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A highly sensitive fluorescence probe for metallothioneins based on tiron–copper complex

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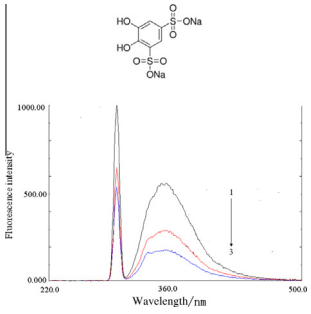
HIGHLIGHTS

- The fabrication of tiron–copper complex as a novel fluorescence probe for metallothioneins.
- The mechanism was studied and discussed in terms of the fluorescence spectra.
- The detection limit was 2.60×10^{-9} mol L^{-1}.
- The method proposed was successfully reliable, selective and sensitive.

GRAPHICAL ABSTRACT



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ABSTRACT

The fabrication of tiron–copper complex as a novel fluorescence probe for the sensitive directly detection of metallothioneins at nanomolar levels was demonstrated. In Britton–Robinson (B–R) buffer (pH 7.50), the interaction of bis(tiron)copper(II) complex cation $[Cu(tiron)_2]^{2+}$ and metallothioneins enhanced the fluorescence intensity of the system. The fluorescence enhancement at 347 nm was proportional to the concentration of metallothioneins. The mechanism was studied and discussed in terms of the fluorescence spectra. Under the optimal experimental conditions, at 347 nm, there was a linear relationship between the fluorescence intensity and the concentration of the metallothioneins in the range of 8.80×10^{-9}–7.70×10^{-7} mol L^{-1}, with a correlation coefficient of $r = 0.995$ and detection limit 2.60×10^{-9} mol L^{-1}. The relative standard deviation was 0.77% ($n = 11$), and the average recovery 94.4%. The method proposed was successfully reliable, selective and sensitive in determining of trace metallothioneins in fish visceral organ samples with the results in good agreement with those obtained by HPLC.

Introduction

Metallothioneins (MTs) are a class of inducible metal-binding proteins characterized by low molecular weight (1–10 kDa), a high cysteine content (about 30%), lack of aromatic acid residues and heat stability. Under normal physiochemical conditions, MTs are composed of two metal clusters (α and β). MTs are found to occur in a widespread variety of organisms, including vertebrates, invertebrates, microorganisms and various kinds of plants [1,2]. It is believed that these proteins play important roles in protecting cells from oxidative stress through metal binding/release dynamic mechanisms [3,4]. Moreover, MTs are also involved in other important biology functions, such as detoxification, transport and storage of heavy metals such as cadmium and mercury by sequestering and preventing them from binding to sensitive biochemical sites in cells [5], as well as in the homeostasis and storage of essential

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metals like zinc and copper, which act as scavengers of free radicals and reactive oxygen metabolites.

The induction of MTs in a variety of aquatic animals in response to increased exposure to heavy metals has been extensively reported. Owing to their significant responses to heavy metal exposure, and a statistically significant correlation between the content of MTs in aquatic animals and the levels of environmental pollution from metals, MTs have also been proposed as potential biomarkers for metal pollution in the environment [6]. In the 1950s and 1960s, Minamata disease and bone ache disease were due to mercury and cadmium contamination, respectively, in Japan. Today international organizations are seriously concerned about aquatic environmental disruption and the dangers to humans resulting from heavy metal contamination, especially from cadmium, mercury, and lead from industrial sources [1]. Other studies have shown a statistically significant correlation between the content of MTs in human urine and the levels of environmental pollution due to metals. Accordingly, in regard to the widespread applications of MTs, the study of MTs as biomarkers for metal contamination has already become a major focus in many fields such as environmental science, biological science, medical science, toxicology and so on [7–9].

Due to the low molecular mass and unique primary structure of MTs in modern bioanalytical chemistry, detection and quantification of MTs are not easy tasks. Over the past 10 years, several analytical methods for the determination of MTs have been reported [10], including metal saturation assays [11], atomic absorption spectroscopy (AAS) [12], and so on [13]. These methods are primarily indirect and based on the content of coordinated metal ions in MT molecules or the high content of sulfhydryl groups, even having many deficiencies, such as insufficient specificity and sensitivity. Other quantification methodologies like enzyme immunoassay (ELISA) have also been proposed to detect MTs directly based on the use of specific antibodies [14,15]. The main obstacles to the use of ELISA methods are the need to avoid cross reactivity of polyclonal antibodies. The quantification of MTs by electrochemical methods, based on the reactivity of the –SH group, allows quantification at very low concentration levels [16,17]. Unfortunately, only a few of groups adopt this method for the purpose of quantification. In recent years, capillary zone electrophoresis (CZE) [18], real time PCR, high-performance liquid chromatography (HPLC) [19], and ICP-mass spectrometry [20] methods have been reported. However, these methods require specialized instrumentation, and the limits of detection of some assays are still not sufficient to measure MTs in biological samples. Therefore, the development of a convenient and reliable method for direct determination of MTs is urgently needed for monitoring environmental quality and for assessing occupational and environmental health risks. In addition, direct monitoring of MT fluctuations under physiological conditions could play an important role in investigations related to the pathophysiological and toxicological effects of cellular exposure to additional metal ions or reactive species.

