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Investigations on the thermotropic phase behaviour of lipids A from *Brucella* and other Gram-negative bacteria

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Abstract

The phase behaviour of the lipopolysaccharide lipid moiety, free lipid A, of *Salmonella typhimurium, S. minnesota, Shigella flexneri, Escherichia coli* and, especially, *Brucella melitensis* and *B. abortus* has been investigated by applying differential scanning calorimetry. Working with samples in the solid state and a N_2 atmosphere, we have obtained results that could lead to a redefinition of the main temperature of the gel $\leftrightarrow$ liquid crystalline transition of the hydrocarbon chains to between 30 and 41°C (instead of around 45°C).

The fluidity of the acyl chains and the lyotropic behaviour in the $\beta \leftrightarrow \alpha$ melting transition, which are important parameters with respect to the expression of biological activities, are also discussed for the lipids A from the different bacterial species.

INTRODUCTION

Lipopolysaccharides (LPS) form a major component of the outer membrane of Gram-negative bacteria. LPS have been known to exhibit endotoxic activities, including fever and lethal properties, making LPS an interesting and useful tool in the investigation of biological systems and their functions. In general, LPS macromolecules consist of three genetically, biochemically and antigenetically distinct regions or domains: the O-side chain, the core oligosaccharide and the lipid A moiety. Of these three regions, the O-side chain is the most phylogenetically diverse and the most antigenetically exposed. The core and lipid A structures, in contrast, are relatively conserved among different bacteria, and are less accessible to antibody attack by virtue of the overlying sugars contained in the O-side chain or outer core.

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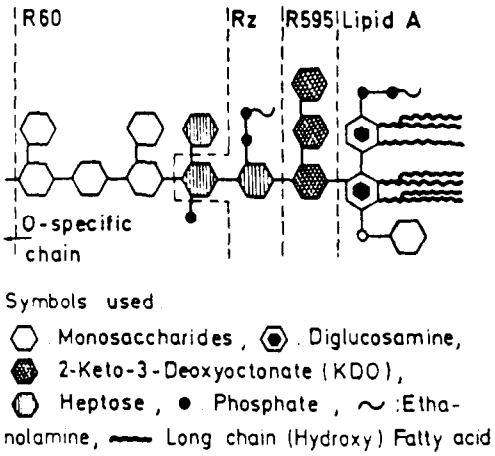

Fig. 1. Schematic structure of lipopolysaccharide from different mutants of *Salmonella minnesota* (after ref. 9).

The composition of the various LPS O-chains is reported in the literature [1–5]. The lipid A structure, in general, consists of a disaccharide backbone to which up to seven acyl chains are ester- or amide-linked and two organic phosphate groups in the 1- and 4′-positions. The length of the polysaccharide side chain linked to lipid A characterizes the mutant strains within one bacterial species (Fig. 1).

In a previous paper on O-chains [6], we reported valuable thermal data on the structure–activity–thermal stability relationships for bacterial LPS. Now we describe the thermal features of the lipids A of six bacterial species (focusing on *Brucella abortus*, *Brucella melitensis* and *Salmonella minnesota*) within the research field that concerns the thermotropic behaviour of these compounds.

EXPERIMENTAL

Free lipids A from *Brucella melitensis* 16M (biovar 1) and *Brucella abortus* 544 (biovar 1) were isolated from LPS by acid hydrolysis [7] of phenol-extracted LPS [8]. The lipids A from *Salmonella minnesota* Re 595 (R mutants), *Salmonella typhimurium* SL684 (Rc mutant), *Shigella flexneri* and *Escherichia coli* F583 (Rd mutant) were purchased from Sigma Chemical Co.

Differential scanning calorimetry measurements were performed on a Perkin-Elmer DSC-7 apparatus in the range −50 to 60°C, according to the usual procedures.

RESULTS AND DISCUSSION

In Fig. 2, DSC heating scans are plotted for the lipids A from six bacterial species. A complete description of their endotherms (onset and

TABLE 1

Temperatures and enthalpy changes of the $\beta \leftrightarrow \alpha$ acyl chain melting transition for various lipids A from Gram-negative bacteria

Lipid A from	Thermal effects										
	Onset (°C)	Peak (°C)	ΔH ($J\,g^{-1}$)	Onset (°C)	Peak (°C)	ΔH ($J\,g^{-1}$)	Onset (°C)	Peak (°C)	ΔH ($J\,g^{-1}$)	Peak (°C)	ΔH ($J\,g^{-1}$)
Salmonella typhimurium											
SL684 (Rc mutant)	–	–	–	10.3	15.5	3.2	–	(24.5)	15.4		
Salmonella minnesota											
Re 595 (R mutants)	−26.3	−15.8	11.3	–	–	–	31.4	32.8	7.2		
							39.1	40.7	4.5		
Shigella flexneri	−9.6	−4.6	28.8	–	–	–	23.7	29.7	45.6		
Escherichia coli F583 BPL	–	–	–	–	23.0	–	–	34.0	–	52.3	
Escherichia coli F583 MPL	–	–	–	(19.1)	20.2	–	29.8	33.8	10.4	54.8	18.2
Brucella melitensis											
16M (biovar 1)	−23.1	−20.5 [a]	8.4	(5.5)	(8.0) [a]	–	34.4	36.0	49.6		
Brucella abortus											
544 (biovar 1)	−41.0	−23.0	21.5	5.1	5.8	20.5	36.5	37.3	31.5		

BPL = biphosphoryl (data from Brandenburg and Blume [10]).
MPL = monophosphoryl.
[a] Tg instead of peak.

