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# A simple and efficient fluorescent sensor for histidine†

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**A simple coordination complex terpyridine–$CuCl_2$ is found to be an efficient fluorescent sensor for histidine in aqueous solution with up to 1004 fold fluorescence enhancement.**

Histidine is an essential amino acid for human growth.[1] It can also act as a neurotransmitter in the central nervous system of mammals.[2] Recently, it was found that the impaired nutritional state of patients with chronic kidney disease could be attributed to the deficiency of histidine.[3] Histidine-rich proteins are found to play many important roles in humans and their abnormal level could indicate a variety of diseases.[4] Therefore, detection of histidine in biological fluids has become an important goal and a number of methods have been developed for this purpose.[5–7] In these studies, several molecular fluorescent sensors have been prepared.[5] These studies have demonstrated that the high sensitivity of fluorescence, the easy availability of instruments and the potential for remote observation make the use of fluorescence sensors very attractive in the detection of L-histidine in biological analysis. Herein, we report our discovery that a simple $TpyCu^{2+}$ (Tpy: 2,2′,2″-terpyridine) complex is an efficient fluorescent sensor for histidine in aqueous solution. No ligand synthesis is required for the use of this sensor.

$TpyCuCl_2$ is a classical coordination complex first prepared by Morgan and Burstall in 1937 from the reaction of Tpy with $CuCl_2$ in water.[8,9] The distorted trigonal bipyramidal structure of this complex in solid state was later established by X-ray analysis.[10] The formation constant of the 1 : 1 complex of $Cu^{2+}$ with Tpy in aqueous solution was found to be $\sim 10^{13}$.[11] In 50% (v/v) ethanol–water solution, $TpyCu^{2+}$ can bind to amino acids such as glycine, valine and isoleucine to form TpyCu(II)–amino acid complexes.[12] Mass spectroscopic study also showed the formation of the TpyCu(II)–histidine complex from the reaction of $TpyCu^{2+}$ with histidine in a 50/50 water/methanol solution.[13] In spite of these studies, however, no fluorescence response of the $TpyCu^{2+}$ complex in the presence of amino acids was investigated before.

We have studied the fluorescence properties of the TpyCu(II) complex. In aqueous solution at neutral pH, titration of Tpy with $CuCl_2$ led to diminished fluorescence at 1 equiv. $CuCl_2$ (Fig. 1a). That is, the formation of the TpyCu(II) complex almost completely quenched the fluorescence of Tpy. However, when the aqueous solution of $TpyCuCl_2$ was treated with L-histidine, a large fluorescence enhancement was observed (Fig. 1b). For example, in the presence of $1.0 \times 10^{-4}$ M L-histidine, the fluorescence intensity of $TpyCuCl_2$ ($2.0 \times 10^{-5}$ M in hepes buffer solution at pH = 7.35) at $\lambda$ = 352 nm was increased to 712 times of the original value. The fluorescence enhancement reached saturation (1004 times of the original value) when the concentration of L-histidine was greater than 12 equiv. of $TpyCuCl_2$.

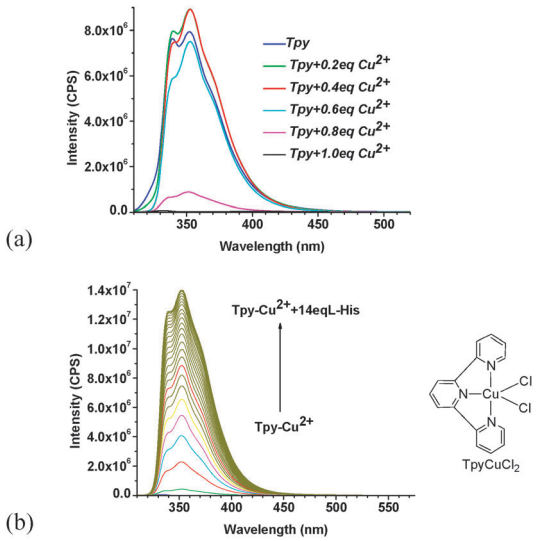


**Fig. 1** Fluorescent responses of Tpy ($2.0 \times 10^{-5}$ M) toward $CuCl_2$ (a), and those of $TpyCuCl_2$ ($2.0 \times 10^{-5}$ M) toward L-histidine (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.5, 12.0, 13.0 and 14.0 equiv.) (b) in 25 mM hepes buffer solution (pH = 7.35) ($\lambda_{exc}$ = 298 nm, slits: 5 nm/5 nm).

