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Interaction between lipopolysaccharide and detergents detected by differential scanning calorimetry

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Abstract

The Gram-negative bacteria can cause very serious diseases. One of their pathogenetic factors is the endotoxic lipopolysaccharide (LPS). This molecule is a component of outer membrane of Gram-negative bacteria. The LPS is released from the living bacteria in a modest measure, but after killing off bacteria by antibiotics a larger quantity of endotoxin is released into the surrounding. This molecule can induce different biological effects including pyogenicity and toxic shock. The LPS is an amphiphilic molecule: it has a hydrophobic and a hydrophilic part of molecule. In water, the LPS can aggregate. This aggregation process has been followed in our experiment by calorimetry. When we mixed the LPS extracted from *Shigella sonnei* Re 4350 with different detergents (polymyxin B (PmB), deoxicholat (DOC)) in different concentration, we could diminish the measure of aggregation. The heat treatment process was reversible within the temperature range and detergent concentration used by us.

Keywords: *Shigella sonnei* Re 4350 LPS; Polimyxin B; DOC; Thermal treatment (DSC)

1. Introduction

The bacterial endotoxin or lipopolysaccharide (LPS) is one of the components of Gram-negative outer membrane. So we can find it in *Escherichia coli*, *Shigella sonnei*, *Salmonella typhi* and so on strains cell walls. The LPS may be extracted from there. The purified molecule consists of two parts: lipid A and polysaccharide. The lipid A contains among them fatty acids, aminosugars, etc. and it is responsible for the biological effects of endotoxin. This is the hydrophobic part of LPS. The polysaccharide chain contains O-specific epitopes and is hydrophilic. So the LPS is an amphiphilic molecule. It can bind both hydrophobic and hydrophilic compounds. This binding may be important in development of the structure and function of the Gram-negative outer membrane and in generation of endotoxic biological effects. The LPS may be aggregated in distilled water. This aggregation process is influenced by different ions and organic molecules. The differential scanning calorimetry is a sensitive and reproducible method to follow this aggregation. In our recent experiments, we have made some effort to study the thermal stability of purified LPS — prepared from *S. sonnei* Re 4350 strain (9) — with different agents in distilled water by differential scanning calorimetrical method in the temperature range of 0–100°C.

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2. Materials and methods

2.1. Sample preparation

S. sonnei Re 4350 LPS was used in our experiments. The rough mutant was isolated[1] from *S. sonnei* phase I [1]. This strain is a so called absolute (Re) rough mutant. The bacterium was cultivated in a Braun-Melsungen U30 fermentor. The LPS was extracted by PCP (phenol–chloroform–petrolether) method after Galanos et al. [2]. The freeze-dried LPS was checked by gas chromatographic and photometric methods. Its LPS consists of lipid A and KDO (keto-deoxioctonic acid). This LPS was dissolved in distilled water in different concentrations: 0.5; 1.0; 2.0; 5.0; 10.0 and 20.0 mg/ml. This pure LPS had an endothermic melting in its thermal treatment with a maximum around 22°C. The temperature range and transition enthalpy depended on the concentration of LPS.

Polymyxin B (PmB) (supplied by Sigma, St. Louis, MI, USA) is a peptide antibiotics. It can bind to LPS both in vivo and in vitro. It can neutralize some of the biological activity of LPS. This binding process was detected by differential scanning calorimetry.

Deoxicholat (DOC) (supplied by Sigma, St. Louis, MI, USA) is a detergent. It can inhibit, depending on its concentration, the aggregation of LPS.

2.2. DSC measurements

The thermal treatment of samples was carried out with the aid of a SETARAM Micro DSC-II scanning calorimeter in temperature range of 0–100°C with a scanning rate of 0.3°C/min. Conventional Hastelloy batch vessels were used with an average sample volume of 850 μl. The sample and reference vessels were equilibrated with a precision of ±0.1 mg to minimalize the heat capacity difference. The samples underwent several heating–cooling cycles to check the reversibility of heat treatment process.

