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Coordination induced supramolecular assembly of fluorescent C-Phycocyanin for biologic discrimination of metal ions

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ABSTRACT

Living microbes can accumulate and exhibit distinct responses to different metal ions by strong coordination between bio-macromolecules and metal ions. Thus carefully selected bio-macromolecules could possibly serve as sensitive biologic probes to discriminate toxic metal ions. C-Phycocyanin (C-PC), a water-soluble phycobiliprotein widespread in algae, was selected as a unique biologic probe. Heavy-metal ions coordinated with C-PC, altered greatly secondary structures and fluorescence of C-PC, and also induced supramolecular fibrillation, in contrast to inert response of other metal ions.

1. Introduction

During million years of natural evolution, living organisms get along with various metal ions abundant in their living circumstances (e.g. ocean and soil), and further accumulate part of them (e.g. Na^+, Ca^{2+} *etc.*) to accomplish specific biologic functions. However, as for metal ions scarce in ecosystems (e.g. Hg^{2+}, Pb^{2+}, Ag^+ *etc.*), when entering the biosphere through the alimentary chain, they may also accumulate in living organisms and coordinate to bio-macromolecules [1]. An excessive buildup many cause a series of health effects. On the other hand, with rapid scientific and industrial development, large quantities of metal ions from mining, industry, daily chemical products and scientific activities are discharging continuously into water body without adequate control treatment. In view of wide applications of metal ions yet with distinct biologic responses, it is urgent to search efficient and inexpensive methods to detect and distinguish toxic metal ions from metal ions inert or essential for biologic systems.

Optical detection (colorimetric and fluorescent) is considered as one of the most convenient and quickest methods to detect metal ions. The colorimetric approach allows naked-eye detection based on colour changes, and the fluorescent approach relies on the quenching or strengthening response of fluorescent sensors to specific ions [2]. Nevertheless, most of these techniques concentrated mainly either on selectively detecting one or two specific metal ions, or relied on expensive instrumental equipment and delicate procedures. Considering that living microbes could accumulate and exhibit distinct responses to different metal ions, microbial biosensors were constructed by utilizing pre-engineered genetic circuits of live microbes [3]. However, the microbial biosensors relied strongly on delicate genetic engineering of synthetic biology and continuous nutrient environment in bioreactor systems to optimize microbial growth rates.

In principle, distinct responses of living organism to metal ions were determined by strong coordination of their bio-macromolecules and metal ions. Thus carefully selected bio-macromolecules could possibly serve as sensitive biologic probes to discriminate toxic metal ions. In this study we selected C-Phycocyanin (C-PC), a water-soluble phycobiliprotein widespread in algae, as a unique biologic probe. Metal ions could be discriminated into two groups according to their abilities of coordination with C-PC. Heavy-metal ions have strong tendency of coordination with C-PC, which thus alter greatly secondary structures and fluorescence of C-PC and also induce supramolecular fibrillation, in contrast to inert response of other metal ions.

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2. Experimental

Specific salt was added dropwisely into C-PC solution to reach the final concentrations of metal ions (5 mM) and C-PC (5 mg/mL). The solution was stirred for 12 h at room temperature to test the response of C-PC.

The antimicrobial activity was evaluated through a spread plate method with Colibacillus [4]. 2 mL of antimicrobial material (6.1 mg/mL) were added to agar plates (diameter 90 mm) and dried at 60 °C. After inoculating ~10^5 colony forming units of Colibacillus, the incubation was performed at 37 °C for 12–48 h. Pure C-PC was performed as control.

Microstructures of samples were recorded by SEM (Hitachi S-4800) and TEM (Hitachi H-7650). FT-IR (Nicolet 6700), fluorescence spectrum (Hitachi F-4600), UV–Vis (DU800 UV–vis), Far-UV CD (BioLogic MOS-450/AF-CD) and Malvern Nano S90 laser particle size analyzer were used to characterize the samples.

3. Results and discussion

Structurally a C-PC molecule contains 12 subunits and 18 chromophores (i.e. liner bilin-phycocyanobilin, Fig. 1a), promising strong absorbance coefficients and funnelling light energy to the photosynthetic reaction centre [5]. The liner bilins contain multiple electron donors, along with high content of negatively-charged amino acid residues and cysteinyl residues in C-PC molecule, enabling their powerful ability to coordinate with metal ions.

In order to study the response of C-PC to metal ions, Ag^+ was first tested because of its broader applications as well as severe ecological and health influence. Upon exposing to Ag^+, C-PC solution changed from transparent blue to turbid brown (Fig. 1b). Meanwhile, both the strong fluorescence emission at ~649 nm and UV absorbance at ~617 nm, resulting from high absorbance coefficients of multiple chromophores in C-PC, declined rapidly (Fig. 1c & d), and a new UV absorbance peak at ~415 nm appeared, indicating that coordination occurred between Ag^+ and liner bilins.

