Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 135 (2015) 669–675

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Investigation on the interaction between an antimicrobial in aquaculture, malachite green and hemocyanin from Mud Crab *Scylla paramamosain*

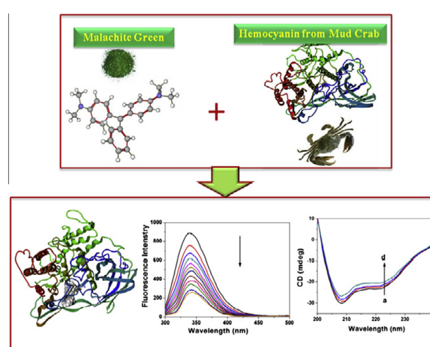
Zhenxing Li [a], Boping Tang [a,*], Hongmei Zhang [b]

[a] *Jiangsu Provincial Key Laboratory of Coastal Wetland Bioresources and Environmental Protection, Yancheng City, Jiangsu Province 224002, People's Republic of China*
[b] *Institute of Applied Chemistry and Environmental Engineering, Yancheng Teachers University, Yancheng City, Jiangsu Province 224002, People's Republic of China*

HIGHLIGHTS

- Binding interaction exists between MG and Hc.
- MG binds at the binding cavity of Hc.
- Multi- noncovalent interactions stabilize MG–Hc complex.
- MG induces some conformational changes of Hc.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 May 2014
Received in revised form 7 July 2014
Accepted 17 July 2014
Available online 29 July 2014

Keywords:
Malachite green
Hemocyanin
Spectroscopy measurement
Binding mode
Binding site
Molecular modeling

ABSTRACT

Interaction between malachite green and hemocyanin of crab plays a crucial role in the metabolism, distribution, and efficacy of toxic dyes in aquaculture. The mechanism of interaction between malachite green and Hc from mud crab was studied by using multi-spectral methods and molecular modeling in this work. The spectroscopic and thermodynamic data show that the interaction is a spontaneous process with the estimated enthalpy and entropy changes of $-14.85(\pm 1.86)$ kJ mol^{-1} and $30.38(\pm 5.21)$ J mol^{-1} K^{-1}, respectively. The binding sites of malachite green in hemocyanin mainly locate in the interface of protein. The hydrophobic and electrostatic forces are the primary contributors to the interaction between hemocyanin and malachite green. The results of ultraviolet–vis absorbance, circular dichroism, and synchronous fluorescence spectroscopy suggest that the binding of malachite green to hemocyanin induces some conformational changes of protein.

© 2014 Elsevier B.V. All rights reserved.

Introduction

Malachite green (MG) is a cationic triarylaminnethane dye (Fig. 1). It was widely used in the textile, paper, leather, and farming industries [1,2]. Now, many studies have shown that MG is harmful to human and others due to its reported toxic properties that are known to cause carcinogenesis, mutagenesis, teratogenecity and respiratory toxicity [3]. Therefore, MG is not permitted for use as an aquaculture veterinary drug in many countries and areas including the United States, European Union, and China, etc [4,5]. Unfortunately, illegal use of MG continues worldwide in aquaculture due to its low cost, very availability, and powerful antimicrobial activity [6]. Numerous incidences of MG misuse in

* Corresponding author. Tel./fax: +86 515 88233991.
E-mail address: tangwang2006@126.com (B. Tang).

http://dx.doi.org/10.1016/j.saa.2014.07.062
1386-1425/© 2014 Elsevier B.V. All rights reserved.

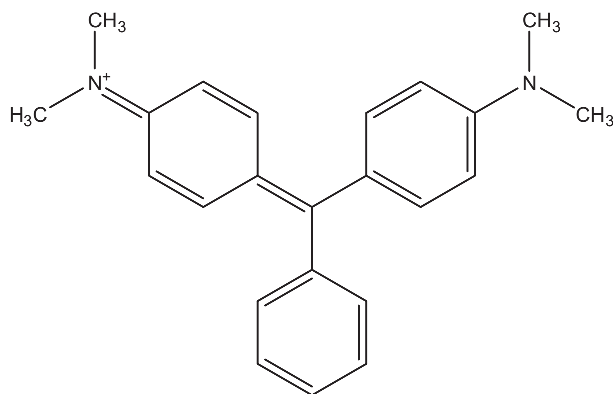


Fig. 1. The molecular structure of malachite green.

aquaculture have also occurred [7]. Owing to MG becomes a highly controversial compound and has potential toxic risk, it is very important to investigate the interaction of MG with proteins, as it can provide an insight into the transport and metabolism process of the toxic dye [8]. Considerable amounts of research are being devoted to work out the wide spectrum of biological effects it exerts on different animals and humankind [9]. Ding and colleagues have studied the interaction of MG with lysozyme [10]. Zhang et al have probed the interaction of MG with bovine serum albumin [11,12], yet the studies on binding interaction of MG with proteins of mollusks in aquaculture have not been reported.

