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THERMODYNAMIC INVESTIGATIONS ON MONO- AND BILAYER MEMBRANE SYSTEMS MADE FROM LIPID COMPONENTS OF GRAM-NEGATIVE BACTERIA

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ABSTRACT

The phase behaviour of lipopolysaccharide, a major lipid component of the outer membrane of Gram-negative bacteria, was studied and compared with the respective behaviour of synthetic and natural phospholipids. The investigations were performed with optical and calorimetric techniques on the bilayer and with film balance measurements on the monolayer system. From the presented results a model is derived for the physical state of the outer membrane of Gram-negative bacteria under physiological conditions, i.e. 37°C.

INTRODUCTION

A major characteristic of the cell envelope of Gram-negative bacteria (e.g. Escherichia-coli) is the existence of a second - the outer - membrane, which acts as an additional permeability barrier especially against larger hydrophobic molecules. The architecture of this outer membrane differs considerably from that of the cytoplasmic membrane with respect to the lipid components: While the latter membrane consists of two monolayers built up from phospholipids (PL's), the inner leaflet of the outer membrane is made from PL's and the outer leaflet from lipopolysaccharide (LPS). LPS consists of a covalently bound lipid component - called lipid A - which anchors the LPS in the membrane, and a polysaccharide moiety - the core and the O-chain. Lipid A, in general, consists of a disaccharide backbone to which up to 7 acyl chains are ester- or amide-linked and - as a rule - two organic phosphate groups in the 1- and 4'-position. The length of the polysaccharide side chain characterizes the mutant strains within one bacterial species.

It is reported on investigations of the physical structure of membrane systems (mono- and bilayer) made from LPS, especially of their phase behaviour, to get information on the state of order of such systems.

MATERIALS AND METHODS

The fluorometric and calorimetric techniques applied on the lipid bilayer as well as the film balance measurement on the monolayer system have been described in detail elsewhere (re. 1). Briefly, fluorescence measurements were performed

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on an Aminco-Bowman spectrophotofluorometer with N-phenylnaphtylamine (NPN) as a probe to get information on the phase transition range and its midpont, the phase transition temperature T_c, and the van't Hoff enthalpy ΔH_{vH} of the gel-liquid-crystalline phase transition of the hydrocarbon chains. The true enthalpy ΔH_m was determined from calorimetric scans (upward endotherms) at heating-rates of 0.5 to 1 K/min with calibration of enthalpy values by using dodecanoic acid as reference (downward endotherms). Monolayer measurements at the air-water interface were done on a film-balance from Krüss (Hamburg, FRG) scanning the pressure-area curves at constant temperatures (isotherms).

For fluorometric and calorimetric measurements all lipids were prepared - unless otherwise stated - by vortexing a 10^{-3} M lipid solution in distilled water for some minutes at $T > T_c$, while monolayers were formed by spreading a 10^{-3} M lipid solution in chloroform on a 0.1 M NaCl subphase.

The various lipopolysaccharides, differing in the length of the polysaccharide side chains (fig. 1, the lengths of the saccharide moiety are indicated by dashed lines for LPS's from mutants R60, Rz, and R595) were isolated from bacterial mutants of Salmonella minnesota (ref. 3, 4). Free lipid A was isolated from LPS by acid hydrolysis. Phospholipids were extracted from E. coli according to the methanol/chloroform procedure (ref. 5), and phosphatidylethanolamine (PE), the main component of E. coli-LPS, was isolated by thin-layer chromatography. Synthetic PL's, like dimyristoyl-L-α-phosphatidylethanolamine (DMPE), were purchased from Sigma (München, FRG) and Serva (Heidelberg, FRG).