Recently, fluorescence spectroscopy (FS) has been increasingly applied to the study and determination of some biological macromolecules, organic substances, inorganic ions, etc [21–25]. Compared with other analytical methods, FS has the distinct advantages of being less time-consuming, less instrumentation requirement, and high sensitivity and stabilization [26,27]. Furthermore, it can be accomplished with a common fluorescence spectrometer using inexpensive and safe reagents. Although florescence method allows sensitive analysis of bio-macromolecule with fluorophore, it is difficult to carry out in those proteins such as MTs which have no native fluorescence. Thus, it is very significant to select a fluorescence probe for the detection of MTs [28].

4,5-Dihydroxy-1,3-benzenedisulfonic acid (Tiron) [29] is very soluble in water and have a structure good for coordination with metal ions as shown in Fig. 1. To the best of our knowledge, there is no report on the determination of MTs using the tiron–Cu complex as a fluorescence probe.

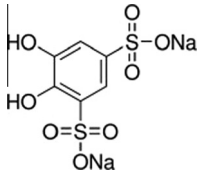

Fig. 1. 4,5-Dihydroxy-1,3-benzenedisulfonic acid monohydrate (tiron) structure.

In the present work, we report a highly sensitive probe based on tiron–Cu complex combined with FS technique to determine trace MTs in fish visceral organ samples. Our investigation shows that the high sensitivity of FS can provide a promising potential for the detection of protein in biological samples and can extend the applications of FS. Furthermore, the proposed method has some advantages over others, such as greater sensitivity, wider linear ranges, and lower limits of detection. In addition, the interaction between MTs and tiron–Cu complex, as well as the mechanism of the FS enhancement were investigated.

Experimental

Reagents and chemicals

All experiments were performed with analytical reagent grade chemicals and doubly distilled water. MTs was purchased from Sigma. The stock solution (1.54×10^{-5} mol L^{-1}) was prepared by dissolving an appropriate amount of MTs in water. The standard working solution was prepared by diluting the stock solution with water in a 100 mL volumetric flask to give a final concentration of 7.70×10^{-6} mol L^{-1}. Tiron was obtained from Shanghai Chemistry Reagent Company (China). To make the tiron solution, 0.0332 g tiron was weighed into a beaker and an appropriate amount of doubly distilled water was added to dissolve. The solution was transferred to a 100 mL volumetric flask and diluted to the mark with doubly distilled water to give a concentration of 1.0×10^{-3} mol L^{-1}. Then, 10.0 mL of the solution was transferred to a 100 mL volumetric flask and diluted to the mark with water to give a final concentration of 1.0×10^{-4} mol L^{-1}. The B–R buffer was prepared by dissolving phosphoric acid (1.410 mL), acetic acid (1.150 mL) and boric acid (1.227 g) and diluting to the mark with water in a 500 mL volumetric flask. The three-acid buffer solution concentration was 0.040 mol L^{-1}. The pH was adjusted by adding a known volume of 0.200 mol L^{-1} NaOH. The copper sulfate solution was prepared by accurately weighing 0.025 g $CuSO_4 \cdot 5H_2O$ dissolving in a small amount of water, transferring to a 100 mL volumetric flask, and diluting to the mark to give a concentration of 1.0×10^{-3} mol L^{-1}. Then, 10.0 mL of the solution was transferred to a 100 mL volumetric flask, and diluted to the mark to give a final concentration of 1.0×10^{-4} mol L^{-1}.

Apparatus

All fluorescence spectra were measured on a Shimadzu RF-5301 fluorescence spectrofluorometer (Shimadzu, Japan). Excitation and emission bandwidths were 5 nm, and the scanning speed was very fast. Model PB-20 pH meter (Sartorius AG, Germany) was used for pH adjustment and calibrated before each measurement. The electronic analytical balance (Sartorius AG, Germany) was used for determining the weights of all the reagents needed in the course of the experiments. All experiments were carried out at laboratory temperature (±25 °C).