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Communication

Bioinspired fluorescent dihydroxyindoles oligomers

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ABSTRACT

In this paper we report the desgin and synthesis of dihydroxyindoles oligomers based reversible fluorescence sensor. We find dihydroxyindoles-2-carboxylic acid derived oligmer (P-DHICA) has the highest selectivity and sensitivity for Cu^{2+} detection. This work provide a highly efficient, environmentally friendly biosensor for potential use in medical testing.

Melanin is a ubiquitous biomacromolecule that is widely distributed in the animal and plant kingdoms. They are not only function as the pigment, but also involved in various biological activities, such as sequestering metal ions [1–3], free radicals scavenging [4–6], photocatalysts [7,8], and photoprotection [9,10]. Eumelanin is the most intensively studied melanin that produced from the 3,4-dihydroxyphenylalanine precursor. The large number of studies have confirmed the structure of eumelanin as essentially an amorphous heterogeneous biopolymer composed primarily of random assembles of 5,6-dihydroxyindoles (DHI) and 5,6-dihydroxyindoles-2-carboxylic acid (DHICA) [11].

Biomacromolecules, includes nucleic acids and proteins are made from building blocks of nucleobase and amino acid, of which the sequence and composition variety leads to diverse structures and functions that are vital to life [12,13]. Researchers construct synthetic materials made of these building blocks in a range of fields including in biotechnology, programmable structural synthons, and catalysis [14–16]. In each application, the function of material is highly dependent on the chemistry and assembly of the building blocks [17]. The structure complexity of melanin hindered the efforts in exploring its building blocks and use for constructive purpose. Recent advances in construction of melanin-like materials by oxidation polymerization of dopamine showed great potential in revealing fundamental structure information and related applications [18,19]. However, the use of building blocks, 5,6-dihydroxyindole and its derivatives, originated from melanin to construct functional materials is relatively unexplored.

Recently, the development of chemical probes for detection of Cu^{2+} has attracted considerable interest worldwide [20–25]. Note that Cu^{2+} plays an important role in human homeostasis, but also toxic to living organisms at high concentration [26–29]. Therefore, it is imperative to seek rapid, sensitive and efficient methods for detecting Cu^{2+}. In the past, a large number of organic fluorescent probe, including cyanines [30], phthalocyanines [31], rhodamines [32] and coumarins derivatives [33], with excellent sensitivity and selectivity has been developed for Cu^{2+} detection [34–36]. However, the efforts of using biological building blocks to build fluorescence sensor is rare [37–39]. Herein, we report a new class of melanin-inspired dihydroxyindoles oligomers-based reversible fluorescence sensor for Cu^{2+} detection. We chose 5,6-dihydroxyindoles (DHI), 5,6-dihydroxyindoles-2-carboxylic acid (DHICA) and its derivatives 5,6-dihydroxyindoles-2-carboxylic acid methyl ester (DHICMe) and 5,6-dihydrocy indole-2-carboxylic acid ethyl ester (DHICEt) as the building blocks for constructing fluorescence probes. Notably, we find that these building blocks can be oxidized and further polymerized under alkaline condition to form fluorescent oligomers (Scheme 1). Though the structure complexity of melanin-based material hindered the efforts in revealing their structural information, the polymerization process can be monitored *via* the fluorescence spectrum.

Moreover, we found these fluorophores were quenched specifically by Cu^{2+} association over other metal ions, including Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Co^{2+}, Cr^{3+}, Fe^{3+}, Mn^{2+}, Sr^{2+} and Zn^{2+}. In addition, fluorescence intensity can be switched between ON and OFF states by the Cu^{2+} association and disassociation process. This

