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# Kinetic analysis of interaction between lipopolysaccharide and biomolecules

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**Abstract** Lipopolysaccharide (LPS) is a major component of the outer membrane of all gram-negative bacteria. It interacts with some biomolecules and triggers a toxic reaction. In this paper, we studied the interaction between LPS from *Salmonella Minnesota* and some biomolecules using a surface plasmon resonance (SPR) biosensor. Biomolecules were immobilized on a CM5 sensor chip using the amino coupling method and LPS was injected over the immobilized surfaces. The affinity constant  $K_A$  of LPS with serum albumin, hemoglobin, chitosan and lysozyme was  $2.36 \times 10^7$ ,  $2.03 \times 10^8$ ,  $7.58 \times 10^6$ ,  $2.82 \times 10^4$  L·mol<sup>-1</sup>, respectively. However, LPS could not interact with ferritin.

**Keywords** Lipopolysaccharide, biomolecule, surface plasmon resonance, interaction

## 1 Introduction

Lipopolysaccharide (LPS), the major component of the outer membrane of Gram-negative bacteria, is also called bacteria endotoxin. It can be released from the bacteria surface slowly. When bacteria undergo disintegration, LPS is usually released in larger amounts.

In recent years, many LPS preparations have been purified and characterized both biologically and chemically. LPS molecules consist of a bisphosphorylated lipid (lipid A) forming the matrix of the outermost membrane leaflet, and a hydrophilic polysaccharide (PS), extending outward from the bacterium. The lipid and polysaccharide moiety of LPS are all involved in its biological activities [1,2]. Intact LPS from various bacteria were able to bind specifically to macrophages and human

monocytes [3]. Specific binding of intact LPS molecules to monocytes was detected only in the presence of serum factors and occurred through the CD14 molecule, and intact LPS could not bind to CD14-deficient cells [4].

It has been shown that an inflammatory reaction caused by LPS was induced by its interaction with the host cell, and the susceptibility of the host to LPS was mediated and adjusted by soluble binding proteins and proteins on the cell surface [5]. LPS may interact with many proteins in body fluid circulation, and these interactions are closely related to its biological activities.

The biosensor technique based on the principle of surface plasmon resonance (SPR) has been used to study biomolecular interactions in real time without labeling. In this paper, we analyzed interactions between LPS from *Salmonella Minnesota* and several biomolecules, and studied their kinetic characters using SPR.

## 2 Experimental

### 2.1 Instruments and reagents

LPS from *Salmonella Minnesota*, human serum albumin (HSA) and lysozyme (egg white) were purchased from Sigma. Human hemoglobin (Hb) was obtained from Shanghai Sangon Biological Engineering Technology & Service Co. Ltd (China). Human spleen ferritin was obtained from Fitzgerald. Chitosan with a molecular weight of 4200 (degree of deacetylation 90%) was purchased from Haidebei (Jinan, China). CM5 (Carboxymethyl dextran) Sensorchip was obtained from Pharmacia Biosensor AB, Sweden. N-hydroxysuccinimide (NHS), N-ethyl-N'-(3-diethylaminopropyl) carbodiimide (EDC), and ethanolamine hydrochloride were obtained from BIACORE, Sweden. HBS-EP (BIA-certified) (10 mmol/L HEPES, pH7.4, 0.15 mol/L NaCl, 3 mmol/L EDTA, 0.005% (v/v) Surfactant P20) was used as running buffer. All other chemicals were of analytical grade and used as received. Highly purified 18.2 MΩ distilled water was obtained from an ultrapure water

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purification system (Mini-Q Plus, Millipore Inc) and was used in the preparation of all solutions.

## 2.2 Experimental methods

The interaction analysis and kinetic determination were carried out with an SPR biosensor BIACORE 1000 (Pharmacia Biosensor AB, Sweden). A CM5 sensor chip (a carboxymethyl dextran hydrogel coupled to a gold-coated glass surface) was used.

Lysozyme, ferritin and HSA were dissolved in 10 mmol·L<sup>-1</sup> NaAC-HAC (pH4.7) with a concentration of 50 mg/L. Hb was dissolved in 10 mmol·L<sup>-1</sup> NaAC-HAC (pH5.2) with a concentration of 50 mg/L. Chitosan was dissolved in 10 mmol·L<sup>-1</sup> PBS (pH5.5) with a concentration of 20 mg/L. All these solutions were used for immobilization.

