Experimental report

16/08/2021

Proposal: 9-13-911 **Council:** 10/2019

Title: Effect of cations on bacterial lipopolysaccharides (LPS) self-assembled structures

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: LPS Ra
LPS Rd
electrolytes

Instrument	Requested days	Allocated days	From	To
D11	3	2	23/09/2020	25/09/2020
D22	3	0		

Abstract:

Lipopolysaccharides (LPS) is a key structural component of the outer membrane of gram-negative bacteria. LPS can trigger lethal septic shocks when released into a host’s bloodstream (i.e. the endotoxic effect). Cations can complex with LPS and lead to LPS aggregates structure transformation, which affects bacterial membrane integrity and LPS virulence. However, the effects of different cations on LPS aggregate structures are not well understood. Here, we propose a small-angle neutron scattering study (SANS) of self-assembled structures formed by different LPS chemotypes in solutions of different cations at near physiological conditions. We will vary the cation valency (comparing monovalent (Na+), divalent (Ca2+) and multivalent cations (La3+)), as well as the LPS architecture, i.e. its carbohydrate head group length (LPS-smooth, LPS-Ra and LPS-Rd). We also propose to investigate the temperature effect on these systems, as it has been shown in preliminary studies to have a profound effect on the self-assembled structure.

EXPERIMENT OBJECTIVES

Lipopolysaccharides (LPS) is a key structural component of the outer membrane of gram- negative bacteria. LPS can trigger lethal septic shocks when released into a host's bloodstream (i.e. the endotoxic effect). Cations can complex with LPS and lead to LPS aggregates structure transformation, which affects bacterial membrane integrity and LPS virulence. Here, we propose a small-angle neutron scattering study (SANS) of self-assembled structures formed by different LPS chemotypes in solutions of different cations at near physiological conditions. We will vary the cation valency (comparing monovalent (Na^+), divalent (Ca^{2+}) and multivalent cations (La^{3+})), as well as the LPS architecture, i.e. its carbohydrate head group length (LPS-smooth, LPS-Ra and LPS-Rd). We also propose to investigate the temperature effect on these systems.

EXPERIMENT REPORT

In addition to the empty cell and control D_2O sample, 27 samples have been measured at 25℃, 40℃ and 60℃, which is lower and above the acyl chain melting temperature of LPS Ra, Rd and smooth mutant.