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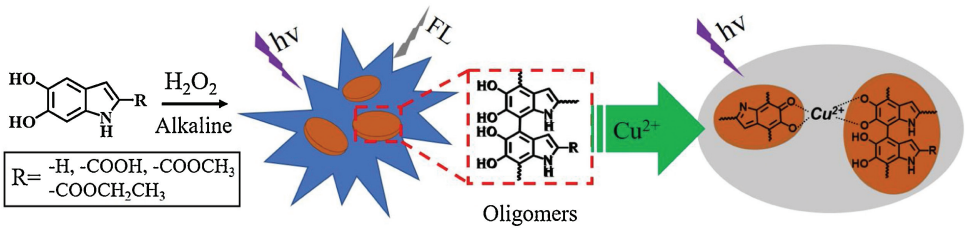

Scheme 1. Schematic illustration of fluorescence generation and quenching.

work suggests a new approach for constructing fluorescent probe originated from nature, which helps to reveal the fundamental information of melanin and potentials in biosensing.

DHICA were synthesized *via* ferricyanide oxidation of L-3,4-dihydroxyphenylalanine (L-dopa), followed by rearrangement of the resulting dopachrome under anaerobic conditions [40]. DHICMe and DHICEt were prepared by the esterification DHICA and alcohols (Scheme S1 in Supporting information). The resulting products were confirmed by ^{1}H, ^{13}C NMR spectra (Figs. S1–S3 in Supporting information), and the electrospray ionization mass spectrometry (ESI-MS) (Fig. S4 in Supporting information). The oxidative polymerization of DHI, DHICA, DHICMe and DHICEt under alkaline conditions yield corresponding oligomers namely, P-DHI, P-DHICA, P-DHICMe and P-DHICEt (Scheme 1). The successful polymerization was evidenced by the disappearance and shift of peaks from aromatic rings in ^{1}H NMR spectra, indicating the coupling between dihydroxyindoles units (Fig. 1a). To further characterize the extent of polymerization, ESI-MS was applied to detect the molecular weight of the possible oligomers. As shown in Fig. 1b, P-DHICA consisted of several oligomers, including dimer (compound **D**), trimers (compounds **E**, **F** and **G**) and tetramers (compounds **H** and **I**) with *m*/*z* of 388.2 [**D**+3OH+H]$^{+}$, 488.2 [**E**+H]$^{+}$, 588.3 [**F** + OH + K]$^{+}$, 593.0 [**G** + OH+H]$^{+}$, 672.9 [**H** + K]$^{+}$, and 783.9 [**I** + OH+H]$^{+}$, respectively. Meanwhile, P-DHI, P-DHICMe and P-DHICEt also consisted of serval dimers, trimers and tetramers, as suggested by ESI-MS (Figs. S5–S7 in Supporting information).

The fluorescence properties of P-DHI, P-DHICA, P-DHICMe and P-DHICEt were first evaluated in water by two-dimensional contour maps to estimate the optimal excitation-emission wavelength. As shown in Fig. S8 (Supporting information), the thickest contour regions of the spectrum can be clearly seen by the contour maps, that corresponded to the maximum fluorescence emission intensity of the fluorophores. Therefore, the optimal fluorescence excitation-emission wavelength can be obtained accordingly. In addition, the darker color in the spectrum indicated the higher fluorescence intensity. All of the two-dimensional spectrum showed a broad color distribution, indicating that all samples exhibited an excitation-dependent emission wavelength behaviour [41], due to the presences of multiple oligomers. With the increase of excitation wavelength, the maximum emission wavelength of fluorophore shifted to the longer wavelength. The emission intensity increased with increasing excitation wavelengths and then attenuated gradually, showing a maximum fluorescence emission intensity in the excitation wavelengths range of 330 nm and 370 nm, set as the optimal excitation wavelength. Therefore, the optimal excitation wavelength was determined to be 365 nm, 359 nm, 360 nm and 340 nm for P-DHI, P-DHICA, P-DHICMe and P-DHICEt, respectively.