We have examined the fluorescence response of $TpyCuCl_2$ toward a variety of natural amino acids in aqueous solution. As shown in Fig. 2a and b, unlike L-histidine, all the other amino acids could not enhance the fluorescence of $TpyCuCl_2$ except L-cysteine when excited at 298 nm. Because L-cysteine can be easily oxidized to cystine, the dimer of L-cysteine with a disulfide bond, which has very low solubility in water at neutral pH and gives much smaller fluorescence enhancement on $TpyCu^{2+}$, the effect of L-cysteine can be readily distinguished from that of L-histidine (see Section IV in ESI†). In Fig. 2a, we also showed that imidazole and its methyl derivatives had little

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† Electronic supplementary information (ESI) available: Detailed experimental procedures, additional plots of UV, fluorescence, CV and mass analysis data. See DOI: 10.1039/c2cc17156b

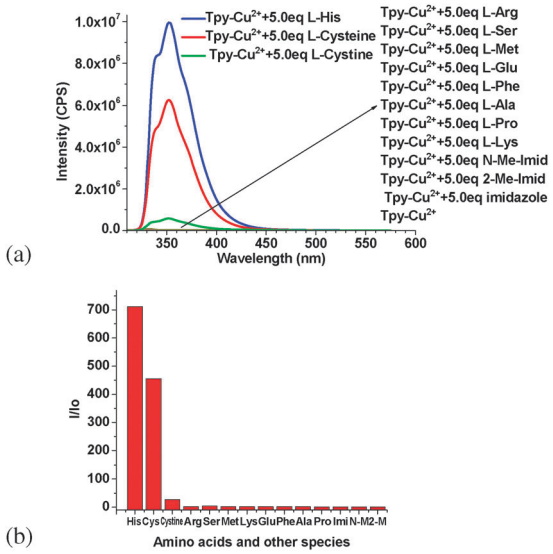


**Fig. 2** Fluorescent responses of $TpyCu^{2+}$ ($2.0 \times 10^{-5}$ M in 25 mM hepes buffer solution, pH = 7.35) toward amino acids and others ($1.0 \times 10^{-4}$ M) (a), and the bar graphs of the fluorescence intensity at $\lambda$ = 352 nm (b) ($\lambda_{exc}$ = 298 nm, slits: 5 nm/5 nm).

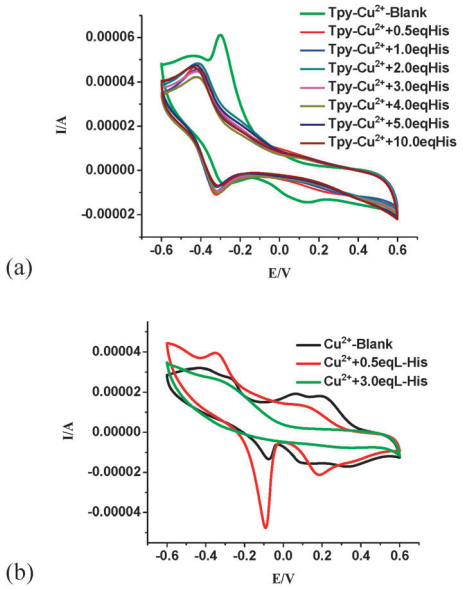


**Fig. 3** CV titration profiles of (a) $TpyCu^{2+}$ (1 mM) with various amounts of L-histidine and (b) $Cu^{2+}$ (1 mM) with 0, 0.5 and 3.0 equiv. L-histidine.

