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Communication

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Fluorescence Detection of Adenosine Triphosphate in an Aqueous Solution Using a Combination of Copper(II) Complexes

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Supporting Information

ABSTRACT: Fluorescent ligands have been designed to form ternary complexes with a Cu^{II} cation and phosphates in a buffer solution at physiological pH 7.4. It has been shown that a combination of two different ligands and $CuCl_2$ allows one to achieve high adenosine triphosphate/adenosine diphosphate, adenosine 5'-monophosphate selectivity, and ratiometric fluorescence sensing, while separately each ligand complex does not have such properties.

The recognition and sensing of biologically important phosphate-containing molecules using artificial receptors has attracted considerable attention in recent years.[1] Highly selective receptors have great potential in bioanalytical applications.[2] Among phosphates adenine containing nucleotides are of particular interest because they are a universal energy source and are intracellular mediators in many biological processes. In cells, adenosine triphosphate (ATP) is enzymatically hydrolyzed to PPi (pyrophosphate) and adenosine 5'-monophosphate (AMP) or Pi (orthophosphate) and adenosine diphosphate (ADP). This imposes certain conditions on a sensor to have good selectivity between these anions.[3] The incorporation of several different binding sites in one receptor or the use of an array of different receptors has allowed one to achieve good selectivity between phosphates,[4] e.g., hydrogen bonding,[5] ion−ion interaction,[6] $\pi-\pi$ interaction,[7] or combinations of them.[8] Dizinc(II)-containing sensors possess excellent affinity for phosphates among others.[6,9] Copper(II)−phosphate interactions are stronger; however, there are only a few reports about their use in recognition of nucleotides.[10] Although metal-based receptors have a good affinity for phosphates in an aqueous solution, they lack the desired selectivity. Recently, we have shown that a combination of up to four zinc(II) binding sites in one receptor leads to high affinity ($\log K > 7$) of the receptor for oligopeptides containing several carboxylate groups.[11] The selective recognition of molecules containing different functional groups will then require the presence of different binding sites in the structure of the receptor. In this work, we present a new approach for fluorescent sensing of phosphates based on the design of relatively simple fluorescent ligands that can form ternary complexes with copper and phosphates. In such ligand−metal−analyte complexes, the ligand functions as a key component that determines the selectivity and generates an analytical signal. We show that the combination of two different ligands and a copper(II) salt allows one to achieve high ATP/ADP, AMP selectivity, and ratiometric sensing, while separately each ligand complex does not have such properties.

Ligands **1** and **2** (Figure 1) were suggested to possess the required properties. Our approach is based on the combination

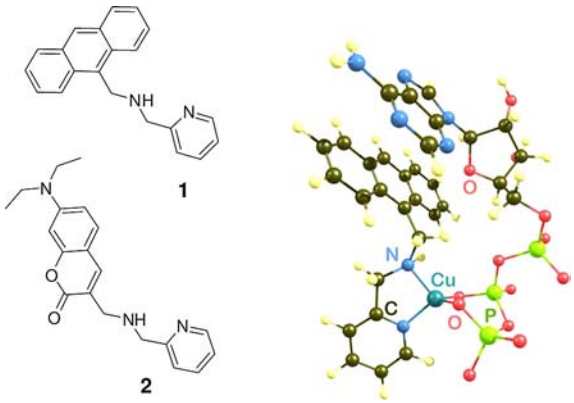

Figure 1. Structures of investigated ligands **1** and **2**. The structure of complex $[Cu(\mathbf{1})ATP]^{2-}$ according to DFT calculations, showing the interaction of adenine with anthracene.

of two strong interactions between a sensor and adenine nucleotide, namely, the copper(II)−phosphate interaction and $\pi-\pi$ interactions between adenine and anthracene. 2-(Aminomethyl)pyridine serves as a coordination site for Cu^{II} cations;[12] however, it occupies only two coordination places of the metal. Thus, ligands **1** and **2** leave the possibility for copper to coordinate analytes. Density functional theory (DFT) calculations[13] were performed to optimize the linker between the Cu^{II} coordination site and anthracene. The structure of the optimized complex is shown in Figure 1, where anthracene and adenine form $\pi-\pi$ interactions. The size of ligand **1** better matches ATP than AMP or ADP because Cu^{II} likes to coordinate two phosphate residues.[14] These two particular dyes were chosen because anthracene is known to form $\pi-\pi$ interactions with adenine[15] and the coumarine dye has additional coordinating heteroatoms. The presence of the weakly coordinating heteroatom in the structure of the ligand is important for realization of the "turn-on" mechanism of fluorescence sensing of phosphates.[16] The direct interaction of a fluorophore with Cu^{II} cations usually leads to the

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quenching of fluorescence because of the energy-transfer process.[17] The coordination of an analyte can cleave the bond between Cu^{II} and the weakly coordinating heteroatom, thus resulting in the recovery of fluorescence. Such a mechanism of intramolecular displacement was recently realized by us for amine recognition, where not only cleavage of the bond but also considerable rearrangement of the receptor structure was observed.[18]

