

Ammonium-Bearing Dinuclear Copper(II) Complex: A Highly Selective and Sensitive Colorimetric Probe for Pyrophosphate

Wenxiang Yu,[†] Jian Qiang,[†] Jun Yin,[‡] Srinivasulu Kambam,[†] Fang Wang,[†] Yong Wang,[†] and Xiaoqiang Chen^{*,†}

[†]State Key Laboratory of Materials-Oriented Chemical Engineering, College of Chemistry and Chemical Engineering, Nanjing Tech University, Nanjing 210009, P. R. China

[‡]Key Laboratory of Pesticide and Chemical Biology, Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, P. R. China

Supporting Information

ABSTRACT: Two dinuclear copper complexes with and without ammonium moieties were synthesized. The complexes exhibited selective binding affinity to pyrophosphate in aqueous solution. The dinuclear copper complex, with ammonium arms, showed a ca. 527-fold enhancement in pyrophosphate binding affinity compared with its analogue without ammonium units.



The development of recognition systems for anions has L attracted increasing attention in recent years because of their potential application in clarifying the function of anions in biological processes.¹ Pyrophosphate (P₂O₇⁴⁻, PPi) plays an important role in bioenergetic and metabolic processes.² Metal complex based chemosensors for PPi in conjunction with indicator displacement assays (IDAs) are highly interesting to researchers because the sensing assays can be carried out in aqueous media.³ Among various indicators, pyrocatechol violet (PV) is the most popular for anion detection. PV exhibits a remarkable color change between blue and yellow, which corresponds to associated and disassociated forms with metal complexes, respectively, thus allowing naked eye recognition and sensing. This IDA strategy is based on the displacement of the indicator attached onto the metal complex by a noncovalent model of the analyte ion, thus avoiding the synthesis through covalent linking with the receptor and signal reporter. In the past, most of the receptors based on zinc complexes bearing a dipicolylamino structure as phosphate-coordinated pockets have been reported,⁴ where the bis-dipicolylamino-based zinc complexes exhibited high affinity and selective binding with PPi and other phosphates. Conversely, few transition-metal complexes derived from bis-2-((pyridin-2-ylmethylamino)methyl)phenol were used as receptors for PPi even though the related dinuclear Cu, Zn, and Fe complexes have been reported.5

In nature, the high affinity and reactivity of nucleases is attributed to cooperation between metal ions and several functional groups of the amino acid side chains present in the active site.⁶ The positively charged residues of amino acids are thought to assist and stabilize the binding status between metal ion centers and phosphates through electrostatic interactions, hydrogen bonding, and proton transfer.⁷ Therefore, introducing some hydrogen-bonding donors to a dinuclear metal complex as the PPi receptor is a desirable strategy to enhance the binding affinity with PPi. In this study, we report two dinuclear copper complexes, namely, $Cu-L_a$ and $Cu-L_b$. We used bis-2-((pyridin-2-ylmethylamino)methyl)phenol as the coordinated unit (Figure 1) for the detection of PPi using





the IDA method. The ligand based on bis-2-(((pyridin-2-ylmethylamino)methyl)phenol is easier to modify by approach compared with the bis-dipicolylamino-based donors. The design of the new receptor complex $Cu-L_b$ with two ammonium arms in the scaffold was inspired by nucleases. We expected $Cu-L_b$ to show stronger binding affinity with PPi through electrostatic interactions, hydrogen bonding, and proton transfer.

 L_a was synthesized according to a previous report.^{5b} The synthesis of L_b is depicted in Scheme 1. The synthesis starts with 2-aminomethylphenol, stirred with phthalic anhydride to

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Scheme 1. Synthesis Procedure for L_b



yield phthalimidoyl group labled as compound 1. After formylation, reduction, and chlorination, compound 4 was obtained. The reaction of 4-methyl-2,6-bis((pyridin-2ylmethylamino)methyl)phenol with compound 4 resulted in compound 5. The target ligand L_b was acquired with deprotection treatment using N₂H₄·H₂O. These compounds were characterized by NMR and high-resolution mass spectra (see Figures S1–S15 in the Supporting Information). Cu–L_a or Cu–L_b complexes was obtained by mixing the L_a or L_b with 2 equiv of Cu(ClO₄)₂ in aqueous solution.⁸ The formation of dinuclear copper(II) complexes was supported by the corresponding mass spectra (see Figures S16 and S17 in the Supporting Information).