influence on the fluorescence of $TpyCu^{2+}$. This indicates that the interaction of $TpyCu^{2+}$ with the imidazole ring of L-histidine *alone* cannot produce the observed large fluorescence enhancement. Because the amino acids L-tryptophan and L-tyrosine have absorptions at $\leq$310 nm, we have compared the fluorescence responses of $TpyCuCl_2$ toward these two amino acids with those toward L-histidine by using a longer excitation wavelength $\lambda_{exc}$ = 320 nm. When excited at 320 nm, $TpyCuCl_2$ also exhibited much greater fluorescence enhancement with L-histidine than with L-tryptophan and L-tyrosine (see Fig. S5–S11 in ESI†).

There are two possible mechanisms for the observed fluorescence enhancement: (a) L-histidine displaces the $Cu^{2+}$ center off $TpyCu^{2+}$ to release free Tpy and restore its fluorescence; (b) L-histidine coordinates to $TpyCu^{2+}$ to form a highly fluorescent ternary TpyCu(II)–histidine complex. In order to distinguish these two mechanisms, we have obtained the cyclic voltammogram (CV) of $TpyCu^{2+}$ in the presence of L-histidine. Fig. 3a gives the CVs of $TpyCu^{2+}$ upon treatment with various equivalents of L-histidine. The CVs of the $Cu^{2+}$ aqueous solution treated with L-histidine in the *absence* of Tpy are given in Fig. 3b. Because the CV waves of $TpyCu^{2+}$ after treating with excess L-histidine are very different from those of $Cu^{2+}$ with L-histidine in the *absence* of Tpy, it indicates that L-histidine does not displace $Cu^{2+}$ off the Tpy ligand in the aqueous solution. Fig. 3a gives a reversible redox couple for the ternary TpyCu(II)–histidine complex which is assigned to the Cu(II)/Cu(I) couple with $E_{1/2}$ = −0.366 V [reference electrode: saturated Hg(l)/$Hg_2Cl_2$(s), supporting electrolyte: 0.1 M NaCl solution; scan rate = 50 mV $S^{-1}$].[14] Formation of this ternary complex is also supported by the mass spectroscopic analysis of the reaction mixture which showed ion peaks corresponding to the association of TpyCu with histidine (Fig. S24–S28, ESI†).[13] The fluorescence titration data of $TpyCu^{2+}$ with L-histidine in water (25 mM hepes buffer, pH = 7.35) follow the Hildebrand–Benesi equation. This supports the formation of a 1 : 1 complex between $TpyCu^{2+}$ and L-histidine and gives the association constant of the complex as $8.94 \times 10^3$ (Fig. S4, ESI†). Using quinine bisulfate as the standard, we determined the fluorescence quantum yield of Tpy as 4.1% and that of TpyCu(II) + histidine (12 equiv.) as 2.7%.

The UV absorption spectroscopic data for the interaction of $TpyCu^{2+}$ with L-histidine have provided further support for the formation of the TpyCu(II)–histidine ternary complex in aqueous solution. As shown in Fig. 4, coordination of Tpy with $Cu^{2+}$ led to a large red shift in the UV absorptions. However, the addition of L-histidine to $TpyCu^{2+}$ only caused small changes in the longest wavelength absorption. No evidence of free Tpy formation was observed, that is no displacement of Tpy by histidine occurred.

We also studied the CV of $TpyCu^{2+}$ upon treatment with L-alanine. As shown in Fig. 5, a reversible couple was observed for the reaction of $TpyCu^{2+}$ with L-alanine which could be assigned to the redox of Cu(II)/Cu(I) with $E_{1/2}$ = −0.297 V for the TpyCu(II)–alanine complex. The significantly different reduction potential of TpyCu(II)–histidine *versus* that of TpyCu(II)–alanine demonstrates that it is harder to reduce the TpyCu(II)–histidine complex. That is, the Cu(II) center in TpyCu(II)–histidine is more electron rich than that in TpyCu(II)–alanine. This could

