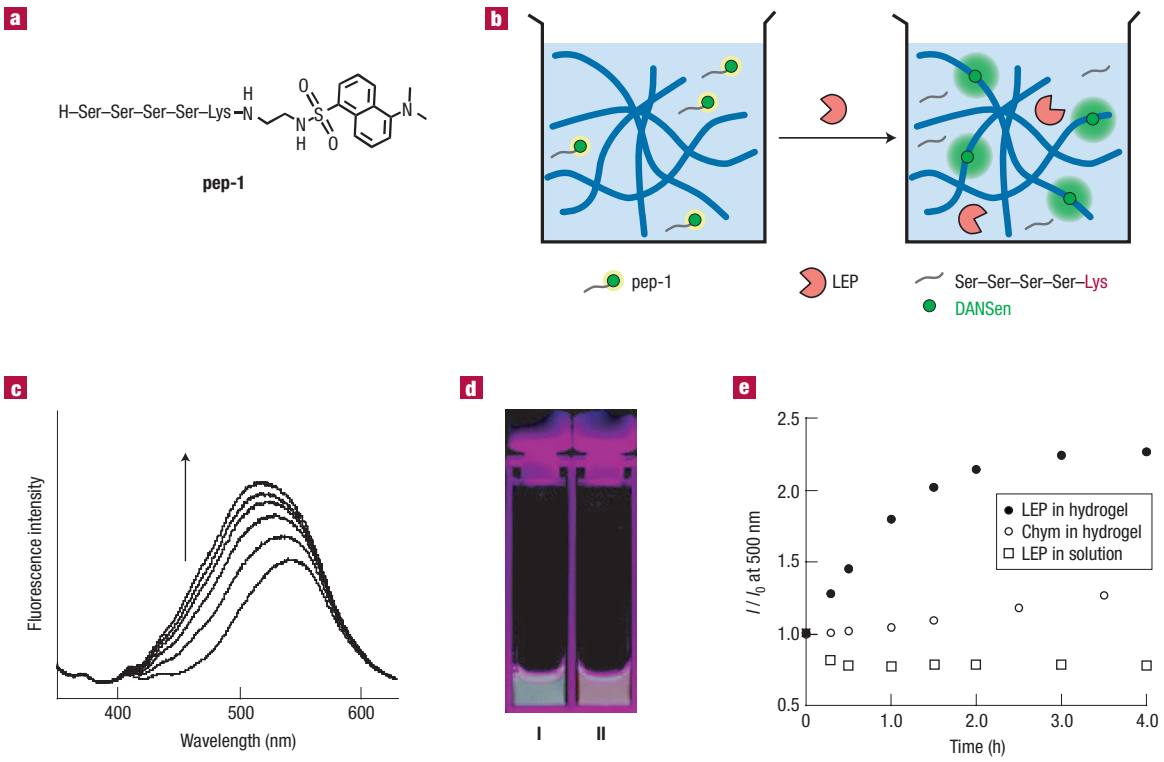

Figure 5 **Fluorometric activity assay of an enzyme entrapped in a supramolecular hydrogel. a**, Chemical structure of a peptide substrate (**pep-1**) for LEP. Ser = serine; Lys = lysine. **b**, Illustration of an enzyme activity assay using a supramolecular hydrogel containing **pep-1**. **c**, Fluorescence change by enzymatic hydrolysis. The arrow represents the direction of fluorescence spectral change. **d**, Direct observation of fluorescence change with the naked eye. (I) **pep-1** after cleavage by LEP in the hydrogel; (II) **pep-1** in the hydrogel (without LEP). **e**, Time course of the fluorescence spectral change. Conditions: [**pep-1**] = 20 μM, [LEP] = 500 nM in 50 mM Tris buffer (pH 8.5) at 37 °C, [gelator] = 0.25 wt%.