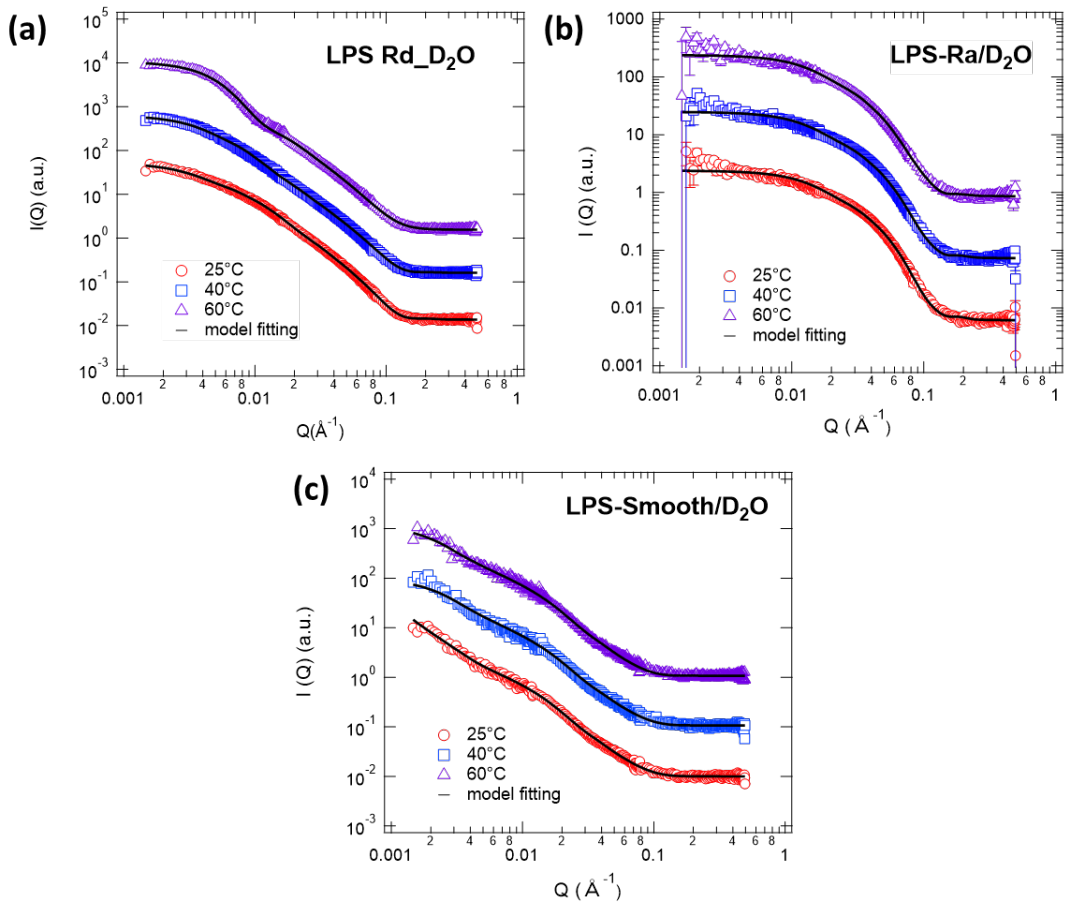

Figure 1. SANS data for LPS Rd, Ra and Smooth in D2O at different temperature.

Table 1. The fitting results from SANS data of different samples

		radius_minor	axis_ratio	radius_major	length
Rd	25ºC	22.161	8.0163	177.65	1512.3
	40ºC	20.701	12.805	256.08	1335.7
Ra	25ºC	24.756	1.8627	46.113	334.42
	40ºC	24.142	1.9681	47.514	354.8
	60ºC	23.495	2.141	50.303	341.23
Smooth	25ºC	20.026	7.0052	140.29	538620
	40ºC	20.238	6.4514	130.56	3998.9
	60ºC	22.164	6.0264	133.57	4539.2

The effects of different LPS chemotypes and temperature on the structure of LPS aggregates were studied by SANS. As shown in Fig.1, we can see the curve shape of different LPS chemotypes are different. According to the model fit, Rd and Ra form short rigid elliptical cylinder micelles and Smooth forms worm like micelles. For Rd sample, the structure of aggregates changes form elliptical cylinder (25^{o} C and 40^{o} C) to vesicle (60^{o} C) with the increase of T. We also find the decrease of bilayer thickness with the increase of T. For Ra and Smooth, there is no shape changes with the increase of T. However, the bilayer thickness of Smooth sample increase with the increase of T which is different from Rd and Ra sample. The fitting results from SANS data of samples are shown in Table 1.

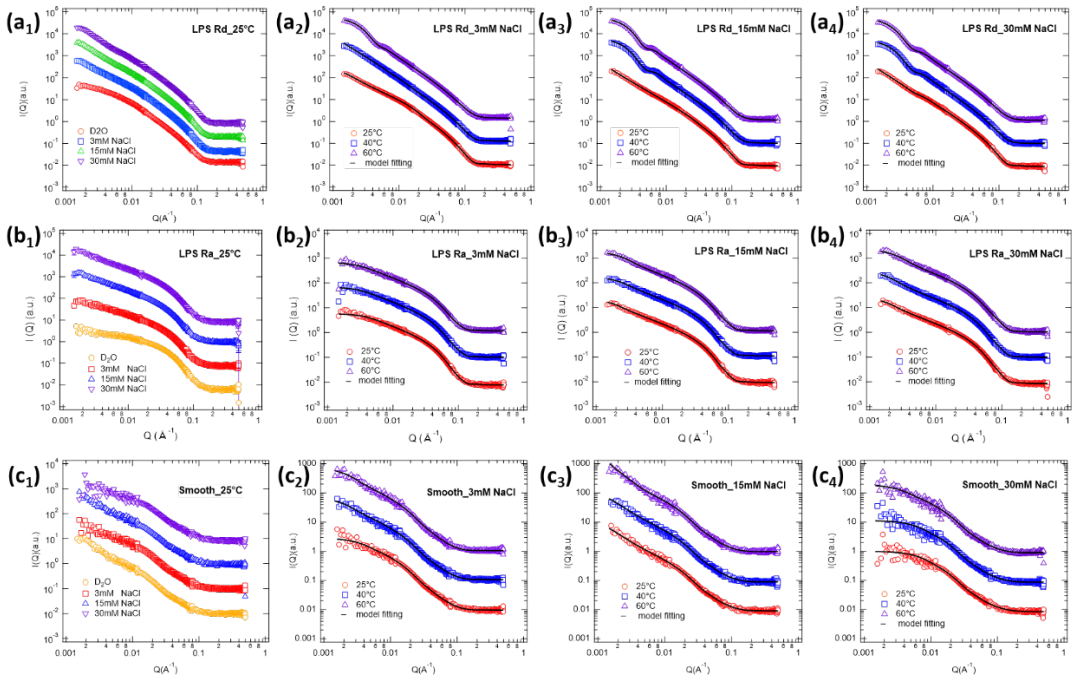

Figure 2. SANS data for LPS Rd, Ra and Smooth in different NaCl concentration at various temperature.

