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Influence of Cationic Antibiotics on Phase Behavior of Rough-Form Lipopolysaccharide

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ABSTRACT

The rough-form lipopolysaccharide (LPS) interacted with cationic antibiotic polymyxin B and gramicidin S in solution, and showed altered thermotropic phase behavior and viscoelasticity. The phase behavior was measured by differential scanning calorimetry and quartz crystal microbalance (QCM). Addition of polymyxin B of up to 0.5 mg/mL to the 5.0 mg/mL LPS solution increased gel-to-liquid crystalline phase transition enthalpy (ΔH) and raised the transition temperature (t_{max}). The further addition of polymyxin B reduced the ΔH value. Gramicidin S produced a different effect, whereby a minor addition reduced t_{max} and ΔH value of the LPS. The LPS film on the platinum electrode of the QCM indicated a downward shift of resonant frequency and an upward shift of resonant resistance when in contact with the antibiotic solution. An interpretation of these variations is that the LPS on the QCM electrode changed not only film weight, but also viscoelasticity owing to contact with the antibiotic solution. The different effects between the antibiotics between polymyxin B and gramicidin S on the LPS are induced by the difference of the governing effect. Polymyxin B interacts with the LPS electrostatically, whereas gramicidin S interacts by hydrophobic moieties.

Index Entries: Lipopolysaccharide; antibiotic; DSC; quartz crystal microbalance; LPS-antibiotic interaction.

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INTRODUCTION

Lipopolysaccharide (LPS) is an outer membrane constituent of Gram-negative bacteria that constructs the permeability barrier and arranges membrane proteins on the cell surface (*1*). Since LPS covers the cell surface, it interfaces with materials in the environment, such as antibiotics (2) and phage (3). LPS is a macromolecular amphiphile, consisting of three distinct regions; *O*-antigenic polysaccharide, core oligosaccharide, and lipid A moiety. The hydrophilic, hydrophobic, and charge moieties are included within each molecule, which shows many functions, e.g., the polysaccharide part acts as an antigen to interact with protein (4), fatty acid interacts with membrane proteins by hydrophobic interaction (*5*), and charge groups phosphate and carboxylic acid interact electrostatically with metal ion and other materials (*6,7*). The fluidity and permeability of the LPS membrane are affected by those interactions (*8*). The calorimetric approach for the study of LPS has the drawback of requiring a large sample for measurement.

Quartz crystal microbalance (QCM) is used for measurement of weight change, viscosity, and microrheology of a thin film in contact with a liquid (*9,10*). The resonant frequency change of the QCM by interaction of the lipid with foreign materials is dependent on the molecular structures of each (*11*). Since LPS contains many functional groups, it is able to interact with many kinds of materials, not only by hydrophobic interaction, but also hydrophilic and electrostatic interactions (*12,13*). The study of interaction on the cell surface is important to elucidate the action of antibiotics on bacteria and other chemical interactions (*14*). However, research into LPS–material interaction with respect to membrane behavior has not been studied extensively. In this article, we describe the interaction of R-LPS with antibiotics by thermotropic phase behavior and resonant frequency and resistance change of the QCM.

MATERIALS AND METHODS

Materials

Rough-form lipopolysaccharide from *Escherichia coli* EH100 (Ra mutant, Sigma Chemical Co., St. Louis, MO) and *Erwinia carotovora* FERM P-7576 (*15*) were used. Gram-negative bacterial-acting antibiotics used herein for LPS interaction were polycationic oligopeptide polymyxin B sulfate (Wako Pure Chem. Co. Ltd, Tokyo, Japan) and gramicidin S hydrochloride (Sigma).

Calorimetric Measurement of the LPS

Calorimetric scans were obtained with an MC-2 differential scanning calorimeter (DSC, Microcal Inc., Northampton, USA) with a sample cell volume of 1.2584 mL. An appropriate amount of the antibiotic solution or water was added to aliquots of the *E. carotovora* LPS solution. The concentration of LPS was adjusted to 5.0 mg/mL. The sample solutions were agitated and sonicated, and then degassed using an aspirator for 10 min at ambient temperature. DSC measurements were performed at a heating scan rate of 45°C/h in the range 5–50°C under applied pressure of 1.5×10^5 Pa of nitrogen. The scans were repeated 10 times in each sample after the sample was cooled in the calorimeter. The reference cell was filled with an antibiotic solution of identical concentration to that of the sample cell, but without the LPS.