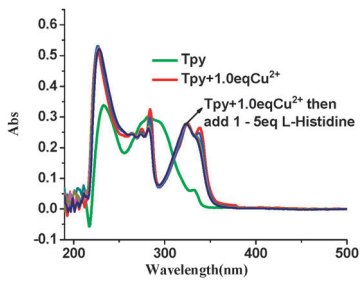


**Fig. 4** UV spectra of Tpy and $TpyCu^{2+}$ ($2.0 \times 10^{-5}$ M in 25 mM hepes buffer solution, pH = 7.35) with 0, 1.0, 2.0, 3.0, 4.0 and 5.0 equiv. L-histidine (The absorptions of the stoichiometric amount of L-histidine were subtracted from the corresponding plots).

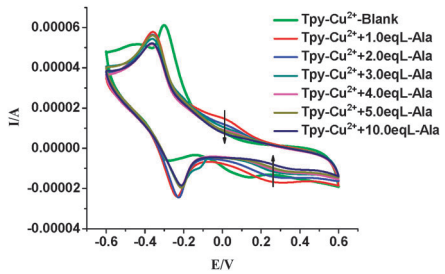


**Fig. 5** CV titration profile of $TpyCu^{2+}$ (1 mM) with various amounts of L-alanine.

be used to explain their dramatic difference in fluorescence. The fluorescence of Tpy is quenched by $Cu^{2+}$ in the $TpyCu^{2+}$ complex by either electron or energy transfer from the excited state of Tpy to the $Cu^{2+}$ center. The more electron rich Cu(II) center generated upon coordination of L-histidine to $TpyCu^{2+}$ should make the electron or energy transfer from the excite state of Tpy more difficult, leading to the large fluorescence enhancement. However, the Cu(II) centers in the complexes of $TpyCu^{2+}$ with other amino acids such as L-alanine should be still electron deficient enough to accept the electron or energy transferred from the excited state of Tpy, providing efficient fluorescence quenching for Tpy.

A DFT calculation was conducted on TpyCu(II)–histidine before.[15] Two structures **A** and **B** were found to have the lowest energy, with **B** slightly lower than **A** (Fig. 6). It is expected that the coordination of the amino acids like L-alanine to $TpyCu^{2+}$ should be similar to **A**, involving the bonding of an amine and a carbonyl to the metal center. However, for the interaction of L-histidine with $TpyCu^{2+}$, we propose that the structure **B** may be more important in order to account for the observed large difference in the fluorescence response of $TpyCu^{2+}$ toward L-histidine over the other amino acids. The chelate coordination of both the more basic imidazole nitrogen (*versus* the carbonyl group) and the α-amine group to the Cu(II) center in **B** should increase the electron density on the Cu(II) center and make it harder to be reduced. The structure **B** has a resonance form **B′** in which the positive charge is delocalized from the metal center to the imidazole ring to make the metal center less electron deficient. The more electron rich metal center in **B** and **B′** should contribute to the inhibition of the fluorescence quenching of Tpy by Cu(II) in TpyCu(II)–histidine, giving rise to the observed large fluorescence enhancement. The chelate coordination of histidine with $Cu^{2+}$ in B is also supported by our observation of little fluorescence enhancement when $TpyCuCl_2$ is treated with a histidine derivative whose amine group is protected with Boc (see Fig. S12–S14 in ESI†).

In summary, we have discovered that the classical coordination complex $TpyCuCl_2$ is a highly efficient and selective fluorescent sensor for histidine in aqueous solution at neutral pH. It is potentially useful for practical application.

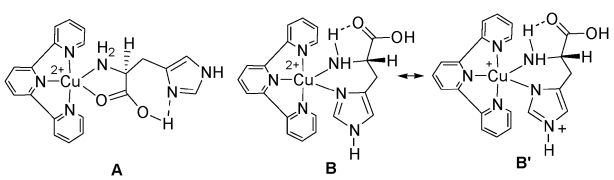


**Fig. 6** Two calculated structures for TpyCu(II)–histidine.

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