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A benzothiazole alkyne fluorescent sensor for Cu detection in living cell

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Abstract

A new type of alkyne dye, 6-dimethylaminobenzothiazole alkyne (1), was developed for Cu sensing in biological system. Dye (1) offered excellent selective over a panel of ions, only Cu (I) could change the fluorescence of dye (1) by forming copper acetylide between the terminal alkyne and Cu (I). Its potential of detecting Cu in biological system was demonstrated in cell culture.

Keywords

Fluorescent sensor; copper; benzothiazole alkyne

Copper is an essential trace element in most higher plants and animals^{1–3}. It participates in many fundamental physiological processes⁴ including iron and zinc utilization⁵, elimination of free radicals⁶, the development of bone⁷, and the production of skin⁸ and hair pigments⁹. Copper deficiency or over-accumulation in vivo can cause various diseases³, such as Menkes disease¹⁰, Wilson's disease¹¹, and cancer¹². Thus, a convenient method to detect copper ions with high sensitivity would be valuable. Recently, a few fluorescent probes have been reported that can detect Cu (II) and Cu (I) in aqueous solution. These probes are typically designed based on stable chelation between a copper ion and an electron-donating group, such as an amino^{13,14}, sulfane^{15,16}, or carboxylate group¹⁷ on a fluorophore, resulting in an 'on' or 'off' fluorescent signal. Besides, a fluorogenic Cu (I)-catalyzed azide–alkyne cycloaddition reaction were reported to detect Cu (II) indirectly based on allosteric effectors^{18,19}. Recently, while working on fluorogenic 'click-on' dyes^{20,21}, we observed that the fluorescence of 6-dimethylaminobenzothiazole alkyne (1) was quenched by Cu (I) in aqueous solution, presumably due to the formation of copper acetylides (2) between the terminal alkynes and Cu (I) (Scheme 1). This quenching effect was also found to be only responsive to Cu (I), not to Cu (II). This suggested that these alkyne-containing molecules could be used in detecting copper ions in living cells.

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Supplementary Material

Supplementary material that may be helpful in the review process should be prepared and provided as a separate electronic file. That file can then be transformed into PDF format and submitted along with the manuscript and graphic files to the appropriate editorial office.

Dye 1 was synthesized according to our previous report using 2-chlorobenzo[d]thiazole as the starting material, via nitration with a mixture of concentrated sulfuric acid and fuming nitric acid, followed by iron powder reduction and reductive amination with formaldehyde and triacetoxyborohydride, cross-coupling a terminal alkyne with catalytic quantities of $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ under basic conditions, finally, deprotection, to yield 6-dimethylaminobenzothiazole alkyne 1 (Scheme S-1). The absorption and emission maxima were 345 nm and 500 nm, respectively, and the Stokes shift was 157 nm ($\Phi = 0.46$ in MeOH) in pH 7.2 PBS buffer²¹.

The possibility of measuring Cu (I) in aqueous condition was explored using a known concentration of Cu (I). A standard stock solution was prepared by dissolving $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{PF}_6$ in acetonitrile. The fluorescence intensity of dye 1 (10 μM) in water decreased significantly as the concentration of Cu (I) increased (Figure S-2B); however, the fluorescence intensity could not be quenched completely even if a high concentration of the $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{PF}_6$ solution was added (Figure S-2C). This is probably due to the instability of $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{PF}_6$ in aqueous solution. Only part of the released Cu (I) reacted specifically with dye 1, resulting in the decrease in fluorescence intensity, but most of Cu (I) was quickly oxidized or disproportionated to Cu (II) and lost its quenching capacity with the alkyne dye 1. Because Cu (I) is not stable in aqueous solution, to correctly measure Cu concentration, an in situ reduction approach might be useful to generate fresh Cu (I) and initiate the quenching process, as used in the ‘click’ reaction.

The quantitative correlation of Cu (I) and dye 1 was then calibrated in the presence of a reducing agent, sodium ascorbate (NaAsc). To an aqueous solution of NaAsc (1 mM) and dye 1 (10 μM), different amounts of Cu (II) were added. The reaction was fast; the fluorescence changes were near instantaneous. Assisted by NaAsc, dye 1 reacted with the freshly prepared Cu (I) effectively, and showed an excellent response between fluorescent signal and Cu (II) concentration (Figure 1A). The plots showed a near linear correlation ($R = 0.997$) between relative fluorescence intensity and the molar concentration of Cu (II) (Figure 1B). The linear regression equation was $F = 226.7 \times [\text{Cu (II)}] + 2274$, suggesting the mole complexing ratio of dye and Cu (II) was 1:1. Under UV light (365 nm), the fluorescent intensity changes could be clearly seen by the naked eye (Figure 1C).