3. Results and discussion

During the heating phase, we have got low temperature transitions between 20 and 40°C which were affected by antibiotic treatment. *S. sonnei* Re 4350 LPS dissolved in distilled water showed an endothermic peak around 24°C, the transition enthalpy strongly correlated with its concentration (Fig. 1 and Table 1). At the concentrations 0.5 and 1 mg/ml, the effect was below the detection limit. Administrating PmB (cc: 0.5; 1; 2 and 5 mg/ml), the transition temperatures decreased compared to the aqueous solution of *S. sonnei* Re 4350 LPS and varied between 23 and 24°C for *S. sonnei* Re 4350 LPS having 5 and 10 mg/ml concentration (Fig. 2), while at 20 mg/ml concentration, we detected increasing transition temperatures (Table 2). In contrast to this finding, Fukuoka and Karube [3] reported increasing transition temperatures in *E. carotovora* R-LPS solutions with increasing PmB concentrations. Our calorimetric enthalpies decreased with increasing PmB concentration for *S. sonnei* Re 4350 LPS with concentration of 5 mg/ml and they were greater than in distilled water up to 1 mg/ml PmB, further addition of PmB reduced the ΔH similarly to [3]. This latter is in good agreement with the results which showed that addition of PmB to LPS increased gel-to-liquid crystalline phase transition enthalpy [3] and displayed a large negative ΔC_p for binding to LPS, indicating that non-polar forces are primarily responsible for this recognition. This in turn suggests that the segregation of non-polar and charged polar groups on the opposite faces of the PmB molecule are responsible for its observed biological activity [4]. Addition of 5 mg/ml or more PmB caused the endothermic peak to disappear. At a concentration of 10 mg/ml of *S. sonnei* Re 4350 LPS, the transition enthalpy had a maximum value with PmB of

Table 1
The denaturation (D) and renaturation (R) melting temperatures (T_m) as well as the transition enthalpy changes (ΔH, round of average) of *S. sonnei* Re 4350 LPS dissolved in deionized water in different concentration[a]

S. sonnei Re 4350 LPS, c (mg/ml)	T_m (°C)		ΔH (mJ/g)	
	D	R	D	R
20.0	24.2	23.0	−70.0	74.0
10.0	23.9	22.8	−36.0	39.0
5.0	24.0	22.5	−12.0	16.0
2.0	24.3	21.6	−3.0	2.0

[a] The standard deviation of enthalpy values were below 10%.

[1] Report on this strain is in preparation.

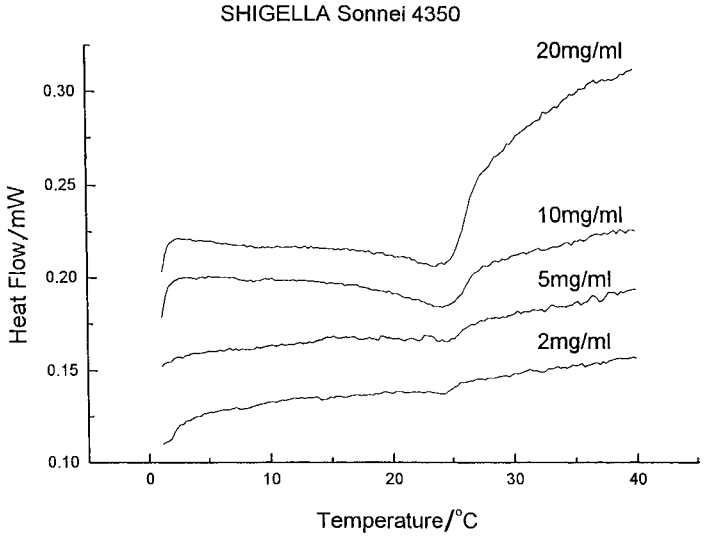

Fig. 1. Thermal stability of *S. sonnei* Re 4350 LPS in deionized water at different concentration (the curves are shifted from each other for better presentation in Figs. 1–3).

1 mg/ml, while in 20 mg/ml *S. sonnei* Re 4350 LPS, the enthalpy values showed oscillation as function of antibiotic concentration (see Table 2).

The *S. sonnei* Re 4350 LPS in 10 mg/ml concentration treated with DOC exhibited increasing transition temperatures with decreasing enthalpies as function of concentration of DOC (see Fig. 3).

During repeated heating–cooling cycles (up to four or five) the *S. sonnei* Re 4350 LPS dissolved in distilled water showed very high degree of reversibility practically with the same melting temperatures and transition enthalpies which is in good agreement with the earlier observations referring to the great thermal stability of *S. sonnei* Re 4350 LPS.

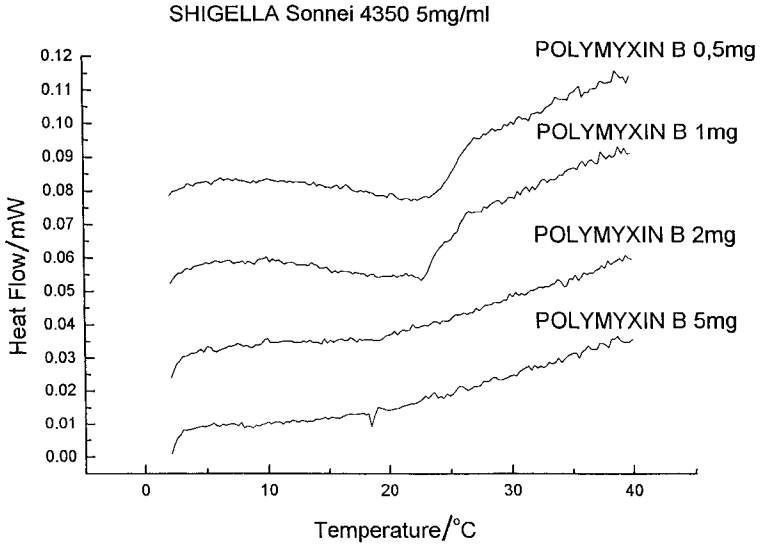

Fig. 2. Effect of PmB on the membrane stability of *S. sonnei* Re 4350 LPS.

