Biological Glucose Metabolism Regulated Peptide Self-Assembly as a Simple Visual Biosensor for Glucose Detection

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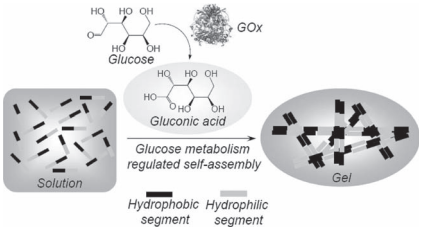
A glucose oxidase (GOx)-mediated glucose metabolism was in vitro mimicked and employed to regulate the self-assembly of peptide-based building blocks. In this new stimuli-responsive self-assembly system, two peptide-based building blocks, respectively, having aspartic acid (gelator **1**) and lysine (gelator **2**) residues were designed and prepared. When adding glucose and GOx to the aqueous solution of gelator **1** or the self-assembled fibrillar hydrogel of gelator 2 to construct glucose metabolism system, the metabolic product (gluconic acid) can trigger the protonation of the peptide molecules and induce the phase transitions of **gelators** 1 (sol-gel) and 2 (gel-sol). Because this glucose metabolism regulated peptide self-assembly is built on the oxidation of glucose, it can be used as a simple visual biosensor for glucose detection.



1. Introduction

Self-assembly of molecular building block into ordered nanostructures not only plays a vital role in various biological phenomena but also provides an important guidance for design and fabrication of novel nanomaterials. In particular, peptide-based self-assemblies have drawn much attention because of their unique properties including well biocompatibility, chemical versatility, and biological recognition abilities.[1–4] To date, self-assembled peptide-based materials have become a class of very important biocompatible materials that have found various applications in tissue engineering,[5–7] drug delivery,[8–10] wound healing,[11,12] and inhibitor screening.[13,14] The self-assembly of peptide can be generally divided into two categories: spontaneous self-assembly and induced self-assembly. The latter one has attracted significant attention because the properties of the self-assembled peptide can be adjusted in response to the local environmental stimuli. In the past few decades, various strategies such as heating–cooling cycle,[15] light irradiation,[16–18] pH,[19–22] and enzymatic triggeration[23–26] have been reported to regulate the self-assembly of peptides and their derivatives. Recently, disulfide bond reduction with a similar principle as enzymatic triggeration has been also developed.[27–29] Of the above strategies, enzymatic triggeration is more beneficial than the other ones for biomedical applications because of the mild self-assembling conditions and low level of damage to body tissues. It is known that enzymes widely involve various biological reactions in organism. Therefore, developing of a self-assembling system in response to enzyme triggered biological reactions is of great importance to construct new materials for biomedical applications. In this study, we reported a new type of peptide-based self-assembly in response to biological glucose metabolism. Glucose is well recognized as the energy source of biological metabolism. The glycolysis of glucose is reported to be a complicated metabolic pathway that converts glucose into pyruvic acid with the aid of various kinds of enzymes including kinase, aldolase, isomerase, and enolase.[30] Here, we constructed a biological glucose metabolism system by using glucose oxidase (GOx) and investigated its influence on

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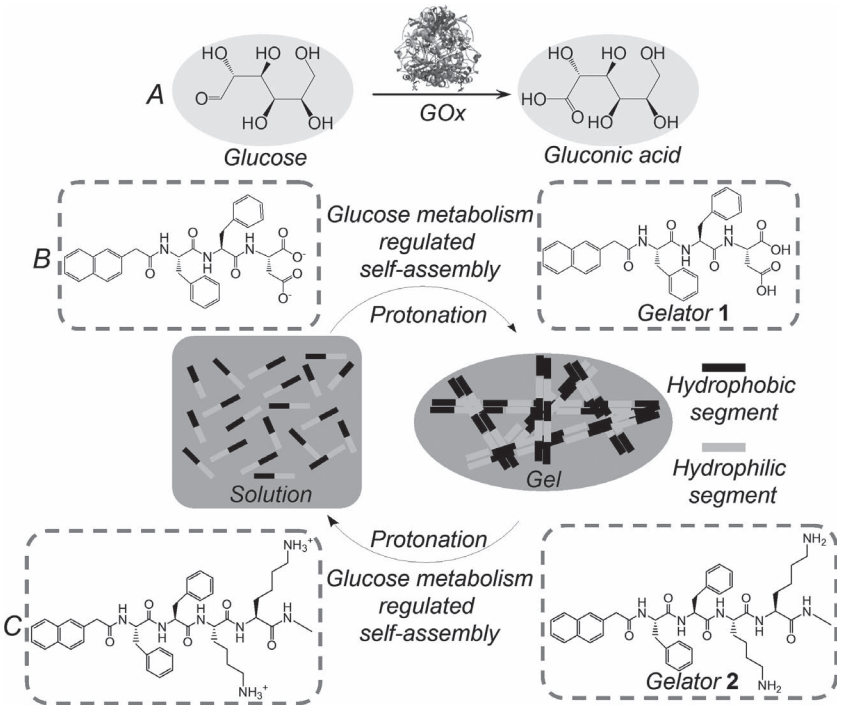