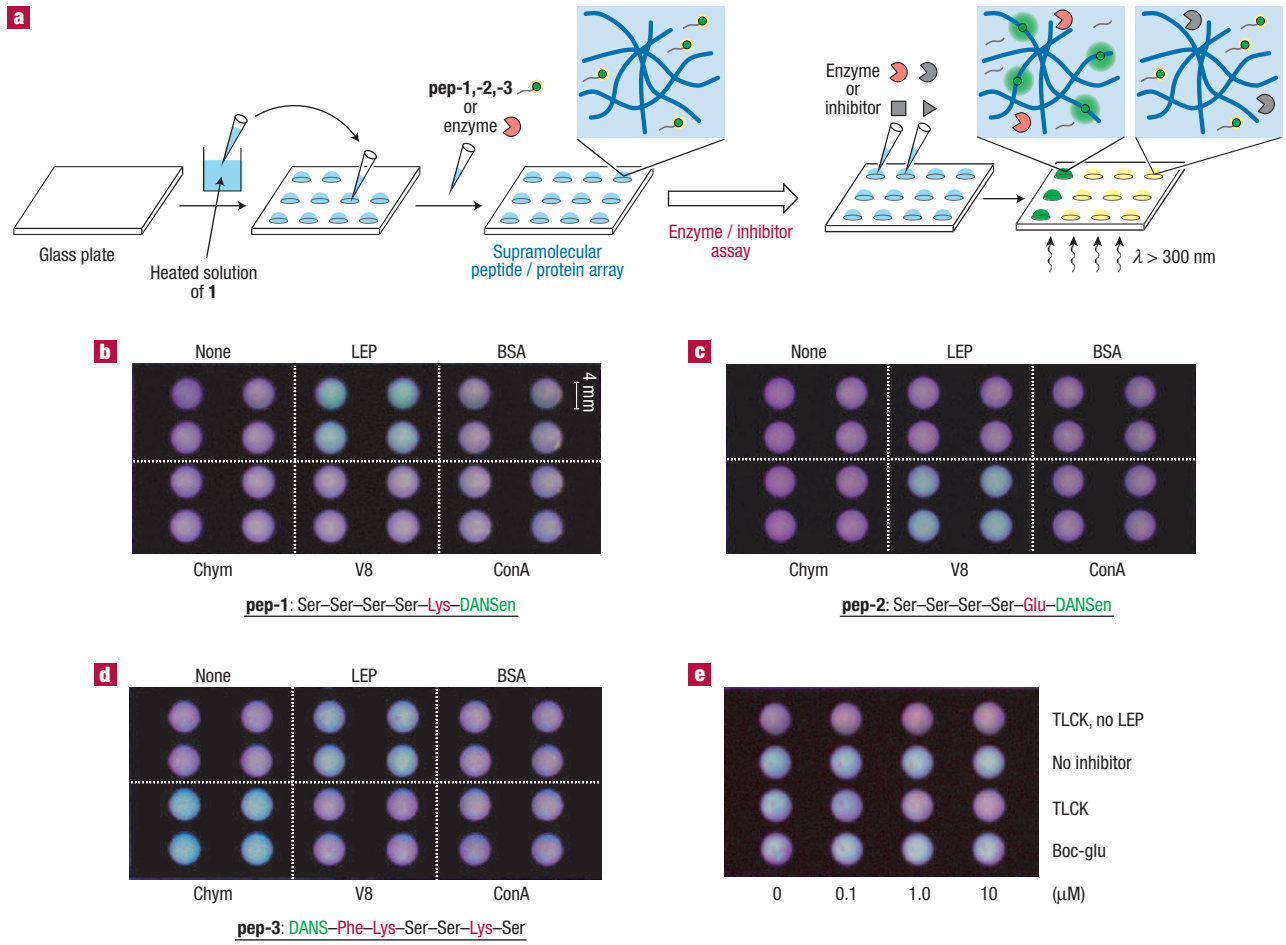

Figure 6 Semi-wet peptide/protein chip using the supramolecular hydrogel. a, Preparation scheme of the supramolecular peptide/protein array. **b–d**, Fluorescent enzyme activity assay using a supramolecular hydrogel-based peptide chip. Conditions: [peptide] = 20 μM, [gelator] = 0.25 wt% in 50 mM Tris buffer (pH 8.0) at room temperature. [enzyme or protein] = 0.5 μM. **e**, Assay of LEP inhibitors using supramolecular hydrogel-based protein chip. Conditions: [LEP] = 1.0 μM, [gelator] = 0.25 wt% in 50 mM Tris buffer (pH 8.5) at room temperature. [inhibitor] = 0, 0.1, 1.0, 10 μM. [**pep-1**] = 40 μM.

hydrogel[25]. In addition, the present structural analysis clarifies that nanometre-sized hydrophobic domains are included in the self-assembled gel fibres. On the basis of their amphiphilicity, it is possible that an aqueous cavity immobilized in the gel would provide a suitable semi-wet reaction medium for an enzyme, whereas the hydrophobic domain of the hydrogel could be used as a unique site for monitoring the reaction.