Lipopolysaccharide Film and Interaction with Antibiotics

AT cut quartz crystal of resonant frequency 8.9 MHz with platinum electrode (5 mm diameter) QCM was used. This QCM varies in resonant frequency by approx 1 Hz/1 ng weight change on the electrode. Of the LPS solution (LPS 0.5 mg/mL), 10 μL was put on one side of the electrode and air-dried to obtain a 0.5-μg LPS film. The LPS-coated QCM was put on a Teflon™ cell with a well (6-mm diameter) to expose it to only on one side of the electrode. Distilled water was poured into the well, and the resonant frequency and resonant resistance measured using a Quartz Chemical Analyzer QCA917 (Seiko Instrument Inc. Tokyo, Japan) under ambient temperature (25°C). After the water was removed, the antibiotic solution was charged.

RESULTS

Influence of Antibiotics and Phase Behavior of the LPS

Thermotropic phase behaviors of the LPS in the presence of cationic antibiotics are illustrated in Fig. 1. All the data are the tenth measurement of repeated scans. The phase behavior was reversible, and the shapes of the curves were equilibrated at that scans. ΔCp was defined as necessary energy for raising the sample temperature for 1°C. The endothermic curve of gel-to-liquid crystalline phase transition of the LPS was affected by the antibiotics and the amount of the additives. As listed in Table 1, the addition of polymyxin B up to 0.5 mg/mL shifted the phase transition temperature (maximum temperature t_{max}) from 24.5°C in distilled water to 26.3°C,

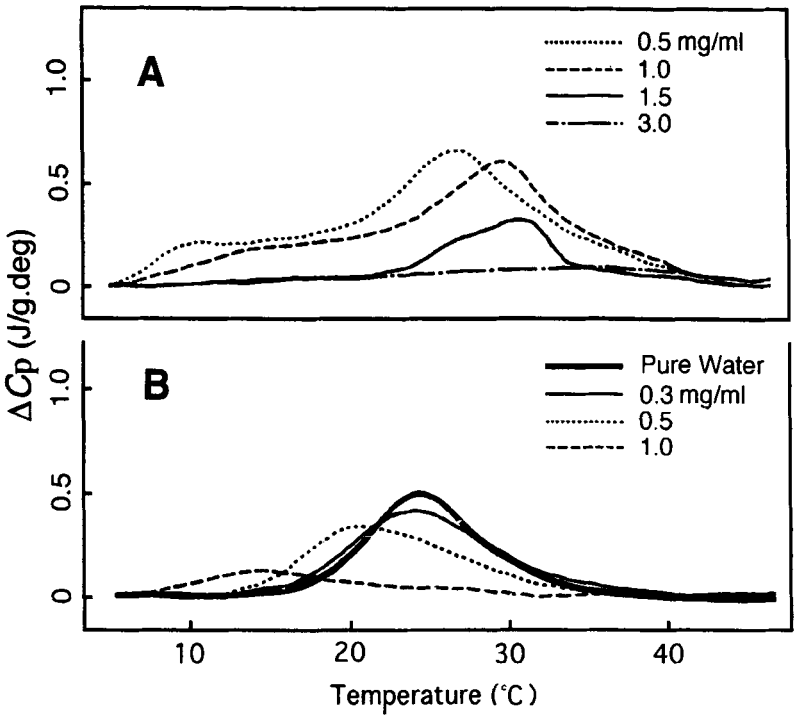

Fig. 1. DSC heating scans for *E. carotovora* R-LPS solution in the presence of the antibiotics. (**A**) Polymyxin B. (**B**) Gramicidin S.

Table 1
Effect of the Antibiotic Concentration on Temperature and Enthalpy Changes Associated with the Gel-to-Liquid Crystal Phase Transition for *E. carotovora* R-LPS[a]

Antibiotic	Concentration, mg/mL	Peak, t_{max}, °C	ΔH, $J \cdot g^{-1}$
Polymyxin B	0.5	26.3	10.4
	1.0	29.3	8.8
	2.0	30.8	3.1
	3.0	—	—
Gramicidin S	0.3	24.0	4.6
	0.5	20.5	3.9
	1.0	13.3	1.4

[a]The LPS concentration was set at 5.0 mg/mL for DSC measurement.

and caused the phase transition enthalpy (ΔH, including shoulder peaks) to increase to 10.4 $J \cdot g^{-1}$ (Fig. 1A), i.e., the ΔH value was approximately two times that of the LPS preparation in distilled water. Further addition of polymyxin B reduced the ΔH value, but t_{max} shifted upward. Addition of 3.0 mg/mL polymyxin B caused the endothermic peak to disappear. In the presence of gramicidin S, the t_{max} and ΔH values of the LPS reduced in proportion to the ratio added (Fig. 1B). Addition of 1.0 mg/mL gramicidin S produced a broad thermotropic peak with t_{max} around 13°C and

