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Supramolecular Hydrogels Based on the Epitope of Potassium Ion Channels

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Abstract

Imparting aromatic-aromatic interaction to potassium binding epitope affords a supramolecular hydrogelator that response to K⁺ concentration by self-assembly into nanofibers of different widths and crosslinking pattern, which illustrates a simple approach to generate biomimic materials based on tunable, hierarchical self-assembly of small molecules.

This paper reports a novel supramolecular hydrogelator, which consists of fluorene and the pentapeptide epitope (TIGYG) of potassium ion (K⁺) channels,¹ to self-assemble in water to form the tunable, hierarchical nanostructures dictated by the concentration of K⁺ ([K⁺]). Supramolecular hydrogels² rely on non-covalent interactions to drive the self-assembly of small molecules in water for forming molecular nanofibers and encapsulating water. As a new type of soft materials,³ supramolecular hydrogels have attracted considerable research attentions because of their promising applications in biomedicine. Although hydrophobic interactions originated from alkyl chains or aromatic rings are essential non-covalent interactions for the formation of ordered nanostructures in water, which afford supramolecular hydrogels, other additional non-covalent interactions also are useful for triggering the self-assembly or enhancing the nanostructures formed by self-assembly. For example, the electrostatic interaction between calcium cations and carboxylate anions renders the simple mixture of cell suspensions and peptide amphiphile molecules into a hydrogel to guide the differentiation of neuron progenitor cells,⁴ provides a convenient method to tailor the mechanical properties of hydrogels,⁵ or offers ionic bridging for significantly affecting the self-assembly of the peptide building blocks.⁶ Zinc ions-triggered folding of β -hairpin results result in the formation of molecular nanofibers and hydrogels.⁷ More interestingly, the binding of G-quartets (G₄) with a cation (e.g. Na⁺ or K⁺) has resulted constitutional dynamic hydrogels that permit multilevel self-assembly processes for phase-organization and phase-transition events.⁸ These seminal works have encouraged us to evaluate the use of selective interactions of K⁺ and small peptides for constructing supramolecular hydrogels.

Although there are scarce reports on the use of K⁺ and small peptides for producing organogels⁹ or hydrogels,¹⁰ the interaction between K⁺ and the peptides is still electrostatic (i.e., attraction between cations and anions), which inherently lacks of selectivity. The selectivity exhibited by G₄ towards K⁺ suggests that it is easier to achieve selectivity toward alkaline metal ions by using a neutral ligand for binding the cations.¹¹ Based on this principle, we choose to learn from nature and use the K⁺-binding epitope of the K⁺

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channels¹ as the building block for the hydrogelator and evaluate the specificity of the hydrogelator towards K⁺. We find that the attachment of a fluorene group to the pentapeptide epitope of potassium ion channels affords a supramolecular hydrogelator that self-assembles into nanofibers whose widths and crosslinking depend on [K⁺] (Scheme 1), but not the concentration of Na⁺ ([Na⁺]), which suggests the selective binding of the nanofibers towards K⁺. As the first example of the generation of a hydrogel by mimicking the functions of sophisticated membrane proteins, this work opens a new venue to develop functional materials that exhibit specific responses to ions of biological importance.

Fig. 1A and B show the key structural feature of the K⁺ channel,¹ which serves as the base of the molecular design of the hydrogelator. In a typical potassium channel, four parallel K⁺ binding epitopes (TIGYGs) form a K⁺ filter that allows the selective in-flow of K⁺ ions.¹ The electro-negative and cation-attractive carbonyl oxygen atoms of the epitopes align towards the axis of the filter that favours the entry of K⁺ and disfavours the entry of Na⁺. This feature suggests that the arrangement of multiple TIGYGs in bundles may offer the selective binding to K⁺. Therefore, we decided to use aromatic-aromatic interaction from fluorene for promoting the self-assembly of TIGYG. There are several additional advantages for this choice. (i) Aromatic-aromatic interactions can effectively enable the self-assembly of pentapeptides to form nanofibers¹² so that epitopes can arrange in a close proximity. (ii) Aromatic-aromatic interaction is orthogonal to the attraction of carbonyl oxygen to K⁺. (iii) As part of protecting group for amino acids, it is easy to add fluorene to TIGYG. Thus, we connected fluorenylmethoxycarbonyl (Fmoc) group¹³ at the N-terminal of TIGYG to generate molecule **1** (Fig. 1 C and D) via standard solid phase peptide synthesis and obtained **1** in a yield about 90%.

