Phenanthroline bridged bis(β-cyclodextrin)s/ adamantanecarboxylic acid supramolecular complex as an efficient fluorescence sensor to Zn$^{2+}$†

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A water-soluble fluorescent Zn$^{2+}$ sensor, 1,10-phenanthroline bridged bis(β-cyclodextrin) (1), was synthesized by “click chemistry”, and its fluorescence sensing behavior toward Zn$^{2+}$ against various metal ions was investigated under physiological conditions. Significantly, 1 showed high selectivity and sensitivity toward Zn$^{2+}$ with a limit of detection (LOD) down to 10$^{-7}$ M. Moreover, the spectrophotometric studies demonstrated that after complexation with 1-admantanecarboxylic acid sodium salt (AdCA), the 1/AdCA complex gave much stronger binding affinity and lower LOD value toward Zn$^{2+}$ through a cyclodextrin/substrate/Zn$^{2+}$ triple recognition mode. The fluorescence stopped-flow experiments also indicated that the association rate of complex 1/AdCA to Zn$^{2+}$ was much faster than compound 1 to the same ion. Furthermore, the fluorescence intensity of 1 and 1/AdCA was greatly enhanced after binding Zn$^{2+}$ in living cells, and thus 1 and complex 1/AdCA could be considered as a biosensor for Zn$^{2+}$ at the cellular level.

Nonetheless, among these fluorescent Zn$^{2+}$ chemosensors, only few of them are totally water-soluble and can detect Zn$^{2+}$ without disturbance from Cd$^{2+}$.5

Cyclodextrins (CDs), a class of cyclic oligosaccharides possessing a hydrophobic cavity, are widely used as drug carriers and solubilizers.8 The modification of the CD backbone with chromophoric substituents has been proven as a more powerful strategy in the fluorescence sensing of transition- or heavy-metal ions. For instance, we have constructed a series of CD-based switch-on fluorescent sensors for Zn$^{2+}$, Cd$^{2+}$, and Hg$^{2+}$, showing satisfactory molecular selectivity and enhanced binding abilities in aqueous solution, thin film, and living cells.7 In the present work, phenanthroline is connected with the resultant CD cavities in 1,10-phenanthroline bridged bis(β-CD) 1 by “click chemistry”, which showed satisfactory water solubility and high fluorescence sensing efficiency for Zn$^{2+}$. The two adjacent CD cavities in 1 could not only act as an ideal solubilizer, but also endowed the phenanthroline core with cell permeability through the interaction of β-CD with the phospholipids and cholesterol on the cell membrane.9 Moreover, due to the strong association of the β-CD cavity with the adamantyl skeleton, 1 could form a stable inclusion complex with 1-admantanecarboxylic acid sodium salt (AdCA), and the resultant 1/AdCA complex exhibited much stronger binding ability, lower limit of detection (LOD) value, and faster reaction rate toward Zn$^{2+}$. Our obtained results will energize the potential use of CD-based bioactive nanosupramolecules in the construction of efficient fluorescence sensors with actual device implementation.

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Results and discussion

Synthesis

The synthetic route to compound 1 is described in Scheme 1. Compound 1 was synthesized via “click chemistry” between 2,9-dipropargyl-1,10-phenanthroline and mono(6-deoxy-6-azido)-β-CD in 28% yield, and was sufficiently characterized by NMR spectroscopy, MALDI-MS, and elemental analysis (Fig. S1–S6 in the ESI†). Benefitting from the good solubilization ability of two adjacent β-CD units, the host compound 1 showed a satisfactory solubility up to 0.05 M in water.

Fluorescence sensing of Zn²⁺ by 1

The fluorescence sensing behavior of 1 toward Zn²⁺ was investigated by means of fluorescence spectroscopy in HEPES buffer solution (10 mM, pH = 7.2) at 25 °C. As shown in Fig. 1, compound 1 showed a good fluorescence sensing ability for Zn²⁺. The emission intensity of 1 at 377 nm gradually increased upon addition of Zn²⁺, accompanied by an obvious bathochromic shift from 368 to 377 nm. Furthermore, a maximum peak at a molar fraction of 0.5 was clearly observed in the Job plot, corresponding to a 1 : 1 coordination stoichiometry between 1 and Zn²⁺ (Fig. 2). After validating the 1 : 1 1/Zn²⁺ stoichiometry, the binding constant (log K) between 1 and Zn²⁺ could be calculated as 5.95 using a nonlinear least-squares curve-fitting method by analyzing the sequential changes in fluorescence intensity (ΔF) of 1 in the presence of varying concentrations of Zn²⁺. In addition, the limit of detection (defined as LOD value) of 1 to Zn²⁺ was measured as 4.92 × 10⁻⁷ M by multiplying the standard derivation of 11 groups of blank measurements by 3 and then dividing by the slope of the linear calibration curve at the lower concentration range of Zn²⁺.