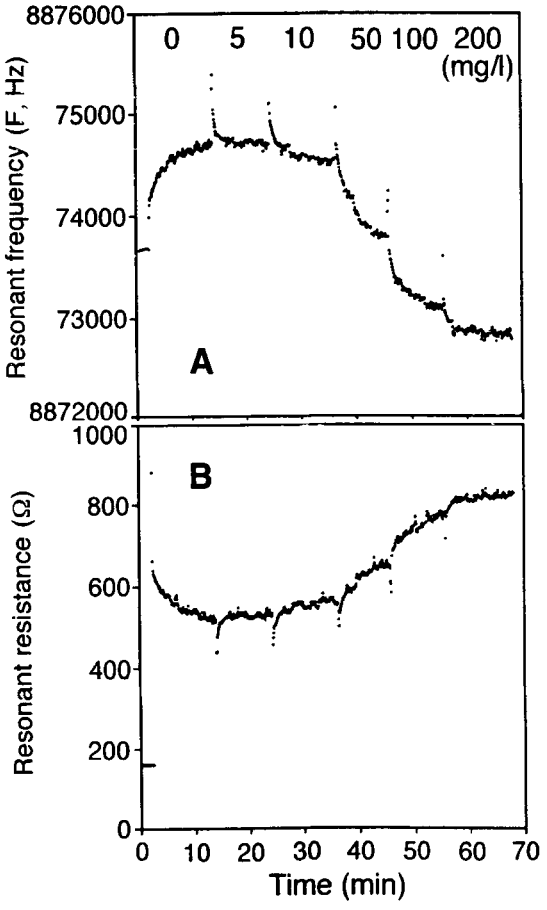

Fig. 2. Resonant frequency and resistance profile for *E. carotovora* R-LPS-coated QCM by the polymyxin B solution. (**A**) Resonant frequency. (**B**) Resonant resistance.

ΔH of 1.4 $J \cdot g^{-1}$, suggesting that excessive addition destroys the membrane structure of the LPS.

Resonant Frequency and Resistance Change of the QCM by LPS–Antibiotic Interaction

The time-course of resonant frequency of the *E. carotovora* LPS-coated QCM during contact with the antibiotic solution is plotted on Fig. 2A. Contact with distilled water shifted the resonant frequency up to 8,874,600 Hz. The resonant frequency stabilized within 15 min. By charging the polymyxin B solution, the resonant frequency shifted downward in proportion to the concentration of the material. A 10 mg/L solution shifted the resonant frequency down to 8,874,400 Hz, and a 50 mg/L solution to 8,873, 800 Hz. The LPS free QCM, however, indicated little difference in the degree of shift of resonant frequency between contact with antibiotic solutions and distilled water (data not shown).

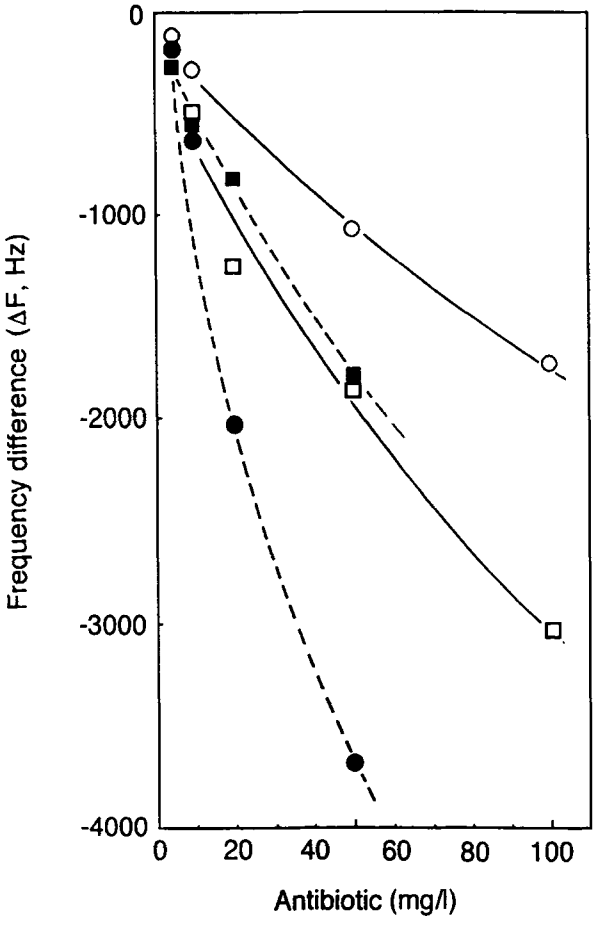

Fig. 3. Resonant frequency change of *E. coli* or *E. carotovora* R-LPS-coated QCM by the antibiotic solution. Open symbols are R-LPS from *E. carotovora* and filled symbols with dashed lines are that of from *E. coli*. Circles are resonant frequency change by the polymyxin B solution, and squares are that of the gramicidin S solution.