Scheme 1. The schematic illustration of the constructed biological glucose metabolism (a) and the biological glucose-metabolism regulated self-assembly of gelators **1** (b) and **2** (c).

the peptide-based self-assembly. GOx, as one of the most widely used biorecognition elements, can specifically oxidize glucose into gluconic acid during the glucose metabolism of some bacteria such as *Pseudomonas aeruginosa*[31] and *Aspergillus niger*.[32] As shown in Scheme 1 a, gluconic acid can be generated from GOx-mediated glucose metabolism. Because of the presence of carboxylic acid or amine functional groups, the generated gluconic acid is capable of triggering the protonation of the peptide building blocks and thus regulating their self-assembly (sol–gel and gel–sol transitions, Scheme 1 b and c). Because this glucose metabolism regulated self-assembly is based on the oxidation of glucose, it can be used a simple visual biosensor for glucose detection.

2. Experimental Section

2.1. Materials

N-Fluorenyl-9-methoxycarbonyl (FMOC)-protected amino acids [FMOC-Phe-OH, FMOC-Asp(OtBu), FMOC-Lys(Boc)-OH], 2-chlorotrityl chloride resin (100–200 mesh, loading: 1.32 mmol g^{-1}), *N*-hydroxybenzotriazole (HOBt), benzotriazole-*N,N,N′,N′*-tetramethyluroniumhexafluorophosphate (HBTU), and piperidine were purchased from GL Biochem Ltd. (China) and used as received. GOx (100–250 U mg^{-1}) was provided by Sigma–Aldrich and used directly. 2,2,2-Trifluoroethanol, trifluoroacetic acid (TFA), dichloromethane (DCM), *N,N′*-dicyclohexylcarbodimide (DCC), *N*-hydroxysuccinimide (NHS), acetic acid, methylamine aqueous solution (25–30 wt%), and glucose were provided by Shanghai Reagent Chemical Co. (China) and used as received. Dimethylformamide (DMF), diisopropylethylamine (DiEA), and tetrahydrofuran (THF) were obtained from Shanghai Reagent Chemical Co. and distilled prior to use. All other reagents and solvents were of analytical grade and used directly.