The polymerization process was analyzed by its fluorescence change over time. DHICA has two active sites (positions a and c in Fig. 1) for polymerization, that resulted in the formation of linear oligomer P-DHICA. As shown in Fig. S9a (Supporting information) and Fig. 2a, the fluorescence intensity increased with polymerization time at first 10 min and then attenuated gradually, suggesting the oxidative degradation of hydroxyl radical at late stage [42,43]. The trend stayed true for DHICMe and DHICEt, both formed linear oligomer during polymerization (Figs. S9b and c in Supporting information and Figs. 2b and c). In contrast, for DHI, its oxidative polymerization process is a little bit different from other dihydroxyindoles derivatives, probably due to an extra active site

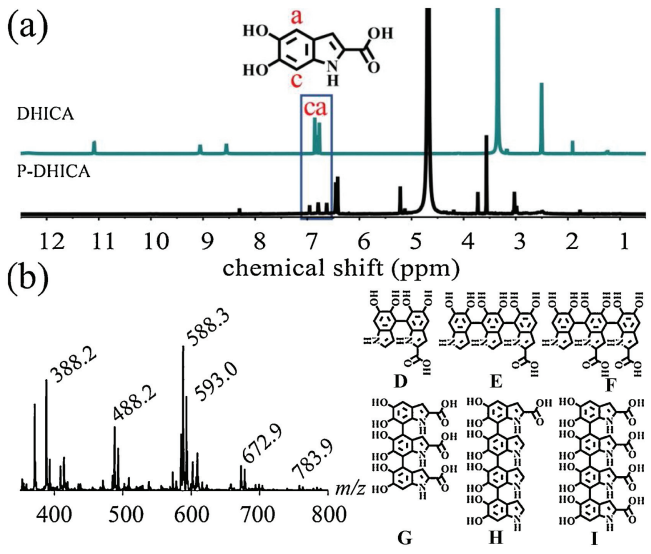

Fig. 1. (a) ^{1}H NMR spectra of DHICA (in DMSO-d_6) and P-DHICA (in D_2O); (b) The ESI-MS spectrum and possible chemical structures of P-DHICA.

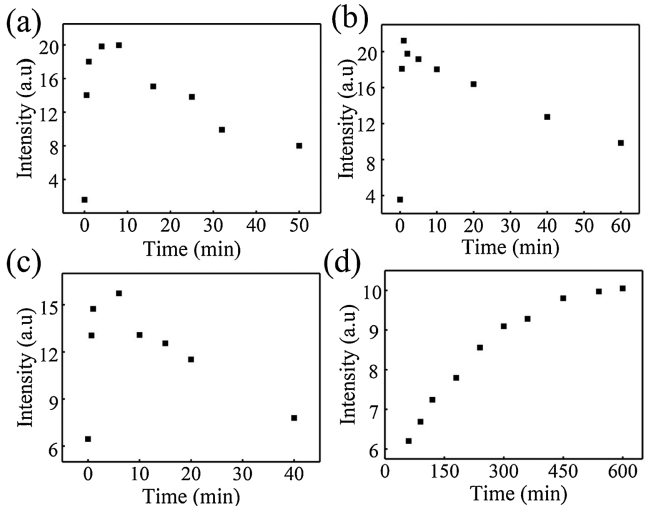

Fig. 2. Oxidation time dependent fluorescence intensity for dihydroxyindoles oligomers. A plot of the fluorescent intensity value at excitation wavelength 359 nm *versus* the oxidation time for P-DHICA (a); at excitation wavelength 360 nm for P-DHICMe (b); at excitation wavelength 340 nm for P-DHICEt (c); at excitation wavelength 365 nm for P-DHI (d).

at position b of DHI (Fig. S5). The first 30 min of oxidative polymerization, the P-DHI emission wavelength showed a blue shift and the fluorescent intensity gradually decrease (Fig. S9d in Supporting information), then the emission wavelength kept constant and the fluorescence intensity increased continuously during the polymerization (Fig. 2d). We hypothesized that oxidative polymerization at the initial stage tends to form oligomer with a high degree of π conjugation, which will contribute to the delocalization of electrons. As the increase of polymerization degree of oligomers and the introduction of hydroxyl groups results in a decrease in the degree of conjugation [44]. Therefore, the fluorescence blue-shift phenomenon occurs at the initial stage of oxidation [45]. With these data in hand, we can selectively prepare the dihydroxyindoles-based fluorescent oligomers with desirable emission wavelength and intensity.

