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Dinuclear zinc complex for fluorescent indicator-displacement assay of citrate

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ABSTRACT

Two zinc complexes based on 9,10-distyrylanthracene, 1-Zn and 2-Zn, were synthesized for fluorescence indicator-displacement assay of citrate. The fluorescence of the two complexes can be quenched significantly by energy transfer mechanism upon the binding of a common indicator – phenol red (PR). By using the chemo-ensemble of 2-Zn and PR, a linear fluorescence enhancement is observed upon the addition from 0 to 10 μM citrate ($R = 0.9947$). The detection limit is evaluated to be 0.1 μM on the basis of the $3\sigma_b$/slope. This approach showed good selectivity over other nine anions including succinate and glutarate. The association constant of 2-Zn with citrate was evaluated to be 9.2×10^5 M^{-1} by UV-titration. To our knowledge, this affinity was higher than the reported citrate receptors based on electrostatic interaction, hydrogen bonding and copper complexes. Many other diamagnetic metal complexes could be employed in this approach to detect various analytes with improved affinity.

1. Introduction

Anion sensing is one of major fields in supramolecular chemistry with potential applications in anion exchange and transport, biomedical and environmental monitoring [1–3]. For example, citrate is an important intermediate in the citric acid cycle, which is the central metabolic pathway in the cell for energy transfer and biomolecule synthesis [4]. Citrate levels in some tissues provide direct biochemical information for diagnosis of certain diseases [5–8]. Citrate concentrations in prostatic fluid samples decrease from the normal range of ~50–200 mM in healthy males to cancer levels of ~2–20 mM [8]. Thus, it is important to develop citrate sensors. In classical chemosensors, anion receptors are functionalized with chromophores to obtain absorption or fluorescent signal upon the binding of anions [9–23] including citrate [24–27]. Recently, assembled chemosensors noncovalently formed by receptors and chromophores have been widely used in anion detection [28–38]. The chromophore, usually a commercial indicator, can be displaced by an analyte that results in a change of its absorption or fluorescent spectrum. So it is also called indicator-displacement assay. Compared with "classical" chemosensors with integrated binding and signaling components, indicator-displacement assays have two major advantages [35]: (i) they are easy to generate because the indicator is attached by noncovalent interactions; (ii) the affinity of the receptor with the indicator and the indicator–receptor ratio can be varied according to specific needs.

Based on this approach, many receptors based on electrostatic interaction and hydrogen bonding have been developed for citrate detection [39]. However, since water is the most active competitor in the recognition process by strongly solvation effects and hydrogen bonding [9–12], most of these citrate receptors gave low affinities in aqueous solution, thus many of them were tested in organic solvents and their analytical applications were very limited [39–44]. As alternative, receptors based on metal–ligand coordination interaction with large enthalpies worked more effectively in aqueous solutions than other non-covalent interactions [9–23]. But only a few metal complexes were developed for citrate detection [7,8,45,46], and it is still necessary to develop citrate receptors that can work in aqueous solutions with high affinities. Moreover, most fluorescent chemo-ensembles consist of fluorogenic indicator and receptor moieties with a quencher such as a paramagnetic metal ion [28–34] or chromogenic azo-compound [36,37]. Only a few chemo-ensembles consisting of receptors and indicators as fluorophores and quenchers, respectively, have been developed [38] (Scheme 1). These reverse-type ensembles have advantages over fluorogenic sensors because that the sensitivity can be increased by eliminating the background fluorescence of the receptor, and the selectivity can be enhanced by choosing a quencher with the proper affinity for the receptor. Furthermore, comparing with those ensembles using paramagnetic metal ion such as Cu^{II}, this approach can easily

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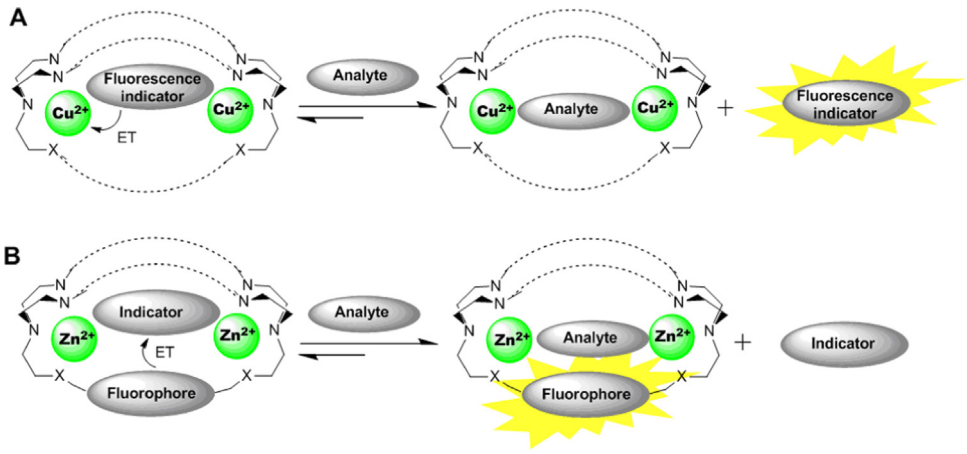

Scheme 1. Schematic of indicator-displacement assay by using Cu^{II} (A) and Zn^{II} (B) complex.

employ many other diamagnetic metal ions with improved affinity that could not quench any proximate fluorophores, and do not need other covalently linked quenchers.