The possible mechanism for the enhanced fluorescence of 1/Zn²⁺ complex may originate from a controlled intramolecular photo-induced electron transfer (PET) process; that is, before coordination with Zn²⁺, the PET process takes place from the 1,2,3-triazole moiety as an electron-rich donor to the phenanthroline moiety as an electron-deficient acceptor. After coordination with Zn²⁺, the nonradiative channel in the PET process was synchronously suppressed to a great extent,

Fig. 1 Fluorescence spectral changes of 1 (1.5 × 10⁻⁵ M) upon addition of Zn(ClO₄)₂ (0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, and 36 × 10⁻⁶ M, from a to m) in HEPES buffer solution (10 mM, pH = 7.2). Inset: the nonlinear least-squares analysis of the differential fluorescence intensity (ΔF) with the concentration of Zn²⁺ to calculate the binding constant (K) (λₑₓ = 272 nm and λₑₘ = 377 nm).

Fig. 2 Job plot of 1/Zn²⁺ system in HEPES buffer solution (10 mM, pH = 7.2) at 25 °C ([1] + [Zn²⁺] = 2.0 × 10⁻⁵ M).

Scheme 1 Synthetic route of compound 1.
ultimately leading to a pronounced emission intensity of the phenanthroline fluorophore. Moreover, a favorable size/shape fit was achieved between the ionic radius of Zn$^{2+}$ and the semirigid structure of the phenanthroline and triazole units to form a five-membered chelating ring.

Comparatively, the fluorescence sensing selectivity of 1 toward various metal cations was also studied in a physiological environment. As shown in Fig. 3, the fluorescence of 1 showed a 2.1-fold fluorescence enhancement in the presence of 2 equiv. of Zn$^{2+}$, but its IIB homologue Cd$^{2+}$ only gave a negligible change under the same experimental conditions. Moreover, an obvious fluorescence quenching was observed when Ag$^{+}$, Co$^{2+}$, Cu$^{2+}$, Hg$^{2+}$, and Pb$^{2+}$ were added into the solution of 1. These phenomena may be ascribable to the electron or energy transfer from the phenanthroline moiety of 1 to the unfilled electronic orbitals on these cations.

Fluorescence sensing of 1/AdCA to Zn$^{2+}$

It is well-documented that there is a high affinity between the cavity of β-CD and adamantane derivatives with $K_S$ values up to $10^7$ M$^{-1}$. In the present work, 1-admantanecarboxylic acid sodium salt (AdCA) was employed to investigate the enhanced Zn$^{2+}$ binding stability and sensing ability of the 1/AdCA inclusion complex (Fig. 4). In comparison to the fluorescence quenching with Cd$^{2+}$, it was found that the fluorescence intensity of 1/AdCA was exclusively enhanced with Zn$^{2+}$ (Fig. S7†). Similar to the 1/Zn$^{2+}$ system, the 1 : 1 coordination stoichiometry between 1/AdCA and Zn$^{2+}$ was confirmed by a Job plot (Fig. S8†). Therefore, using the fluorescence titration method, the log $K_S$ value of 1/AdCA with Zn$^{2+}$ was calculated as 7.74, which was almost 100 times higher than the corresponding value of 1 with Zn$^{2+}$. Meanwhile, a relatively lower LOD value was also obtained as $3.38 \times 10^{-7}$ M in 1/AdCA to Zn$^{2+}$.

Binding mode

The binding modes of 1 and 1/AdCA with Zn$^{2+}$ were studied by means of $^1$H NMR, fluorescence spectroscopy, and circular dichroism spectroscopy. As shown in Fig. 5, the protons of the phenanthroline and triazole moieties of 1 exhibited a downfield shift ($\Delta \delta$) in the presence of Zn$^{2+}$ ($\Delta \delta_{a1}$ = 0.37 ppm, $\Delta \delta_{b1}$ = 0.04 ppm, $\Delta \delta_{c1}$ = 0.29 ppm, and $\Delta \delta_{d1}$ = 0.18 ppm), indicative of the electron deshielding effect of metal ions on the adjacent protons. Thus, we could deduce that the phenanthroline and triazole moieties of 1 participated in the coordination process with Zn$^{2+}$. Moreover, by employing a Hill plot,\textsuperscript{11} the binding stoichiometry and log $K_S$ value between 1 and AdCA could be fitted as 2 and $4.2 \times 10^6$ M$^{-1}$, respectively, indicating that two AdCA units were concurrently encapsulated in the CD cavities to form a 2 : 1 complex with high affinity (Fig. S9 and S10 in the ESI†). Based on this binding constant, we can calculate that more than 90% of 1 was changed to the 1/AdCA complex under our experimental conditions, and both CD cavities of 1 were occupied by AdCA to jointly coordinate with...
Zn\(^{2+}\). In addition, the fluorescence sensing of Zn\(^{2+}\) was investigated with ca. 50% and 90% 1/AdCA encapsulation ratios. As the AdCA encapsulation ratio increases, it is found that the fluorescence intensity of the 1/AdCA/Zn\(^{2+}\) ternary complex decreases accordingly (Fig. S11 in the ESI†). This result indicates that as compared with free host 1 and the 1:1 1/AdCA complex, the introduction of more AdCA can induce a slight fluorescent quenching in the coordination process.