2.2. Synthesis of Gelator 1

The peptide building block of gelator **1** was synthesized manually in 1.98 mmol scale on the 2-chlorotrityl chloride resin employing a standard FMOC solid phase peptide synthesis (SPPS) technique. Before the reaction, the resin was washed with DCM (three times) and DMF (three times) and then immersed in DMF for 30 min. After draining off DMF solution, a DMF solution of the mixture of FMOC-Asp(OtBu)-OH (4 equiv relative to resin loading) and DiEA (6 equiv) was added to the resin and shaken for 2 h at room temperature. After removing the reaction solution, the resin was washed with DMF (three times). Subsequently, 20% piperidine/DMF (V/V) solution was introduced to the resin to remove the FMOC protected groups. After shaking for 30 min at room temperature, the reaction solution was drained off and the resin was washed with DMF (three times). The presence of free amino groups was indicated by blue color in the Kaiser test. Thereafter, a DMF solution of the mixture of FMOC-Phe-OH (4 equiv), HBTU (4 equiv), HOBt (4 equiv), and DiEA (6 equiv) was added. After shaking at room temperature for 1.5 h, the reaction solution was drained off and the resin was washed with DMF (three times). The absence of free amino groups was indicated by yellow color in the Kaiser test. After the repetition of the deprotection and acylation reaction, a DMF solution of the mixture of naphthyl acetic acid (4 equiv), HBTU (4 equiv), HOBt (4 equiv), and DiEA (6 equiv) was added. After shaking at room temperature for 1.5 h, the resin was washed with DMF (three times) and DCM (three times). Cleavage of the peptide from the resin was performed using a mixture of TFA and DCM in the ratio of 5:5. After shaking at room temperature for 2 h, the cleavage mixture and DCM washing were collected. The combined solution was concentrated to a viscous solution by rotary evaporation. Cold ether was then added to precipitate the product. After washing with cold ether (five times), the crude product (Nap-Phe-Phe-Asp-OH) was obtained after drying the precipitation under vacuum for 24 h. The purification of the crude product was performed by high-performance liquid chromatography (HPLC) with a C18 column and using a linear gradient of acetonitrile and deionized water containing 0.1% NH_4OH. ESI–MS (see Figure S1, Supporting Information): 594.4 $(M-H)^-$.

2.3. Synthesis of Gelator 2

Similarly as the synthesis of gelator **1**, the precursor of gelator **2** [Nap-Phe-Phe-Lys(Boc)-Lys(Boc)-OH] was also synthesized manually in 1.98 mmol scale on the 2-chlorotrityl chloride resin employing a standard FMOC SPPS technique. Cleavage of the precursor from the resin was performed using a mixture of 2,2,2-trifluoroethanol, acetic acid, and DCM in the ratio of 2:1:7. After shaking at room temperature for 2 h, the cleavage mixture and DCM washing were collected. The combined solution was concentrated to a viscous solution by rotary evaporation. Cold ether was then added to precipitate the product. After washing with cold ether (five times), the precursor of gelator **2** was collected after drying the precipitation under vacuum for 24 h.

To synthesize gelator **2**, the precursor (0.468 g, 0.5 mmol), DCC (0.124 g, 0.6 mmol), and NHS (0.069 g, 0.6 mmol) were dissolved in 10 mL dried THF and stirred under ice-bath for 4 h. Thereafter, the precipitation was removed and 3 mL methylamine aqueous solution was added dropwise. After stirring for 24 h at room temperature, the solution was concentrated and cold ether was added to precipitate the product [Nap-Phe-Phe-Lys(Boc)-Lys(Boc)-$CONHCH_3$]. The dried precipitation was subsequently dissolved in a 10 mL mixture of DCM and TFA in the ratio of 2:8. After stirring for 2 h, cold ether was added to precipitate gelator **2** (Nap-Phe-Phe-Lys-Lys-$CONHCH_3$). The crude gelator **2** was purified by HPLC with a C18 column and using a linear gradient of acetonitrile and deionized water containing 0.1% TFA. ESI–MS (see Figure S2, Supporting Information): 750.5 $(M + H)^+$.

2.4. Glucose Metabolism Regulated Self-Assembly

The solution of gelator **1** (2.5 mg mL^{-1}, 4.2×10^{-3} M, pH 10) and the hydrogel of gelator **2** (5 mg mL^{-1}, 6.67×10^{-3} M, pH 10) were prepared. Subsequently, 1 mg GOx and different amounts of glucose were, respectively, added to the solution of gelator **1** and hydrogel of gelator **2** to construct the biological glucose metabolism system. The glucose metabolism regulated self-assembly of the peptide-building blocks was evaluated based on the phase transition of the gelators. The phase transition time of gelator **1** was measured as the time needed to form a stable hydrogel from a solution. With respect to gelator **2**, the phase transition time was determined as the time needed to transform from the hydrogel into a clear solution.

