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Kinetics and thermodynamics of interaction between sulfonamide antibiotics and humic acids: Surface plasmon resonance and isothermal titration microcalorimetry analysis

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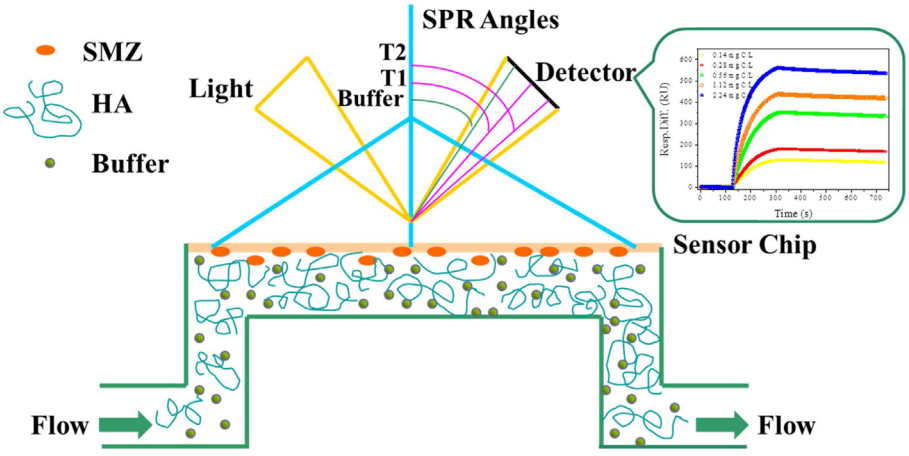
HIGHLIGHTS

- HA would significantly affect the migration and transformation of SMZ.
- Kinetics and thermodynamics of HA–SMZ interactions were studied using SPR and ITC.
- The interaction is enhanced by increasing ionic strength and decreasing temperature.
- Hydrogen bond and electrostatic interaction play important roles in the process.

GRAPHICAL ABSTRACT



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ABSTRACT

The presence of sulfonamide antibiotics in the environments has been recognized as a crucial issue. Their migration and transformation in the environment is determined by natural organic matters that widely exist in natural water and soil. In this study, the kinetics and thermodynamics of interactions between humic acids (HA) and sulfamethazine (SMZ) were investigated by employing surface plasmon resonance (SPR) combined with isothermal titration microcalorimetry (ITC) technologies. Results show that SMZ could be effectively bound with HA. The binding strength could be enhanced by increasing ionic strength and decreasing temperature. High pH was not favorable for the interaction. Hydrogen bond and electrostatic interaction may play important roles in driving the binding process, with auxiliary contribution from hydrophobic interaction. The results implied that HA existed in the environment may have a significant influence on the migration and transformation of organic pollutants through the binding process.

1. Introduction

Antibiotics residues in the environment are considered to be emerging pollutants due to their acute and chronic toxic effects on public health [1–3]. Among the major classes of antibiotics, sul-

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fonamide antibiotics are produced in large quantities and heavily used in human therapy and livestock production [4]. They have been widely detected in the effluents from municipal wastewater treatment plants, natural waters and soils [5–8]. Immigration of these sulfonamides into environments would induce spread of antibiotic resistance in response to increased selective pressure, potentially leading to proliferation of resistant pathogens [5]. Furthermore, it was reported that up to 90% of sulfonamides were excreted within 1–2 days into a wastewater treatment system or in the natural environment, and some metabolites of sulfonamides could convert back to parent compounds [9]. As a result, the present of these residual sulfonamides in the environment may arouse possible environmental risks.

Humic acids (HA) are main component of natural organic matters presented in the natural environment. They are high molecular weight and heterogeneous organic materials with various functional groups [10], and can affect the transport, persistence and bioavailability of pollutants in the environment [11,12]. Thus, understanding the interaction between sulfonamides and HA is essential for assessing the potential of sulfonamides to leach into the natural environment. However, little information is available about the kinetics and thermodynamics for such interactions. Furthermore, due to the solubility of HA in aqueous solutions, it is difficult to study their binding characteristics for pollutants. Several methods have been developed to analyze the binding characteristics of HA, such as solubility enhancement [13,14], reverse phase separation [15,16] and dialysis [17]; however, these approaches are generally time-consuming and insensitive. Novel sensitive methodologies are still needed to effectively characterize the interaction between HA and pollutants.

In this study, surface plasmon resonance (SPR) combined with isothermal titration calorimetry (ITC) was used to investigate the interaction between HA and sulfamethazine (SMZ), a typical sulfonamide widely found in the environment [18]. SPR is a widely used sensor technology to measure molecular interaction affinities between soluble analytes and ligands immobilized on the metal sensor surface [19–21]. It is a sensitive and quantitative biophysical approach that can measure binding affinity and kinetics simultaneously [22–24]. ITC, as a principal microcalorimetric technique, can be used to directly obtain thermodynamic information about biochemical binding processes at a constant temperature [25,26]. With the integration of these simple and effective techniques, the kinetics and thermodynamics of the interaction between HA and SMZ could be determined readily and accurately, allowing for a better understanding of the migration and transformation of organic pollutants in the environment.

