A supramolecular gel formed by Zn$^{2+}$ coordination with adenosine monophosphate (AMP) is reported. The adenine base, the monophosphate, and Zn$^{2+}$ are all important for gel formation. Mechanically disrupted gels can re-form upon centrifugation; applications of this gel for guest molecule entrapment are explored.

Supramolecular gels have received considerable attention in recent years both as intriguing examples of self-assembly and as a means of creating smart materials.1-5 These gels are formed by noncovalent interactions to assemble supramolecular networks that trap solvent.6 Among the various types of supramolecular gels, those crosslinked by metal coordination are particular interesting. A few types of ligands have been developed for this purpose. For example, Craig and co-workers reported organopalladium–pyridine coordination.7,8 Bipyridine and pyridine-based hydrogels crosslinked by Co$^{2+}$, Cu$^{2+}$, and Fe$^{3+}$ were developed by Lewis and Miller.9,10 Chujo et al also employed the bipyridine chemistry to include polyoxazoline into the gel matrix.11,12 Cu$^{2+}$/carboxyl,13 Fe$^{3+}$/catechol,14,15 and N$i$$^2$$^+$/histidine interactions were also employed.16,17 Self-healing is a very interesting and useful feature of metal-coordinated gels; broken gel pieces can seal together with a mild mechanical force.

Most of previously reported gels have to use specially designed ligands (e.g. incorporating a segment of PEG chain in addition to the metal ligand) to facilitate solvent trapping and gelation. Few reports are based on pure small molecule ligands. We reason that small molecule ligands will allow a higher metal density and thus may display different properties.

Adenine, a purine nucleobase, is an important ligand in supramolecular chemistry. Adenine and its derivatives can form many complexes by coordination (with metal ions) and hydrogen bonding (with organic molecules).18-20 With metal ions, it may form metal-organic frameworks,21 and nanoparticles.22-25 Recently, the gelation of adenine with tricarboxylic acid compounds was reported based on hydrogen bonding and π-π stacking.26,27 We are interested in exploring whether it is possible to achieve gelation by metal coordination with adenine derivatives. Guanine and its derivatives have been mixed with monovalent metal ions to form gels based on quadruplex formation.28-31 However, little has been done on adenine derivatives. Nanoparticles are the most common products.22,25 Compared to nanoparticles, hydrogels are more attractive materials for making stimuli-responsible materials.32-39 In this work, we communicate such an example, its property in guest molecule encapsulation, and healing.

Since most previous work focused on adenine nucleobase and nucleoside as ligands, we started with nucleotide coordination in this work. Zn$^{2+}$ was chosen as the metal because it is a good Lewis acid that can be coordinated by a diverse range of ligands. In addition, it is a relatively biocompatible metal with low toxicity. We first studied the reaction between Zn$^{2+}$ and three kinds of nucleotides (AMP, GMP and CMP). TMP was not included since thymine has very weak coordination ability at neutral pH. Zn$^{2+}$ was respectively titrated into the solutions of AMP, GMP and CMP, and the UV-vis extinction at 550 nm was recorded. Since none of the products are colored, the growth of light extinction at 550 nm is an indication of light scattering and thus formation of precipitation. The UV-vis results (Figure 1A) indicate that all the three samples formed particles that strongly scattered light. With increasing Zn$^{2+}$ concentration, scattering became stronger and saturated signal was observed at high Zn$^{2+}$ concentrations. AMP saturated at ~1:1 ratio between AMP and Zn$^{2+}$, suggesting the stoichiometry of coordination. Binding of Zn$^{2+}$ by AMP is stronger than that by the other two nucleotides since much more Zn$^{2+}$ is required to saturate the binding of GMP and CMP. To study the property of these coordination complexes, we prepared samples using a ratio of 1:1 between Zn$^{2+}$ and each nucleotide. The samples all turned cloudy, which is consistent with the UV-vis results. All the samples were centrifuged to collect the precipitated products. White solid materials of Zn/GMP and Zn/CMP were obtained, while very interestingly a gel was observed with Zn/GMP (Figure 1C). To understand this gel, the precipitated samples were re-dispersed in pure water by vortex mixing, and the suspensions were used for DLS measurement to obtain the hydrodynamic size and z-potential information. After vortexing, the Zn/AMP gel is broken into small pieces, showing an average size of a few micrometers (Figure 1B). The hydrodynamic sizes of Zn/CMP and Zn/GMP complexes are relatively smaller (<300 nm). The surface
charge of Zn/AMP is close to zero, while the other two are more negatively charged. This could explain the formation of large gel pieces by Zn/AMP due to a lack of strong charge repulsion. Considering the differences of nucleobase structure in GMP, CMP and AMP, the coordination of Zn\(^{2+}\) with adenine is likely to be critical for the formation of the gel complex. For example, our UV-vis data indicated the Zn/AMP binding is the strongest of the three (Figure 1A).

