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Thermal analysis of lipopolysaccharides from *Brucella* and other Gram-negative bacteria

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Abstract

DSC curves of chromatographically purified lipopolysaccharides (LPS) of Gram-negative bacteria show very similar thermal features between (I) *Brucella melitensis* and *Salmonella typhimurium*; between (II) *Salmonella enteritidis* and *Pseudomonas aeruginosa*; and among (III) *Escherichia coli, Shigella flexneri* and *Serratia marcescens*. The order of thermal stabilities is III > II > I, in agreement with results previously obtained from the whole bacteria.

The established relative strength for linkage types $(1 \rightarrow 3) > (1 \rightarrow 6) > (1 \rightarrow 4) > (1 \rightarrow 2)$ has been useful to justify the thermal lability of *Brucella abortus* (with only $(1 \rightarrow 2)$ linkages in its O-chain) and *Brucella melitensis* (one $(1 \rightarrow 3)$ and four $(1 \rightarrow 2)$ linkages) versus other Gram-negative bacteria (with O-chains rich in α/β-$(1 \rightarrow 3)$, α/β-$(1 \rightarrow 4)$ or α-$(1 \rightarrow 6)$ linkages).

INTRODUCTION

A major characteristic of the cell envelope of Gram-negative bacteria is the existence of a second, outer membrane which acts as an additional permeability barrier especially against larger hydrophobic molecules. The inner leaflet of the outer membrane is made from phospholipids and the outer leaflet from lipopolysaccharides (LPS).

A LPS (Fig. 1) consists of a carbohydrate portion (the core and the O-chain) covalently bound to its lipid moiety (named lipid A) which anchors the LPS in the membrane. Lipid A, in general, consists of a disaccharide backbone to which up to 7 acyl chains are ester- or amide-linked and two phosphoryl groups in the 1- and 4′-positions. The core region (proximal to lipid A) contains an outer and an inner core, the latter being composed of L-glycero-D-manno-heptopyranose and 3-deoxy-D-manno-2-octulopyranosonate (KDO). The O-chain (O antigen), that corresponds to the "whole" polysaccharide, occurs as repeating units of the tetra-, penta-, or hexasaccharide (*N*-acyl derivatives of 4-amino-4,6-dideoxy-D-manno-pyranosyl residues (ADMP) in the case of *Brucella*).

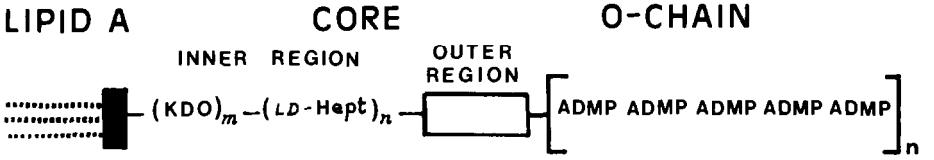


Fig. 1. Schematic representation of the structure of the lipopolysaccharides of Gram-negative species.

To obtain information on the relative thermal stability of such systems in *Brucella* and other Gram-negative bacteria, DSC curves of chromatographically purified LPS were compared with one another and in relation with their corresponding whole cells.

This study does not overlap previous investigations centred on the thermotrophic phase behavior of LPS at temperatures below 50 °C and those regarding the lipid A [1–3]. Our interest centres on the polysaccharide moiety at temperatures above 100 °C.

EXPERIMENTAL

Apparatus

TG analyses were performed in air using Perkin Elmer TGS-2 and DTA 1700 instruments, at heating rates of 5 and 10 °C min^{-1}. DSC curves were obtained with a Perkin Elmer DSC 7 apparatus, in dynamic N_2 (20 cm^3 min^{-1}), at a heating rate of 10 °C min^{-1}, and with sealed capsules of aluminium as sample containers.

Samples

Chromatographically purified LPS extracts from *Escherichia coli* 0111:B4 (L 3012); *Shigella flexneri* 1A (L 9018); *Serratia marcescens* (L 2512); *Salmonella enteritidis* (L 2012); *Pseudomonas aeruginosa* (L 8643); and *Salmonella typhimurium* (L 2262) were purchased from Sigma Chemical Co. Lypopolysaccharides from *Brucella melitensis* and *Brucella abortus* were isolated and purified by one of the authors (A.O.-D.).

RESULTS

For a series of Gram-negative bacteria, the thermolysis of their LPSs showed a TG–DTG effect at 270 ± 30 °C (Fig. 2); two DTA effects near 315 and 470 °C (Fig. 3); and a clearly well defined endothermic effect in DSC at 160 ± 40 °C (Fig. 4). As regards the DSC thermal effect, the obtained onset and peak temperatures and the enthalpy change values are summarized in Table 1.