2. Material and methods

2.1. Chemicals

HA were purchased from RCNC Corp., China and were purified prior to use according to the method proposed by Stevenson [27]. SMZ was purchased from Sigma–Aldrich Corp. In this work, HA were dissolved in phosphate buffers (PBS) to the desired concentration, and the actual concentration was measured by total organic carbon (TOC) Analyzer (Vario TOC, Germany) and expressed as TOC concentration.

2.2. SPR analysis

The interaction between HA and SMZ was measured using an SPR device with dual-channel detection (Biacore 3000 system, GE, USA). Before analysis, SMZ was firstly immobilized on the commercial CM5 (carboxymethylated dextran) sensor chip. The CM5 chip was activated using an amine coupling reagent mixture containing 0.4 M EDC [1-ethyl-3-(3-dimethylaminopropyl) carbodi-imide-HCl] and 0.1 M NHS (*N*-hydroxysuccinimide) at a flow rate of 5 μL/min for 12 min. SMZ solution with a concentration of 0.5 mg/mL in PBS buffer (50 mM, pH 7.0) was injected into channel 1 on the CM5 chip at a flow rate of 5 μL/min for 20 min, while PBS buffer without SMZ was injected into channel 2 as reference. The remaining *N*-hydroxysuccinimide was blocked by a 2 min pulse of ethanolamine (pH 8.5, 10 μL/min). After immobilization, the stability of the SMZ immobilized surface was confirmed by three washes with regeneration solution (50 mM NaOH contained 0.05% SDS followed by 50 mM NaOH).

HA with various concentrations (0.13–2.88 mg C/L) in PBS buffer passed over the SMZ-immobilized SPR chip surface (10 μL/min, 30 μL injection) for 3 min to allow association. Then PBS buffer passed over the surface for 7 min for dissociation. Afterwards, the chip surface was regenerated. The signal, in resonance units, was monitored with respect to time for real-time monitoring. Each analyte solution was injected into both channel 1 and channel 2. The specific response of the SMZ surface was obtained by subtracting the channel 2 response from the channel 1 response.

All SPR sensorgrams were processed using BiaEvaluation 4.1 software. Sensorgrams were firstly zeroed on the *y*-axis and then *x*-aligned at the initial injection. Kinetic parameters, such as the dissociation rate constant (k_d) and the association rate constant (k_a), were determined by fitting the sensing curves at dissociation stage and association stage based on 1:1 molecular binding model. As these kinetic parameters were independent of the HA concentrations used, the average values of kinetic parameters were calculated from five curves with gradient HA concentrations.

Thus, the dissociation rate constant (k_d) could be derived from the equation:

$$R_d = R_0 e^{-k_d(t_1 - t_{1,0})} + R_{offset} \quad (1)$$

where R_d is the SPR response at time t_1 at dissociation stage; R_0 is the SPR response at time $t_{1,0}$ at dissociation stage; and R_{offset} is the residual response at infinite time.

The association rate constant (k_a) could be derived from the equation:

$$R_a = \frac{k_a C R_{max}}{k_a C + k_d}\left[1 - e^{-(k_a C + k_d)(t_2 - t_{2,0})}\right] + RI \quad (2)$$

where R_a is the response at time t_2 at association stage; R_{max} is the maximum response related to analyte binding capacity; C is the molar concentration of HA; $t_{2,0}$ is the fitting start time during the association stage; and RI is the bulk shift. The value of the equilibrium affinity constant (K_A) for the binding reaction could be calculated from the quotient k_a/k_d.

2.3. ITC analysis

The thermodynamics of the interaction between HA and SMZ was investigated using a VP-ITC calorimeter (MicroCal, Northampton, MA). HA and SMZ solutions were prepared at concentrations of 595.2 mg-C/L (measured as TOC) and 500 mg/L, respectively, in PBS buffer (50 mM, pH 7.0). In order to achieve a better resolution for the binding test using the ITC technique, high concentrations of SMZ and HA solutions were used due to the low heat release during binding process. All Solutions were previously degassed for 15 min under vacuum before titration. Experiments were carried out with a working volume of 1468.5 μL at 25 °C under a stir rate of 306 rpm. Titrations of SMZ into the buffer and HA solution were completed in 13 μL aliquots injected over 26 s with 180 s between injections to ensure complete equilibration. Analysis of the data was performed using Origin 7.0 as described in previous work [28].