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Quest for the binding mode of malachite green with humic acid

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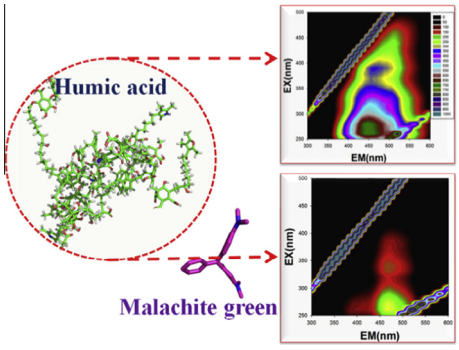
HIGHLIGHTS

- Spectroscopic methods combination with molecular modeling were used.
- MG located in the hydrophobic cavity of HA.
- The π–π stacking and hydrophobic forces played a key role in the binding.
- HA binding caused coplanar conformation changes of MG.

GRAPHICAL ABSTRACT



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ABSTRACT

The association of malachite green (MG) with humic acid (HA) was investigated by using fluorescence, UV–vis spectroscopy and molecular Modelling method. The fluorescence spectral results indicated that the binding between MG and HA occurred by mainly hydrophobic and electrostatic forces with association constants of K_A (298 K) = 6.24 × 10^5 L/mol and K_A (310 K) = 10.20 × 10^5 L/mol. There were more than one binding sites on HA to bind with MG. The binding sites of MG with HA primarily located at the aromatic rings of HA. MG could enter into the hydrophobic cavities of HA to quench the fluorescence of HA. On the contrary, HA binding caused MG to a coplanar conformation with more extended π bond distribution by π–π stacking interactions. The experiment and calculation data both showed that the hydrophobic binding cavities in HA played a key role in its binding with MG.

Introduction

Malachite green (MG, Scheme 1), a triphenylmethane dye, is the most widely used dye for colouring purpose among all other dyes of its category [1]. Furthermore, MG is also extensively used as a parasiticide and antibacterial in the commercial aquaculture due to its high effectiveness against oomycete Saprolegnia and ichthyophthirius in freshwater aquaria [2,3]. Thus, significant amounts of MG have the potential to enter natural environment through dye wastewater from textile dyeing and dye manufacturing and aquaculture industry [4]. MG has some hazardous and carcinogenic effects on human including acting as a liver tumour promoter, decreasing food intake capacity, causing damage to spleen, kidney and heart, etc [5]. In addition, it also has acute toxicity to a wide range of aquatic and terrestrial animals [3]. Hence, understanding how MG binds with matters in natural environment is important for assessment of the negative ecotoxicological effects and bioaccumulation of MG in wildlife.

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Humic substances, a kind of important matter in natural environment, represent the mainly dissolved organic matter found in soil, water and sediments. The sorption and desorption of organic compounds with HA can affect the environmental behaviours of them [6–7]. Therefore, the studies on the interactions of organic compounds or metal ions with HA are still hot research topics [8]. For instance, Wang et al. have examined the sorption mechanisms of phenanthrene, lindane, and atrazine with HA to find the different interaction mechanisms of apolar and polar organic compounds with humic substances [9]. Bedard et al. have studied the binding interactions between 17β-estradiol and estriol with HA via NMR and biochemical analysis to yield a more comprehensive model of binding of HA with estrogens [10]. Zhao et al. have investigated the binding behaviour of phenanthrene by humic acids, proteins and their complexes using a passive dosing technique [11]. Especially the debate on the structural components of HA in organic compound sorption and desorption is also still ongoing [9]. In addition, the debate on the importance of the structural components of HA in organic compound sorption and desorption is still ongoing [9].

In this article, we present the binding characteristic of MG with HA by using fluorescence, UV–vis spectroscopy and molecular modelling methods. We quantified the binding energy, binding constant, binding sites, and binding forces of the association of MG with HA as a function of solution conditions by spectra experimental data. In addition, we used the optimal model of HA to deep insight into the nature of the intermolecular forces of MG with HA by using docking software. This combination of spectral and theoretical methods was employed to find a more important understanding of binding interactions of the triphenylmethane dyes with HA.

