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Metalloprotein-Inspired Thermo-Gene for Thermogels

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Physical hydrogels and many chemical hydrogels usually melt at temperatures higher than 40~50°C, we show that by introducing a coordinating hydrogel as the thermo-gene, heat-melting hydrogels can be transformed into thermogels that remain stable at temperatures over 80°C. Devices made with this gel work stably at the high temperature of 80°C. In combination with previous knowledge of anti-freezing hydrogel, the current work allows to remove the bottle-neck that limits the practical application of hydrogel-based flexible devices under different weather conditions.

Introduction

Hydrogels composed of fixed water in the physically cross-linked networks of self-assembled fibers or polymer chains, namely physical hydrogels, have emerged as powerful materials in a wide range of fields, such as biology1-3, pharmacy4-5 and material science6-8. Recent studies revealed that physical hydrogels are suitable materials for various flexible devices including wearable sensors9-12, stretchable displays13-14, and flexible supercapacitors15-17 owing to their good flexibility, stretchability and conductivity. Unfortunately, the devices made of physical hydrogels can work only in a very narrow temperature range because most hydrogels will freeze as the temperature falls below 0°C, and melt into viscous solution at above gel-to-sol temperature (Tgel) around 40~50°C18. These situations cause fragile or collapse of the physical networks, losing their original flexibility and mechanical strength. Therefore, it is very urgent to improve both the cold and heat resistance of physical hydrogels for practical application.

Recently, scientists have developed a number of anti-freezing hydrogels upon using binary solvent system (ethylene glycol – water or glycerol – water19-20) or inorganic salt21, which are inspired from the anti-freezing mechanism of peeper frogs and rainbow fish in extremely cold weather. However, stable physical hydrogels that can tolerate high temperatures, which we named thermogels (TG), still remain insurmountable. Actually, in nature there are organisms capable of tolerating extremely hot conditions by evolving desired substances in their body. For example, thermophile and heat-loving microbes employ metalloproteins to resist high temperature22-24. When most proteins denatured at temperatures beyond 40~50°C, the structure of metalloproteins remains stable. Recent study shows that coordination mediated cutinase can even catalyze the hydrolysis of plastics at the high temperature of 72 ° C25. This inspires that coordination interaction may also be employed in physical hydrogels and other heat-melting gels in a planned way to endow the gel with high temperature resistance. Some metallo-hydrogels in literature indeed show excellent thermo-resistance26-28. However, so far the role of metal coordination on the thermal stability of hydrogels hasn’t been systematically studied.

Herein we report a metalloprotein-inspired design of TG that would endow thermal stability to heat-melting hydrogels. We found that upon introducing a metallo-hydrogel into any conventional hydrogels, the resultant hydrogel would inherit the thermo-resistance of the metallo-hydrogels. In this sense, the metallo-hydrogel behaves like a ‘gene’ that expresses itself in other gels, and we named it as ‘thermo-gene’. The thermo-gene is the coordinating hydrogel formed with folate and zinc ions(Scheme 1a). Previous work verified that the coordination interaction may also be employed in physical hydrogels and other heat-melting gels in a planned way to endow the gel with high temperature resistance. Some metallo-hydrogels in literature indeed show excellent thermo-resistance26-28. However, so far the role of metal coordination on the thermal stability of hydrogels hasn’t been systematically studied.

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In combination with the anti-freezing technique developed in previous study19-21, the working temperature window of the DNTG can be extended from sub-zero degrees to around 90°C. As such, we expect this metalloprotein-inspired thermo-gene
would solve the poor heat resistance problem in the development of hydrogel-based flexible devices, paving ways for their practical application at both the extremely cold and extremely hot weathers.

![Diagram](Image)

Scheme 1. Illustration of the folate-Zn\(^{2+}\) hydrogel thermo-gene (a) and the formation of thermogel by introducing the thermo-gene into a heat-melting gel (b).

