A Novel Fluorescent Aminoacid Designed for Fluorometric Detection of Cu(II)

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A fluorescent aminoacid was designed for selective and sensitive detection of Cu(II) in aqueous solution. The designing of this Cu(II) fluorescent chemosensing molecule, *N*-(1-naphthyl)-aminoacetic acid (NAA), was based on the binding of Cu(II) to aminoacetic acid and the novel charge transfer photophysics of 1-aminonaphthalenes. The fluorescence of NAA was found quenched by Cu(II) and several other metal ions of similar electronic structure such as Co(II), Ni(II) and Zn(II). The quenching was shown to occur via electron transfer within the metal-NAA complex, which required an optimal combination of high binding affinity and favorable redox properties of the components in the metal-NAA complex and hence afforded selective fluorometric detection of Cu(II). The calibration graph obeyed Stern-Volmer theory and was shown for Cu(II) over the range of 0—2.75×10^{-4} mol/L. The quenching constant of Cu(II) was measured as 8.0×10^{3} mol/L that was two orders of magnitude higher than those of Co(II), Ni(II) and Zn(II). The 3SD limit of detection for Cu(II) was 8.00×10^{-6} mol/L with a coefficient of variation of 1.65%. Linear range for quantitative detection of Cu(II) was 2.67×10^{-5}—2.75×10^{-4} mol/L. The method was applied to synthetic sample measurements which gave recoveries of 105%—112%.

Keywords *N*-(1-naphthyl)aminoacetic acid, fluorescence quenching, Cu(II), electron transfer

Introduction

It has been well known that α-aminoacid could efficiently chelate Cu(II).[1-3] Applications based on this novel binding character have been reported, for instance, the chiral discrimination of *D*- and *L*-aminoacids in a Cu(II)-cyclodextrin complex[4,5] and the recovery of pyrene fluorescence by aminoacid from a pyrene-Cu(II)-cyclodextrin composite.[6] It was thus envisaged that a rational design would lead to an aminoacid derivative that could work as a novel ligand for efficient detection of Cu(II). A combination of the aminoacid with a fluorophore would be the first choice. In this fluorescent aminoacid the aminoacid moiety should retain its Cu(II) chelating capacity while the fluorophore moiety working as a fluorescent signal reporting group, as in a fluorescent chemosensor of the structure fluorophore-spacer-receptor.[7] In that molecular binding of a Cu(II) ion should lead to substantial change in the fluorescence properties, thus assuming a highly sensitive response to the presence of Cu(II). It is hence required that the fluorophore should be able to communicate with the aminoacid moiety in the ground and/or excited state. Researches into the photophysics of 1-aminonaphthalenes have been a subject of current interest,[8-11] as a thermally activated internal conversion (IC) occurred with *N*,*N*-substituted 1-aminonaphthalenes and this IC process was shown to be the consequence of the coupling of the proximate S_1 and S_2 states, of which the S_1 state is the emissive state of intramolecular charge transfer (ICT) character. We therefore decided to construct the fluorescent aminoacid as *N*-(1-naphthyl)aminoacetic acid (NAA, Chart 1) and investigate the possibility of using NAA as the chelating reagent for sensitive and selective detection of Cu(II). We expected that in the Cu(II)-NAA complex the coupling of the S_1 and S_2 states would be modified because of the coordination of amino nitrogen to Cu(II) and therefore the fluorescence emission of NAA changed, allowing for a fluorometric detection of Cu(II). The experimental results showed that NAA could indeed work as an efficient fluorescent reagent for Cu(II) determination and the designing principle outlined here worked successfully.

Materials and methods

NAA was synthesized by reaction of 1-aminonaphthalene (AN, Chart 1) with chloroacetic acid in aqueous NaOH solution. The product was purified by repeated recrystallizations from ethanol and its structure was characterized by IR and ^{1}H NMR spectra. *N*-(1-Naphthyl) ethylenediamine (NEDA, Chart 1) was received as an AR reagent from Shanghai Chemicals Group Company.