2.5. Characterizations of the Self-Assembly

The morphology of the self-assembled gelators was examined using Tecnai G20 S-TWIN transmission electron microscopy (TEM) without staining. Before the TEM measurements, a small volume of the aqueous solution of the self-assembled gelators was applied to a copper grid with Formvar film and dried before the observations. To obtain the circular dichroism (CD) information of the self-assembled gelators, the aqueous solution of the self-assembled gelators was fixed in a 0.5 mm quartz cell and analyzed on a Jasco J-810 spectropolarimeter (Japan) with 4 s accumulations every 1 nm and averaged over three acquisitions. Fourier transform infrared spectroscopy (FTIR) spectra of the self-assembled gelators were collected on a Perkin-Elmer spectrophotometer by pressing the lyophilized samples into KBr pellets. Fluorescence emission spectra of the self-assembled gelators were recorded on a LS55 luminescence spectrometry (Perkin-Elmer) with excitation at 272 nm and emission data range between 280 and 500 nm.

3. Results and Discussion

3.1. Glucose Metabolism Regulated Self-Assembly

The peptide building block (gelator **1**) was synthesized by SPPS technique and its molecular structure is illustrated in Scheme 1 b. Gelator **1** is consisted of an aspartic acid residue and a naphthalene–diphenylalanine (Nap–FF) segment that is prone to self-assembly.[33] The pK_a of gelator **1** was determined as ≈5.8 (see Figure S3a, Supporting Information) via classic acid–base titration. Prior to the self-assembly, gelator **1** was first suspended in distilled water and then dissolved at a pH of 10 by adding a small amount of concentrated NaOH (6 M) to form a homogeneous solution (2.5 mg mL^{-1}, 4.2×10^{-3} M). After the addition of GOx (1 mg, 100–250 U mg^{-1}) and glucose (10 mg, 0.056 mmol) to construct a biological glucose metabolism system, a self-assembled hydrogel was obtained ≈40 min later (Figure 1b) and pH of the self-assembling system declined to ≈4.2. The viscoelastic properties (see Figure S4, Supporting Information) indicate that the storage modulus (G′) is higher than loss modulus (G″), implying a gel characteristic rheological behavior of the solid-like materials. The interior morphology of the self-assembled hydrogel was examined by

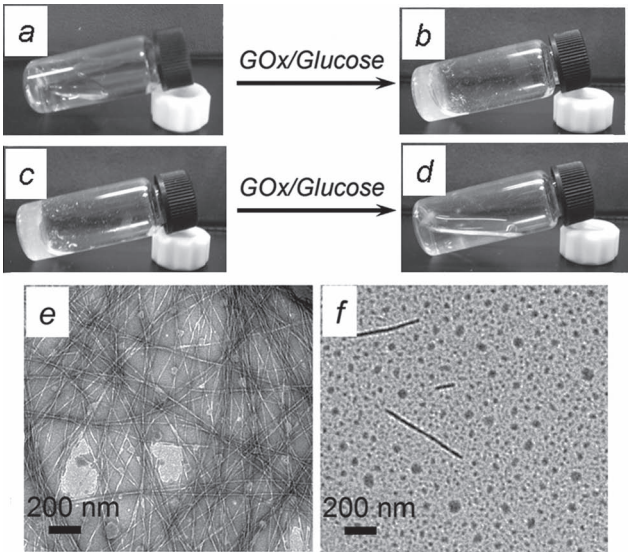

Figure 1. Glucose metabolism regulating the self-assembly of gelator **1** from aqueous solution (a) to hydrogel (b); glucose metabolism regulating the self-assembly of gelator **2** from hydrogel (c) to aqueous solution (d); TEM images of the hydrogel of gelator **1** (e) and aqueous solution of gelator **2** (f) resulted from glucose metabolism regulated the self-assembly.