In order to evaluate the fluorescence response in the presence of the phosphates, ligands **1**, **2**, and $CuCl_2$ were mixed in a 50 mM Tris buffer (8 vol % MeOH, 100 mM NaCl, pH 7.4) in a 1:1:1 ratio and the fluorescence response for each phosphate was recorded (Table S2 in the Supporting Information, SI). We also tested zinc(II) salts, but the affinity of the metal for the ligands was not enough to form stable complexes in such a competitive media. After the addition of ATP to the mixture of the ligands and $CuCl_2$, the produced fluorescence changes were different from those of ADP, AMP, PPi, and Pi. Further variation of the $CuCl_2$ portion in the mixture proved that 0.5 equiv of the salt relative to the ligand amount is optimum to achieve maximum selectivity for ATP among the phosphates. From Figure 2, it can be seen that our

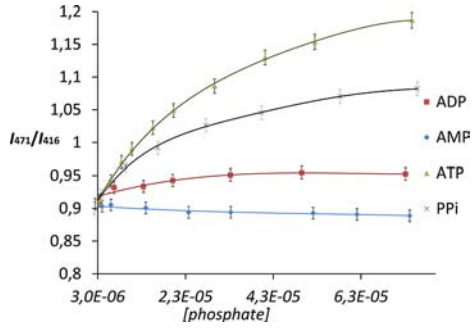

Figure 2. Changes in the ratio of the coumarine and anthracene intensities induced by the addition of phosphates to a mixture of **1**, **2**, and $CuCl_2$ (10^{-5} M); excitation at 366 nm.

sensing system has high ATP/ADP and AMP selectivity in the analyte concentration range 10^{-6}–10^{-4} M. The interaction with the orthophosphate anion (Pi) did not induce any considerable fluorescence changes.

To clarify the origin of high ATP/ADP and AMP selectivity, the properties of each ligand were investigated in the presence of $CuCl_2$ and nucleotides. According to the fluorescence, UV–vis titrations, and Job plot analyses, the ligands coordinate one Cu^{II} cation with log K = 5.80(2) for ligand **1** and 6.40(1) for ligand **2**. Upon coordination of Cu^{II}, the fluorescence of the ligands is quenched (Figure S3 in the SI). The preference for the formation of complex $Cu(\mathbf{1})Cl_2$ was observed also in a methanol solution, and we could obtain single crystals suitable for X-ray structure determination (Figure 4). Ligand **2** was crystallized from acetonitrile with copper(II) perchlorate in a 2:1 stoichiometry. Unfortunately, there was no possibility to obtain crystals for both complexes in the same solvent because of the low solubility of the complexes produced from ligand **1**.

Potentiometric titrations were carried out in water with ligands **1** and **2** separately to prove the fact that both ligands form 1:1 complexes with $CuCl_2$ and $Cu(ClO_4)_2$. The amount of complex L_2Cu^{2+} present in solution is considered rather low; e.g., for **1**, β_{11} = 7.36(3) and β_{21} = 9.87(8). Because ligands coordinate $CuCl_2$ relatively fast according to the fluorescence titrations, we prepared complexes in situ prior to titration experiments with analytes ATP, ADP, AMP, and PPi. Upon the addition of ATP and ADP to complex $[Cu(\mathbf{1})]^{2+}$ in a Tris buffer, fluorescence quenching was observed (I_0/I is 1.7 and 1.2 for 10 equiv of ATP and ADP, respectively), while the addition of AMP and PPi led to negligible quenching of fluorescence (Table S2 in the SI). This behavior is attributed to the strong π–π interactions between adenine and anthracence, which is the result of the good geometry match in ternary complex $[Cu(\mathbf{1})ATP]^{2-}$ (Figure 1). Although the degree of quenching for the analytes was different (Table S2 in the SI), the binding constants did not differ more than 1 order of magnitude: log K values for ATP, ADP, AMP, and PPi are 5.52(1), 4.42(2), 4.50(8), and 4.01(4), respectively. Interestingly, for the complex with ligand **2**, an opposite situation was observed: log K values for ATP, ADP, AMP, and PPi are 3.71(1), 4.34(2), 4.98(2), and 4.30(2), respectively. The fluorescence response for ATP, ADP, and AMP was small, while for PPi, a considerable increase of fluorescence was detected. As can be seen from Figure 2, the ATP/PPi selectivity is lower than that of ATP/ADP and AMP. This fact is explained by the different responses of ligands **1** and **2** toward phosphates. In the case of ATP addition, the anthracene fluorescence is decreased and the coumarine fluorescence is increased (Figure 3). The addition of

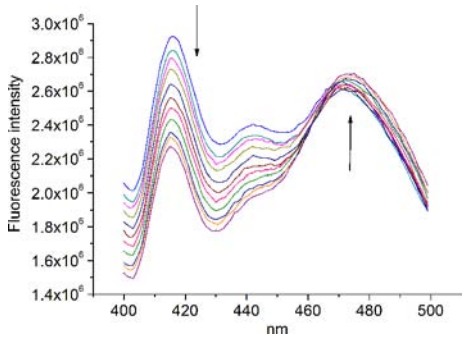

Figure 3. Fluorescence changes induced by the addition of ATP to a 1:1:1 mixture of ligands **1**, **2**, and $CuCl_2$ in a buffer solution. The concentration of each component is 10^{-5} M.