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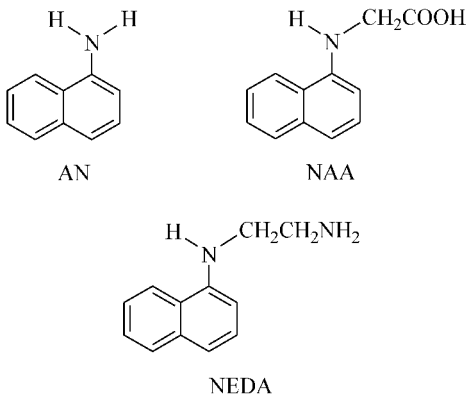
Ethanol was redistilled before use and ensured that there was no fluorescent impurity. The inorganic salts were of the highest purity available and existed in their chlorides or sulfates. Twice de-ionized water was further distilled in the presence of $KMnO_4$. All experiments were conducted in 30% ethanol aqueous solution at room temperature of *ca*. 20 ℃.

Chart 1



Corrected fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer and absorption spectra were taken on a Shimadzu UV-2401-PC absorption spectrophotometer. Fluorescence lifetime of NAA in aqueous solution was measured using the time-correlated single photon counting (TCSPC) setup in Göttingen.[9,10] The fluorescence intensity was calculated from the area under the spectrum. Cyclic voltammetry experiments were carried out on a CHI-832 Electrochemical Analyzer in a three-electrode cell using glass-carbon electrode as working electrode, Ag/AgCl electrode as reference electrode, and platinum wire as the counter electrode. 0.5 mol/L of HOAc-NaOAc (1 : 1) was used as the supporting electrolyte.

Results and discussion

Quenching of NAA *fluorescence by* Cu(II) *in aqueous solution*

The fluorescence spectra of NAA in aqueous solutions were recorded in the presence of Cu(II), Co(II), Ni(II) and Zn(II). The maximum excitation and emission wavelengths were found to be 320 and 437 nm, respectively. It was found that, while the spectral shape was not changed, the fluorescence of NAA was quenched to different extents by these transition metal ions, of which the quenching by Cu(II) was substantially higher than those of other metal ions of similar electronic structure. Fig. 1 shows the examples of fluorescence quenching by Cu(II) and Ni(II). In Fig. 2 the Stern-Volmer plots of the fluorescence quenching are given. It is obvious that, within the investigated metal ion concentration range, the quenching obeys Stern-Volmer theory described in Eq. (1),

$$I_0/I = 1 + K_{SV}[Q] \quad (1)$$

in which I_0 and I represent fluorescence intensities in the absence and presence of the quencher of concentration [Q], respectively, and K_{SV} is the quenching constant. It was found that the quenching constant of Cu(II) was at least two orders of magnitude higher than those of the other ions (Table 1).

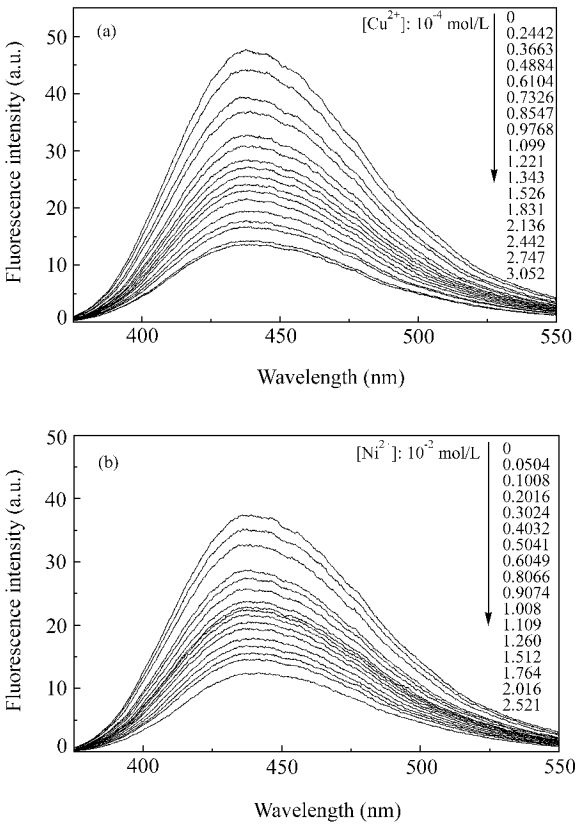


Fig. 1 Fluorescence spectra of NAA in 30% ethanol aqueous solution in the presence of (a) Cu(II) and (b) Ni(II). [NAA] is 1.007×10^{-5} mol/L, Cu^{2+} and Ni^{2+} concentrations are given in the figures.