To test the minimum gelation concentration (mgc) of **1**, we evaluate the hydrogelation of **1** at different concentration of **1**, but at the constant ratio of [K⁺]/[**1**] = 2.33 because the adjustment of the pH (by adding 1.0 N KOH) inevitably introduces K⁺. To produce the hydrogel, we first adjust pH of the solutions of **1** to 8.0, then increase the temperature of the solutions to 50 °C before adding in 1.0 N HCl to lower the pH to 4.0. We observe the formation of the hydrogels after placing the solutions of **1** at room temperature for overnight and determine that the mgc of **1** is at 0.10 wt% (1.37 mM). Below this value, **1** in water only results in a viscous solution (Fig. S1).

Since the event of hydrogelation itself can serve as a simple visual assay for ligand-receptor interactions,¹⁴ we use the formation of the hydrogels to evaluate the interaction between K⁺ or Na⁺ and hydrogelator **1** by mixing the solution of KCl or NaCl at different molar ratio to **1** when the concentration of **1** is at 0.05 wt% (below the mgc). If there is a sufficient interaction between K⁺ or Na⁺ and TIGYG, it will increase the crosslinking of the nanofibers and result in hydrogelation. As shown in Fig. 2, 0.05 wt% of **1** remains as a solution regardless of the increase of [Na⁺] from [Na⁺]/[**1**] = 2.33 up to [Na⁺]/[**1**] = 82.33, indicating that Na⁺ unlikely binds strongly with **1**. Contrary to the case of Na⁺, the increases of [K⁺]/[**1**] from 2.33 to 12.33 triggers hydrogelation of the solution of **1** at 0.05 wt%, suggesting that K⁺ likely binds to **1** to promote the crosslinking of the nanofibers of **1** to form a sufficient network for the hydrogelation. When [K⁺]/[**1**] reaches 22.33, the hydrogel becomes softer. Upon the further increase of [K⁺] to [K⁺]/[**1**] = 42.33 and 82.33, the hydrogels appear too soft to maintain a perfect self-support. Plotting the density of the gels or the solution versus [M⁺]/[**1**] (Fig. S2), we find that the difference in weight of the solutions or hydrogels of **1** with the increase of the amount of K⁺ or Na⁺ is negligible, suggesting that factors other than weight must affect the mechanical strength of the K⁺ containing hydrogels.

To explain the influence of K^+ on **1**, we used TEM to reveal the nanoscale structures of the hydrogels or viscous solutions of **1** at 0.05 wt% of **1** and at different $[K^+]$ or $[Na^+]$. As shown in Fig. 3A–D, although long extended nanofibers are the major feature of both the solution and hydrogels of **1** at 0.05 wt% and pH 4.0, the increase of $[K^+]$ clearly changes both the width and the aggregation of the nanofibers. At $[K^+]/[1] = 2.33$, the nanofibers have a width at 11.3 ± 1.2 nm and exhibit limited crosslinking. The increase of $[K^+]/[1]$ to 12.33 changes little on the width of nanofibers (11.4 ± 0.6 nm) but dramatically increases the crosslinking between nanofibers. The interaction between TIGYG epitopes and K^+ likely causes the increase of crosslinking. As the nanofiber comprises many monomers of **1**, significant numbers of TIGYG epitopes likely present on the surfaces of the nanofibers. Among their possible conformations, some of the epitopes may adopt the conformation of TIGYG in the potassium filter, thus binds to K^+ . Although the binding to K^+ can also happen within the nanofiber, the binding of K^+ with multiple epitopes (mimicking the nature structure) on the surface is more accessible and naturally favoured. Thus, K^+ should be able to bind with TIGYGs from different nanofibers, serving as the potassium bridges which, when concentration is high enough (e.g., $[K^+]/[1] = 12.33$), are capable to “stick” the nanofibers together and dynamically crosslink the nanofibers. Multiple crosslinking leads to a stronger network and consequently enables formation of hydrogel. At $[K^+]/[1] = 22.33$, bundles of parallel aligned nanofibers that have the width of 9.9 ± 1.2 nm become the dominate feature. The reduction in width of the nanofibers produces more surface area for the protruding TIGYG to bind with K^+ . The alignment of nanofibers leads to less capacity to withhold water and the decrease of the width of the nanofibers weakens the strength of the network, the two phenomenon add up to give a hydrogel that is softer than that at $[K^+]/[1] = 12.33$. Further increase $[K^+]/[1]$ to 42.33 and 82.33, the width of nanofibers continue to decrease to 6.3 ± 0.7 and 7.2 ± 0.5 nm, respectively (Fig. S3) thus generating more surface area. As thinner nanofibers have weaker mechanical strength, the hydrogels become softer at high $[K^+]$. On the other hand, the width of the nanofibers and the aggregation of the nanofibers exhibit little difference at low or high $[Na^+]$ (widths are 8.3 ± 0.6 and 8.0 ± 1.0 nm at $[Na^+]/[1] = 2.33$ and 82.33, respectively), suggesting that there is little interaction between **1** and Na^+ .