The UV-vis spectra of PV with the increasing addition of complexes are illustrated in Figure 2. The absorption band



Figure 2. (a) Changes in absorbance of PV (20 μ M) upon addition of various concentrations of **Cu–L**_a (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 28, 32, 36, and 40 μ M). (b) Changes in absorbance for PV (20 μ M) upon addition of various concentrations of **Cu–L**_b (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 28, 32, 36, and 40 μ M). The spectra were obtained in the HEPES buffer (10 mM, pH 7.0).

centered at 444 nm decreased, whereas the absorption peaks at 685 nm for $Cu-L_a$ and 630 nm for $Cu-L_b$ increased when $Cu-L_a$ or $Cu-L_b$ complex was added to HEPES buffer (10 mM, pH 7.4) containing PV (20 μ M). This result indicated that free PV bonded with the complexes. Clear color changes from yellow to light-green for $Cu-L_a$ and yellow to blue for $Cu-L_b$ were observed by the naked eye when the complexes were added to the solution containing PV (see Figure S18 in the Supporting Information). Using the Job's plot method, the stoichiometry of the binding between PV and Cu-La or Cu- L_b was determined to be 1:1 (see Figure S19 in the Supporting Information). The absorption at 444 nm of PV depended on the concentration of Cu-L_a or Cu-L_b and described as Figure S20 in the Supporting Information. After fitting of the titration curves, the association constants (K_a) for PV were found to be 3.54×10^5 M⁻¹ for Cu–L_a and 1.90×10^7 M⁻¹ for Cu–L_b, thus

Afterward, $\mathbf{Cu}-\mathbf{L}_{a}-\mathrm{PV}$ and $\mathbf{Cu}-\mathbf{L}_{b}-\mathrm{PV}$ ensembles were utilized in anion assays. $\mathbf{Cu}-\mathbf{L}_{a}-\mathrm{PV}$ and $\mathbf{Cu}-\mathbf{L}_{b}-\mathrm{PV}$ ensembles were prepared by mixing PV with the equivalent $\mathbf{Cu}-\mathbf{L}_{a}$ or $\mathbf{Cu}-\mathbf{L}_{b}$ complex, and then different anions (10 equiv), such as PPi, HPO₄²⁻, Ac⁻, SO₄²⁻, CO₃²⁻, F⁻, Cl⁻, Br⁻, and I⁻, were added to the solution containing $\mathbf{Cu}-\mathbf{L}_{a}-\mathrm{PV}$ (20 μ M) or $\mathbf{Cu}-\mathbf{L}_{b}-\mathrm{PV}$ (20 μ M). The absorption spectra of the $\mathbf{Cu}-\mathbf{L}_{a}-\mathrm{PV}$ and $\mathbf{Cu}-\mathbf{L}_{b}-\mathrm{PV}$ ensembles showed that PPi induced a remarkable change whereas other anions did not induce a significant change (Figure 3). PPi also caused a color



Figure 3. Changes in the absorbance of $\mathbf{Cu}-\mathbf{L}_{a}-\mathrm{PV}$ and $\mathbf{Cu}-\mathbf{L}_{b}-\mathrm{PV}$ ensembles upon the addition of various anions, such as PPi, HPO₄²⁻, Ac⁻, SO₄²⁻, CO₃²⁻, F⁻, Cl⁻, Br⁻, and I⁻. The spectra were obtained in the HEPES buffer (10 mM, pH 7.0): (a) $\mathbf{Cu}-\mathbf{L}_{a}-\mathrm{PV}$ (20 μ M) in the presence of concentrations of 10 equiv of anion; (b) $\mathbf{Cu}-\mathbf{L}_{b}-\mathrm{PV}$ (20 μ M) in the presence of concentrations of 10 equiv of anion.

change from blue to yellow in the $Cu-L_b-PV$ ensemble, and $Cu-L_a-PV$ showed a color change from light-green to yellow. In contrast, other anions did not induce any color change (see Figure 4 and Figure S21 in the Supporting Information). These results suggested that both $Cu-L_a-PV$ and $Cu-L_b-PV$ have good selectivity for PPi detection.



Figure 4. Color of the $Cu-L_b-PV$ ensemble (20 μ M) with and without the addition of various anions (200 μ M). From left to right: no anion, PPi, HPO₄²⁻, Ac⁻, SO₄²⁻, CO₃²⁻, F⁻, Cl⁻, Br⁻, and I⁻.

