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Tb^{3+}-triggered luminescence in a supramolecular gel and its use as a fluorescent chemoprobe for proteins containing alanine†

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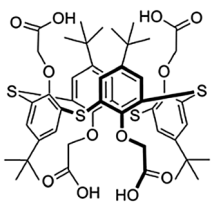
A tetracarboxylic acid-appended thiacalix[4]arene-based ligand with Tb^{3+} formed a supramolecular gel which showed novel fluorogenic sensor capability for probing alanine and proteins containing alanine.

Amino acids are key intermediates of primary metabolism in all biological processes.[1] In general, HPLC, MS and NMR methods, advanced microchips, portable instrumentation, and other rapid sensing systems have recently been applied in studies related to proteomics, metabolism, astrobiology, the food industry, and other fields.[2] Because all amino acids exhibit a variety of important functions, new chemoprobes for detection of specific amino acids would be useful tools in basic science and applied technology. For example, histidine, a polar amino acid, has been investigated by various detection methods that use chemoprobes composed of functionalized nanoparticles and chromogenic receptors.[3] In contrast, there have been few reported detection methods for alanine, a typical hydrophobic amino acid, and for proteins containing alanine. D-alanine has been determined by using high-performance liquid chromatography and gas chromatography.[4] These methods are very sensitive, reliable and reproducible, but they require long measuring times, use specialized techniques for sample preparation and analysis, and are very expensive to perform. Indeed, a selective detection method for alanine and proteins containing alanine has never been reported by using a chemoprobe.

Lanthanides (Tb^{3+}) are of specific interest for such applications, owing to their unique magnetic and photophysical properties, which can be modulated through ligand design by taking advantage of their high coordination requirement/number (*ca.* 8–12).[5] Consequently lanthanides have found applications in sensing,[6] medical diagnostics,[7] and telecommunications.[8] To date, Tb^{3+}-containing hydrogels and organogels have been obtained by noncovalently incorporating these compounds as complexes into a gelator matrix, *i.e.*, as independent coordination complexes. Tb^{3+} can also be introduced into self-assembled structures of organic molecules where it acts as a bridging unit that enables the formation of branched 2D and 3D coordination polymers and networks. We recently demonstrated that a tetracarboxylic acid-appended thiacalix[4]arene formed a supramolecular gel with a network structure, which acted as a chemoprobe for specific gases containing chlorine atoms.[9] In this communication, we show that a tetracarboxylic acid-appended thiacalix[4]arene-based gel with Tb^{3+} acts as a selective fluorogenic chemoprobe for alanine and alanine-containing proteins. Herein, we report the preparation of a luminescent supramolecular gel with Tb^{3+} and its applications as a novel fluorogenic chemoprobe for alanine and alanine-containing proteins.



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The tetracarboxylic acid-appended thiacalix[4]arene-based ligand **1**, which has a 1,3-alternating structure, was prepared in two steps (Scheme S1 in the ESI†). Compound **3** was reacted with 3-bromo ethyl bromoacetate in the presence of anhydrous cesium carbonate in acetone. Then, compound **2** was treated with KOH to yield desired product **1**, as confirmed by ^1H, ^{13}C NMR spectroscopy, ESI-MS, FT IR and elemental analysis. For example, the ^1H NMR spectrum of ligand **1** showed the presence of carboxyl acidic protons as broad singlets at 11.5 ppm, indicating that the ligand has C_2 symmetry. The formation of ligand **1** was also confirmed by HR-ESI MS with *m*/*z* 953.43, which matched the calculated isotopic distribution pattern of $[\mathbf{1} + H]^+$.

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The absorption spectrum of ligand **1** (5.0 wt%) was obtained in DMF/water (6 : 1 v/v%) at pH = 11 as shown in Fig. S1 (ESI†). The absorption spectrum of **1** with Tb^{3+} exhibited a band centered at approximately 272 nm ($\log \varepsilon = 3.609$), assigned to the π–π^* transition. Ligand **1** could gelate a DMF/water mixture (6 : 1 v/v%) in the presence of Tb^{3+}, indicative of the formation of a supramolecular polymer (Fig. S2A, ESI†). The formation of supramolecular gel **1** was undertaken at various concentrations of Tb^{3+} (0.5–5.0 equiv.), in which the gel was maintained for several months at room temperature. As shown in Fig. S2B, the green photoluminescence emission of the gels under UV irradiation gradually decreased as the concentration of Tb^{3+} increased. In contrast, sol **1** with Tb^{3+} was non-emissive under the same conditions (Fig. S3, ESI†). The loss of photoluminescence intensity of ligand **1** with increasing concentration of Tb^{3+} in the gel state was attributed to the coordination geometry.