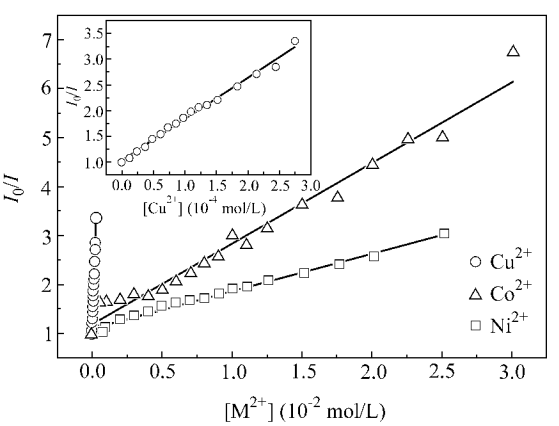


Fig. 2 Stern-Volmer plots of NAA fluorescence quenching by Cu(II), Co(II) and Ni(II). Inset shows the extended quenching plot of Cu(II).

Table 1 Fluorescence quenching constants K_{SV} and metal-NAA complex stability constants K

	$K_{SV\ Cu(II)}$ [a]	$K_{SV\ Co(II)}$ [a]	$K_{SV\ Ni(II)}$ [a]	$K_{SV\ Zn(II)}$ [a]	$K_{Cu(II)}$ [b]	$K_{Ni(II)}$ [b]
NAA	8012	81	76	36	2.04×10^6	3.63×10^6
NEDA	75	18	43	~0	—	—

[a] As of L/mol in 30% ethanol (V/V) aqueous solution; [b] as of L/mol in 30% ethanol (V/V) aqueous solution containing 0.1 mol/L $NaClO_4$.[1]

Analytical characteristics

The much higher sensitivity for detection of Cu(II) against other ions of similar electronic structure, such as Co(II), Ni(II) and Zn(II), in a Stern-Volmer quenching mode indicated a high selectivity for Cu(II) over these transition metal ions. Based on the quenching constants, it was found that the selectivity of NAA for Cu(II) against other ions was higher than 10^2.

The analytical characteristics of NAA for Cu(II) were assessed. A calibration curve was shown in the Stern-Volmer plot (inset in Fig. 2) over 0—2.75×10^{-4} mol/L, with a detection limit of 8.0×10^{-6} mol/L based on $3SD/k$ ($n=10$), in which SD represents the standard deviation for NAA intensity measurements in the absence of Cu(II) and k represents the slope of the calibration curve. The coefficient of variation for measurements of a sample with 1.83×10^{-4} mol/L Cu(II) was determined as 1.65% for 10 times of parallel measurements. The linear range of quantitative detection for Cu(II) was 2.67×10^{-5}—2.75×10^{-4} mol/L. The presence of less than 1 equivalent of Fe(III), 20 equivalents of Ni(II) and Pb(II), and 50 equivalents of Co(II), Zn(II) and Hg(II), respectively, did not lead to interference of the fluorometric detection of Cu(II) by 10% change in the emission intensity. Anions such as Cl^-, NO_3^- and SO_4^{2-} had no influence on the assays. The analytical assay was also applied to synthetic samples, which gave satisfactory recoveries of 105%—112%.

Possible mechanism for fluorescence quenching

Because of the well-known efficient binding of aminoacid to Cu(II), it was natural to assume that the stronger quenching of NAA fluorescence by Cu(II) was due to the binding of Cu(II) to NAA, *i.e.* a static quenching mechanism might be assumed. Absorption spectra (Fig. 3) ineed indicated the binding of Cu(II) to NAA. The maximum absorption wavelength of NAA was found to shift to blue with increasing Cu(II) concentration with an isosbestic point at 317 nm, supporting a 1:1 binding stoichiometry in Cu(II)-NAA complex. Other metal ions investigated here also showed in absorption spectra evidence of binding to NAA.

Control experiments were carried out on quenching by Cu(II) of the fluorescence of AN that was expected not to bind Cu(II). Results indicated that no observable quenching occurred. It was suggested that there was no appreciable dynamic quenching of Cu(II) to the aminonaphthalene moiety in NAA. This is understandable because of the short lifetime of AN in aqueous solution (estimated to be *ca.* 20 ns[13]). The lifetime of NAA in aqueous solution was also measured and it was quite short either (18.3 ns). This may also suggest that the quenching of NAA fluorescence by Cu(II) observed here is not dynamic. Therefore static quenching could be assumed for the observed quenching. This was apparently supported by a decreased quenching at higher temperature (40 ℃) with a quenching constant of 731 L/mol.