Results and discussion

Figure 1a shows the temperature scanning rheology measurement for the thermo-gene of folate-Zn\(^{2+}\) hydrogel. Characteristic gelling behavior was observed in the wide temperature range of 30°C~80°C. Higher temperature is not adopted to avoid drying of the gels in the process of measurements. At all the tested temperatures, the shear modulus does not drop. Instead, even a slight increase of the shear modulus was observed in the temperature range of 30°C~50°C, indicating the physical network is even strengthened. This strengthened state is maintained at higher temperatures, which is evidenced by the constant shear modulus in the temperature range of 30°C~80°C. In fact, the gel still remains solid-like at 90°C (Inset in Figure 1a). Detailed rheological measurements suggest that the storage modulus (elastic modulus, G') is 1 order higher than the loss modulus (viscous modulus, G'') at all the tested temperatures (Figure S1), confirming that the network in the hydrogel keeps stable. In line with the rheological results, the circular dichroism (CD) spectra of the hydrogel were not affected by the variation of the temperature, too (Figure S2), indicating that the chiral stacking of petrin tetramer remains unchanged. However, SAXS measurements reveal that the distance between the folate tetramers has increased from 3.18 Å to 4.08 Å as the temperature increases from 30°C to 70°C, which is evidenced by the shift of the scattering peak from q = 1.97 Å\(^{-1}\) to 1.54 Å\(^{-1}\) (Figure 1b). On the meanwhile, the \(^1\)HNMR peaks are broadened at all the tested temperatures, indicative of the presence of effective π-π stacking at both low and high temperatures. However, the chemical shifts of the protons on the petrin ring in the \(^1\)HNMR spectra move toward lower field (Figure 1c), which means the electron density on the petrin ring is reduced. In combination with the SAXS and HNMR measurements, it is obvious that the π-π interaction has been weakened by elevating temperature. It is then clear that the coordination interaction has played a crucial role in preserving the physical network in the hydrogel, just as metalloprotein in thermophiles.

To further confirm the role of coordination in endowing thermal stability to the folate-Zn\(^{2+}\) hydrogel, variation of the extent of coordination was conducted in the gel system. Figure 1d shows that as the molar ratio of folate: Zn\(^{2+}\) varies from 1:1.4 to 1:1.9, all the resulting hydrogels display thermal resistance. Increased fraction of Zn\(^{2+}\) leads to higher shear modulus, verifying that the increased extent of coordination linkages would strengthen the networks in the hydrogel. It is noteworthy that the shear modulus in all the tested hydrogels increases in the temperature range of 30-50°C, then levels off at higher temperatures. According to the analysis in previous text, this temperature-triggered increase of shear modulus indicates that the coordination interaction is promoted upon increasing temperature. Indeed, TEM observation reveals that the diameter of the nanofibers at 80°C is larger than that at room temperature (Figure S3).

![Figure 1](Image)

Figure 1. (a) Rheological measurements of folate-Zn\(^{2+}\) hydrogel in the temperature range of 30°C~80°C. Higher temperature is not adopted to avoid drying of the gels in the process of measurements. The inset is the photo of the gel at 90°C. (b) SAXS spectra of the folate-Zn\(^{2+}\) hydrogel upon rising temperature. The numbers in legend are temperatures in °C. The arrow is to guide the direction of the peak shifting. (c) \(^1\)HNMR spectra of folate-Zn\(^{2+}\) hydrogel upon rising temperature. Sample conditions in SAXS and \(^1\)HNMR: [folate] = 15 mM, [folate]: [Zn\(^{2+}\)] =1:1.7. (d) Temperature dependent rheological measurements of folate-Zn\(^{2+}\) hydrogels at a given [folate] of 15 mM but varied [folate]: [Zn\(^{2+}\)] molar ratios.

Since the coordination interaction between folate and Zn\(^{2+}\) is very strong, it would not be interfered by non-coordinating component. We therefore expect that the folate-Zn\(^{2+}\) network...
would act as a ‘thermo-gene’ for heat-melting gels to render them superior thermal stability. To this end, the stock solutions of folate and Zn\(^{2+}\) were introduced to the melts of three types of heat-melting hydrogels covering both physically and chemically cross-linked ones. Two physically cross-linked hydrogels were employed, including (1) the freezing-thawing gel formed by polyvinyl alcohol (PVA), and (2) the heating-cooling gel of agarose. The last one (3) is the chemically cross-linked hydrogel of in-situ polymerized polyacrylamide (PAM).