Biomolecules were immobilized on the CM5 sensor chip by amine coupling. HBS-EP buffer was used as running buffer and flow rate was set at 5 µl/min. The carboxymethylated dextran was activated with EDC/NHS for 7 mins. Biomolecule solutions were then injected across the activated surface. After coupling, 35 µl, 1 mol/L ethanolamine, pH8.5, was used to block the remaining NHS-esters and remove unspecific binding material

All experiments were carried out in the HBS-EP buffer. A flow rate of 5 µl/min was used for all experiments. The LPS was dissolved in the HBS-EP buffer and placed on the biomolecule-immobilized sensor surface. After each measurement, the sensor surface was regenerated with 10 µl, 10 mM HCl and then used for the next cycle. The real time sensor grams for kinetic analysis were evaluated using Evaluation 3.0 software to obtain kinetic parameters.

## 3 Results and discussion

### 3.1 Immobilization of biomolecules

We immobilized biomolecules on the CM5 sensor chip using the amine coupling method and then analyzed their

interactions with LPS in solution. The immobilization amounts of biomolecules are HSA 6000 RU, Hb 12000 RU, ferritin 5500 RU, lysozyme 6000 RU and chitosan 4400 RU.

### 3.2 Kinetic analysis of interaction between LPS and serum proteins

The experimental results showed that LPS could interact with HSA and Hb, but could not bind with ferritin. The kinetic determination curves are shown in Fig. 1A and 1B, and kinetic parameters are listed in table 1.

LPS is usually considered to be the main component of endotoxins, which can cause toxic reactions such as fever, shock and even death. It is the main component of pyrogen in injection preparations. It is of great theoretical and practical significance to study interactions of LPS and biomolecules.

Many researches have shown that LPS could bind certain substances in blood after entering blood circulation. Benbark discovered that heat-treated plasma could not promote the activities of LPS to human neutrophils [6]. Takayama found that bovine serum albumin played an important role in the activation of LPS from E. Coli to B lymphocytes [7]. Shapira [8] demonstrated that the presence of plasma was not an exclusive condition for the activation of human neutrophils by LPS, but accelerated the binding of LPS to the membrane receptors of neutrophils by way of LPS-LBP complex formation. Serum albumin is the most abundant protein in blood. Our experiment showed that HSA could bind with LPS with relatively high affinity.  $K_A$  is  $2.36 \times 10^7$  L·mol<sup>-1</sup>. The binding of LPS with HSA may favor LPS conversion to a monomer from an aggregation state, and the binding may protect LPS from hydrolysis by enzymes.

LPS binds with human Hb with high affinity. The affinity constant  $K_A$  is over  $10^8$  L·mol<sup>-1</sup>. Hb has excellent oxygen delivery properties and a long shelf life, and therefore is a potentially ideal red blood cell substitute. However, Hb has not yet been used clinically because of significant toxicity problems. This is mainly due to the contamination of endotoxins such as LPS. In this study,

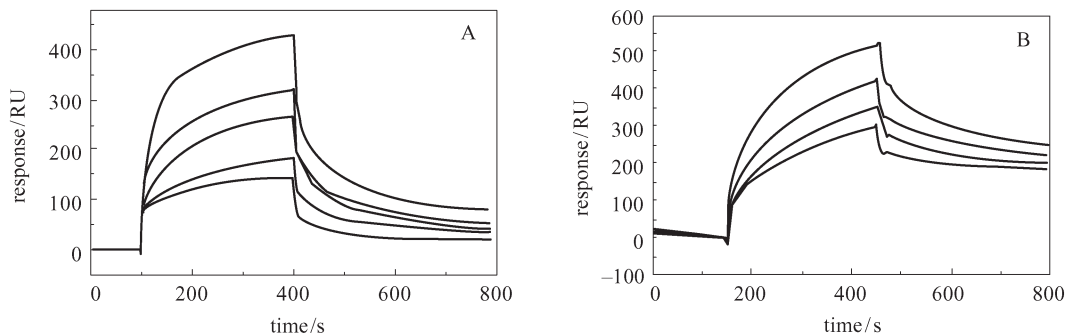


Fig. 1 Kinetic curves of the interactions between LPS and some biomolecules. A: HSA. B: Hb

**Table 1** Kinetic parameters of interactions between LPS and biomolecules

parameters	association rate constant $k_a/\text{L}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$	dissociation rate constant $k_d/\text{s}^{-1}$	association constant $K_A/\text{L}\cdot\text{mol}^{-1}$	dissociation constant $K_D/\text{mol}\cdot\text{L}^{-1}$
HSA-LPS	$6.85 \times 10^4$	$2.9 \times 10^{-3}$	$2.36 \times 10^7$	$4.24 \times 10^{-8}$
Hb-LPS	$1.91 \times 10^5$	$9.37 \times 10^{-4}$	$2.03 \times 10^8$	$4.91 \times 10^{-9}$
CHI-LPS	$2.80 \times 10^3$	$3.70 \times 10^{-4}$	$7.58 \times 10^6$	$1.32 \times 10^{-7}$
LZM-LPS	53.5	$1.89 \times 10^{-3}$	$2.82 \times 10^4$	$3.54 \times 10^{-5}$

our results demonstrated that LPS binds with Hb with very high affinity, and is not easy to eliminate. Evidence for synergistic toxicity of LPS and Hb has been reported [9]. The analysis of the interaction between LPS and Hb is of great significance for the clinic application of Hb.

