An insulin-sensing sugar-based fluorescent hydrogel†

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We have prepared a small library of amphiphiles, each comprising a polar carbohydrate head group attached through an N-terminal amino acid to a nonpolar pyrene tail group. One of these derivatives is sensitive to the presence of insulin in aqueous media.

Gels are important materials for a diverse range of applications.[1] The synthesis of supramolecular self-assembled gelators, a so-called "smart" or "intelligent" approach in gel chemistry, was first reported by Tanaka *et al.*[2] Since then, much effort has been exerted to develop related materials.[3–6] Recently, supramolecular self-assembled gelators have raised a great deal of interest for their applications in fields such as drug delivery, charge transport, fluorescence, and sensing,[7] but so far, very few of these materials have been identified as having the ability to sense biological entities. Glucose, which is one of the most important energy sources for human cells, is transported from the blood through its binding with insulin. The mode of interaction between any form of glucose and insulin is quite complex and involves the interaction of many metabolic and regulatory factors.[8] In some cases insulin binds to the hydroxy groups at the C4, C3 and C2 positions of glucose through hydrogen bonding.[9] It can also bind with various derivatives of D-glucose,[10] aromatic compounds,[11] acyclic alcohols,[11] cubane[12] and divalent metal ions.[11] The binding mode of various ligands with insulin depends on the concentration of insulin as well as the ligands.[11] Our aim for this study was to design a supramolecular assembly that could sense important biological receptor.

In this paper, we report the synthesis of monosaccharide-based fluorescent hydrogelators that possess the ability to sense insulin. Although many saccharide-based hydrogelators have been reported,[13] to the best of our knowledge none of them has been demonstrated to possess the ability to sense insulin. The gelation abilities of our newly synthesized (Scheme 1) gelators in distilled water and in pH 7 buffer solution were examined by adopting the "stable-to-inversion-of-the-container" method (see electronic supplementary information†). We observed that most of our gelators (**4a**, **4b**; **5b–d**; **6a**, and **6c**) are able to form stable robust gels in water (Table 1), even at concentrations below 0.1%. Gelator **5d** displayed the best performance of the series: it restricted the flow of water to a concentration of only 1.04 mM; *i.e.*, 53,400 molecules of water are constrained by one molecule of **5d**. Very few gelators have been reported to possess such a strong gelating ability.[14] The gelation abilities of our gelators vary with respect to their amino acid and saccharide units (Table 1). The gels formed from gelators **4b** and **5d** were transparent; the rest were translucent.

We used SEM to analyse the textures of the hydrogels of these gelators (Fig. 1). The images of their xerogels[15] reveal closely packed fibre network structures having thicknesses of 20–60 nm. It has been reported that fiber gels are more stable than are lamellar gels;[16] our observation are consistent with those previously reported.

Since none of the gelators formed single crystals, we are unable to clarify the exact mechanism of their self-assembly, although we

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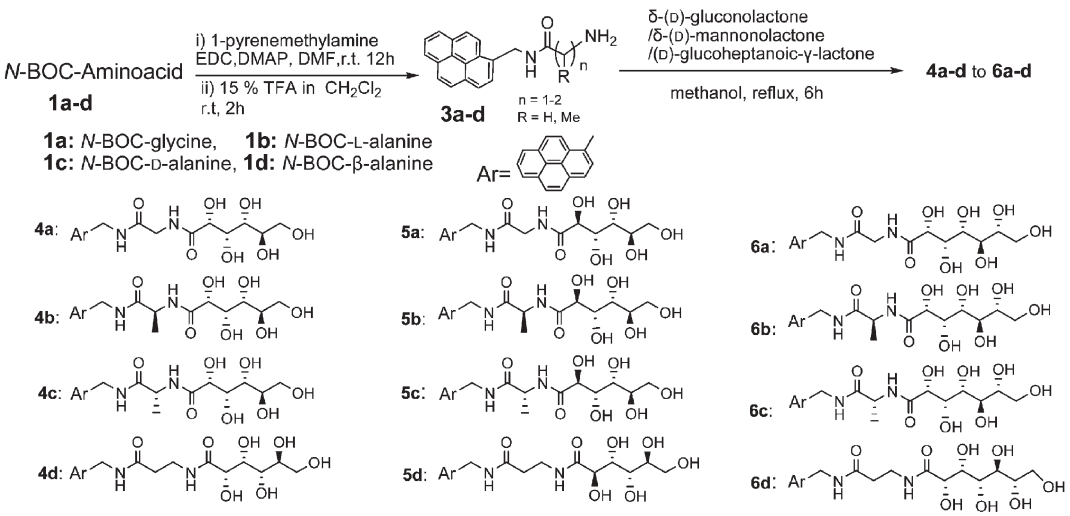

Scheme 1 Synthesis of hydrogelators.

