*Thermochimica Acta*, 215 (1993) 227–233
Elsevier Science Publishers B.V., Amsterdam



# Low temperature thermal behaviour of lipopolysaccharides from *Brucella* and other Gram-negative bacteria

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(Received 29 May 1992)

**Abstract**

In lipopolysaccharides (LPSs) from *Brucella* and other Gram-negative bacteria, cooling to temperatures between −13 and −36°C gives rise to a phase transition between different ordered supramolecular arrangements. During a subsequent heating program in DSC, the thermal effect temperatures that show the phase transition for each LPS are in inverse relationship with their respective thermolysis temperatures. Both effects, thermolysis and cooling phase transition, have been related to the relative strength of the linkage types in the *O*-change structure and to the facilities for intermolecular hydrogen bonding.

## INTRODUCTION

Lipopolysaccharides (LPSs) of Gram-negative bacteria (*Brucella, Salmonella, Escherichia,* etc.) constitute part of the outer membrane of the bacterial cell wall. Their exposed position on the cell surface wall allows the investigator to isolate them from bacteria with relative ease.

Chemical investigations on purified LPSs have shown that they consist of two main components with different physicochemical characters: a heteropolysaccharide and a covalently bound lipid (lipid A) [1]. A schematic representation of a *Brucella* lipopolysaccharide is shown in Fig. 1.

The polysaccharide component in the LPSs from enterobacteriaceae and *Brucella* is made up of an *O*-specific chain and the core oligosaccharide. The *O*-specific chain, in general, is represented by a polymer of oligosaccharide units (in *Brucella abortus* S-type LPS it appears as a

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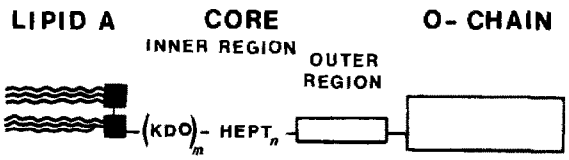


Fig. 1. Schematic representation of the structure of the lipopolysacchardies of Gram-negative species.

linear homopolymer of 1,2-linked 4,6-dideoxy-4-formamido-α-D-manno-pyranosyl units). It carries the antigenic determinants responsible for the serological specificity of LPS and of the respective bacteria [2]. The core oligosaccharide (proximal to lipid A) contains an outer and an inner core, the latter being composed of L-glycero-D-mannoheptopyranose and 3-deoxy-manno-2-octulopyranosonate (KDO) [3].

In a previous report on the thermal study of these LPSs at temperatures between 120 and 200°C, we found valuable results about their structure–activity–thermal stability relationships [4].

The present investigation led to identification of a phase transition at low temperatures for all the LPSs. This phase transition has been put in relation to the sequence of thermal stabilities established in the earlier paper.

## EXPERIMENTAL

### *Apparatus*

DSC curves were obtained with a Perkin-Elmer DSC 7 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 *Shigella flexneri* 1A (L 9018), *Escherichia coli* (L 2637), *Serratia marcescens* (L 2512), *Salmonella abortus* (L 1887), *Salmonella enteritidis* (L 2012), *Salmonella typhi* (L 2387) and *Vibrium cholerae* (L 5262) were purchased (Sigma). LPSs from *Brucella melitensis* M 16 and *Brucella abortus* 544 were isolated and purified by one of the authors (A. O.-D.).

## RESULTS

Figure 2 shows the low temperature DSC curves of the LPS series studied. All the curves exhibit a common endothermic effect, although the onset and peak temperatures and the enthalpy changes are characteristic for each lipopolysaccharide. In Table 1, it can be observed that such thermal data vary considerably. It can easily be seen that the peak