Fig. 4 shows the storage moduli of the hydrogels or viscous solutions formed by **1** (0.05 wt %, 0.68 mM) with K^+ or Na^+ , indicating that the change of G' with $[K^+]$ agrees with the morphological change. The values of G' of the hydrogels with different $[K^+]/[1]$ peaks at $[K^+]/[1] = 12.33$. When the $[K^+]/[1]$ is above 12.33, G' gradually decreases with increase of $[K^+]$, agreeing the appearance of softer hydrogels. In the control test, in which **1** exhibits little interaction with Na^+ , the values of G' only show a trend of slight decline, likely resulted from salting-in effect¹⁶ that weakens mechanical strength of the system. These rheological behaviours agree with that **1** selectively binds K^+ .

In conclusion, we designed and synthesized the first example of hydrogelator with functional epitope (TIGYG) from a natural ion channel protein. Gelation of the hydrogelator **1** occurs exclusively at specific ratio of $[K^+]/[1]$, proving the conservation of inherent ability of the epitopes to bind with K^+ . In a control experiment to demonstrate the importance of structure of epitope to K^+ binding, we change the structure of **1** by alternation of the pentapeptide sequence into TGGIY (**2**) which losses the hydrogelation ability upon the addition of either K^+ or Na^+ at 0.05 wt% of **2** (Fig. S6). These results imply that self-assembly effectively generates repeats of epitopes for interacting with specific targets in the form of soft nanomaterials when multiple epitopes are necessary for the specific interaction with the target(s).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. (A) Front and (B) side view of the crystal structure of the epitopes (TIGYG) interact with K⁺ in the K⁺ channel.¹ Purple sphere is the K⁺, red is O, and gray is C, and the tubes represent the backbone of TIGYG. (C) The chemical structure of **1** and (D) molecular model of **1** in which the TIGYG adopts the conformation in potassium ion channel.

Fig. 2. Optical images of **1** (0.05 wt%, 0.68 mM) with different concentrations of K⁺ or Na⁺ at pH 4.0.

Fig. 3. TEM images obtained by negative staining¹⁵ the hydrogels or viscous solutions formed by **1** (0.05 wt%, 0.68 mM) with K⁺ or Na⁺. Molar ratio [K⁺]/[**1**] at (A) 2.33, (B) 12.33, (C) 22.33 and (D) 82.33 and molar ratio [Na⁺]/[**1**] at (E) 2.33 and (F) 82.33. Insert is enlarged image of aligned fiber bundle. Scale bar = 20 nm.

Fig. 4.

The values of the storage modulus (G') in frequency sweep oscillation at frequency = 0.1 rad/s of the hydrogels or solutions formed by **1** (0.05 wt%, 0.68 mM) with different concentrations of Na^+ or K^+ .

Scheme 1.

A scheme illustrates the morphology change of the nanofiber network in the hydrogel of **1** changing with the increase of $[K^+]$.