In a proof-of-principle experiment, lysyl-endopeptidase (LEP)[31,32] was used as an enzyme catalyst. A substrate for LEP was designed that is a hydrophilic pentapeptide (**pep-1**) bearing lysine (Lys) and DANSen at the *C*-terminal as shown in Fig. 5a. When LEP cleaves a peptide bond between Lys and DANSen, the resultant DANSen is reasonably expected to shift from the aqueous cavity of the hydrogel to the hydrophobic domain because of DANSen's strong hydrophobicity, causing the fluorescence increase in the environmentally sensitive DANSen (Fig. 5b). Figure 5c shows a typical fluorescence change in the hydrogel containing **pep-1**. Before hydrolysis, the emission maximum is at 540 nm, which is almost comparable to that in aqueous solution (545 nm). After the LEP-catalysed hydrolysis, the emission maximum was shifted to 508 nm, and the emission intensity increased by twofold. This colour change can be visually monitored as shown in Fig. 5d, that is, a pinkish-yellow hydrogel (II) turned into light green (I) by LEP addition. The time course of the fluorescence change is evaluated in Fig. 5e. In the case of LEP, the emission increase completed within two hours. The addition of chymotrypsin (Chym) instead of LEP did not induce such a fluorescence change because Chym requires an aromatic or hydrophobic side chain on the amino acid (phenylalanine (Phe), tyrosine (Tyr) or tryptophan (Trp)) of the scissile bond. In contrast, a negligible fluorescence change was observed for the reaction in a homogeneous aqueous solution (that is, in the absence of hydrogel). These results clearly imply that the present hydrogel provides a unique reaction medium for sensing enzymatic activity.

This assay system can be minimized so that one can construct a supramolecular gel array including a peptide/protein in an active state (Fig. 6a). After a homogeneous solution containing the gelator **1** and **pep-1** was prepared by gentle heating, a small amount of the solution was dropped onto a glass plate and incubated to yield a supramolecular peptide gel array by spontaneous gelation. The subsequent injection of the corresponding protein solutions (less than 1 μM) into the gel enabled us to assay the protein's activity as displayed in Fig. 6b. A bright green emission was observed only at spots injected with LEP. The addition of Chym, V8 protease (glutamyl endopeptidase)[33], BSA (a carrier protein) and ConA (a saccharide-binding protein), all of which are not capable of cleaving **pep-1**, did not cause any fluorescence changes. When we used

pep-2 bearing DANSen at the proximity of the *C*-terminal of glutamic acid (Glu), a substrate specific for V8-protease, a bright emission appeared only at spots injected with V8-protease (Fig. 6c; see Supplementary Information, Fig. S3). In case of **pep-3** having both Phe and Lys at the *N*-terminal as a substrate that shows a cross-reactivity for Chym and LEP, a bright green emission was observed at hydrogel spots injected with Chym or LEP (Fig. 6d; see Supplementary Information, Fig. S3). These results indicate that the present peptide-hydrogel array can distinguish the protein character based on its enzymatic activity. The present study uses the partition coefficient difference between substrate and product to monitor the protease activity fluorometrically, and it is reasonably envisioned that other mechanisms such as using fluorogenic or FRET (fluorescence resonance energy transfer) substrates may operate in the present supramolecular peptide array[34,35].

Alternatively, a protein array can be prepared by injection of LEP into the supramolecular hydrogel before addition of **pep-1**. Using this protein chip, we can successfully assay the inhibitor for LEP (Fig. 6e). In the absence of inhibitors, the gel spot showed a bright green emission, whereas the emission from spots containing the inhibitor (TLCK (N^{α}-tosyl-lysine chloromethylketone)) became smeared, depending on the inhibitor concentration. In contrast, spots containing another inhibitor (Boc-glu, where Boc is *t*-butoxycarbonyl), which is not potent for LEP, remained brightly fluorescent. Thus, the protein-gel chip is expected to be potentially useful not only for screening inhibitors from non-inhibitors, but also for discriminating levels of the inhibition ability in a high-throughput manner.