The design of fluorescent sensors for metal ion detection is based on the phenomena of fluorescence response on the metal chelation [46,47]. Dihydroxydoles derivatives possess serval active site, such as catechol, carboxylate acid and quinone imine groups, for Cu^{2+} binding (Scheme S2 in Supporting information) [48,49]. We examined the fluorescence quenching behaviour of P-DHI, P-DHICA, P-DHICMe and P-DHICEt at different concentration of Cu^{2+}. As shown in Fig. 3a, the fluorescent intensity of the P-DHICA decreased continuously with the increase of Cu^{2+} concentration from 0 to 50 μmol/L. A plot of the $((I_{F0}-I_F)/I_{F0})$ value *versus* the Cu^{2+} concentration also showed a positive correlation with the concentration of Cu^{2+}, where I_{F0} and I_F are the fluorescence intensities in the absence and presence of Cu^{2+} (Fig. 3b). Notably, the $((I_{F0}-I_F)/I_{F0})$ value showed a linear increase with the concentration of Cu^{2+} (0–20 μmol/L) ($R > 0.997$, Fig. 3b). The detection limit for Cu^{2+} was estimated to be about 57 nmol/L according to the 3σ per slope, which is lower than maximum contamination level (20 μmol/L) of Cu^{2+} in drinking water permitted by the U.S. Environmental Protection Agency (EPA) [50]. In contrast, the change of fluorescence intensity becomes slower at higher Cu^{2+} concentration, suggesting the saturation of Cu^{2+} binding. The similar trend was also observed for P-DHI (Figs. S10a and b in Supporting information), P-DHICMe (Figs. S11a and b in Supporting information) and P-DHICEt (Figs. S12a and b in Supporting information). More interestingly, the significant fluorescence quenching of P-DHICA was exclusive to Cu^{2+} (Fig. 3c). Other metal ions, including Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Co^{2+}, Cr^{3+}, Fe^{3+}, Mn^{2+}, Sr^{2+} and Zn^{2+} showed little changes in the fluorescence intensity at the same concentration of 50 μmol/L. It is noted that the structural complexity of P-DHICA hindered the detailed investigation of the quenching mechanism. But we reasoned that Cu^{2+} is an effective fluorescent quenchers due to its paramagnetic nature by electron or energy transfer [51,52]. Another two paramagnetic ion, Co^{2+} and Fe^{3+}, exhibited much less ability towards fluorescence quenching. So it was suggested that carboxylic acid group of P-DHICA has high affinity for Cu^{2+} over other metal ions [53], which caused a more dramatic fluorescence quenching. This conclusion was in accordance with the affinity study of nature melanin [54]. Other metal ions showed a fluorescence enhancement effect towards P-DHICA, possibly *via* blocking of photoelectron transfer (PET) process [55,56]. In comparison, P-DHI, P-DHICMe and P-DHICEt presented a similar fluorescence quenching behaviour, but the selectivity for Cu^{2+} was less significant (Figs. S13a–c in Supporting information). Meanwhile, the effect of pH on fluorescence intensity was evaluated at a wide range of pH. The result revealed that the fluorescence intensity of P-DHICA was stable in a wide range of pH from 2 to 10 (Fig. 3d). Compared to P-DHICA, the fluorescence intensity of other oligomer fluorophore has some fluctuations, suggesting the weaker association with Cu^{2+} (Figs. S14a–c in Supporting information). Lastly, we gave a quantitate evaluation of this selectivity by calculating the specific selectivity parameter W, defined as relative fluorescence intensity difference, of P-DHI, P-DHICA, P-DHICMe and P-DHICEt towards Cu^{2+}. As showed in Table S1 (Supporting information), P-DHICA has the highest W values over all the metal ions, and therefore the best candidate for Cu^{2+} sensing. Again, this emphasize the importance of carboxylic group for the selective quenching of fluorescence.

