Electronic Supplementary Material (ESI) for RSC Advances
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Electronic Supplementary Information (ESI)

MC-LR@HSA: non-covalent interaction and effect

Chao Song, Yan-Qin Zi, and Hong-Wen Gao

Experimental

Apparatus and chemicals

A P/ACE MDQ capillary electrophoresis system (Beckman, Coulter, Fullerton, CA, USA) with a UV detector was used in the capillary electrophoresis experiment. The uncoated fused-silica capillary (75 µm i.d.) was purchased from Polymicro Technologies Inc. (Phoenix, AZ, USA), and capillary length to detector was 50 cm. All the fluorescence experiment was carried out on the F-4500 fluorophotometer (Hitachi High-Technology, Tokyo, Japan) equipped with 1 cm quartz cell and a thermostat bath. The Isothermal Titration Calorimeter (ITC) experiments were carried out on a Model VP-ITC system (MicroCal, USA). A Model J- 715 CD spectropolarimeter (Jasco Instruments, Tokyo, Japan) with secondary structure estimation-standard analysis software was used to determine the conformation of HSA. The Model RC 30-5K semi-permeable membranes (Molecular Weight Cut Off 5 kDa, Shanghai Green Bird STD, China) were used for equilibrium dialysis. An electronic thermoregulating water bath (NTT-2100, EYELA, Japan) was used to control the temperature of the samples. Millipore MilliQ water-purification system (Millipore Corp., Bedford, OH, USA) was applied to produce ultrapure water used throughout the experiment.

MC-LR (CAS101043-37-2, 95-99% by HPLC) was purchased from GenBank, Taiwan and HSA (CAS A3782, fatty acid free <0.05%) from Sigma-Aldrich Co. (USA). A boric acid (0.2 mol L^{-1}) - borax (0.05 mol L^{-1}) buffer was used to adjust pH of the solution at 7.4, and NaCl (1.0 mol L^{-1}) solution to keep ionic strength at 0.15 mol L^{-1}. A HSA stock solution, 1.0×10^{-5} mol L^{-1} was prepared with the boric acid - borax solution and a stock MC-LR solution (1.0 mmol L^{-1}) with ultrapure water. All other chemicals were of analytical grade, and ultrapure water was used throughout the experiments. All stock solutions were stored at 4 ºC.

CE measurement

The capillary electrophoresis was performed under following conditions: the running buffer contained 0.20 mol L^{-1} boric acid and 0.05 mol L^{-1} borax, and the pH was maintained at 7.4. The temperatures of the sample room and cartridge were set at 25 ºC. Samples were injected with the pressure injection mode at 0.5 psi for 5 s at the positive side and detected at 214 nm. The separation was conducted under the voltage of 18 kV. After each run, the capillary was rinsed with ultrapure water, 1 mol L^{-1} NaOH, ultrapure water, and the buffer for 3 min each. All the solutions injected in the capillary were filtered through 0.22 μM nylon membrane before use. Series of mixtures with different concentrations of MC-LR in the range between 5.0 and 200.0 μmol L^{-1} and a constant HSA concentration of 7.5 μmol L^{-1} were prepared by dilution of the stock solutions to the required concentrations with the running buffer.

Fluorescence measurement

A 2.0 ml of solution containing 5.0 μmol L^{-1} HSA was added to the quartz cell, and then titrated by successive addition of 10 μL of the 1.0 mmol L^{-1} stock solution of MC-LR with the trace syringes to give a concentration ranging from 0 to 120 μmol L^{-1}. The fluorescence spectra were recorded at 26 ºC with recycle water keeping the temperature constant. The excitation and emission slits of the passage of band were set at 10 and 20 nm, respectively, and the scanning speed was set at 12000 nm min^{-1}. The fluorescence spectra were recorded at excitation wavelength of 295 nm and the emission wavelength from 290 to 500 nm.

The 3D fluorescence spectra were conducted as follows: the emission wavelength was scanned in the range from 200 to 500 nm with the increment of 10 nm, and the excitation wavelength was set between 200 and 350 nm with the increment of 10 nm. The scan speed was set at 12000 nm min^{-1} and the excitation and emission slits with a band pass of 5 nm were used in the 3D fluorescence spectra. All experiments were performed at the temperatures of 26 ºC. The 3D fluorescence spectra of HSA (5.0 μmol L^{-1}) were recorded in the presence of MC-LR in 0, 10, 40 and 90 μmol L^{-1}, and the data were analyzed in the golden software surfer 8.0.