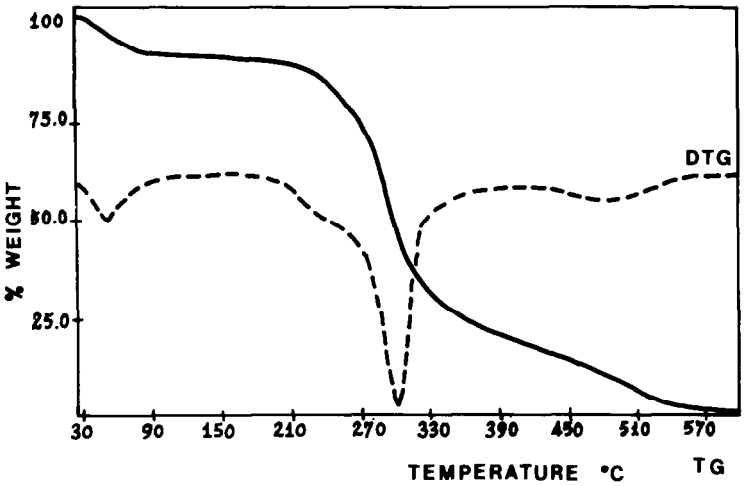


Fig. 2. TG and DTG thermograms for LPS S-form from *Brucella melitensis* in air at 10 °C min^{-1}.

It can easily be seen that the decomposition temperatures of LPSs from *E. coli, S. flexneri* and *S. marcescens* are approximately equal, and higher than those from *S. enteritidis* and *P. aeruginosa* (also quite closely grouped) and much higher than those from *B. melitensis* and *S. typhimurium* (in turn, both very similar). Within same group, the enthalpy changes ΔH fluctuate considerably due to variations of the supramolecular structure.

The order of thermal stabilities is in agreement with that previously obtained for whole bacteria [4], *Morganella morganii* > *Proteus mirabilis* >

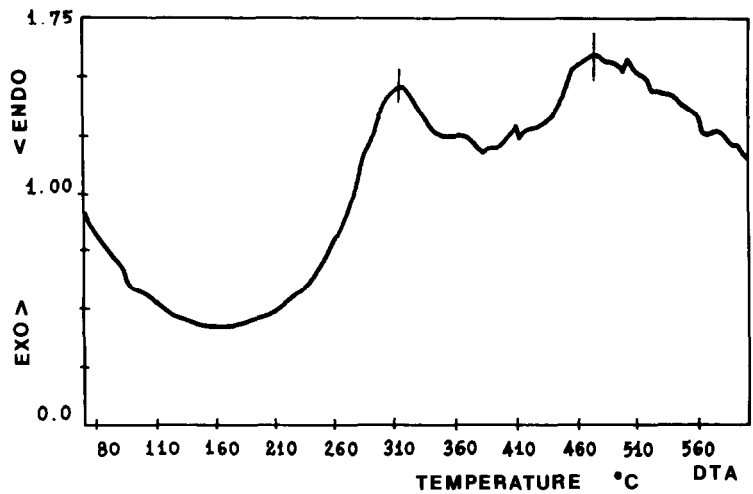


Fig. 3. DTA thermogram for LPS from *Shigella flexneri* in air at 5 °C min^{-1}.

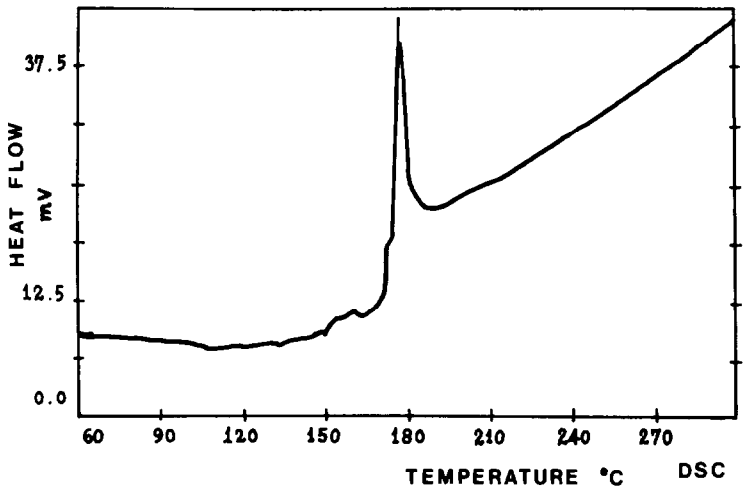


Fig. 4. DSC curve for LPS from *Pseudomonas aeruginosa* at 10 °C min^{-1}.

Neisseria meningitidis = *Neisseria lactamica* = *Neisseria gonorrhoeae* > *Escherichia coli* = *Streptococcus pneumoniae* = *Staphylococcus aureus* > *Klebsiella pneumoniae* = *Pseudomonas aeruginosa* > *Salmonella typhimurium* = *Enterobacter cloacae* = *Streptococcus agalactiae* = *Streptococcus faecalis*, as is to be expected from the fact that the LPS is the major constituent of the cell wall of Gram-negative bacteria. Thus, the thermal behaviour of the bacteria seems to be largely independent of the presence of proteins and other components.

Searching for a structural reason for the reported differences in thermostability, attention was directed towards the nature of the polysaccharide residues and, above all, to the predominant linkage and configuration present in the LPS O-chains of the studied species (Table 2).