ADP or AMP leads to small and equal changes of both bands. Upon the addition of PPi, the coumarine band grows faster than that of the anthracene ligand (Figures S6 and S7 in the SI).

In contrast to the anthracene-containing complex, the coumarine-containing complex increases the fluorescence upon the addition of ATP, even though the stability constant between **2** and $CuCl_2$ is higher. This fact implies that ligand **2** is not displaced by ATP. The higher complex stability for **2** can be attributed to the additional coordination of the carbonyl oxygen on the Cu^{II} ion, as we observed in the X-ray structure (Figure 4). It is proposed that by the addition of phosphates the coordination bond between the carbonyl group and Cu^{II} ion is cleaved; thus, the energy transfer between fluorophore and the transition-metal cation is less efficient, and an increase of fluorescence is observed. Additional evidence of the stability of the ternary complex $[Cu(\mathbf{2})(ATP)]^{2+}$ was obtained from UV–vis titrations, where the band responsible for the coumarine dye was not shifted toward the position of the free ligand upon the addition of 10 equiv of ATP (Figure S11 in the SI).

To assess the stability constants in the metal–ligand–analyte complexes, potentiometric titrations were carried out. Similar to

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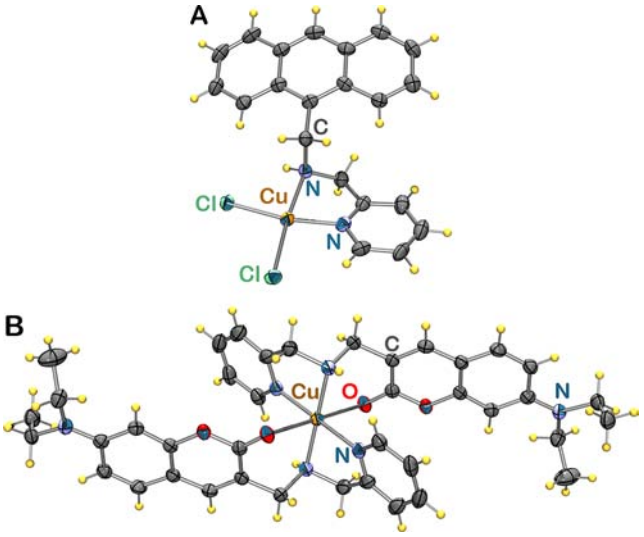

Figure 4. ORTEP Pov-ray-rendered molecular structure of complexes Cu(**1**)Cl_2 (A) and Cu(**2**)$_2$(ClO_4)$_2$ (B) according to the single-crystal X-ray analysis. Perchlorate anions are omitted for clarity.

fluorescence titrations, the stability between complex $[Cu(\mathbf{1})]^{2+}$ and ATP is higher than that between $[Cu(\mathbf{2})]^{2+}$ and ATP: log β_{111} = 16.04(12) and log β_{111} = 13.59(12) for **1** and **2**, respectively. Typical potentiometric titration curves for the mixture of ligand **1**, $CuCl_2$, and ATP are shown in Figure 5.

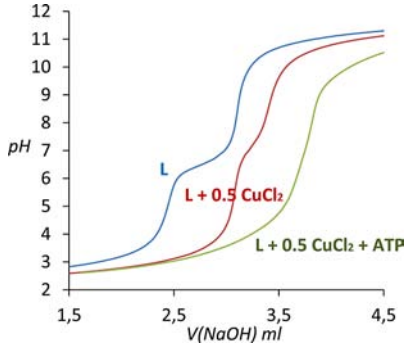

Figure 5. Potentiometric titrations of ligand **1** (0.002 M), complex of **1** with copper(II) chloride, and complex of **1** with copper(II) chloride and ATP.

Ternary complexes with nucleotides were possible to observe using electrospray ionization mass spectrometry. The samples for the analysis were prepared as mixtures of a ligand, $CuCl_2$, and ATP in a ratio 1:1:2. In both cases, we observed the formation of ternary complexes $[Cu(L)NaH_2(ATP)]^+$ with m/z 889.06 and 928.05 for ligand (L) **1** and **2**, respectively (Figures S19−21 in the SI).

In summary, we have successfully designed two fluorescent ligands that can form ternary complexes with the Cu^{II} cation and phosphates (ATP, ADP, AMP, and PPi) in aqueous solution at physiological pH 7.4. The fluorescence responses of in situ prepared complexes from ligands and $CuCl_2$ towards phosphates are different for each ligand because of the different mechanisms of signal transduction: fluorescence quenching through the $\pi-\pi$ interactions and the intramolecular displacement mechanism induced by analyte coordination. Currently, we are testing the design, where two dyes are covalently connected to each other. This will bring robustness to the sensor and will allow one to achieve new selectivities.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

X-ray crystallographic data in CIF format (CCDC 875398 and 875399), synthesis of compounds, potentiometric data, and UV−vis and fluorescence data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

This article is dedicated to Prof. Yuri A. Ustynyuk on the occasion of his 76th birthday.

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