CD measurement

The 40 μl HSA (20 μmol L^{-1}) were mixed with 0, 5, 20 μmol L^{-1} MC-LR in 2 ml flasks. Each sample was allowed to equilibrate for 15 min before measurement. CD spectra were taken with a spectropolarimeter with a 0.1cm light path cell at 26 ºC. The mean residue ellipticity (θ) of HSA was measured between 190 and 250 nm with 0.1 nm data pitch, and averaged over three scans with the scanning speed of 100 nm min^{-1}. From θ curves, the relative contents of secondary structure forms of HSA including α-helix, β-pleated sheet, β-turn and random coil, were calculated by the secondary

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structure estimation-standard analysis software.

ITC characterization of the MC-LR/HSA interaction

ITC experiments were carried out as follows. The MC-LR solution (2 mmol L^{-1} was injected about 10 times in 10-μl increments at 270-S intervals into the isothermal cell containing HSA (10 μmol L^{-1}). The cell temperature was kept at 26 ºC. In each experiment, an exothermic heat pulse was detected following every injection. Its magnitude progressively decreases until a plateau is reached indicating the saturation of binding. The heat involved at each injection was corrected for the heat of dilution, which was determined separately by injecting the MC-LR solution into the B-R buffer and then divided by the number of moles injected and then analyzed. The *N*, enthalpy change (ΔH), and entropy change (ΔS) of the reaction were calculated by the Gibbs free energy (ΔG) equation.

Effect of MC-LR on the physiological function of HSA

Equilibrium dialysis was conducted to reveal the effect of MC-LR on the physiological function of HSA transporting vitamin B_2 (VB_2) with a special dialysis device of equilibrium dialysis designed by Gao's group [49](Zhang et al., 2009). 3.0 ml of solution containing 1.5 ml B-R buffer, 0.15 mmol L^{-1} NaCl, 0.02 mmol L^{-1} HSA, and the gradient concentrations of MC-LR were added into the dialysis bag, which was merged in 24.0 ml of dialysate solution containing 0.15 mmol L^{-1} NaCl and B-R buffer. After dialysis for 10 h at 26 ^{o}C, 2.5 ml of dialysate solution was sampled, and the VB_2 concentration determined with a fluorospectrophotometer. The excitation (λ_{ex}) and emission (λ_{em}) wavelength were at 440 and 525 nm, respectively. The fluorescence intensity of VB_2 in the dialysis solution was recorded to quantify the content of VB_2. The equilibrium dialysis for VB_2 as well as that for VB_2-HSA solution was confirmed to be attained at 10 h. The V_{B2} standard curve for equilibrium dialysis was made at 26 ºC and the binding number of VB_2 was calculated.