Table 1 Minimum gelation concentrations (MGCs, wt%)[a] of hydrogelators in different solvent systems

Gelator	In distilled water	In buffer[b]	Stability
4a	0.25	0.30	stable
4b	0.15	0.18	stable
4c	—[c]	—	—
4d	0.20	0.25	unstable
5a	0.25	0.30	unstable
5b	0.20	0.15	stable
5c	0.25	0.12	stable
5d	0.05	0.08	stable
6a	0.15	0.15	stable
6b	—	—	—
6c	0.07	0.12	stable
6d	0.30	0.20	unstable

[a] The MGC is the lowest gelator concentration at which gelation was observed to restrict the flow of the medium. [b] 0.08 M MOPSO buffer (pH 7). [c] A dash indicates that no gel is formed.

could establish the driving force behind the self-assembly from FTIR and fluorescence spectroscopy studies.† The carbonyl (C=O) stretching band of the amide moiety of each hydrogelator shifted significantly (to *ca.* 1640 cm^{-1}) in the xerogel state relative to its location in the solid amorphous state (*ca.* 1650 cm^{-1}). These spectral changes suggest that hydrogen bonding interactions of the amide units are one of the driving forces behind the self-assembly. Although we observed no significant change in the position of the OH stretching bands, it has been reported that the hydroxy groups play a role during self-assembly.[13] The emission spectra of the gel states of the gelators display their maxima at 393 nm and a broad band centred at 480 nm. The former peak indicates the presence of monomeric pyrene moieties, and the latter emission suggests dimerization of pyrene groups through π–π stacking.[17] These spectroscopic findings suggest that the self-assembly of the gels is governed, at least in part, by hydrogen bonding and π–π stacking interactions.

It is well known that insulin interacts strongly with D-glucose in biological systems, especially in human blood. There are many methods for estimation of insulin, *e.g.*, glucose tolerance test (GTT), insulin tolerance test (ITT), insulin sensitivity test (IST) and continuous infusion of glucose with model assessment (CIGMA).[18] Unfortunately all of these methods require multiple venipunctures, making them relatively impractical for office assessment. Even today, commonly used oral glucose tolerance test (OGTT) does involve several venipunctures and 2 to 4 h of patient and technician time.[18] Herein we performed a series of simple experiments to study the ability of gelators **4b** and **5d**[19] to sense insulin in the gel state.† We found that in the case of gelator **4b**, the intensity of the signal at 393 nm in the emission spectrum gradually decreased upon increasing the insulin concentration (Fig. 2). As a result, the originally deep-blue-coloured gel turned greenish-blue† under UV light when the concentartion of insulin was 5 μg. However, in the case of gelator **5d** the spectral change was inconsistent with the insulin concentration.† This observation indicated that only gelator **4b**, which is a derivative of D-gluconolactone is responsive to the insulin concentration. We also observed from the SEM image in Fig. 3 that the fibre thickness increased to 60–100 nm after the addition of insulin to the gel of gelator **4b**, relative to that of the gel alone. It has been reported that in some cases the ligand–receptor interaction has destroyed the self-assembly[16,20] and in some cases ligand–receptor interaction remold the gel fiber and increases the gel strength.[17] We believe that in this case due to interaction between sugar moiety and insulin, the order of gel fiber changed (Fig. 3), and as a consequence the pyrene moiety has been dislocated. That means