To prepare this chemical hydrogel, the solutions of folate, Zn(NO\(_3\))\(_2\), and acrylamide (AM) monomer were premixed, followed by addition of initiators [Ammonium persulfate (APS) and tetramethylethylenediamine (TEMED)]. Double-network 33-34 thermogels (DNTG) were formed in all the above systems (Figure 2a-c), and they all displayed solid-like characteristics at temperatures up to 90 °C (Figure 2d-f). In contrast, the corresponding single polymer gels all melted at temperatures around 60°C (Figure 2g-i). DNTGs would come into formation by replacing 10% the heat-melting gel with the thermo-gene folate-Zn\(^{2+}\) gel, and increasing the concentration of the thermo-gene(Figure 3) or the fraction of Zn\(^{2+}\) (Figure 2j) would increase the shear modulus of the DNTG considerably. These results unambiguously confirm that the coordination interaction is critical for the temperature resistance.

In order to understand how the thermo-gene folate-Zn\(^{2+}\) hydrogel enhanced the temperature resistance of the DNTG, the microstructure of the DNTG is compared with that of the single hydrogels. Figure 3 shows the SEM images of the three freeze-dried gels of folate-Zn\(^{2+}\), PVA, and the DNTG of folate-Zn\(^{2+}\)/PVA. The dried thermo-gene folate-Zn\(^{2+}\) is continuous structure with large pores(Figure 3a), where the wall of the pores is composed of fibers(Figure S5). Different from these porous structures, the dried PVA gel is composed of individual fibers (Figure 3b). It is interesting to find that the DNTGs perfectly inherit the characteristics of each single component in a manner of hosting fibers in large pores(Figure 3c). Elemental mapping of the DNTG reveals that the Zn\(^{2+}\) is full of the field, but some holes are discernable, as indicated by the red circles in Figure 3d. This means that the thermo-gene folate-Zn\(^{2+}\) has formed an entire network in the DNTG, which acts as a strong skeleton to maintain the DNTG as temperature increases. Actually, the PVA network tends to melt in the DNTG upon heating, which can be inferred from the sharpening and down-field shifting of the \(^1\)HNMR signals for the PVA protons (Figure 3e). However, the extent of the PVA network melting in the DNTG is reduced, since the chemical shifts in the DNTG system are much smaller than that in the single PVA gel(Figure S6). It is clear that the presence of folate-Zn\(^{2+}\) not only endows the system with a thermal resistant network, but also enhances the stability of the PVA network. These two facts co-function as the DNTG is heated, which finally endows excellent temperature tolerance to the DNTG.

![Figure 2](image_url) Impact of the thermo-gene folate-Zn\(^{2+}\) on different heat-melting hydrogels. (a-c) The DNTG of PVA/folate-Zn\(^{2+}\) (wt% 9:1a), agarose/folate-Zn\(^{2+}\) (wt% 9:1b), and PAM/folate-Zn\(^{2+}\) (wt% 9:1c) at 25°C. (d-f) The corresponding DNTG at 90°C. (g-i) The single gel of 10% PVA, agarose, PAM at 60 °C. (j) Rheological measurements of the DNTG with varied molar ratio of [folate]:[Zn\(^{2+}\)]. [PVA]= 5%, [folate]=15 mM, the concentration of [Zn\(^{2+}\)] is 0, 3, 9, 21 mM.