Previously developed protein/peptide chips involve inevitable chemical processes for covalently attaching the protein/peptide to a substrate. In addition, the amount of immobilized protein/peptide is limited to a two-dimensional area, causing a low sensitivity and a low signal/noise level. Furthermore, as the conventional protein/peptide chip is a dry system, the denaturation of immobilized proteins is problematic. In contrast, the present supramolecular hydrogel entraps enzymes in three-dimensional and semi-wet cavities without substantial loss of activity. Although a polymer-based three-dimensional protein-gel chip was reported very recently to improve sensitivity[7], the gel-preparation process, including the photo-polymerization of each spot, is tedious. The supramolecular gel formation reported here is completely spontaneous, and does not require the addition of any polymerization. In addition, the gel is sufficiently mechanically robust that it can be spotted on glass. This will probably permit a more densely packed protein-chip-array than that presently possible using 96-well plates. These unique features are intriguing for pharmaceutical and proteome research.

In conclusion, we clearly demonstrated the hierarchal structural characteristics of supramolecular hydrogel based on a glycosylated amino acid ester. The gel fibres are self-assembled and stabilized by multiple interactions such as β-sheet-like hydrogen-bonding networks, van der Waals packing, and hydrophobic interactions. The spontaneous gel-formation and amphiphilic property of the present hydrogel have been successfully applied to supramolecular semi-wet peptide/protein gel arrays that are compatible with enzyme activity assays and the screening of enzyme inhibitors. Supramolecular chemistry is now a powerful strategy for developing new molecularly defined materials in materials science. Most of these successful examples, however, are limited to a dry (that is, solvent-free) system[36], and wet materials have rarely been reported. As semi-wet biomaterials, protein- or peptide-based hydrogels have been reported by several groups[37–44]. Compared with them, the present example is novel in that it is a purely synthetic small molecule existing as a single component of a supramolecular hydrogel, and thus is expected to be applicable to various biomaterials by flexible molecular design.

METHODS

GENERAL INFORMATION

Reagents for peptide synthesis were obtained frm Watanabe Chemical Industries (Japan). Fluorescence probes were obtained from Molecular Probes, USA. Proteins and enzymes were from Wako, USA (LEP and V8 protease) or Funakoshi (ConA) or Sigma (Chym and BSA). Other chemicals were from Wako, TCI (Japan) or Aldrich. **1** was prepared according to our methods reported previously[24–26]. Mass spectra were recorded on MALDI-TOF-Mass spectrometer (PE Biosystems Voyager DE-RP). Fluorescence spectra were recorded on Hitachi F-4500.

OBSERVATION OF GEL STRUCTURE BY MICROSCOPY

TEM measurements were carried out using a Hitachi H-600 electron microscope. A piece of the gel (0.25 wt%) was placed on a carbon-coated copper grid and dried for 3 h under vacuum. Without staining, the grid was observed using an acceleration voltage of 90 kV. Confocal laser scanning microscopy observation was carried out using Carl Zeiss LSM 510 META equipped with a UV laser (351/364 nm).

X-RAY POWDER DIFFRACTION

X-ray powder diffraction was obtained using a MAC Science M18XHF. The hydrogel (0.5 wt%, 5 ml) was rapidly frozen in liquid nitrogen and dried under vacuum for 24 h at –5 °C. The xerogel thus obtained was put into a glass capillary tube (diameter $\phi = 0.7$ mm). The X-ray diffractogram was recorded on an imaging plate using Cu radiation (1.54178 Å) at a distance of 15 cm at room temperature.

FT-IR MEASUREMENT OF THE HYDROGELATOR

FT-IR was measured with a Perkin Elmer Spectrum One on ATR mode. The hydrogel sample was prepared using D_2O (2.0 wt%). An amorphous solid sample was prepared from the homogeneous solution of the gelator dissolved in $CDCl_3/CD_3OD$ (4/1 (v/v)).

X-RAY CRYSTALLOGRAPHIC ANALYSIS

Single crystals of **1** were obtained by aging the hydrogel (2.0 wt% in water) at room temperature for several days. X-ray diffraction data was collected on a Rigaku R-AXIS RAPID diffractometer with a 2D area detector using graphite-monochromatized Cu-Kα radiation($\lambda = 1.5418$ Å) at about 120 K. Lattice parameters were obtained by least-squares analysis from reflections for three oscillation images. Direct methods (using the SIR92 program[45]) were used for the structure solution. All calculations were performed using the TEXSAN crystallographic software package (Molecular Structure Corporation, The Woodlands, Texas, 1985). The structure was refined by the full matrix least-squares procedure using observed reflections $F^2 > 2.0\sigma(I)$, where $\sigma(I)$ and F are the standard deviation of the observed intensity I of each reflection and the scattering factor, respectively. All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were placed in idealized positions with isotropic displacement parameters relative to the connected non-hydrogen atoms, and were not refined. Crystallographic parameters are summarized in the Supplementary Information, Table S1.