Bacteria require iron as an essential element during growth. A critical component of bacterial virulence is the capacity to obtain iron from its hosts. In the extracellular tissue fluids of human and animals, most iron was found to complex with proteins such as transferrin and lactoferrin. Pathogenic bacteria can use these host molecules as an iron source via production of iron carriers or the presence of binding proteins on their cell surface. Hb is another iron-containing protein, but is usually not available for bacteria because of its intracellular localization. However, some pathogenic bacteria may secrete hemolysins which can lyse erythrocytes and thus release hemoglobin [10]. Our results indicated that LPS could bind with human Hb. Because LPS is the main component of the bacteria outer membrane, the interaction between LPS and Hb may favor bacteria binding and Hb use. Another human iron-containing protein, ferritin, cannot bind with LPS.

Under physiological and experimental conditions, LPS and HSA are negatively charged, while Hb is nearly neutral. The binding is driven mainly by hydrophobic action and other forces, not electrostatic attraction.

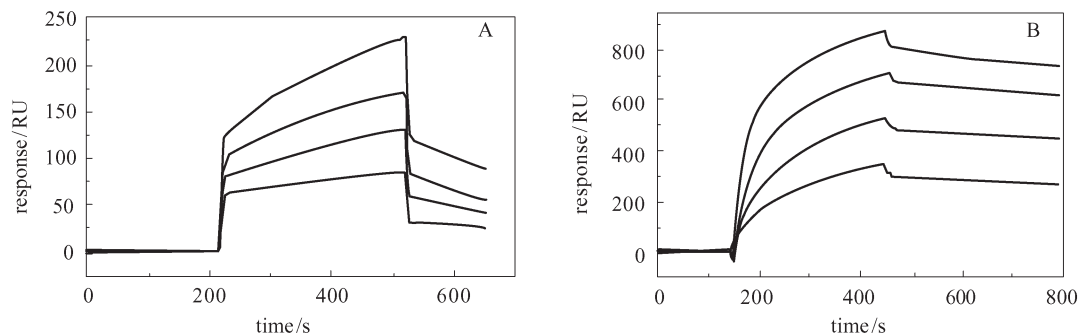
### 3.3 Kinetic analysis of interactions of LPS with lysozyme and chitosan

Chitosan is a non-toxic, natural polysaccharide exhibiting antibacterial activity [11–13]. Chitosan is positively charged, which can be expected to interact with the

predominantly anionic components (LPS, proteins) of the Gram-negative bacteria surface. Binding of chitosan molecules has been shown to disrupt the integrity of the outer membrane, resulting in loss of the barrier function [12]. Antibacterial activity of chitosan was affected by pH, with higher activity at acidic pH value [13]. Under neutral conditions used in this study, we found that low molecule weight chitosan could bind with LPS (binding curves were shown in Fig. 2A). Affinity constant is  $7.58 \times 10^6 \text{ L}\cdot\text{mol}^{-1}$  (see Table 1), corresponding to that of the antigen-antibody interaction. We think that the hydrophobic interaction between the N-acetyl group on the chitosan backbone and lipid moiety of LPS, along with electrostatic attraction, are the main driving forces of the interaction. Under neutral conditions, although chitosan can bind to the outer surface of Gram-negative bacteria, the electrostatic force is too weak to cause a dramatic change of membrane permeability.

Lysozyme can hydrolyze the cell wall of gram-positive bacteria and thus kill the bacteria. In this study, our results demonstrated that LPS could bind with lysozyme (binding curve is shown as Fig. 2B) with high affinity. Udea [14] analyzed the interaction between lysozyme and its substrate analogues, and their affinity is in the same magnitude as that of LPS-lysozyme, but with different rate constants  $k_a$  and  $k_d$ .

The formation of an LPS-protein complex usually affects the biological activities of LPS and protein. Ohno [15] has shown that the presence of LPS might result in the loss of lysozyme enzymatic activities. The association of LPS with lysozyme may prevent the binding of lysozyme with its substrate and influence its activity. With the involvement of a complement and Ig A, lysozyme may hydrolyze the cell wall of gram-negative

**Fig. 2** Kinetic analysis of the interactions between LPS and biomolecules. A: lysozyme. B: chitosan

bacteria such as *E. coli*, although the action mechanism is currently unknown [16]. But the high affinity association of lysozyme with LPS on the cell surface of gram-negative bacteria may favor the hydrolysis of the cell wall.

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