The above comparison has highlighted the importance of the nucleobase. To further understand gel formation, we next tested the Zn\(^{2+}\)/adenine and its derivatives. Gel was formed only in the AMP tube. Among the tested metals, only Zn\(^{2+}\) can interact with the phosphate and AMP. Only Zn\(^{2+}\) produces the gel.

Finally we varied the metal (Figure 1E). In addition to Zn\(^{2+}\), a few other divalent and trivalent metals were tested. We did not include lanthanides, which are known to form nanoparticles instead of gels with AMP.\(^2^2\) Among the tested metals, only Zn\(^{2+}\) induced gel formation. This might be explained that Zn\(^{2+}\) can interact with both the phosphate and adenine base with similarly high affinity. Other metals may interact with mainly with the base (e.g. the transition metals), or mainly with phosphate (alkaline earth metals), but not both.\(^4^0\) Cd\(^{2+}\) is a softer metal and do not interact with phosphate strongly, which can be seen from phosphorothioate modification studies.\(^2^3\) This leaves Zn\(^{2+}\) a unique metal for this purpose. This study also suggests a design principle for making metal coordinated gels, where the ligand should have appropriate affinities for at least two binding sites.

While carrying out the above experiment, an interesting observation is the mechanical disrupted gels can be easily re-formed. For example, with mechanical agitation by a vortex mixer, the Zn/AMP gel was transformed into small gel pieces (like a sol phase), yielding a turbid suspension. Upon centrifugation, the gel is formed again. This mechanically induced gel transition can be repeated many times. Since nucleotide-coordinated nanoparticles have been observed before, we focused this study on the gel system.

To quantitatively understand the property of this new gel, Zn\(^{2+}\) was mixed with an equal mole of AMP at different concentrations (Figure 2A). Below 1 mM, no gel was formed. Therefore, 1 mM is the minimal gelation concentration. The mass of the resulting gels were measured (Figure 2A). The gel weight reached the maximum at 5 mM of Zn\(^{2+}\) and AMP (Figure 2B, black trace). Further increase of their concentrations decreased the gel weight, and the gels also became more opaque. Therefore, the amount of entrapped water decreased significantly when the monomer concentrations were too high. It needs to be noted that although the 5 mM sample has the highest gel mass, the normalized gel mass is the highest for the 1 mM sample (normalized by the monomer mass, blue trace in Figure 2B). With 1 mM Zn\(^{2+}\) and AMP, and assuming all these monomers went into the gel, the monomer mass is 0.41 mg and the gel can trap 68.6±6.4 mg of water, reaching a swelling ratio of ~156. This swelling ratio is comparable with many other types of gels. The percentage of this 1 mM gel is ~0.6%.

**Figure 1.** (A) UV-vis extinction monitored at 550 nm as a function of Zn\(^{2+}\) concentration. The volume of the reaction was 1 mL and the nucleotide concentration was fixed. (B) Particle sizes and \(\zeta\)-potential of the complexes formed by Zn\(^{2+}\) and the three nucleotides after redispersing the precipitated complexes in water. (C) A photograph of the products formed with different nucleotides after centrifugation and flipping the microcentrifuge tubes. A hydrogel was formed with the Zn-AMP system in the red box. (D) A photograph showing the reaction products between Zn\(^{2+}\) and adenine and its derivatives. Gel was formed only in the AMP tube. (E) Effect of metal ions in forming gels with AMP. Only Zn\(^{2+}\) produces the gel.

**Figure 2.** (A) A photograph showing the gels formed by the 1:1 Zn\(^{2+}\)/AMP mixture at different monomer concentrations. No gel formed below 1 mM Zn\(^{2+}\)/AMP. (B) The final gel weight and normalized gel weight of the Zn/AMP gels prepared with different concentration of Zn\(^{2+}\) and AMP.