Table 2
Thermal treatment data for *S. sonnei* Re 4350 LPS having different concentration in the presence of PmB in aqueous solutions (symbols and S.D. of ΔH are the same as in Table 1)

c_{PmB} (mg/ml)	T_m (°C)		ΔH (mJ/g)	
	D	R	D	R
S. sonnei Re 4350 LPS 5 mg/ml + PmB				
0.5	22.7	21.4	−21.0	20.0
1.0	22.8	20.1	−15.0	13.0
2.0	19.1	18.8	−1.0	2.0
5.0	20.5	–	−1.0	–
S. sonnei Re 4350 LPS 10 mg/ml + PmB				
0.5	23.7	22.2	−24.0	34.0
1.0	23.1	21.7	−76.0	35.0
2.0	21.5	19.6	−50.0	28.0
5.0	–	–	–	–
S. sonnei Re 4350 LPS 20 mg/ml + PmB				
0.5	22.5	22.5	−68.0	65.0
1.0	22.8	22.5	−58.0	93.0
2.0	23.6	21.8	−182.0	73.0
5.0	24.2	19.5	−58.0	36.0

The action of antibiotics on bacteria were widely studied; see, e.g. Bader and Teuber [5], Teuber and Bader [6]. It is known that the polypeptide antibiotics adhere to the LPS of the cell surface and induce cellular reaction [7]. The detailed nature of the interaction between the polypeptide antibiotics and the LPS is still not clear. In our results on the less investigated *S. sonnei* Re 4350 LPS, the DSC scans indicated the effect of PmB and DOC on the membrane phase transition. Minor addition of PmB promoted membrane stability and raised ΔH values. When PmB was absorbed on the LPS membrane, the antibiotic reduced the electrostatic repulsion of the LPS by charge neutralization of the phosphate and carboxylic acid [8], resulting in the increase of the phase transition enthalpy. In the present of DOC, the transition temperature increased and the enthalpy raised up to 2 mg/ml antibiotics and it was greater than in the distilled water solution of *S. sonnei* Re 4350 LPS (Table 3). The standard deviations of ΔH values were greater than it is usual in the case of

Table 3
Thermal characterization of *S. sonnei* Re 4350 LPS in the presence of DOC (symbols and S.D. of ΔH are the same as in Table 1)

S. sonnei Re 4350 LPS 10 mg/ml + DOC, c_{DOC} (mg/ml)	T_m (°C)		ΔH (mJ/g)	
	D	R	D	R
0.5	23.3	21.5	−96.0	71.0
1.0	24.9	22.0	−56.0	83.0
2.0	24.4	23.2	−48.0	35.0
5.0	25.5	25.2	−23.0	18.0
10.0	36.5	31.6	−13.0	10.0

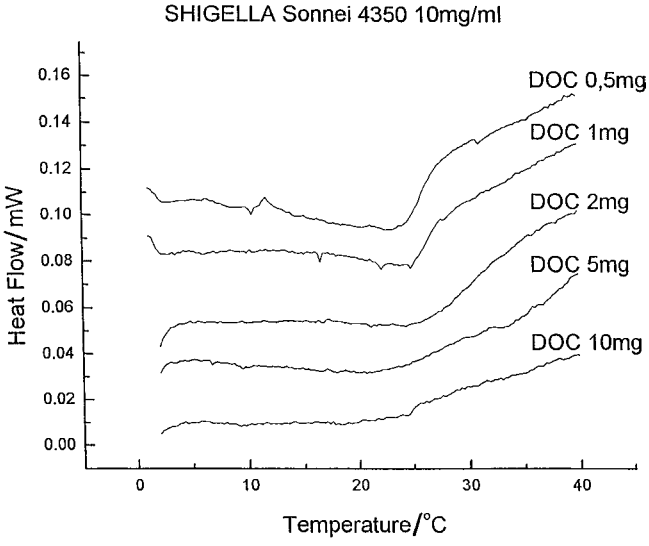

Fig. 3. Thermal stability of *S. sonnei* Re 4350 LPS after treatment with of DOC with of different concentration.

inorganic materials because of the biological variability. These deviations changed slightly from sample batch to batch, but remained below 10%. The concentration dependence of denaturation enthalpy change for *S. sonnei* LPS, *S. sonnei* LPS 5 mg/ml + PmB as well as for *S. sonnei* LPS 10 mg/ml + DOC was proved by curve fitting. The regression coefficients were different from zero which proves the significance.

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