To investigate the quantitative photoluminescence properties of the supramolecular gel with Tb^{3+}, we observed the photoluminescence emission spectra of the gels at different concentrations of Tb^{3+} (Fig. 1). The photoluminescence spectrum of the gel in the presence of a small amount (0.2 equivalents) of Tb^{3+} showed a strong emission, which gave rise to a characteristic Tb^{3+} complex emission at 490, 546, 586 and 623 nm. In contrast, the intensities of the photoluminescence emissions of gels formed upon addition of larger amounts (0.3–1.0 equivalents) of Tb^{3+} gradually decreased. These results indicated that the high geometric number of Tb^{3+} changed into a lower geometric number.[10]

To gain insight into the morphology and possibly to reveal the orientation of the supramolecular polymers within gels, xerogel samples were observed by scanning electron microscopy (SEM) as well as by energy-dispersive X-ray spectroscopy (EDX). The SEM image of xerogel **1** prepared with Tb^{3+} showed a nanorod structure (Fig. 2A), which was approximately 100–120 nm in length. Furthermore, the xerogel prepared with Tb^{3+}, consisting of carbon, oxygen, sulfur and terbium, was also analyzed by EDX (Fig. 2B), and demonstrated that the supramolecular gel has efficiently incorporated Tb^{3+}. Thus, the main driving force to induce the supramolecular gel **1** with Tb^{3+} was the coordination bond.

We investigated the thermally promoted stability of supramolecular gel **1** by measuring the transition temperature ($T_{sol-gel}$) of gel **1** by differential scanning calorimetry (DSC) (Fig. S4, ESI†). Supramolecular gel **1** showed a sharp phase transition at 115 °C, consistent with an endothermic reaction. This endothermic thermogram was due to the transition of the supramolecular gel **1** into the solution phase.

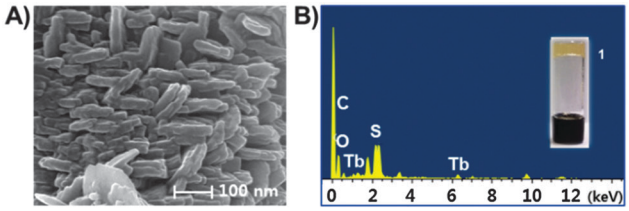

Fig. 2 (A) SEM image of xerogel **1** (5 wt%) with Tb^{3+} (1 equiv.). (B) EDX spectrum of xerogel **1** with Tb^{3+}.

To investigate the selectivity for specific amino acids, the fluorescence intensity changes of supramolecular gels prepared with Tb^{3+} were observed upon addition of various amino acids (0.3 mM). As shown in Fig. S5 (ESI†), with the exception of Ser, Phe and His, addition of other amino acids maintained the stability of the supramolecular gel, indicating that the amino acids coordinated to the Tb^{3+} center. In particular, the supramolecular gel **1** exhibited a strong emission upon UV irradiation with the addition of alanine (Fig. 3A). The addition of other amino acids such as Asp, Val, Leu, Gly, Tyr, Met and Cys, in contrast, has no effect on the fluorescence emission changes of the supramolecular gels. Thus, the supramolecular gel responds selectively to alanine, a response which can be attributed to its coordination to Tb^{3+} by alanine. We also recorded the fluorescence spectra of supramolecular gels prepared with Tb^{3+} upon addition of amino acids (Fig. 3B). Only in the presence of alanine did we observe four narrow emission bands for the supramolecular gel, which were attributed to a $^5D_4 \rightarrow {}^7F_5$ (546 nm) transition. The addition of alanine resulted in a linear enhancement of the fluorescence intensities of the supramolecular gel, indicating that the alanine molecule became coordinated to Tb^{3+}. The supramolecular gel also showed excellent sensitivity with alanine at a very low concentration of 0.3 mM. The main binding force for high selective