FLUORESCENT PEPTIDE SYNTHESIS

pep-1: A commercially available trityl resin with loaded Fmoc-Lys(Boc)-OH was used (Fmoc is 9-fluorenylmethoxycarbonyl). Peptide synthesis was performed by conventional procedures in which the deprotection was performed with 20% piperidine in dimethyl formamide (DMF) and the condensation step was done with Fmoc-aa (aa is amino acid) (4eq) in the presence of HBTU (2-(1-*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate); 4eq) and DIEA (diisopropylethylamine; 8eq) in DMF. After removing Fmoc from the terminal amino group of the peptide, the resultant free amine was protected by Boc_2O (10eq) in DMF. Subsequently, the peptide was cleaved with $CH_3COOH/CH_3OH/CH_2Cl_2 = 4:1:5$ at room temperature for 4 h. The resin was filtered off and removal of CH_3COOH from the filtrate by toluene azeotrope gave the protected peptide. The *C*-terminal carboxylic acid of the peptide was condensed with DANSen using HBTU/DIEA in DMF. The other protective groups were removed by gentle stirring in trifluoroacetic acid (TFA)/thioanisol/*m*-cresol = 90:5:5 at room temperature. The residues obtained by TFA-removal were diluted with methyl-*t*-butyl ether (MTBE), and then the target peptide was extracted with H_2O. The lyophilized crude peptide was dissolved in *N*-methyl-2-pyrrolidinone (NMP)/H_2O = 1:1, and then purified by reverse-phase HPLC (HITACHI-LC series, octadecyl silyl column, eluent was a water/acetonitrile gradient containing 0.1% TFA). The purified peptide was characterized by MALDI-TOF-mass spectrometry (matrix: α-cyano-4-hydroxy cinnamic acid (CHCA)): $m/z = 770.84$ $[M+H]^+$, 792.85 $[M+Na]^+$.

pep-2 was prepared according to the same method as **pep-1**. MALDI-TOF-mass spectrometry (matrix: CHCA) for **pep-2**: $m/z = 794.06$ $[M+Na]^+$, 810.05 $[M+K]^+$.

pep-3 was synthesized by an automated peptide synthesizer (ABI 433A, Applied Biosystems) using the standard Fmoc-based chemistry with Fmoc-amide resin. After the automated solid-phase peptide synthesis, the resultant free amine was condensed with dansyl chloride (10eq) in DIEA/DMF. After cleavage with TFA/thioanisol/*m*-cresol = 90:5:5, crude peptide was precipitated in MTBE, and purified by the same method as **pep-1**. MALDI-TOF-mass spectrometry (matrix: CHCA) for **pep-3**: $m/z = 916.19$ $[M+H]^+$.

ENZYME ASSAY IN THE HYDROGEL

Gelator **1**: 4.5 mg was suspended in 50 mM Tris buffer containing **pep-1** ([**pep-1**] = 20 μM, pH 8.5) 1.8ml, and the suspension was heated until a homogeneous solution was obtained. 1.5 ml of the solution was quickly poured into a quartz cell and the solution solidified into a hydrogel after standing for 1 h at room temperature. To the top of the hydrogel, 15 μl of an enzyme solution ([Enzyme] = 30 μM in Tris buffer (50 mM, pH 8.5)) was added. The fluorescence spectral change was measured at 37 °C.

PREPARATION OF A SUPRAMOLECULAR PEPTIDE/PROTEIN GEL ARRAY

10 μl of a dispersed peptide solution (20 μM in Tris buffer (50 mM, pH 8.0)) containing a gelator (0.25 wt%) was spotted on a 24-well ($\phi = 4$ mm) glass plate. After aging at room temperature for 1 h, a supramolecular peptide gel array was obtained. For the enzyme assay, 1 μl of a protein solution (5 μM in Tris buffer (50mM, pH 8.0)) was injected into each gel spot, and incubated for 15 min at room temperature. On **pep-3** assay, the gel array was incubated for 5 h at 37 °C after the enzyme was injected. The glass plate was irradiated with a UV trans-illuminator (TM-20, Funakoshi), and a photograph was taken using a digital camera equipped with a cut-off filter (cut-off $\lambda < 420$ nm) on the front of the lens. Protein-gel arrays were prepared in a similar manner to the peptide array.

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Competing financial interests

The authors declare that they have no competing financial interests.