The effects of different concentrations of Na^{+} on LPS Ra, Rd and smooth were measured and the results are shown in Fig.2. Fig.2(a_1) shows the SANS results of Rd aggregates formed in different concentration of NaCl. Compared with Rd aggregates

formed in D_2O, we can see the shape change. But there is no significant different among the curves of Rd aggregates formed in different NaCl concentration at 25° C. All the curves can be fitted by flexible elliptical cylinder model. Interestingly, the Rd aggregates formed vesicle at 40° C and 60° C when the NaCl concentration is higher than 15mM. For Ra and Smooth samples, the shape of the aggregates is stable in different NaCl concentration at various T which is due to the protection of its long hydrophilic headgroup.

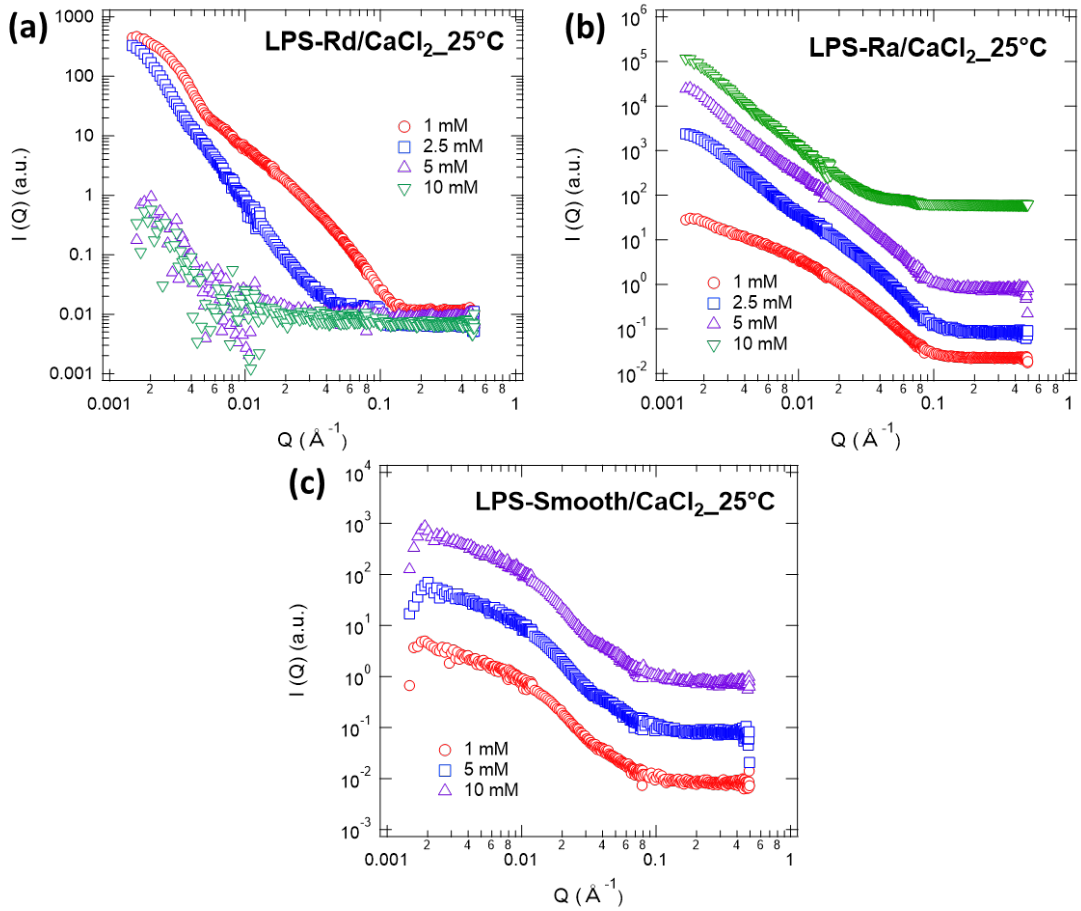

Figure 3. SANS data for LPS Rd, Ra and Smooth in different $CaCl_2$ concentration.

The effects of different concentrations of Ca^{2+} on LPS Ra, Rd and smooth were measured and the results are shown in Fig.3. According to the SANS fitting, for Rd samples (Fig.3(a)), Rd forms vesicles in 1mM $CaCl_2$ solution and mutilamellar structures when the concentration of $CaCl_2$ is higher than 2.5mM. For Ra samples (Fig.3(b)), Ra aggregates change from elliptical cylinder (1mM $CaCl_2$) to unilamellar vesicles (2.5mM $CaCl_2$) and mutilamellar vesicles (5mM and 10mM $CaCl_2$) with the increase of $CaCl_2$ concentration. For Smooth samples (Fig.3(c)), we can see there is no shape changes with the increase of $CaCl_2$ concentration.