In the above discussion, we attributed the fluorescent quenching phenomena to the binding of Cu^{2+}. To validate this, we performed a Cu^{2+} binding and dissociation experiment, in which the fluorescence intensity of the P-DHICA can be switched between ON and OFF states by alternating addition of Cu^{2+} and pyrophosphate as the competition agent for Cu^{2+} (Fig. 4). The results indicating an excellent recyclability and reusability for the detection of Cu^{2+}.

In summary, we successfully prepared fluorescent oligomers (P-DHI, P-DHICA, P-DHICMe and P-DHICEt) through oxidative polymerization of nature derived building blocks. The fluorescent properties were confirmed by two-dimensional fluorescence spectroscopy and useful for revealing the structural information. More importantly, we found P-DHICA has the highest selectivity to the Cu^{2+} detection, which worked over a wide range of pH values and had a good cyclability. We believed that our fluorescence sensors have great potential for use in medical testing.

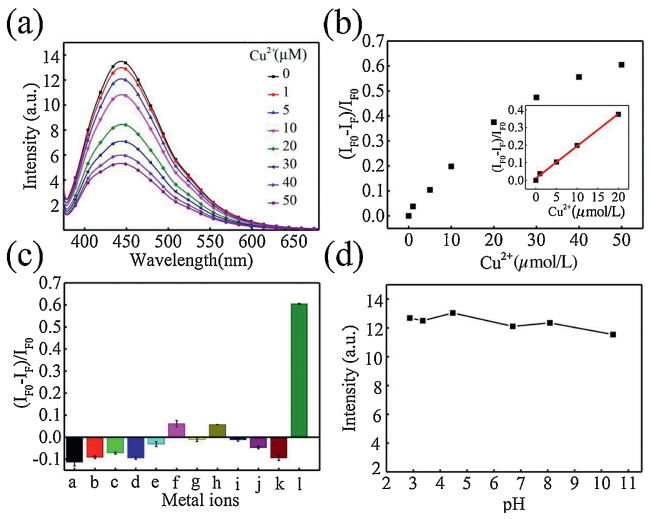

Fig. 3. (a) The change of P-DHICA fluorescence spectra with the increase of Cu^{2+} concentration; (b) A plot of the $(I_{F0}-I_F)/I_{F0}$ value *versus* the concentration of Cu^{2+}; the inserted figure is the linear fit from 0 to 20 μmol/L, $R > 0.998$; (c) The corresponding histogram of $(I_{F0}-I_F)/I_{F0}$ value at 359 nm *vs.* metal ions (a to m: Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Co^{2+}, Cr^{3+}, Fe^{3+}, Mn^{2+}, Sr^{2+}, Zn^{2+} and Cu^{2+}); (d) Effect of pH value on P-DHI florescence intensity.

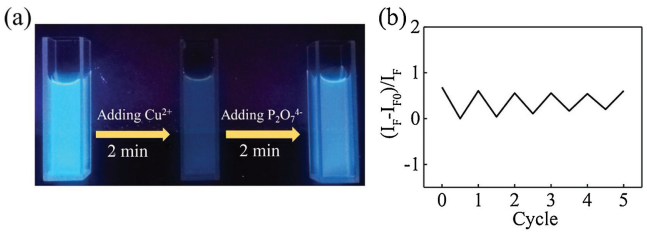

Fig. 4. (a) Reversible switching of P-DHICA between the ON and OFF states through the alternate addition of Cu^{2+} and pyrophosphate; (b) The photograph of P-DHICA solution before and after adding Cu^{2+} and $P_2O_7^{4-}$ under 365 nm light.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2019.05.021.

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