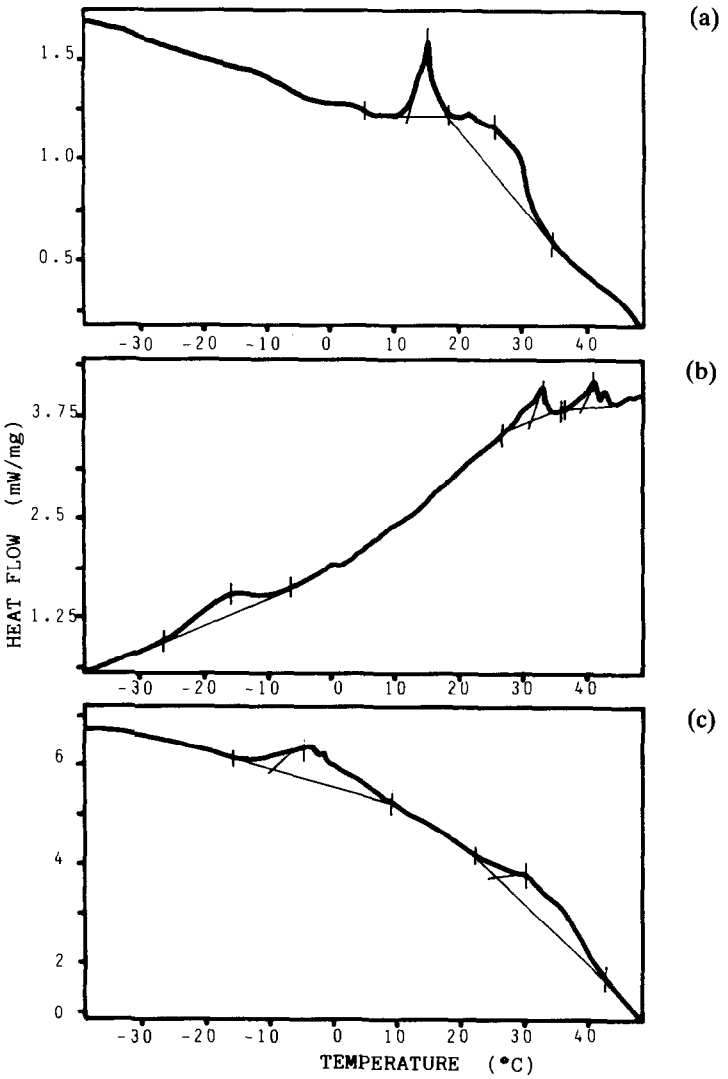

Fig. 2. DSC of lipids A from: (a) *S. typhimurium*; (b) *S. minnesota*; (c) *S. flexneri*; (d) *E. coli* MPL and *E. coli* BPL; (e) *B. melitensis*; and (f) *B. abortus*.

peak temperatures and enthalpy values) is presented in Table 1. From these data, three phase transitions can be clearly distinguished: one between −23 and −4°C; another around 15°C, and the last between 24 and 37°C.

The phase transition around 35°C takes place in all the samples. It correlates with the thermal effect assigned by Brandenburg and co-workers [9–11] to the gel ↔ liquid crystalline (beta ↔ alpha) phase transition of the hydrocarbon chains, although the temperatures of such a transition appear at significantly higher temperatures (around 50°C) than