Figures S1-S3

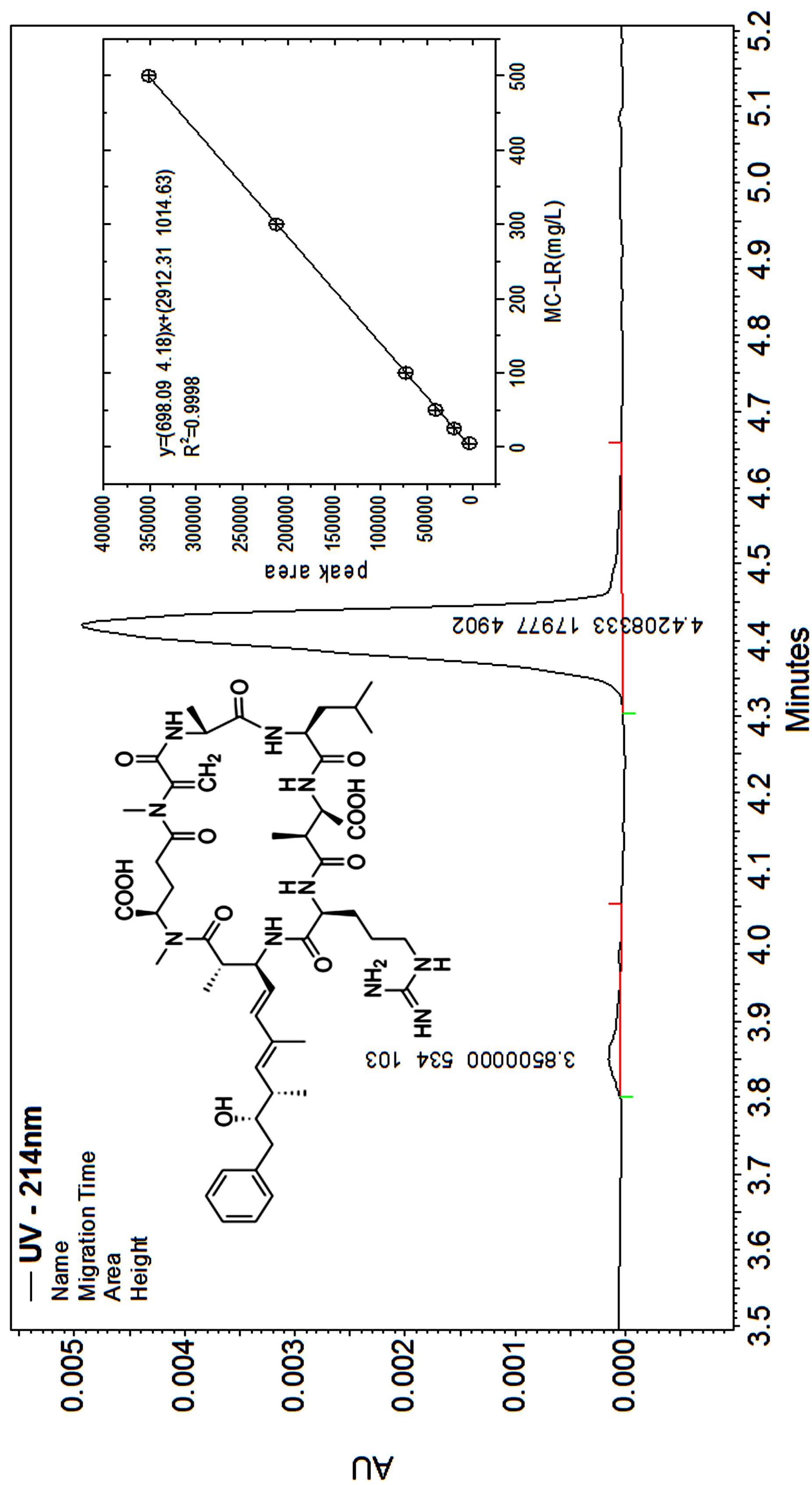

Fig. S1. Calibration curve for determination of MC-LR and CR chromatogram for MC-LR (50 mg·L^{-1}) eluted at 4.42 min at 214 nm with a UV detector.