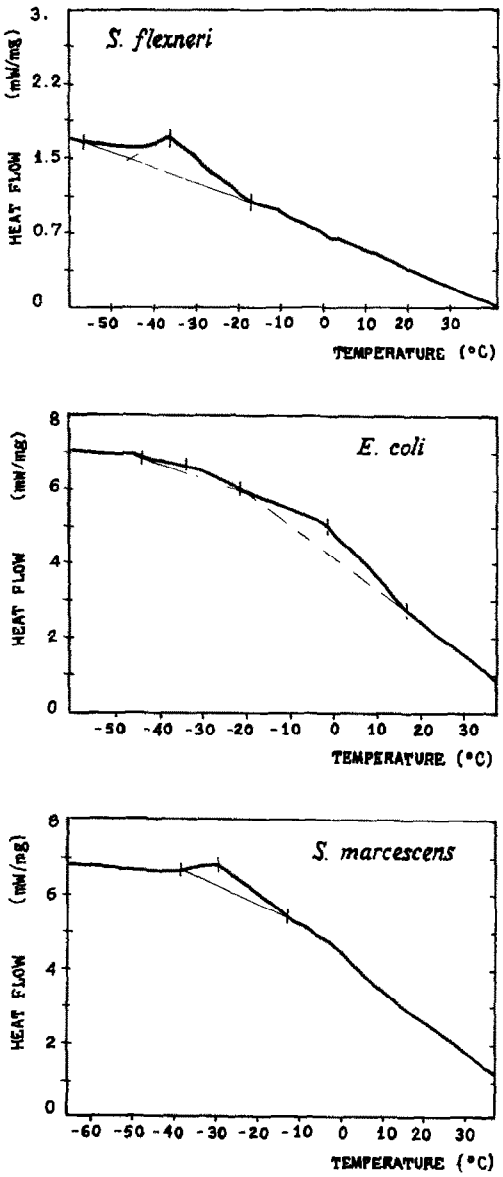


Fig. 2. DSC curves for LPSs from various Gram-negative bacteria.

temperatures of the LPS from *Brucella abortus, Brucella melitensis, Vibrium cholerae* and *Salmonella typhi* are approximately equal, higher than those from *Salmonella enteritidis* and *Salmonella abortus* (also very similar to each other), and much higher than those from *Serratia marcescens, Escherichia coli* and *Shigella flexneri* (in turn, quite closely grouped). Interestingly, this sequence is the inverse of that previously obtained at higher temperatures (between 120 and 200°C) for the same LPSs [4].

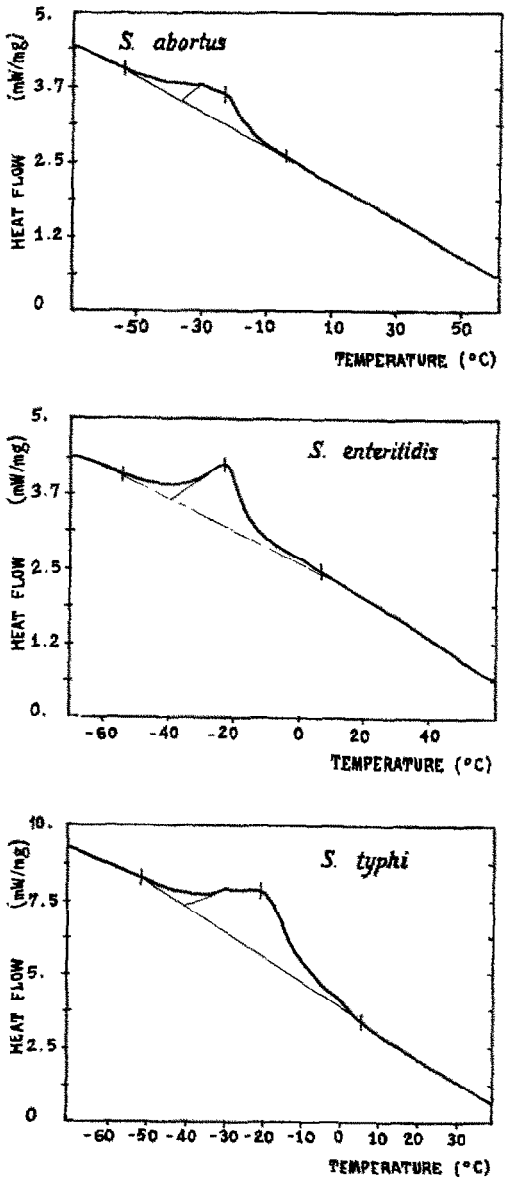


Fig. 2. (continued)

Searching for the reason for the ordering at high temperatures, we suggested an examination of the nature of the polysaccharide residues and, above all, of the predominant linkage and configuration present in the LPS *O*-chains of the species studied [4]. The stabilities of the residues decreased in the order Glc–Glc > Glc–Gal > Glc–Rha > Man–Rha. As regards the linkage types, given that the relative strength for linkage types is $\beta$-(1→3) > $\alpha$-(1→3) > $\alpha$-(1→6) > $\alpha$-(1→4) > $\beta$-(1→6) > $\beta$-(1→4) > $\beta$-(2→1) > $\alpha$-(1→2), the presence of an excess of linkages $\beta$-