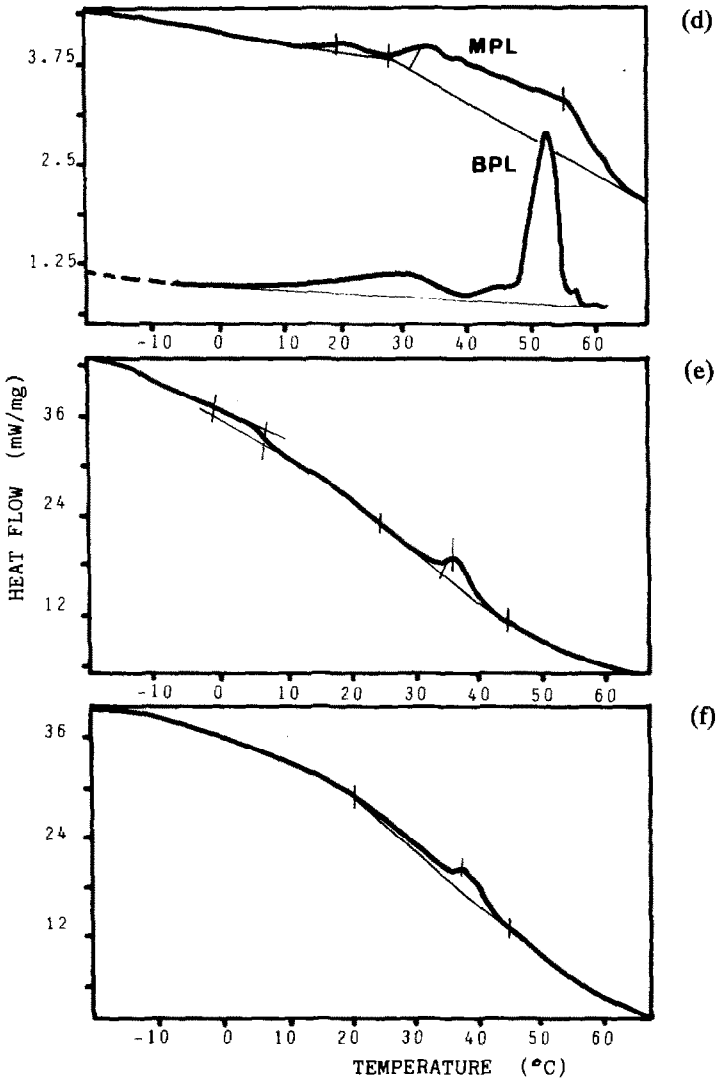

Fig. 2—contd.

that from our study. This feature could arise from a mistake by the above authors in the interpretation of the DSC scan of free diphosphoryl lipid A from *E. coli* [10]: the true transitions around 23 and 34°C (which were called "pretransitions") were discarded as characteristic in favour of the main thermal effect at 52.3°C (which corresponds to the phosphoryl substituent, as evidenced by comparison with the monophosphoryl lipid A from the *E. coli* scan). However, the difference in results could also come from methodological differences, in that the above authors worked in aqueous media whereas our measurements were carried out in a N_2 atmosphere.

In Table 1, the endotherms that appear in the middle temperature range reveal the large differences in temperature between the lipids A from *Brucella* and the phosphoryl and diphosphoryl lipids A from *E. coli*, thus showing that they must be structurally different.

Also, the common phase transitions at lower temperatures evidenced in the scans of *S. minnesota* and *Brucella* species unequivocally show that the lipids A of these bacteria are structurally related and have similar behaviour. Although the chemical analysis of the lipid A in *B. abortus* revealed that *Brucella* species contain both glucosamine and diaminoglucose, whereas the lipid A from *S. minnesota* contain only glucosamine, the pattern of amide-linked 3-hydroxylated fatty acids must be closely similar.

We have attributed this latter transition to an order↔disorder phase transition having to do with the role of the acyl chains which are amide-linked to glucosamine disaccharide on the cross-sections of hydrophilic and hydrophobic portions, by analogy with similar features in the DSC scans of *N*-acetylglucosamine and chitin [12].

Correlation of biological effects

It has been stated that the fluidity of the acyl chains is an important parameter with respect to the expression of biological activities such as induction of leukotriene C4 release, binding of complement factors C1 and C1q, stimulation of polymorphonuclear neutrophil chemiluminescence and inhibition of chemotaxis, etc. [10]. Thus it is expected that the lipid A from *S. flexneri* with the lowest transition temperature, i.e. the highest fluidity at 37°C, induces the highest biological activities; free lipids A from *B. melitensis, B. abortus* and *S. minnesota* with the higher transition, i.e. a lower fluidity at 37°C, have lower activities.

In addition to fluidity, another factor is correlated with the biological effects: the three-dimensional supramolecular (tertiary) structure of the lipid A assemblies. The strong lyotropic behaviour (high lipid:water ratio) of some lipids A, associated with the reduction of expression (*S. minnesota* and *E. coli*) or even the non-occurrence of phase transition, may favour a changed supramolecular (i.e. inverted) structure, thus promoting the fusion process when the bacterial outer membrane reacts with the host cell membrane.

The fact that free lipid A from *S. flexneri* has a low lyotropism (the ΔH values are dependent on the lipid:water ratio, and at lower lipid concentration the enthalpy change increases), but is usually biologically active, strongly suggests that fluidity is the governing parameter in this case.

For *Brucella* species, the transition temperatures indicate higher activities for the lipid A from *B. melitensis* than for that from *B. abortus*,

whereas the enthalpy changes and the tendency to adopt an inverted structure are more favourable for the lipid A from *B. abortus*.

Overall, there is a good agreement with the conclusion of Brandenburg and Seydel [11] that the establishment of a correlation between the observed biological effects and the physical properties requires the determination of not only the lipid acyl chain fluidity but also of its supramolecular structure under physiological conditions.

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