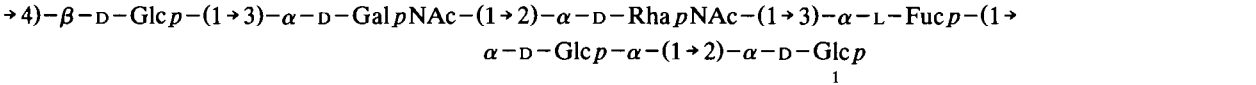
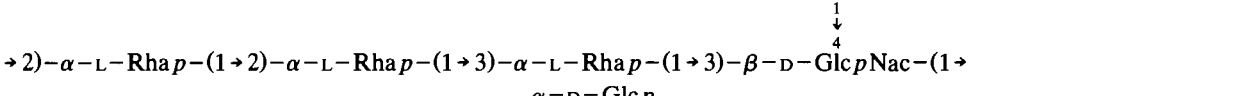
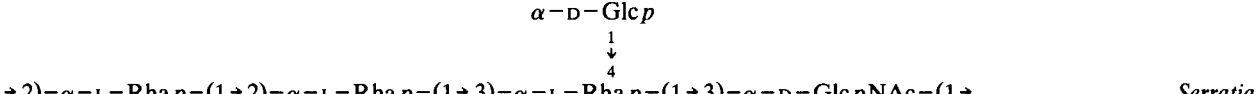
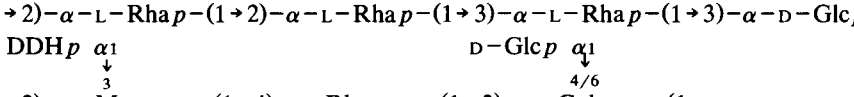
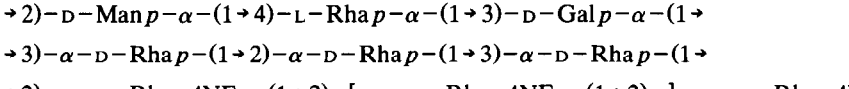
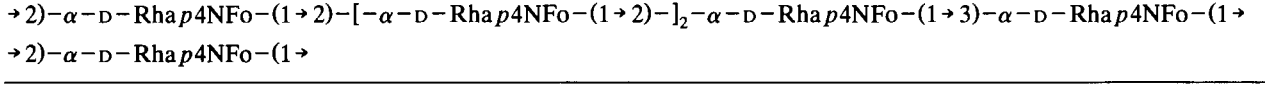
TABLE 1

DSC thermal effects for LPSs of Gram-negative bacteria

Organism	Onset (°C)	Peak (°C)	ΔH ($J\ g^{-1}$)
Escherichia coli O111	199.2	200.9	154.7
Shigella flexneri 1A	197.5	200.6	36.5
Serratia marcescens	196.7	198.6	150.3
Salmonella enteritidis	178.9	180.7	196.7
Pseudomonas aeruginosa	174.1	176.2	247.2
Salmonella typhimurium	120.7	139.5	161.4
Brucella melitensis 16M	123.6	139.3	137.5
Brucella abortus B19	108.7	136.0	165.7

TABLE 2

Partial structures for the repeating units in the LPS O-specific side-chains of Gram-negative bacteria [5–12]

	*Escherichia coli* O111
	*Escherichia coli* O157
	*Shigella flexneri*
	*Serratia marcescens* O22 and O10
	*Salmonella enteritidis*
	*Pseudomonas aeruginosa*
	*Brucella melitensis*
	Brucella abortus

Stability of residues decreased in the order Glc–Glc > Glc–Gal > Glc–Rha > Man–Rha > Rha–Rha.

As regards the linkage types, because it has been stated [13] that the relative strength for linkage types is β-(1 → 3) > α-(1 → 3) > α-(1 → 6) > α-(1 → 4) > β-(1 → 6) > β-(1 → 4) > β-(2 → 1) > α-(1 → 2), the presence of an excess of linkages β-(1 → 3) and α-(1 → 3) in the O-polysaccharide structure must contribute to enhance the stability of the LPS, whereas the quantitative presence of (1 → 2) linkages leads to their lability. Thus, the relative low stability of *Brucella abortus* (with only (1 → 2) linkages in its *O*-chain) and *B. melitensis* (one (1 → 3) and four (1 → 2) linkages) versus other Gram-negative bacteria (with O-chains rich in α/β-(1 → 3), α/β-(1 → 4) or α-(1 → 6) linkages) becomes justified.

CONCLUSION

Thermal analysis is a good method for rapid characterization of bacterial LPS. The method is suitable for identification as well as classification purposes. The resulting highly simplified data handling is of special interest as a screening tool in structural investigation. In addition, it can yield important information pertaining to relating stability–structure–microbiological activity, as the antigenic expressions related to the proportion of one or another linkage types present in the unbranched linear O-chains have been established.

ACKNOWLEDGEMENT

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