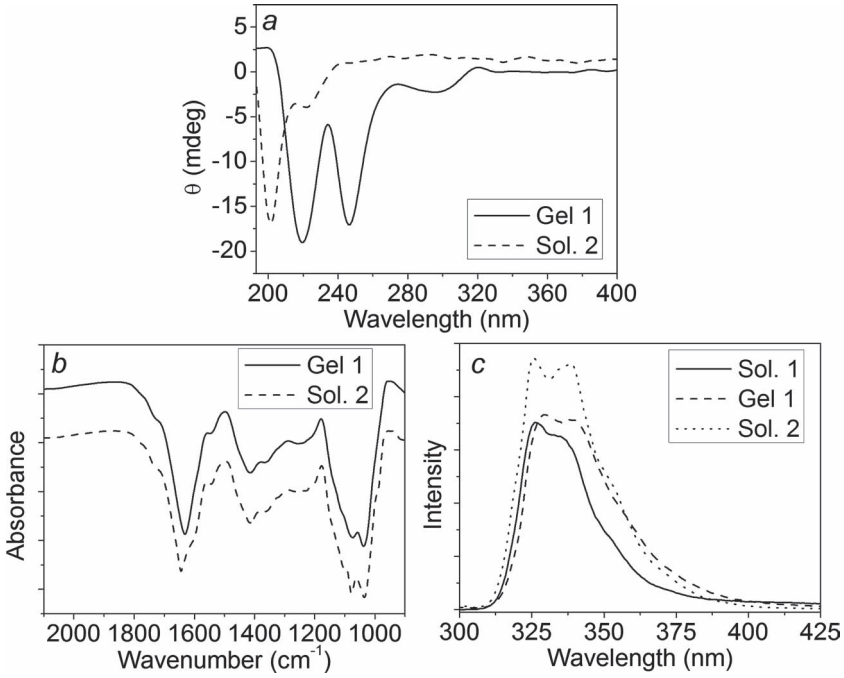

Figure 2. CD (a), FTIR (b), and emission spectra (c) of the self-assembled hydrogel of gelator **1** (Gel 1) and aqueous solution of gelator **2** (Sol. 2) resulted from glucose metabolism regulated self-assembly.

TEM. As shown in Figure 1e, the self-assembly of gelator **1** results in the formation of uniform nanofibers with an average diameter of ≈30 nm. And these nanofibers are entangled with each other to form packed 3D fiber network of the hydrogel of gelator **1**.

The self-assembly of gelator **1** was characterized by CD, FTIR, and fluorescence spectroscopy. As presented in Figure 2a, the negative band at ≈219 nm is a typical CD signal of polypeptide with a β-sheet conformation,[19–22] indicating that the peptide backbone of gelator **1** forms β-sheet-like superstructure in the nanofibers. The negative broad bands ranging from 240 to 320 nm can also be observed, which mainly correspond to π–π* transition of terminal Nap tails.[33] From the FTIR spectrum in Figure 2b, the absorbance of amide I appears at ≈1630 cm^{-1}, implying that β-sheet-like superstructure is formed in the nanofibers.[20,22] The emission spectrum of the self-assembled gelator **1** provides the detailed environment of Nap tails in the nanofibers. As exhibited in Figure 2c, the solution of gelator **1** has the reported two asymmetric emission peaks at ≈326 and ≈336 nm.[33] Whereas, these two asymmetric emission peaks respectively shift to ≈330 and ≈340 nm after the self-assembly. This red shift of ≈4 nm suggests the presence of π-stacking of Nap tails in the nanofibers.[33] Combining the information from CD, FTIR, and emission spectra, the self-assembly of gelator **1** is mainly built on the β-sheet-like arrangement of the peptide backbones via intermolecular hydrogen bonding interaction and π-stacking of the Nap tails. Here, on the basis of the result of pK_a titration, the deprontonated gelator **1** cannot self-assemble in distilled water at a pH of 10 because of the presence of intermolecular electrostatic repulsion. Therefore, the occurrence of self-assembly of gelator **1** and pH decline of the self-assembling system are mainly attributed to the introduction of glucose metabolism system. That is, gluconic acid generated from GOx-mediated glucose metabolism is capable of triggering the protonation of gelator **1** and thus directs its self-assembly.