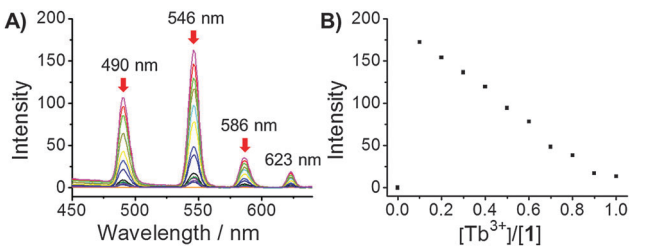

Fig. 1 (A) Photoluminescence spectra of supramolecular gel **1** (5 wt%) with various concentration of Tb^{3+} (0–1.0 equiv.). (B) Plot of photoluminescence intensity against mole ratio of [Tb^{3+}/**1**].

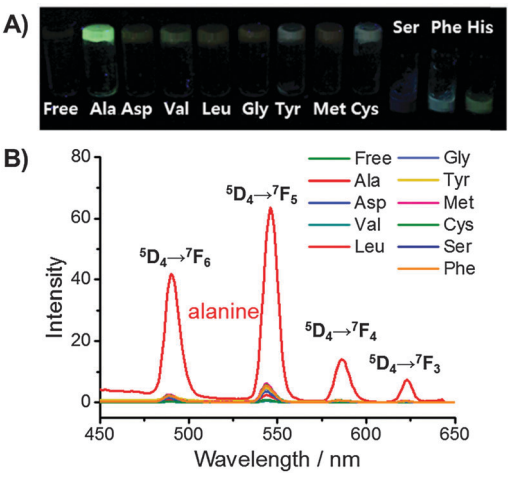

Fig. 3 (A) Photograph of supramolecular gel **1** (5 wt%) with Tb^{3+} (1 equiv.) upon addition of amino acids (3 mM) under a UV lamp. (B) Photoluminescence spectra of supramolecular gel (5 wt%) with Tb^{3+} (1 equiv.) upon addition of amino acids (3 mM).

alanine is the coordination bond between the **1** + Tb^{3+} complex and the carboxyl group of alanine. In addition, the hydrophobic interaction would occur between ligand **1** and alanine.

The SEM image of the supramolecular gel upon addition of alanine showed that the fiber structure connected a sphere and a sphere as a pearl necklace (Fig. S6, ESI†). No significant fluorescence changes of gels were observed upon addition of other amino acids, including Asp, Val, Leu, Gly, Tyr, Met and Cys. In addition, SEM images of supramolecular gel **1** + Tb^{3+} showed no change upon addition of other amino acids such as Asp, Val, Leu and Gly (Fig. S7, ESI†).

To determine if the specificity in the detection of alanine is compromised by complex mixtures of other amino acids, we observed the fluorescence intensity changes of supramolecular gel **1** upon addition of alanine in binary systems (Fig. S8, ESI†). As expected, the fluorescence changes of supramolecular gels were observed when a solution of alanine was added to the mixture. The findings suggest that the highly selective nature of the fluorescence change of supramolecular gels is due to the high selectivity of the assay for alanine. The fluorescence changes of the supramolecular gel were also observed upon addition of various proteins containing alanine such as ferritin, aldolase, thyroglobulin, chymotrypsinogen, catalase and ribonuclease (Fig. 4A), in which the proteins contained different numbers of alanine moieties. As shown in Fig. 4, the addition of chymotrypsinogen induced the largest fluorescence change of the supramolecular gel fluorescence emission, indicating that even though some of the alanine residues are located inside the protein, due to their hydrophobic properties, the alanines within the proteins could be coordinated to Tb^{3+}. Furthermore, the fluorescence intensity changes of the supramolecular gel were dependent on the numbers of alanine moieties in the proteins as shown in Fig. 4B. A linear response between the fluorescence intensities and the concentration of thyroglobulin was observed over the range of 0.22 nM and 1.12 nM and indicated a detection limit of *ca.* 0.1 nM (Fig. 4C), suggesting quantitative binding to Tb^{3+}. As the control experiment for protein, we also observed the fluorescence change of supramolecular gel **1** + Tb^{3+} upon addition of protein (DTG), which is not an alanine moiety. As expected, no significant change was observed upon addition of DTG (Fig. S9, ESI†).