Resonant resistance of the QCM by polymyxin B solution is plotted in Fig. 2B. The value changed according to the concentration of the polymyxin B solution. The time-course curve was symmetrical to that of the resonant frequencies. The resonant frequency and resonant resistance have a linear relation when the concentration of polymyxin B of the solution changes. The resonant resistance in contact with distilled water was 520 Ω. The value was shifted to 560 Ω in the 10 mg/L solution and increased to 630 Ω in the 50 mg/L solution.

In Fig. 3, resonant frequency change curves of the QCM coated with rough-form LPS from *E. coli* or *E. carotovora* by application of the antibiotic solution are illustrated. Each value was measured 10 min after the sample charge. The resonant frequency change differed according to the combination of the LPS and antibiotics. *E. coli* LPS-coated QCM indicated a 3700-Hz

Table 2
Resonant Resistance Difference Against Resonant Frequency of *E. carotovora* R-LPS-Coated QCM Resulting from Antibiotic Adhesion[a]

	E. carotovora R-LPS	*E. coli* R-LPS
Polymyxin B	0.150	0.163
Gramicidin S	0.098	0.159

[a]Expressed by resonant resistance (Ω) per 1-Hz downward shift of resonant frequency.

downward shift in the 50 mg/L polymyxin B solution, whereas *E. carotovora* LPS-coated QCM showed a frequency change of 1010 Hz. Both LPS forms demonstrated similar curves in the gramicidin S solution, the ΔF value obtained being around 1900 Hz in the 50 mg/L solution. *E. carotovora* R-LPS showed a larger resonant frequency shift with the gramicidin S solution than with the polymyxin B solution. The differences in resonant resistance produced by adding a sufficient amount of each antibiotic to cause a 1-Hz downward shift are listed in Table 2. Values for *E. coli* LPS were similar for each antibiotic, whereas those for *E. carotovora* LPS were different.

DISCUSSION

The action of antibiotics on bacteria has been previously studied (*2,16*). The antibiotics adhered to the LPS of the cell surface and induced cellular reaction (*17*). The detailed nature of the interaction between the antibiotics and the LPS, however, is still not clear. In our results, the DSC curves indicated the effect of each antibiotic on the membrane phase transition of *E. carotovora*. In the presence of gramicidin S (1.0 mg/mL for 5.0 mg/mL LPS), the gel-to-liquid crystalline phase transition enthalpy was reduced remarkably to 1.4 $J \cdot g^{-1}$, which suggests that at higher concentrations, the membrane structure of the LPS would be destroyed completely. We can infer that the membrane structure of the LPS is altered by gramicidin S in the same way as that of lipids (*18,19*). Polymyxin B, on the other hand, produced different effects. Minor addition promoted membrane stability, and raised t_{max} and ΔH values. When polymyxin B was adsorbed by the LPS membrane, the antibiotic reduced the electrostatic repulsion of the LPS by charge neutralization of the phosphate and carboxylic acid (*20*), inducing an upward shift of the phase transition enthalpy. Gramicidin S indicated different effect, which bound to the fatty acid region of the LPS, and then reduced the phase transition enthalpy. Since the fatty acid composition and number in *E. coli* and *E. carotovora* are similar (*21,22*), these changes in phase behavior must be related to the membrane permeability change and other features (*8*).

From the similar resonant frequency change between *E. coli* LPS and *E. carotovora* LPS in contact with gramicidin S, we can infer that gramicidin S interacts with LPS mainly in the hydrophobic moieties. This inference coincides with the results obtained by DSC measurements. Both LPSs have similar hydrophobicity, and therefore their resonant frequency changes were similar. *E. coli* LPS is richer in ionic groups, such as phosphate, than is *E. carotovora*. The major interaction force for LPS to polymyxin B is intrinsically electrostatic. The charge moiety rich *E. coli* LPS indicated the greater shift in resonant frequency.

The weight change on the QCM electrode affects resonant frequency, whereas viscoelasticity influences resonant resistance. The viscoelasticity change, which occurred on the QCM, accompanied the upward shift of resonant resistance by decreasing resonant frequency (*10*). In the case of the LPS–antibiotic interaction, the QCM showed increasing resonant resistance with decreasing resonant frequency owing to deposition of the antibiotic on the LPS. The contrast of the resonant resistance and resonant frequency values shows that the LPS–antibiotic interaction causes a viscosity increase on the LPS membrane. In case of *E. carotovora* LPS, the resonant resistance change of the QCM/1 Hz downward shift was 0.098 for gramicidin S and 0.150 for polymyxin B. The different coefficients for polymyxin B and gramicidin S can be interpreted as the difference of the viscoelasticity. The viscoelasticity induced by the former is higher than that of the latter.

The membrane property of the LPS varied in the presence of the antibiotics. The LPS on the QCM altered in resonant frequency and resonant resistance because of contact with the antibiotics. The contrast of resonant frequency and resonant resistance can be interpreted as a viscoelasticity change in the LPS owing to antibiotic adhesion.

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