Furthermore, 2D NMR spectroscopic experiments were performed to obtain structural information on the host-guest complexation. As shown in Fig. S12,† the nuclear Overhauser enhancement (NOE) cross-correlations between the protons of the 1,2,3-triazole ring and the H5 protons of the β-CD (peaks A), as well as those between the protons of the phenanthroline group and the H3 protons of the β-CD (peaks B and C), jointly demonstrate that the phenanthroline moiety of 1 was partially accommodated in the cavity of β-CD from its primary face. After adding 2 equiv. of Zn\(^{2+}\), it was found that the phenanthroline spacer of 1 was still self-included in the cavity of β-CD (Fig. S13 in the ESI). In contrast, clear NOE correlations between the protons of AdCA and the interior protons of β-CD (H3/H5/H6) were observed (peaks A), and all the self-inclusion cross-peaks between phenanthroline and the CD cavity disappeared in the ternary complex of 1/AdCA/Zn\(^{2+}\) (Fig. S14 in the ESI†). In addition, the AdCA protons showed a stronger NOE correlation with H5/H6 than the H3 protons of AdCA†.

As shown in Fig. 6, the host 1 gave two induced circular dichroism (ICD) signals in aqueous solution. Based on the empirical rules for the ICD behavior of CD inclusion complexes,\(^{12}\) the positive Cotton effect peak (Δε = 5.31 M\(^{-1}\) cm\(^{-1}\)) at 230 nm and the negative one (Δε = -6.11 M\(^{-1}\) cm\(^{-1}\)) at 272 nm reveal that the phenanthroline group is partially self-included in the chiral microenvironment of the β-CD cavity, which is in accordance with the intramolecular self-inclusion mode in the 2D NMR spectroscopic experiment. Moreover, it is found that the Cotton peak intensity of 1 slightly decreased in the presence of Zn\(^{2+}\) or AdCA, mainly because the phenanthroline moiety was gradually released from the β-CD cavity. Conversely, when both Zn\(^{2+}\) and AdCA coexisted, the ICD signals of 1 was reversed from a negative Cotton peak to a positive one, accompanied by enhanced signal intensity from Δε = -3.48 M\(^{-1}\) cm\(^{-1}\) to Δε = 27.89 M\(^{-1}\) cm\(^{-1}\) at 278 nm. These phenomena indicate that there is a conformational change upon complexation of 1 with Zn\(^{2+}\) and AdCA. Due to the supramolecular cooperativity between CD and AdCA, the phenanthroline unit of 1 was forced out of the β-CD cavity to facilitate the multivalent binding of the phenanthroline, triazole, and carboxylic sites with Zn\(^{2+}\). Consequently, combining all the characteristic data from NMR and circular dichroism spectroscopy, we can reasonably deduce the possible binding modes of 1/Zn\(^{2+}\) and 1/AdCA/Zn\(^{2+}\), as illustrated in Scheme 2. In addition, the energy minimization structure of the 1/AdCA/Zn\(^{2+}\) system was obtained by a molecular modeling study, which is consistent with the proposed recognition mode (Fig. S15 in the ESI†).

**Binding dynamics**

The binding dynamics for the association of 1 and 1/AdCA with Zn\(^{2+}\) were further measured by fluorescence stopped-flow experiments, in which the dynamic data can be simplified as a pseudo-first-order reaction in the presence of excess amounts of Zn\(^{2+}\). The observed reaction rate constant (k\(\text{obs}\)) could be determined by linear regression at different concentrations of Zn\(^{2+}\).\(^{14}\)

\[
1 + \text{Zn}^{2+} \quad k_+^{-1} \quad 1/\text{Zn}^{2+} \quad (1)
\]

\[
1/\text{AdCA} + \text{Zn}^{2+} \quad k_+^{-2} \quad 1/\text{AdCA}/\text{Zn}^{2+} \quad (2)
\]

\[
k_{\text{obs}} = k_+ [\text{Zn}^{2+}] + k_-^i \quad (i = 1, 2) \quad (3)
\]

where \(k_+^i\) and \(k_-^i\) are the rate constants for the forward and backward reactions for 1 and 1/AdCA with Zn\(^{2+}\), respectively. In the stopped-flow experiments, the values of \(k_+^1\) and \(k_+^2\) were measured as 3.94 × 10\(^{-5}\) M\(^{-1}\) s\(^{-1}\) and 1.46 × 10\(^{-6}\) M\(^{-1}\) s\(^{-1}\), respectively (Fig. 7 and Fig. S16†), indicating that the association rate of 1/AdCA with Zn\(^{2+}\) was much faster than that of 1 with Zn\(^{2+}\). This is reasonable, because there are more binding sites in 1/AdCA to accelerate the coordination of Zn\(^{2+}\) in aqueous solution.