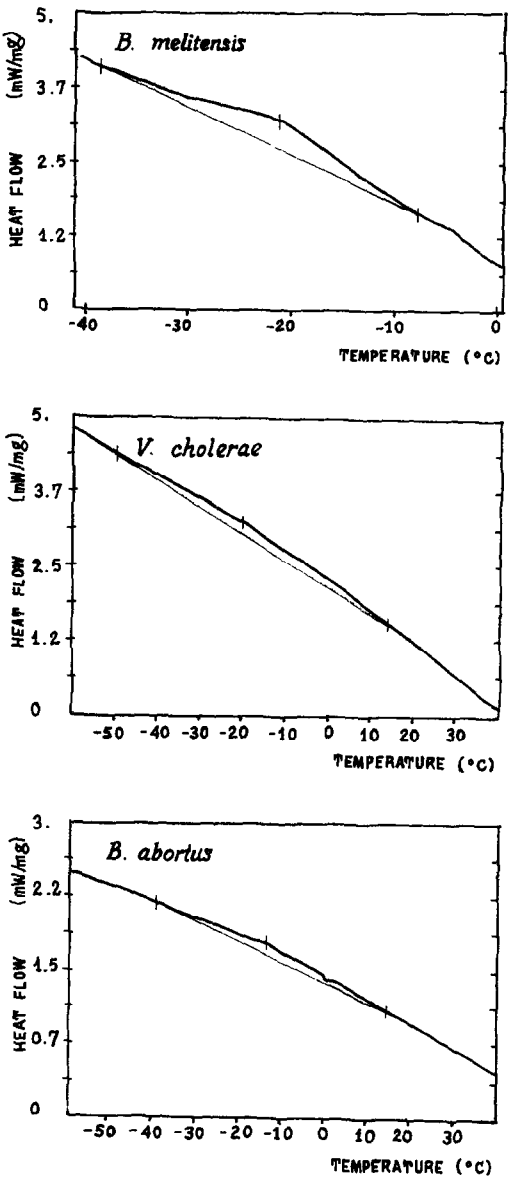


Fig. 2. (continued)

(1→3) and α-(1→3) in the polysaccharide structure must contribute to increasing the stability of the LPS. In contrast, 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 *Brucella melitensis* (one (1→3) and four (1→2) linkages) compared to other Gram-negative bacteria (with *O*-chains rich in α-/β-(1→3), α-/β-(1→4) or α-(1→6) linkages) becomes justified.

Until now, we have not found any articles on the thermal behaviour of

TABLE 1

Low temperature DSC thermal effects for LPSs of Gram-negative bacteria

| Organism | Onset (°C) | Peak (°C) | $\Delta H$ ($J\,g^{-1}$) |
|---|---|---|---|
| *Shigella flexneri* | −55.2 | −36.4 | 34.2 |
| *Escherichia coli* | – | −33.9 | 12.9 |
| *Serratia marcescens* | −37.8 | −29.7 | 72.4 |
| *Salmonella abortus* | −37.8 | −23.9 | 61.8 |
| *Salmonella enteritidis* | −38.8 | −23.0 | 83.6 |
| *Salmonella typhi* | −37.5 | −20.9 | – |
| *Brucella melitensis* M 16 | −34.0 | −20.9 | 23.0 |
| *Vibrium cholerae* | −46.3 | −19.9 | 36.4 |
| *Brucella abortus* 544 | −36.0 | −13.1 | 17.2 |

LPSs or polysaccharides at temperatures below −10°C. The only reference is our exhaustive study on chitins, chitin–glucans and cellulose [5], in which we have shown a phase transition between a distorted structure poor in interchain hydrogen bonding and an undistorted structure rich in interchain hydrogen bonding (in chitin, it seems that the phase transition occurs from the $\beta$ to the $\alpha$ form).

Consequently, we can postulate that in LPS the thermal effect at low temperatures corresponds to a phase transition between structures with different hydrogen bonding networks. The difficulty/ease of this transition is determined by the strength/weakness of the linkages within the polysaccharide moiety of the LPS and by the different susceptibilities of the chains to aggregation by hydrogen bonding [6]. Because cooling to sufficiently low temperatures reduces or breaks linkages [5], the stronger the structure, the greater the cooling which must be applied for labilization and disorder. In DSC, during the subsequent heating program that carries the LPSs to their original state of order, the above feature is apparent in the early or late thermal effect which appears for strong or weak linkages, respectively.

At present, an X-ray study to characterize both the $\beta$-type arrangement of LPS chains at low temperatures and the $\alpha$-type conformation of the LPS chains at high temperatures is in progress.

ACKNOWLEDGMENTS

The authors thank technician N. Chebib-Abuchala for valuable laboratory assistance. The authors are also grateful to the Fondo de Investigación Sanitaria de la Seguridad Social for providing the financial base for this research within the Project 92/0483.

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