The UV-vis absorption spectra were obtained as various concentrations of PPi were titrated into the HEPES buffer (10 mM, pH 7.0) containing $Cu-L_a-PV$ (20 μ M) or $Cu-L_b-PV$ (20 μ M). Upon the addition of PPi, the absorption peak of the ensembles at 444 nm increased while the peaks at 685 nm for $Cu-L_a-PV$ and 630 nm for $Cu-L_b-PV$ decreased, indicating that the free PV was released from the ensembles (Figure 5). We calculated the association constants of complexes Cu-L_a and Cu-L_b with PPi (see Figure S22 in the Supporting Information) based on the curve fittings from the titration data. The association constants were obtained to be $1.09\times10^4~M^{-1}$ for the binding between $\text{Cu}{-}\text{L}_{a}$ and PPi and 5.75 \times $10^{6}~\text{M}^{-1}$ for the binding between $\mathbf{Cu}{-}\mathbf{L}_{b}$ and PPi. The introduction of ammonium moieties to dinuclear copper complex led to a ca. 527-fold enhancement in PPi binding affinity. Higher binding affinity is observed between Cu-Lb and PPi compared with



Figure 5. Changes in absorbance of $Cu-L_a-PV$ and $Cu-L_b-PV$ ensembles upon the addition of various concentrations of PPi (0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 240, 280, 320, 360, and 400 μ M). Spectra were obtained in HEPES buffer (10 mM, pH 7.0): (a) $Cu-L_a-PV$ (20 μ M) in the presence of various concentrations of PPi; (b) $Cu-L_b-PV$ (20 μ M) in the presence of various concentrations of PPi.

 $Cu-L_a$. This finding can be attributed to the cooperation between copper ions and neighboring ammonium moieties (Scheme 2). The positively charged ammonium ions are





thought to assist copper ion to binding with PPi through electrostatic interactions, hydrogen bonding, or proton transfer. PPi can efficiently displace the indicator of $Cu-L_b-PV$ ensemble and demonstrate color change from blue to yellow because of the suitable scaffold and positive effect provided by ammonium moieties. The addition of PPi also induced the IR absorption band of $Cu-L_b$ at 3470 cm⁻¹ shift to 3440 cm⁻¹. We speculated that the binding between ammonium and PPi caused the change of stretching vibration peak of N-H bond (see Figure S23 in the Supporting Information).

Given that PPi has higher selectivity compared with HPO₄²⁻, $Cu-L_b-PV$ was applied to monitor the activity of pyrophosphatase (PPase), which is highly active in catalyzing the cleavage reaction of PPi to HPO₄²⁻ in the presence of Mg²⁺. In the presence of both Mg²⁺ and PPase, the absorbance ratio of 630 and 444 nm increased after a 40 min induction period and reached a plateau at 65 min. Thus, the reaction of PPi cleavage to HPO₄²⁻ was finished after 25 min. By contrast, the absence of Mg²⁺ or PPase caused no change in the absorbance of Cu-L_b-PV (see Figure 6 and Figure S24 in the Supporting Information). These results indicated that Cu-L_b-PV is applicable in PPase activity monitoring.



Figure 6. Time-related ratio of absorption intensity at 444 and 630 nm in the HEPES buffer of Cu–L_b–PV (20 μ M) combined with PPi (200 μ M) and different catalysts: PPase (10 units); Mg²⁺ (20 μ M); PPase (10 units) + Mg²⁺ (20 μ M).

In summary, using ((pyridin-2-ylmethylamino)methyl)phenol as a coordinated unit, we reported two dinuclear copper complexes $Cu-L_a$ and $Cu-L_b$ for the detection of PPi. Both complexes showed good selectivity for binding with PPi. Compared with the complex $Cu-L_a$, the complex $Cu-L_b$ bearing ammonium arms exhibited a 527-fold enhancement in the binding affinity to PPi, which should be attributed to the electrostatic interactions, hydrogen bonding or proton transfer from ammonium ions. Furthermore, $Cu-L_b$ -PV was successful to monitor the cleavage reaction of PPi catalyzed by PPase.

ASSOCIATED CONTENT

Supporting Information

Experimental details; synthesis and characterization of L_b ; spectroscopic data; Job's plot of the binding between $Cu-L_a$ or $Cu-L_b$ and PV; calculation and fitting of K_a . This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: chenxq@njtech.edu.cn.

Notes

The authors declare no competing financial interest.

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(8) *Caution*! Perchlorate salts of metal complexes are potentially explosive. Only small quantities of material should be prepared, and the samples should be handled with care. See: Churchill, D. G. *J. Chem. Educ.* **2006**, *83*, 1798–1803.