![Figure 3](image_url) Microstructure of the DNTG. a-c) SEM image for the microstructure of the folate-Zn\(^{2+}\) gel, PVA gel, and the PVA/folate-Zn\(^{2+}\) DNTG, respectively. d) The elemental mapping of zinc in the dried DNTG. The red circles identify the pores where no Zn\(^{2+}\) presents. e) The \(^1\)HNMR spectra for the protons of PVA single gel and PVA/f-Zn\(^{2+}\) DNTG. F-Zn represents the folate-Zn\(^{2+}\) hydrogel. The arrows are to guide the direction of the chemical shift shifting of the CH\(_2\) proton in PVA.
Along with the superior thermal resistance, the DNTG also displays enhanced mechanical strength\textsuperscript{35-36}. The folate-Zn\textsuperscript{2+} gel is not stretchable, while the PVA/folate-Zn\textsuperscript{2+} DNTG can be stretched to nearly 1000\% with the stress of 1706 kPa\textsuperscript{4}(Figure 4a,b). In contrast, the original PVA gel can only be stretched to 750 \% with the stress of 1000 kPa. This high stretchability allows the PVA/folate-Zn\textsuperscript{2+} DNTG to resist blade cutting (Figure 4c), which is a valuable merit desired for robust flexible devices. It is noteworthy that this excellent mechanical property of PVA/folate-Zn\textsuperscript{2+} gel can extend to very high temperatures. Figure 4d-f shows that the shape, volume, and height of the PVA/folate-Zn\textsuperscript{2+} DNTG can recover after compression at 80°C, manifesting the excellent application potential as thermal stable flexible devices.

Next, a DNTG film was prepared to fabricate flexible sensors. Because the presence of potassium and nitrate ions in the folate-Zn\textsuperscript{2+} hydrogel, the resultant DNTG displays excellent conductivity (Figure S7). The conductivity can be further promoted by preparing the DNTG in the water solution of inorganic salts, such as KNO\textsubscript{3}(Figure S8). By filling the PVA/folate-Zn\textsuperscript{2+} melt in a self-made thin layer mold, a 100 um-thick gel film was obtained (Figure 5a). Figure 5b shows that the film displays excellent flexibility and elasticity, manifesting the great potential in application as stress sensors. Indeed, as a stripe of the gel film (Figure S9a, inset) was introduced to a circuit (Figure S9a) and attached to a human finger (Figure S9b), bending and stretching of the finger can result in significant current change and electronic resistance change (Figure S9c). This change is completely reversible with the repeated finger bending and stretching movements, and the amplitude of the current change is proportional to the extent of the bending action, indicating the excellent sensitivity of the flexible stress sensor.

Most strikingly, this flexible stress sensor is stable even at high temperatures. For the convenience of measurements, we attached the film to a steel plate. To avoid short circuit, the steel plate was covered with an insulated plastic (Figure 5c). Figure 5d (left) shows as the plate is heated to 80°C, the film sensor remains intact, and reversible current change is observed as the steel plate is repeatedly bended(Figure 5e). In contrast, the stress sensor made from PVA gel has melted at 60°C (Figure 5d, right). This result unambiguously verifies that the DNTG can be used as a robust stress sensor that may work in extremely hot environment.

It is noteworthy that the ‘thermo-gene’ does not interfere with the anti-freezing ability of the polymer hydrogels. If the solvent water was replaced by the mixture of glycol and water binary system, the DNTG (Figure S10) also displays anti-freezing ability. Figure S11 shows that the storage modulus...
(elastic modulus, G') and the loss modulus (viscous modulus, G'"") of the DNTG of PVA/folate-Zn are nearly constant in the wide temperature range of -30°C to 80°C, whereas that for the single PVA gel decreases with elevated temperature. This indicates that the strategy of DNTG can be jointly employed with the anti-freezing technique, allowing to fabricate flexible hydrogel devices that can work from sub-zero degrees to temperatures exceed 80°C.

Conclusions

In conclusion, the coordinating folate-Zn\(^{2+}\) hydrogel can be used as thermo-gene to endow heat melting hydrogels with thermal stability via a double network strategy. The resultant double network thermogels (DNTGs) perfectly inherit the thermal stability of the folate-Zn\(^{2+}\) hydrogel, so that the flexible conductive sensor made with the DNTG works stably up to the high temperature of 80°C where most physical hydrogels and some chemical hydrogels melt. In combination with the anti-freezing technique, this work indicates that the working temperature window of hydrogels can be broadened from subzero degrees to temperatures exceed 80°C. We envision that coordinating hydrogels could be universal thermo-genes capable to endow thermal stability to heat-melting hydrogels, which is advantageous for the practical application of smart devices. Relevant work is ongoing in our lab.

Conflicts of interest

There are no conflicts to declare.

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Notes and references