To further confirm the proposed self-assembly mechanism described above, another building block (gelator **2**, Scheme 1 c) comprised Nap–FF segment and two lysine residues was synthesized and its self-assembly in response to glucose metabolism was investigated. Because of the presence of two lysine residues, gelator **2** should theoretically self-assemble under basic condition. In this study, to prevent the influence of the terminal carboxylic acid group on the self-assembly of gelator **2**, it was end capped by using methylamine. On the basis of our experiment, gelator **2** can be dissolved in distilled water due to the protonation of lysine residues (pK_a ≈9.4, see Figure S3b in Supporting Information). And a self-assembled hydrogel can form at a pH of 10 if the solution concentration is higher than 5 mg mL^{-1} (6.67×10^{-3} M) (Figure 3a). From the TEM image in Figure 3a, the self-assembly of gelator **2** results in the formation of nanofibers with an average diameter of ≈30 nm. The negative band at ≈219 nm in the CD spectrum (Figure 3b), absorbance of amide I located at ≈1630 cm^{-1} in the FTIR spectrum (Figure 3c), and red shift of ≈5 nm in the emission spectrum (Figure 3d) indicate that the self-assembly of gelator **2** is mainly attributed to the hydrogen bonding interaction of the peptide backbones and π-stacking of Nap tails.[20,22,33] As shown in Figure 1d, after the addition of GOx (1 mg) and glucose (0.056 mmol) to the hydrogel of gelator **2**, a clear solution can be obtained ≈90 min later and pH of the self-assembling system decreases to ≈5.5, implying that gluconic acid generated from glucose metabolism leads to the protonation of gelator **2** and thus disturbs the corresponding self-assembled hydrogel network. From the TEM image in Figure 1f, only few of residual nanofibers are observed and the glucose metabolism leads to most of nanofibers changing into amorphous aggregates. Noting that, the residual nanofibers suggest that the self-assembly of gelator **2** is not completely

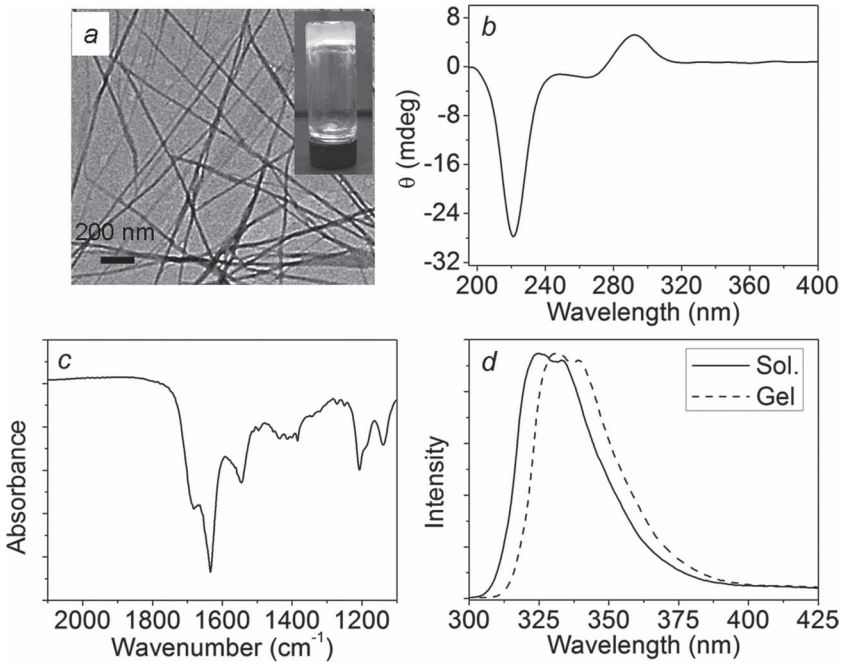

Figure 3. Optical and TEM images (a), CD (b), FTIR (c), and fluorescent emission spectra (d) of the self-assembled hydrogel of gelator **2**.

disturbed even though the appearance of gel–sol transition. The results of CD (Figure 2a) and FTIR (Figure 2b) are consistent with that of TEM measurement. The negative band at ≈200 nm is a typical CD signal of random coil conformation of peptide backbone.[19] In addition, the absorbance of amide I located at ≈1643 cm^{-1} also corresponds to the random coil conformation of the peptide backbone of gelator **2**.[34] Moreover, the typical absorbance located at ≈1720 cm^{-1} belonging to the carboxylic acid group demonstrates the presence of gluconic acid. From the emission spectrum in Figure 2c, the asymmetric emission peaks at ≈326 and ≈336 nm correspond to the monomeric Nap moieties. Similar to gelator **1**, all these results suggest that the gluconic acid produced in the metabolic pathway of glucose could regulate the self-assembly of gelator **2**. Noting that, although the GOx-mediated glucose metabolism constructed here is different from the reported biological glucose metabolism presented in most organisms,[30] the intrinsic essence is similar because their metabolic products are acidic intermediates (the metabolic product of glucose in most organisms is pyruvic acid).[30] Therefore, the peptide building blocks reported here would still present the stimuli-responsive self-assembly behaviors upon the common biological glucose metabolism in organisms.