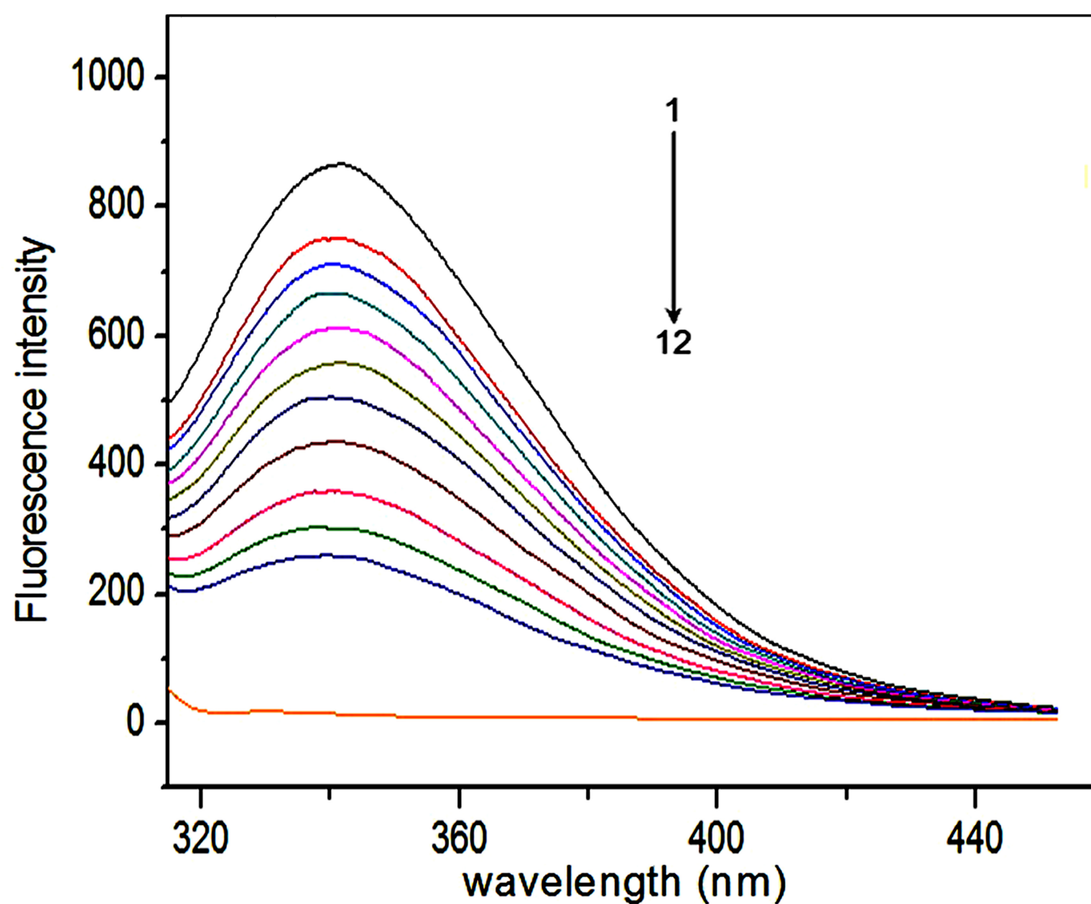

Fig. S2 Effect of MC-LR on the fluorescence spectra of HSA (5 μM) after exposed in the MC-LR solutions (c_0 from 1 to 11: 0, 5, 10, 20, 35, 40, 55, 75, 90, 95 and 110 μM and 12: 110 μM MC-LR without HSA)

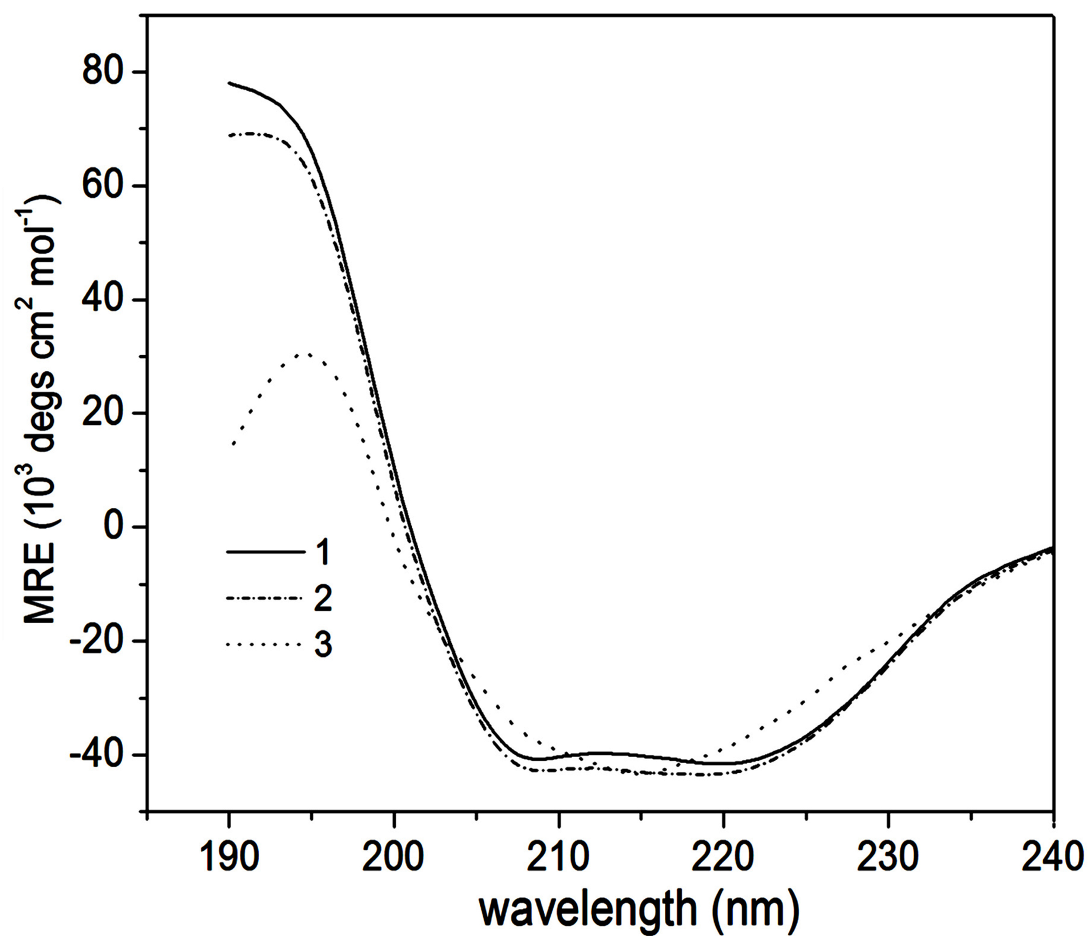

Fig. S3 CD curves of HSA (5.0 μM) in the presence of MC-LR (1 to 3: 0, 5 and 20 μM) at pH 7.4