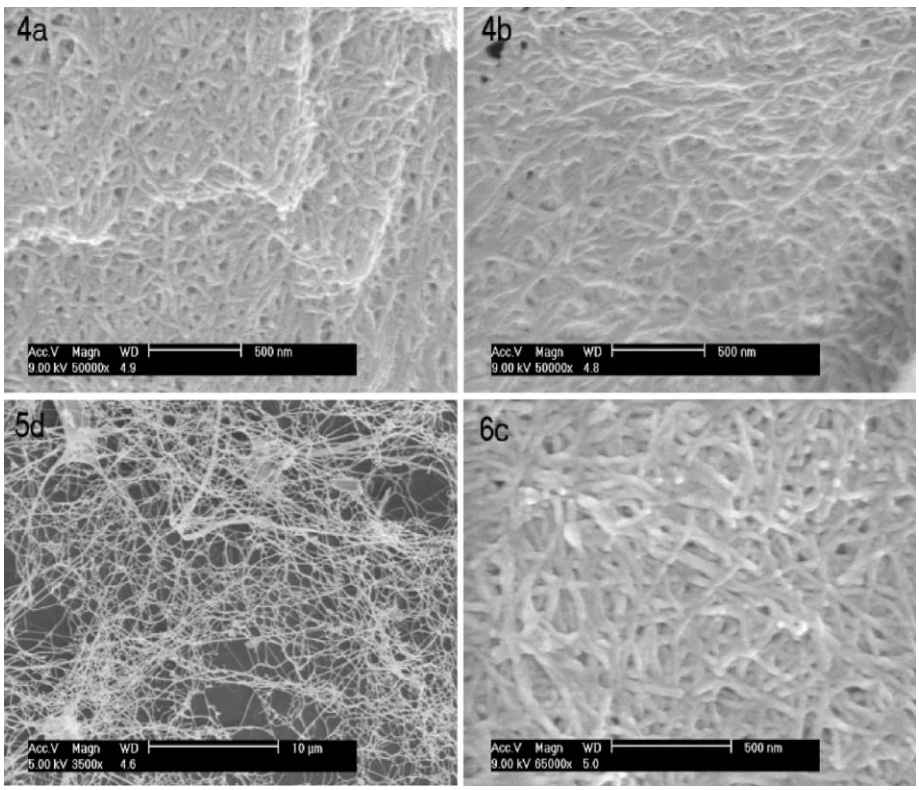

Fig. 1 SEM images of the xerogel of hydrogelators **4a**, **4b**, **5d** and **6c**.

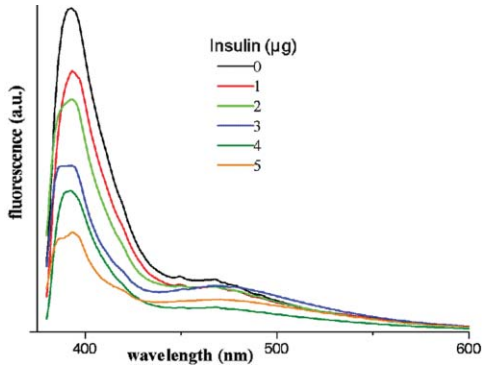

Fig. 2 Emission spectra of gelator **4b** (0.15% w/w) in the presence of insulin at various concentrations (excitation λ_{max} = 370 nm).

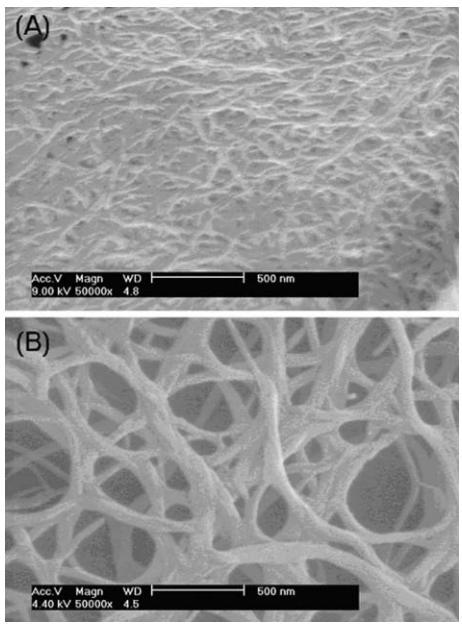

Fig. 3 SEM images of the xerogel of gelator **4b** (A) alone and (B) in the presence of insulin (5 μg).

that the extent of aggregation of pyrene moiety gradually decreases,[21] as a result the quenching at 393 nm increases with the insulin concentration. To our satisfaction, these preliminary experiments suggest that gelators can possess the ability to detect the presence of insulin at concentrations of the microgram level.

In summary, we have synthesized novel and highly efficient fluorescent hydrogelators through a simple series of reactions. These gelators have the ability to gelate water—one of them at a concentration of only 1.04 mM. From a preliminary study, we observed that one such hydrogel has the ability to sense insulin at very low concentrations. These observations encourage us in our quest for materials displaying efficient biosensing properties.

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