The selectivity of dye 1 for various biologically relevant cations, including Ni (II), Mn (II), Mg (II), K (II), Zn (II), Sn (II), Fe (II), Fe (III), Ca (II), Cd (II), and Cu (II), was investigated in parallel (Figure 2). Under the same reaction conditions, dye 1 (10 μM) and NaAsc (1 mM) aqueous solution, no other ion tested was able to quench the fluorescence. Only Cu (II) offered complete fluorescence quenching in the presence of a reducing agent.

Prior to cell culture experiments, potential buffer effects were studied using PBS (pH 7.2), HEPES buffer (pH 7.0), citric acid/sodium citrate (pH 7.0), Tris-HCl buffer (pH 7.0), sodium bicarbonate solution (pH 8.0), and deionized water. The results showed that citrate buffer and PBS had strong inhibitory effects, and Tris buffer had a weak effect. However, interference by PBS and the Tris buffer was less prominent when the buffer concentration was less than 1 mM (Figure S-3A). Citric acid formed a tight complex with Cu (II) at a high concentration (10 mM). This completely blocked the quenching effect. Even at low concentrations, citric acid significantly prolonged the quenching time. In 0.1 mM citric acid buffer at pH 7, the fluorescence quenching lasted more than an hour after adding Cu (II) (Figure S-3B). Thus, HEPES was selected for subsequent studies.

The effects of pH were then investigated with HEPES buffer at pH 4, 5, 6, 7, 8, and 10 (Figure S-4). A consistent result was seen between pH 6 and 10 where fluorescence “on-off” could be achieved with copper (I). When the pH was less than 5, the quenching effect

disappeared, probably due to the instability of copper acetylides in such acidic conditions²². The other possible reason is N,N-dimethylaniline moiety could bind H⁺ to form an ammonium ion.

The potential biological application of the Cu (II) probe developed was then assessed in cell culture. HeLa cells were incubated with Cu (II) (0, 0.1, 10 μ M) and dye 1 (10 μ M) in HEPES buffer for 10 min. After thorough washing, cells were incubated with 0.5 mM NaAsc solution in HEPES buffer for 3 min, followed by washing, and monitored under a microscope. Significant differences in the fluorescent signal were clearly detected using a common filter set for DAPI (4',6-diamidino-2-phenylindole) stain (Figure 3). In the presence of NaAsc (0.5 mM), the fluorescent signal ratios of signal/background for 0, 0.1, and 10 μ M of Cu (II) were 1.88, 1.3, and 1.14, respectively. In contrast, without NaAsc, the fluorescent signal was not weakened (Figure S-5).

In conclusions, to our knowledge, this is the first report of a fluorescence probe with high selectivity for detecting Cu ions based on a terminal alkyne, forming a copper acetylide. The optimal fluorescence response of this Cu chemosensor was within the physiological pH range and it was able to detect trace amounts of Cu quantitatively in aqueous solution. Intracellular Cu in living cells could be determined conveniently using a conventional fluorescent microscope.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

A: Fluorescence quenching responses of probe (10 μM) with different concentrations of Cu (II) (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 μM, from top to bottom) in 1 mM NaAsc aqueous solution. Spectra were obtained with excitation at 345 nm. B: correlation between fluorescence intensity and Cu (II) concentration. C: Fluorescent image of various Cu (II) concentrations under 365 nm UV light.

Figure 2. Quenching effects of different cations. Dye 1 (10 μ M) in water was reacted with various metal cations (10 μ M) in an aqueous solution with (red) or without (blue) NaAsc (1 mM). $\lambda_{\text{ex}} = 345$ nm, $\lambda_{\text{em}} = 500$ nm.

Figure 3.

Confocal fluorescence images of HeLa cells incubated with dye 1 (10 μM) and different concentrations of Cu (II) (0, 0.1, 10 μM), incubated with the addition of 0.5 mM NaAsc. (A) No Cu (II); (B) 0.1 μM Cu (II); (C) 10 μM Cu (II); (D) Quantitation of cellular fluorescence signals (A, B, C) using an open source ImageJ program (v1.44, NIH).

Scheme 1.
Proposed binding mechanism of dye 1 with Cu(I).