Hemocyanin (Hc) is well-known oxygen carrier protein found in mollusks and arthropods. Like hemoglobin, it is efficient dioxygen transporting protein [13]. As an oxygen carrier protein, Hc is often selected as a binding model to investigate the effects of many low molecular weight compounds on its structure and oxygen binding [14–16]. In addition, inducing the conformational change of Hc is used to gain the catalytic activity of protein [17]. However, the interaction mechanism between MG and Hc has not been reported. In this report, we provide investigations on the interaction of MG with Hc using fluorescence, UV/vis absorption, circular dichroism (CD) spectra techniques and molecular modeling method. The studies on the binding mechanism of MG with Hc can provide the basic data for understanding the toxicity mechanism of MG in aquatic animal.

Material and methods

Reagents and chemicals

Hemocyanin from Mud Crab *Scylla paramamosain* were withdraw from the base of the swimming legs of crab by a syringe, and then an equal volume of pre-cooled (4 °C) anticoagulant buffer (18 mM Tris-HCl, 50 mM EDTA, 32.4 g/L NaCl, 1.0 g/L KCl, and 0.3 g/L glucose) was immediately added to the Hc sample. The absorbance coefficient of 93,000 M^{-1} cm^{-1} at 278 nm is used to evaluate the concentration of Hc solution. MG was purchased from Sigma–Aldrich Chemical Company. The Tris, NaCl, etc. were all of analytical purity. The MG solution (2.5×10^{-3} mol L^{-1}) was prepared in pH 7.40 Tris–HCl buffer. Doubly distilled water was used throughout.

Spectral measurements

The fluorescence spectra were recorded on a LS–50B spectrofluorimeter (Perkin–Elmer, Waltham, Massachusetts, USA). The UV/vis spectra were recorded on a SPECORD S600 (Jena, Germany) and the CD spectra were measured by a Chirascan spectrometer (Applied Photophysics Ltd., Leatherhead, Surrey, UK) equipped with temperature control quantum for temperature control.

The steady-state fluorescence spectra were measured with an excitation at 280 nm and emission wavelengths at 300–500 nm with 5.0 nm/5.0 nm slit widths. A 2.5 mL portion of a 5.0×10^{-6} mol L^{-1} solution of Hc was titrated manually by successive additions of MG solution to give final concentrations in the ranges of 5.0×10^{-6} to 9.0×10^{-5} mol L^{-1}. The synchronous fluorescence spectra of Hc in the absence and presence of MG were recorded with the $\Delta\lambda$ values of 15 nm or 60 nm at 288 K under the same conditions as the steady-state fluorescence.

The UV/vis absorbance spectra of Hc, MG, and the difference absorption spectra between Hc and MG system were recorded under the following conditions: wavelength 200–500 nm, slit width 1 nm and scanning speed 200 nm min^{-1}. A fixed concentration of Hc (5.0×10^{-6} mol L^{-1}, 2.5 mL) was prepared in pH 7.40 Tris–HCl buffer in the absence and presence of MG were added to the sample cell.

The CD spectra of Hc in the absence and presence of MG were recorded from 190 to 250 nm at a scanning speed of 200 nm min^{-1}. The Hc solution was prepared in a Tris-HCl buffer of pH 7.40 and stored in a 1 mm quartz cell. During experiment, Each solution in the presence of MG was allowed to equilibrate for 2 h before measurement. Measurements were duplicated three times to obtain an average curve.

Molecular docking

The molecular docking program Autodock 4.2 was used to automatically docked MG to the binding cavity of Hc [18]. The molecular structure of MG was optimized using density functional theory (DFT) at the level of B3LYP/6-311G by the program Gaussian 09 [19]. The crystal structure of Hc(PDB id:1LLA, 2.18 Å) taken from Brookhaven Protein Data Bank (PDB) was used as the initial coordinates for molecular docking [20]. Water molecules were removed, and hydrogen atoms were added. In molecular docking study, the grid maps were set at 0.375 spaces using a grid box of 126–126–126 Å, which includes the entire binding site of Hc and provides enough space for MG translational and rotational walk. The docking parameters used were as follows: Number of GA runs = 100; GA population size = 150; maximum number of evals = 2,500,000 and others used were default parameters. Docking simulations were performed by using of the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method. Cluster analysis was performed on docked results using a root-mean-square deviation (rmsd) tolerance of 2.0 Å. The presented stereo view of the docked best conformation has been prepared by Molegro Molecular Viewer software.

Results and discussion

The interaction between MG and Hc

Hc is one of multi-tryptophan proteins, so the fluorescence emission spectra can be used to analyze the conformational transitions, bio-molecular binding, association, or denaturation of Hc [21,22]. The effect of MG on the fluorescence intensity of Hc was shown in Fig. 2. The intensity of the characteristic broad emission band at 338 nm decreased regularly with the increasing concentration of MG, which indicated that the interaction of MG and Hc occured. In addition, the maximum emission peak at 338 nm indicated that many tryptophan (Trp) residues were buried in the hydrophobic cavities of Hc. There are three kinds of Trp residues. The first kind is fully quencher-accessible Trp that contribute about 35–45% fluorescence of the overall emission; the second kind Trp

Download English Version:

https://daneshyari.com/en/article/1233237

Download Persian Version:

https://daneshyari.com/article/1233237

Daneshyari.com