C-PC showed two minima at 222 and 208 nm associated with the predominant existence of α-helix in Far-UV circular dichroism (CD) spectroscopy. Upon adding Ag^+, the two minima declined and there ultimately remained a weak peak at 219 nm, suggesting the transformation from α-helix to β-sheets [6]. Further increasing of Ag^+ leaded to the formation of Ag nanoparticles and swamped the signals. FT-IR spectroscopy (Fig. 1f) showed that the addition of Ag^+ produced shifts in the characteristic bands of amide I (1655 cm^{-1}) and amide II (1547 cm^{-1}), verified the transformation of α-helix to β-sheet/β-turn, and consistent with the preferable coordination of Ag^+ with bilins and amino acid residues through –NH and C=O regions. Ag^+ interacted with C-PC to form more

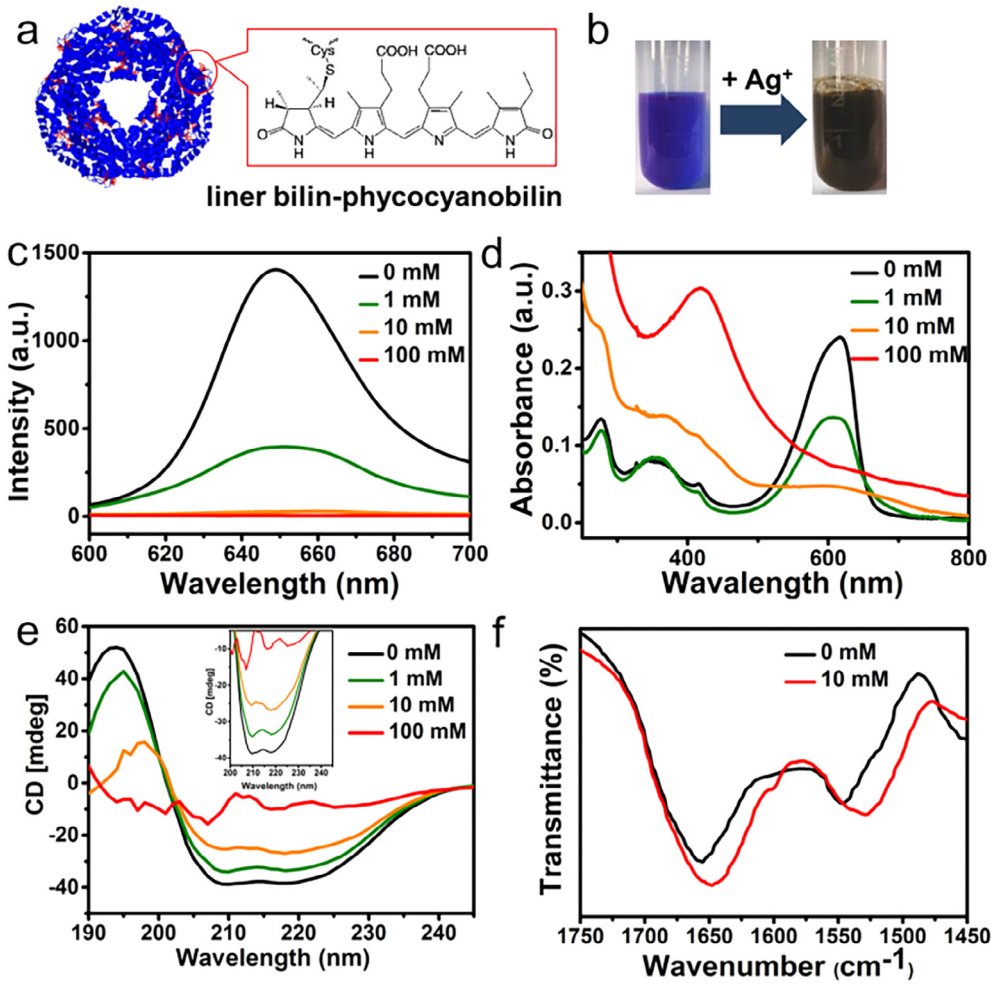

Fig. 1. (a) Schematic representation of C-PC molecular structure and structural formula of its chromophore. (b) Optical images of C-PC coordinating with Ag^+. (c–f) Fluorescence emission spectra excited at 366 nm (c), UV–vis spectra (d), far-UV CD spectra (e) and FT-IR spectra (f) of C-PC coordinating with Ag^+.