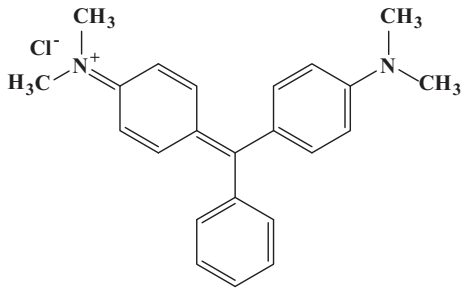
Materials and methods

Materials

MG (purity, ⩾96%) was obtained from Sigma–Aldrich. HA was obtained from Shanghai Jufeng Company (BR, China). KOH, HNO_3, KNO_3, etc. were all of analytical purity. Water (>18.2 MΩ cm^{-1}) was purified with a Milli-Q equipment. The HA solution (30 mg/L) were prepared according to Ref. [12]. The MG solution (2.5×10^{-3} mol/L) was prepared by dissolving MG in purified water.

Fluorescence measurements

Fluorescence data were obtained on a LS–50B Spectrofluorimeter from Perkin–Elmer USA. The excitation wavelength was 325 nm and the emission spectra were recorded between 350 nm and 600 nm for steady-state fluorescence spectra. The slit widths were 5.0 nm/5.0 nm. During the fluorescence experiments, the HA concentration in each sample was 30 mg/L while MG concentrations were varied from 0.0 to 6.0×10^{-5} mol/L. In addition, the three-dimensional fluorescence spectra of HA in absence and presence of MG were performed using the following conditions. The emission wavelengths were set from 300 nm to 600 nm while the excitation wavelength were set from 250 nm to 500 nm with scanning number 26 and increment 10 nm, other parameters were just the same as those of the steady-state fluorescence spectra.



Scheme 1. The molecular structure of MG.

UV–vis absorbance measurements

The UV–vis absorption spectra were measured on a SPECORD S600 spectrophotometer from Jena of Germany. The UV–vis absorbance spectra of MG (2.0×10^{-5} mol/L), the difference absorption spectra between HA–MG and HA (30 mg/L) were recorded.

Molecular modelling

In the present study, Autodock 4.2.3 program was used to perform the molecular modelling study of HA with MG [13]. The 3D structure of MG was built and optimized using DFT/B3LYP/6-311G by Gaussian 09 [14]. For HA model designing, two models with different molecular weight were obtained according Ref. [15,16]. The 3D structures of HA were also generated and optimized with using Gaussian 09. During molecular modelling studies, HA was enclosed in a big grid box of 126–126–126 Å with 0.375 Å grid maps to provide enough space for the translational and rotational walk of MG. Lamarckian Genetic Algorithm, as implemented in AutoDock, was used to perform the blind docking calculations with the following parameters: the number of GA runs = 100; the GA population size = 150; the maximum number of energy evaluation = 2,500,000, and other parameters were default settings. Finally, the lowest energy docked conformation was selected as the best binding mode. The PyMol software was used to analyze the output from AutoDock [17].

Results and discussion

UV–vis absorption studies

The structure changes of MG caused by HA binding were observed through UV–vis absorption spectra. The electrostatic charge distribution across the three rings of MG change when MG enters into the hydrophobic pocket of macromolecule [18]. The effects of HA binding on the UV–vis absorbance spectra of MG were shown in Fig. 1. MG has three absorption peaks,

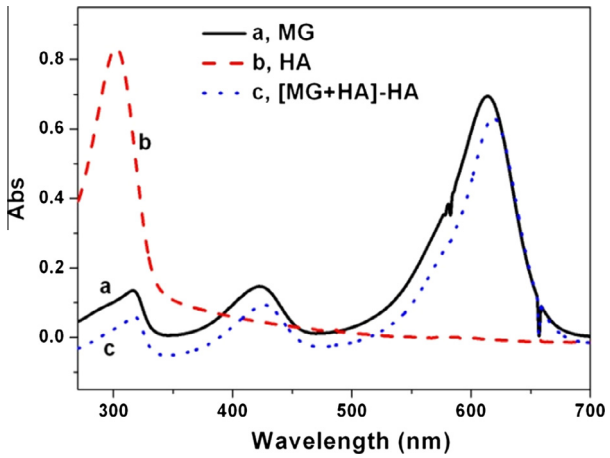


Fig. 1. The UV–vis absorption spectra of MG (2.0×10^{-5} mol/L) (line a), HA (30 mg/L) (line b) and the different absorption spectrum between MG–HA and HA (the concentration of HA: 30 mg/L, the concentration of MG: 2.0×10^{-5} mol/L).