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Methylene blue as a DNA probe for a comparative study of Cd^{2+}, Pb^{2+} and Cr^{3+} ions binding to calf thymus DNA

Changlun Tong *, Zhou Hu

MOE Key Laboratory of Environmental Remediation and Ecological Health, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, Peoples Republic of China

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ABSTRACT

Methylene blue (MB) was developed as a sensitive DNA probe for a comparative study of Cd^{2+}, Pb^{2+} and Cr^{3+} ions binding with calf thymus DNA (ctDNA). The fluorescence intensity of the MB–ctDNA system increased dramatically when heavy metal ions (Cd^{2+}, Pb^{2+} and Cr^{3+} ions) were added, which indicated that some of the bound MB molecules were released from the ctDNA base pairs. To compare the binding affinity of these three different heavy metal ions with ctDNA, the relationships between the fluorescence intensity of the MB–ctDNA–M (Metal ions) system and the concentration ratio of [M]/[DNA(p)] were investigated. The results showed that the order of the binding affinity of heavy metal ions with ctDNA had the following sequence: $Cr^{3+} > Cd^{2+} > Pb^{2+}$. This order was further proved by the effects of heavy metal ions on the number of MB bound to ctDNA, the measurements of binding constants of these heavy metal ions to ctDNA, and the effects of heavy metal ions on the absorption of the MB–ctDNA system. In addition, the interaction mechanisms of Cd^{2+}, Pb^{2+} and Cr^{3+} ions with ctDNA were also discussed in detail. These results indicated that their interaction mechanisms are related to the concentration ratios of heavy metal ions to DNA.

1. Introduction

Heavy metal ions such as cadmium, lead and chromium are ubiquitous environmental pollutants with seriously toxic and carcinogenic effects on human beings. It has been well known that they have deleterious effects on lung [1,2], renal system [3], nervous system [4], immune system [5] and reproductive tissue [6]. It has been suggested that carcinogenicity induced by heavy metal ions may involve direct or indirect interaction of heavy metal ions with DNA [7]. However, the exact molecular mechanism of heavy metal ions-induced carcinogenicity remains largely unclear. Direct interaction may involve covalent binding between heavy metal ions and DNA, whereas indirect interaction may be associated with oxidative damage to DNA, increasing cellular oxidants in the cells and producing free radicals. Indirect interaction may also involve the impairment of DNA repair processes via formation of DNA–protein and DNA–amino acid cross-links. When metal ions bind covalently to nucleobases, it may result in the perturbation of the electron density in the heterocyclic ring and cause weakened phosphodiester bonds, DNA may thus be damaged [8,9]. Nowadays, interactions of heavy metal ions with DNA have attracted more and more attention because of their toxicity and carcinogenicity [10,11]. Therefore, study on the binding properties of heavy metal ions with DNA is an active field of research.

Various techniques have been applied to investigate the interaction of heavy metal ions with DNA. These techniques include UV–visible spectroscopy [12], Raman spectroscopy [13], circular dichroism spectroscopy [14] and Fourier transform IR spectroscopy [15]. However, the spectrofluorometric methods have attracted more attention because of their high sensitivity and high selectivity. Due to the well-known facts that the fluorescence intensity of DNA itself is very weak and heavy metal ions are not luminescent, it is hard to monitor the interactions of these metal ions with DNA by employing direct fluorescence emission method. Generally, fluorescent probes including organic dyes such as ethidium bromide [16], Hoechst 33258 [17] or Phosphin 3R [18] and rare-earth ions [19] are usually employed to investigate DNA. Based on the luminescence characteristics of rare-earth ions, especially Tb^{3+} and Eu^{3+} [20], whose resonance energy levels overlap with ultraviolet light energy [21], they are thus widely used as fluorescent probes to study DNA. In recent years the coordination complexes of metal ions, especially rare-earth ions, as probes to study DNA have been reported [22]. Although many fluorescence probes with high sensitivity can be used for quantitative determination of DNA, only a few of these DNA probes are suitable to be used for the study of the interaction of small molecules with DNA. It is very important that the interaction of the probe itself with DNA should not influence the judgments of the interaction mechanism of small molecules

* Corresponding author. Tel./fax: +86 571 86968415.
E-mail address: cltong@zju.edu.cn (C. Tong).

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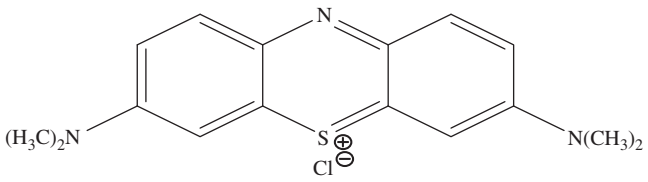

Fig. 1. Molecular structure of MB.

with DNA. For example, the metallic complexes used as fluorescence probes are not suitable for the study of the interaction between small molecules and DNA since in the metallic complexes, both the ligands and metal ions participate in the interaction with DNA to make the interaction mechanisms of small molecules with DNA more complicated. It must also be considered that the possible binding mode of the objective substance with DNA should be consistent with that of probe with DNA. For example, ethidium bromide [16] is an intercalation binding probe of DNA and Hoechst 33258 [17] is a groove binding probe.