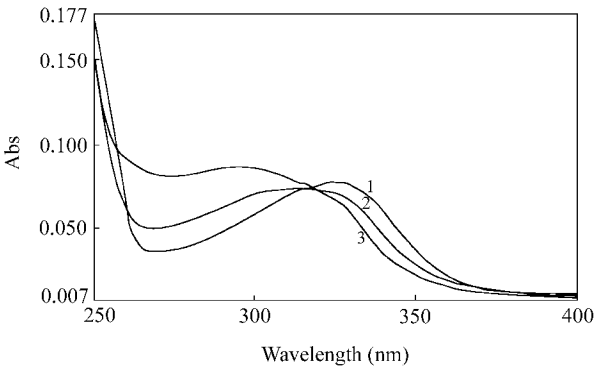


Fig. 3 Absorption spectra of NAA in 30% ethanol aqueous solution in the presence of Cu(II). [NAA] is 1.118×10^{-5} mol/L, $[Cu^{2+}]$ is (1) 0, (2) 1.482×10^{-5} and (3) 2.964×10^{-5} mol/L, respectively.

The much higher quenching constant of Cu(II) over those of other metal ions, however, did not completely correspond to the order of the stability constants of the metal-NAA complexes. For example, the stability constants of Cu(II)-NAA and Ni(II)-NAA were reported to be similar (Table 1).[1] Therefore, the "static quenching" assumed here would actually be a kind of quenching occurred within the complex in which the fluorophore and quencher were brought together like in a "sphere of action".[14] Both electron transfer and energy transfer could be assumed as the quenching mechanism. By cyclic voltammetry, the oxidation potential of NAA was measured to be 0.067 V, and the reduction potentials of metal ions were measured to be −0.15 (Cu^{2+}), −0.91 (Co^{2+}), −0.781 (Ni^{2+}) and −0.71 V (Zn^{2+}) (vs. Ag/AgCl), respectively. The reduction potential of Cu^{2+} is obviously higher than those of the other metal ions. As a consequence, the electron transfer from aromatic amine to metal ion is thermodynamically much more favorable within Cu^{2+}-NAA complex,[15] which is in agreement with the observed much higher quenching constant.

Comparative experiments for the quenching of NEDA fluorescence provided further support for the electron transfer quenching mechanism. As the stability constants of the investigated metal ions with ethylenediamine are similar to those with aminoacetic acid,[16] it is reasonable to assume that the binding properties of the metal ions to NEDA are similar to those to NAA. However, it was found that the quenching constants to NEDA fluorescence (see Table 1) of these ions were all at the same order of magnitude and were much lower than that of Cu(II) to NAA fluorescence. This observation appears not to support the energy transfer mechanism, since the NEDA emission spectrum (maximum emission at 428 nm) is similar to NAA emission with a maximum of 437 nm. On the other hand, the results seems to support the electron transfer quenching mechanism. The oxidation potential of the terminal amine in NEDA was measured to be −0.13 V (vs. Ag/AgCl) that is lower by 0.33 V than that of the aromatic amine in NEDA. In metal-NEDA complexes the electron transfer from the terminal amine to metal ions therefore becomes more favorable, whereas the electron transfer from aromatic amine to metal ion that was assumed to be responsible for the fluorescence quenching would be blocked. Therefore, it was concluded that the quenching occurred within metal-NAA complex was due to an electron transfer mechanism. This electron transfer would compete with the charge transfer from amino group at C-1 to the naphthalene moiety and hence increase the energy of the charge transfer emissive state S_1. The coupling of the S_1 and S_2 states would be enhanced because of the narrowed gap between them, leading to enhanced internal conversion[8-11] that was indicated by the fluorescence quenching.

Conclusions

NAA was designed as a fluorescent chemosensor for selective detection of Cu(II) over Co(II), Ni(II) and Zn(II), in spite of the similar stability constants of Cu(II)-NAA and Ni(II)-NAA complexes. The detection was carried out in the mode of fluorescence quenching that was shown to result from electron transfer within the metal-NAA complex. Both high stability constant of the metal-NAA complex and optimal redox potentials of the components in the complex were required to ensure high sensitivity, which would consequently lead to high selectivity. It was noted that the sensitivity of the present method needed to be improved when compared to the recent examples,[17,18] but the method represented a new responsing mechanism that is different from the reported systems.[17,18] Increasing the stability constant and enhancing the difference of redox potentials would guide the designing of better fluorescent chemosensors for improved sensitivity and selectivity. Works in this direction are currently underway.

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