Herein, we synthesized two regioisomers of dinuclear zinc complexes based on 9,10-distyrylanthracene (DSA): 1-Zn and 2-Zn (Schemes 2 and 3). Zn^{II} does not quench the fluorescence of chromophores by election transfer due to its diamagnetic property. In addition, Zn^{II} is not only low toxic to life, but also exists in vivo for maintaining physiological function [47]. DSA possess a typical aggregation-induced emission property [48–50]. The fluorescence of the complexes could be quenched by phenol red (PR). Upon replaced by target anions, PR would be released, simultaneously the coordination between the dinuclear zinc complexes and target anion would restrict the intramolecular rotation of the DSA that could resulted in fluorescence enhancement. The two regioisomers with coordination centers linked at *para* and *ortho* position of benzene of DSA may bind anions with different mode or affinity which may lead to different fluorescent response. After optimization, the chemo-ensemble of 2-Zn and PR was successfully used for quantitative detection of citrate (Scheme 4).

2. Materials and methods

2.1. General information

^{1}H NMR and ^{13}C NMR spectra were measured on a MECUYRVX300 spectrometer. Elemental analyses of carbon, hydrogen, and nitrogen were performed on a Vario EL III microanalyzer. EI-MS spectra were measured on a ThermoDSQ II 2000 spectrophotometer. ESI-MS spectra were measured on a Waters Micromass ZQ Mass Spectrometer. UV–vis absorption spectra were recorded on a Shimadzu UV-2500 recording spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer. Subsequent fluorescence titration experiments were carried out at room temperature by addition of sodium salts of oxyacid dissolved in distilled water into the buffer solutions. All the solutions were mixed fully and then stood for 5 min before the fluorescence intensity were measured. The solution of sodium pyrophosphate was prepared before measuring.

2.2. Syntheses and characterization

Bis(pyridin-2-ylmethyl)amine (DPA) [23], 3 and 4 [51] were synthesized according to the literature procedure. All the other reagents were commercially available and used without further purification.

2.2.1. Synthesis of 9,10-bis(chloromethyl)anthracene (**3**)

To a mixture of anthracene (1.78 g, 10 mmol), paraformaldehyde (1.5 g, 50 mmol) and $ZnCl_2$ (1.6 g, 12 mmol) in 1,4-dioxane (20 ml) was added dropwise 40 ml of hydrochloric acid, then the mixture was refluxed for 3 h. The precipitate was collected by filtration, washed with water and 1,4-dioxane. The solid was recrystallized from toluene and dried overnight in vacuo to give 1.4 g of **3** (yield: 50%). ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 8.42–8.36 (m, 4H), 7.70–7.63 (m, 4H), 5.62 (s, 4H).

2.2.2. Synthesis of tetraethyl (anthracene-9,10-diylbis(methylene))bis(phosphonate) (**4**)

A mixture of **3** (2.0 g, 7.3 mmol) and 10 ml of triethylphosphite was stirred at 160 °C for 24 h. The excess of triethylphosphite was distilled off under reduced pressure. The crude product was purified by a silica gel column using chloroform/methanol (10:1, v/v) as eluent to give 3.2 g of **4** (yield: 90%). ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 8.41–8.35 (m, 8H), 7.60–7.54 (m, 4H), 4.25 (d, J = 20.1 Hz, 4H), 3.95–3.75 (m, 8H), 1.07 (t, J = 7.2 Hz, 12H).

2.2.3. Synthesis of ***5a*** and ***5b***

The syntheses were similar to the reference [52].

To a mixture of 4-hydroxybenzaldehyde (2.44 g, 20 mmol) and 1,2-dibromoethane (18.8 g, 100 mmol) in acetone (100 ml) was added potassium carbonate (8.7 g, 60 mmol). The mixture was refluxed under stirring for 24 h. After filtration and solvent evaporation, the crude product was purified by a silica gel column using chloroform as eluent to give 4 g of **5a** as white powder (yield: 90%). ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 9.88 (s, 1H), 7.83 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.7 Hz, 2H), 4.37 (t, J = 6 Hz, 2H), 3.67 (t, J = 6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 190.77, 163.00, 132.00, 130.31, 114.83, 67.90, 28.71. EI-MS: m/z $[M]^+$: 229.92.

The synthesis of **5b** was similar to that of **5a**. The product was white powder (yield: 60%). ^{1}H NMR ($CDCl_3$, 300 MHz), δ (TMS, ppm): 10.55 (s, 1H), 7.87 (d, J = 7.5 Hz, 1H), 7.56 (t, J = 7.2 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H), 6.97 (d, J = 8.7 Hz, 1H), 4.43 (t, J = 6 Hz, 2H), 3.72 (t, J = 6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 189.49, 160.31, 135.92, 128.20, 124.98, 121.34, 112.66, 67.09, 29.02. EI-MS: m/z $[M]^+$: 229.90.

2.2.4. Synthesis of ***6a*** and ***6b***

To a mixture of **5a** (1.58 g, 20 mmol) and DPA (1.38 g, 6.9 mmol) in acetonitrile (180 ml) was added potassium carbonate (3.8 g, 30 mmol). The mixture was refluxed under stirring for 24 h. After filtration and solvent evaporation, the crude product was purified by a silica gel column using chloroform/methanol/triethylamine