**Confocal fluorescent images in vitro**

To gain more insight into the practical application of 1 and its corresponding supramolecular complex 1/AdCA, the fluorescence sensing behaviors of 1 and 1/AdCA toward Zn\(^{2+}\) were studied in vitro by confocal fluorescent microscopy using HeLa human cervical carcinoma cells as the model cell line. As shown in Fig. 8a–c, the HeLa cells exhibited a weak blue fluo-
rescence after incubation with 1 alone at 37 °C for 3 h, but a strong blue fluorescence was observed when the same cells were pretreated with Zn²⁺ for 0.5 h and then incubated with 1 for 3 h (Fig. 8d–f). A similar phenomenon was also observed in the case of 1/AdCA/Zn²⁺ system (Fig. 8g–i). These results indicate that, combined with their cell permeability and bio-compatibility, 1 and 1/AdCA could be used as the potential sensors for intracellular Zn²⁺ detection.

Conclusion

In conclusion, we successfully synthesized a water-soluble fluorescent Zn²⁺ sensor 1 by “click chemistry”. As investigated by fluorescent titration, it exhibited high selectivity and sensitivity toward Zn²⁺ with a 1 : 1 binding mode. Study of the binding mode further revealed that the cooperative coordination of phenanthroline and triazole moieties played an indispensable role in the fluorescence sensing of Zn²⁺. Superior to 1, the 1/AdCA system showed much stronger binding affinity, lower LOD value, and faster association rate toward Zn²⁺ in aqueous solution. The confocal fluorescent images demonstrated that 1 and 1/AdCA were cell-permeable and could effectively detect intracellular Zn²⁺ at cellular level. Although the sensitivity toward Zn²⁺ in the present work is moderate, the design strategy may act as a general and versatile platform to create an effective fluorescence Zn²⁺ sensor with a longer emission wavelength. Considering the immense advantages in preparation, water solubility, and sensing specificity for Zn²⁺, we also envision that the 1,10-phenanthroline bridged bis(β-CD) and its AdCA complex may have great application prospects in biological and environmental science and technology.

Experimental section

Synthesis of 1,10-phenanthroline bridged bis(β-CD) (1)

2,9-Bis(hydroxymethyl)-1,10-phenanthroline (90 mg, 0.285 mmol) in 10 mL THF was added to a solution of mono(6-deoxyl-
6-azido)-β-cyclodextrin (1.65 g, 1.42 mmol) in 35 mL water, then CuSO₄·5H₂O (710 mg, 2.84 mmol) and sodium ascorbate (1.4 g, 7.11 mmol) were added into the above solution. The mixture was heated at 50 °C under an atmosphere of N₂ for 2 days. The precipitate was filtered, and the filtrate was dried under reduced pressure. The residue was dissolved in a small amount of water, poured into 300 mL acetone and stirred, and then this process was repeated 3 times. Column chromatography using n-PrOH–H₂O–25% NH₄H₂O (6:3:2 v/v/v) as eluent yielded the crude product. Next, the crude product was further purified by MPLC (reversed phase) with water–ethanol (85:15 v/v) as eluent to give 1 as a pale yellow solid (240.7 mg, 28% yield). ¹H NMR (400 MHz, D₂O, ppm): δ = 3.27–3.98 (m, 84H, H of C-3, C-5, C-6, C-2, C-4 of β-CD); 4.83 (d, J = 4 Hz, 4H, –CH₂–); 4.89 (s, 4H, –CH₂–), 4.97–5.10 (m, 14H, H of C-1 of β-CD), 7.72–7.75 (m, 4H, H of phenanthroline and triazole), 8.10 (s, 2H, H of phenanthroline), 8.31 (d, J = 8 Hz, 2H, H of phenanthroline); ¹³C NMR (100 MHz, D₂O, ppm): δ = 56.1, 64.2, 65.2, 68.4, 76.3, 76.7, 77.0, 78.0, 86.1, 88.0, 106.4, 106.8, 126.8, 131.3, 131.8, 132.9, 142.7, 148.7, 148.8, 162.3 ppm; MALDI-TOF MS: m/z: 2636.88 [M + H]⁺, 2658.87 [M + Na]⁺; elemental analysis calc (%) for C₁₄₉H₁₂₅N₉O₂₀·19H₂O: C 41.94; H 6.50; N 3.76; found: C 41.85; H 6.40; N 4.10.

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References