Methylene blue (MB, Fig. 1), one type of photosensitizer drug, is known to bind to duplex DNA through intercalation as supported by the results of several spectroscopic methods [23,24]. MB is a near infrared (NIR) dye that has a strong absorption band in the long wavelength region and emits fluorescence in the NIR region from 600 to 1000 nm, where most biomolecules have no absorption. Based on its luminescence characteristics, we have reported it as a luminescence probe for quantitative determination of DNA [25]. Furthermore an interesting phenomenon that the binding modes of MB with DNA are related to molar ratio γ (γ=[DNA]/[MB]) was discovered, and the characterization of the interaction between MB and calf thymus deoxyribonucleic acid (ctDNA) has been investigated in detail [26]. In this present work, on the basis of the interaction characterization of MB with DNA, MB as a DNA probe was developed for a comparative study of the binding affinity of heavy metal ions to DNA helix, and its detailed interaction mechanisms were also discussed. This research work should be valuable for ecotoxicology and environmental health study due to the toxicological importance in the binding study of heavy metal ions with ctDNA.

2. Experimental section

2.1. Apparatus

The fluorescence spectra and intensities were measured on a model F-2500 spectrofluorimeter (Hitachi, Japan) with a quartz cell (1×1 cm^2 cross section) equipped with a xenon lamp (150 W) and a dual monochromator. All absorption spectra were recorded with a UV-2401 PC spectrophotometer (Shimadzu, Japan). All pH measurements were made with a MP 220 pH meter (Mettler Toledo, China).

2.2. Reagents

All the chemicals used in this work were of analytical reagent grade and used without further purification. Double distilled water was used for solution preparation. Commercially prepared calf thymus DNA (purchased from Sigma, USA) was suspended directly in water at a final concentration of 100 μg mL^{-1} as stock solution. After establishing the absorbance ratio A_{260}/A_{280} in the range of 1.80–1.90 for DNA, the concentration of DNA was determined according to the absorbance at 260 nm using $\varepsilon_{DNA(p)}$=6600 L mol^{-1} cm^{-1}. The stock solution and its diluted solutions were stored in a refrigerator at 4 °C until use. All diluted solutions of DNA were used within 24 h. A stock solution (1.0×10^{-3} mol L^{-1}) of MB (purchased from Beijing Chemical Factory, China) was prepared by dissolving the corresponding MB in water. The concentration of MB was determined according to the absorbance at 664 nm using ε_{MB} =76,000 L mol^{-1} cm^{-1}. The stock solutions of $CdCl_2$, $PbCl_2$ and $CrCl_3$ were of the same concentration of 0.01 mol L^{-1}. A 0.2 mol L^{-1} tris(hydroxymethyl) aminomethane buffer solution (Tris–HCl) was prepared by dissolving the corresponding Tris in water and adjusting pH to 7.3 with 1:1(v/v) hydrochloric acid to give a final total volume of 500 mL. All the above solutions were further diluted with distilled water as required.

2.3. Fluorometric titration experiments

Fluorometric titration experiments were performed as follows: to a 10 mL colorimetric tube, 1.0 mL of 0.2 mol L^{-1} Tris–HCl buffer (pH 7.3), 1.0 mL of 4.0×10^{-5} mol L^{-1} MB solution and 1.0 mL of 2.75×10^{-4} mol L^{-1} ctDNA solution were added, diluted to 10.0 mL with water and then shaken gently to uniformity. The colorimetric tube containing 10.0 mL mixture solution of 4.0×10^{-6} mol L^{-1} MB, 0.02 mol L^{-1} Tris–HCl buffer and 2.75×10^{-5} mol L^{-1} ctDNA was allowed to stand for 15 min at room temperature. The mixture solution was titrated by successive additions of 0.01 mol L^{-1} stock solutions of Cd^{2+}, Pb^{2+} and Cr^{3+}, respectively; volume of each addition was 0, 10, 20, 40, 60, and 80 μL. All the final concentrations of Cd^{2+}, Pb^{2+} and Cr^{3+} were in the range of 0–80 μmol L^{-1}. For every addition, the mixture solution was shaken and allowed to stand for 5 min at room temperature, and the emission spectra were recorded in the range of 650–750 nm at an excitation wavelength of 630 nm. The entrance and exit slits for all fluorescence measurements were both maintained at 10 nm. A pure MB solution was prepared in a similar manner without ctDNA. After every determination, the residue solution in a 1-cm quartz cell was returned to the colorimetric tube. In the course of successive additions, the stock solutions of heavy metal ions were used so that volume increment (the total increment volume was 0.21 mL) was negligible compared with the 10.0 mL mixture solution. Titrations were done manually by using a trace syringe.

2.4. UV–vis absorption measurements

UV-vis absorption spectra in aqueous solution were all recorded using a 1-cm quartz cell in the wavelength range of 500–800 nm. The experimental procedures and the solutions for the measurements of absorption spectra were the same as those of the measurements of fluorescence spectra except for the titration volumes. The volume of each addition was 0, 20, 40, 60, 80, and 100 μL, respectively. The final concentrations of Cd^{2+}, Pb^{2+} and Cr^{3+} in the mixture solutions were in the range of 0–100 μmol L^{-1}. All absorption spectra had the background absorption subtracted from all the reagents by using a corresponding solution without MB as a reference solution.

3. Results and discussion

3.1. Spectral properties of MB

The primary condition to determine if a fluorescence probe can be used to study the interaction between heavy metal ions and DNA is that heavy metal ions need to have a response on the fluorescence of the probe–DNA system, while heavy metal ions themselves should have no effects or weak effects on the fluorescence properties of the probe. Furthermore, heavy metal ions themselves should have no fluorescence or their fluorescence should have no interference on the fluorescence of the probe.