To further characterize the property of this new gel, we also measured the stability of the Zn/AMP gel at different ionic strength, pH and temperature. First, the Zn/AMP gels were firstly prepared in

\[\text{Zn}^{2+} + \text{AMP} \rightarrow \text{Zn/AMP gel}\]
HEPES buffer (pH 7.4, 10 mM). Then, the buffer was replaced to our designated conditions and the formed gels were broken mechanically by vortex. Then the tube was centrifuged again and the amount of the gel was quantified by weighing. Figure 3A shows an example of such a process. It can also be noticed that the solution became turbid after disrupting the gel since small gel pieces are now suspended at the bottom of the tube. Figure 3B shows the effect of ionic strength, and we repeated the above washing process three times. Most of the gels lost ~30% weight after three washes, and the amount of loss is independent of the salt concentration. Thus, the Zn/AMP gel stability is not affected by ionic strength.

Next the effect of pH was tested. The sol-gel transition occurred at ~pH 4 and pH 13 (Figure 3D). The gel stability is the highest around neutral pH. This experiment also suggests that the N7 position of adenine might be a coordination site since only this position can be protonated at lower pH (pK~a = 3.5). At very high pH, Zn\(^{2+}\) might be extensively hydrolyzed/precipitated and are not available to AMP. A proposed structure of the Zn/AMP complex is shown in Figure 3C. Overall, this gel has excellent stability at physiological conditions. Finally, the effect of temperature was studied and the gel was stable at even 100 °C (Figure S1). The rheology property of the gel was also measured (Figure S2). In the frequency sweep experiment, the storage modulus (G’) values were much higher than the loss modulus (G’’). A linear response was observed over a wide range of frequencies (0.03-30 Hz), confirming its elastic property typical for supramolecular gels.

To further study the coordination materials, we have taken their TEM micrographs. The Zn/AMP complexes appears to be interconnected fibrils (Figure 4A, B), which is consistent with gel networks capable of trapping water. On the other hand, the Zn/GMP (Figure 4C) and Zn/CMP (Figure 4D) samples are consist of aggregated nanoparticles that appear more electron dense. The different microstructures between Zn/AMP and the other two also supports gel formation for the Zn/AMP system.

The fact that the Zn/AMP gel can form reversibly by mechanic agitation indicates the property metal coordination. Metal coordination is not as strong as covalent bonds and can be disrupted more easily. On the other hand, it is a reversible interaction and under the mechanic stress of centrifugation, the sol particles can re-connect to form gels.

Figure 4. TEM micrographs of the coordination complexes formed by mixing Zn\(^{2+}\) with AMP (A, B), GMP (C) and CMP (D).

After understanding the physical property of this Zn/AMP gel, we next studied its molecular encapsulation property. Three kinds of water soluble dyes (fluorescein, rhodamine 6G and rhodamine B) and a protein (fluorescein-labeled bovine serum albumin, F-BSA, pI=4.7) were employed as guest molecules. The reaction solutions of guest molecules (0.01 mM dyes or 50 μg/mL protein) and AMP (5 mM) in 10 mM HEPES (pH 7.4) were mixed with aqueous ZnCl\(_2\) (5 mM), and then centrifuged to induce the gel formation. The amounts of guest molecules incorporated into gels were calculated by the absorption intensities of the supernatant solutions. It was found that incorporation of the protein was the most effective compared with the three dye molecules (Figure 5A). The Zn/AMP gel carries almost no charge and it can interact with guest molecules mainly via hydrogen bonding, aromatic stacking and van der Waals force. In this case, it appears that BSA has the strongest interaction and can be efficiently trapped.

Finally, we tested the healing property of the gels by adding a F-BSA trapped gel to a rhodamine B trapped gel (Figure 5B). The F-BSA gel was first disrupted and then centrifuged on top of the rhodamine B gel. When taken out, a whole piece was formed, indicating that the broken gels can heal. We also showed that two gel pieces can re-joint after a gentle press (Figure S3).
In conclusion, we reported a supramolecular gel formed by mixing AMP and Zn$^{2+}$ coordination, which has the property of mechanically induced gel formation. The base structure, monophosphate, and Zn$^{2+}$ are all critical for the gel formation and replacing any of them by other structures of ions inhibited gelation. The Zn/AMP gel is stable in the neutral, basic and high ionic conditions, but less stable at acidic pH. An advantage of this supramolecular gel is that it can be prepared at a low molecular concentrations and large scale gelation can be triggered simply by centrifugation. Broken gel pieces can also re-form into a large monolithic gel by centrifugation. This property might be useful for entrapping guest molecules in the gels and remove them from water.

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Notes and references