3.2. Stimuli-Responsive Self-Assembly for Glucose Detection

In this study, as the GOx-mediated glucose metabolism regulated self-assembly of the peptide building blocks is based on the oxidation of glucose, it is expected to be used as a simple visual biosensor for glucose detection. As shown in Figure 4a, in the presence of GOx with a fixed amount of 1 mg, increasing the amount of added glucose results in the accelerated phase transition rate of gelators **1** (sol–gel) and **2** (gel–sol). For example, with the addition of 0.167 mmol glucose, gelator **1** can form stable hydrogel ≈32 min later while the hydrogel of gelator **2** can transform into clear solution ≈65 min later. Increasing the amount of glucose to 0.444 mmol induces fast phase transitions of gelators **1** and **2** with transition times of ≈15 and ≈30 min, respectively. This difference in phase transition rate between gelators **1** and **2** is attributed to their different molecular states. In comparison with the self-assembled gelator **2** in the hydrogel state, gelator **1** in solution could be easily accessed by gluconic acid, which results in a relatively rapid phase transition rate. To demonstrate the specification of glucose in the self-assembly, we also used fructose (the isomer of glucose) to replace glucose and investigated the influence of the added fructose on the self-assembly of the peptide building blocks. As exhibited in Figure 4b and c, with the addition of GOx (1 mg) and fructose (0.167 mmol), either the aqueous solution of gelator

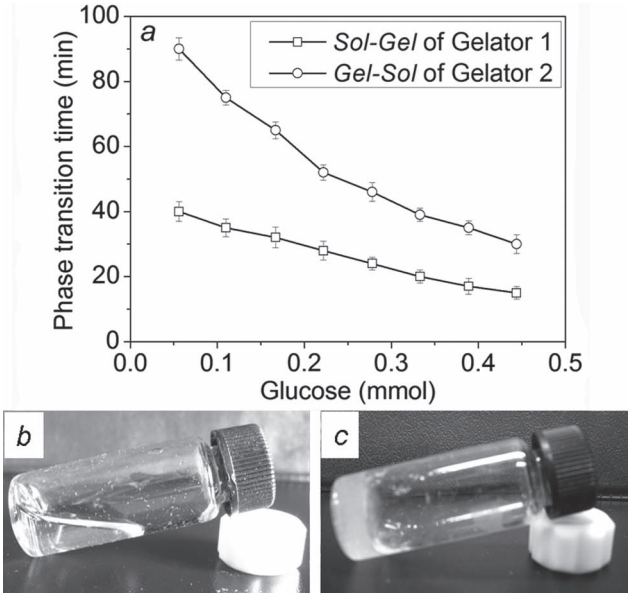

Figure 4. Dependence of the self-assembly of gelators **1** and **2** on glucose concentration (a), photo images of the solution of gelator **1** (b), and hydrogel of gelator **2** (c) with the addition of GOx and fructose.

1 (Figure 4b) or the hydrogel of gelator **2** (Figure 4c) does not present stimuli-responsive phase transition within 24 h. This tendency is easily understood if the specificity of enzyme was taken into account, indicating that the glucose metabolism regulated self-assembly demonstrated here can be specifically used for glucose detection.

4. Conclusions

We have reported a new type of peptide-based self-assembly in response to glucose metabolism. In this self-assembling system, gluconic acid generated from GOx-mediated glucose metabolism can trigger the protonation of the peptide building blocks and thus regulate their self-assembly. Because this glucose metabolism regulated self-assembly is based on the oxidation of glucose, it can be used as a simple visual biosensor for glucose detection.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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