We observed CD spectra of thyroglobulin before and after addition of supramolecular gel **1** + Tb^{3+} as shown in Fig. S10 (ESI†). The CD spectra of thyroglobulin showed the negative signals in the absence and the presence of supramolecular gel **1** + Tb^{3+}, indicating that the structure of thyroglobulin did not change upon addition of supramolecular gel **1** + Tb^{3+}.

We investigated the rheological properties of supramolecular **1** + Tb^{3+} with and without alanine. Fig. S11 (ESA†) illustrates that G' and G'' were almost constant with the increase of the frequency from 0.1 to 100 rad s^{-1}. In addition, G' is *ca.* 3.5 times larger than G'' over all ranges (0.1–100 rad s^{-1}), suggesting that the supramolecular gel **1** + Tb^{3+} is moderately tolerant to external force. Both the storage modulus and the loss modulus of the supramolecular gel **1** + Tb^{3+} upon addition of 1.0 equivalents of alanine were similar to those of the supramolecular gel **1** + Tb^{3+} in the absence of alanine. Furthermore, G' and G'' values of supramolecular gel **1** + Tb^{3+} both without and with alanine were almost constant with the increasing dynamic frequency sweep. These results indicate that the rheological properties of supramolecular gel **1** + Tb^{3+} did not strongly depend on the existence of alanine.

In conclusion, a tetracarboxylic acid-appended thiacalix[4]arene-based supramolecular gel has been prepared in the presence of Tb^{3+} and its sensing properties for proteins containing alanine moieties have been investigated. The supramolecular gel in the presence of 1.0 equivalent of Tb^{3+} exhibited no fluorescence emission when excited with UV light. In contrast, upon addition of alanine to the supramolecular gel, a strong green emission occurred. Interestingly, proteins containing alanine moieties also induced a strong emission from the supramolecular gel. The tetracarboxylic acid-appended thiacalix[4]arene-based supramolecular gel can function as a "turn-on" fluorescent chemoprobe for alanine and proteins containing alanine, which is the first example of detection of alanine with a chemoprobe. Thus, the tetracarboxylic acid-appended thiacalix[4]arene-based supramolecular gel would have potential applications in biological analysis. Furthermore, this concept would be valuable for the development of chemoprobes for proteins.

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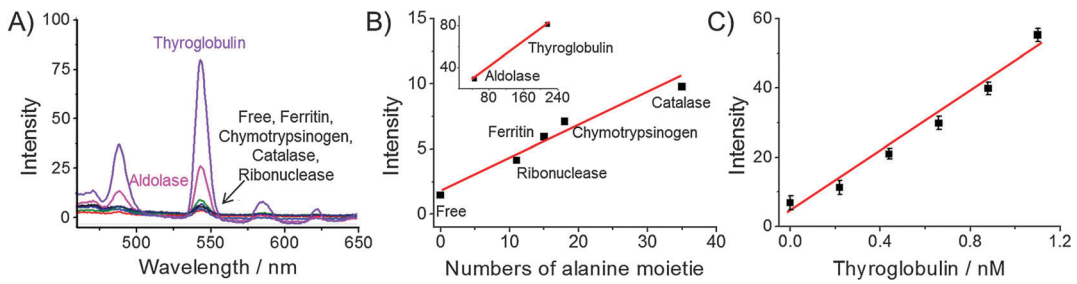

Fig. 4 (A) Photoluminescence spectra of supramolecular gel **1** (5 wt%) with Tb^{3+} (1 equiv.) upon addition of various proteins containing alanine moiety; ferritin, aldolase, thyroglobulin, chymotrypsinogen, catalase and ribonuclease. (B) Plot of photoluminescence intensity of numbers of alanine moiety at 546 nm. (C) Plot of photoluminescence intensity of supramolecular gel with Tb^{3+} upon addition of thyroglobulin (0.22–1.12 nM) at 546 nm.

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