RESULTS AND DISCUSSION

In fig. 2 the NPN-fluorescence intensity I_{425} is plotted vs. temperature T for some PL's and LPS's showing different widths of the transition region. This fact should be due to the varying heterogeneity of the acyl chains. As illustrated for DMPE and LPS Rz, the respective phase transition temperatures T_c are lower in cooling than in heating scans (hysteresis) indicating a cooperative process. However, free lipid A and, to a lower extent, the deep-rough mutant-LPS R595 (not shown) do not exhibit hysteresis behaviour, but the higher level of I_{425} at $T > T_c$ at the end of the heating scan remains unchanged during the subsequent cooling scan. Furthermore, the latter compounds do not show any free lipid A or only a slight LPS R595 phase transition in the 2nd and further scans, indicating irreversibility of their phase transitions at T_c = 42 to 48°C (free lipid A, depending on pretreatment) and T_c = 30°C (LPS R595). As the gel-fluid crystalline transition of the hydrocarbon chains ($L_\beta \rightarrow L_\alpha$) is generally accepted as a reversible first order transition, the transitions of free lipid A and LPS R 595 may be assumed to take place to or within an inverted structure. This possibility is supported by the results of other independent techniques as discussed in ref. 1.

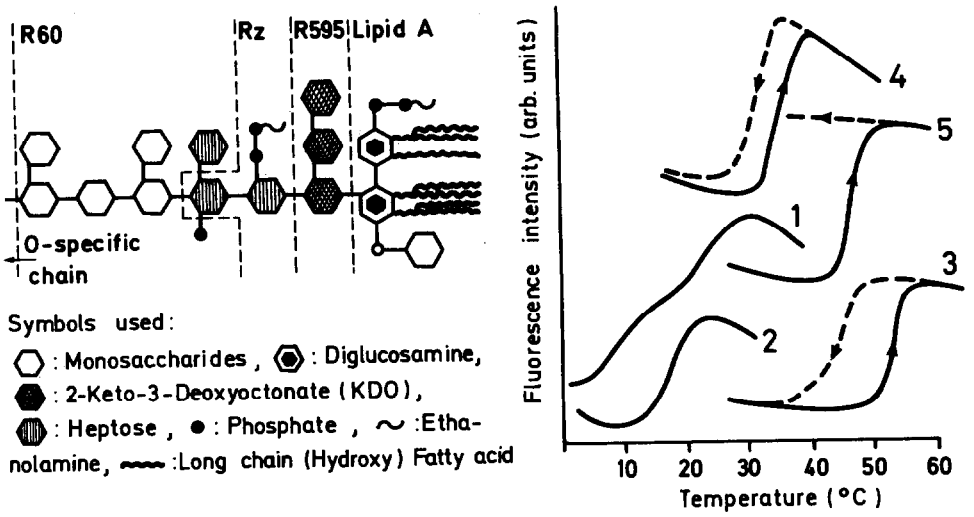


Fig. 1. Schematic structure of lipopolysaccharide from different mutants of Salmonella minnesota (after ref. 2).

Fig. 2. NPN-fluorescence intensity I_{425}-versus temperature T for (1) phospholipid(PL)-extract, (2) phosphatidylethanolamine (PE) from PL-extract, (3) DMPE, (4) lipopolysaccharide from Rz, and (5) free lipid A

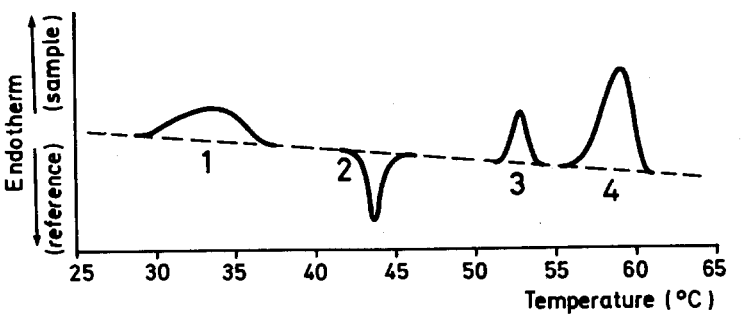
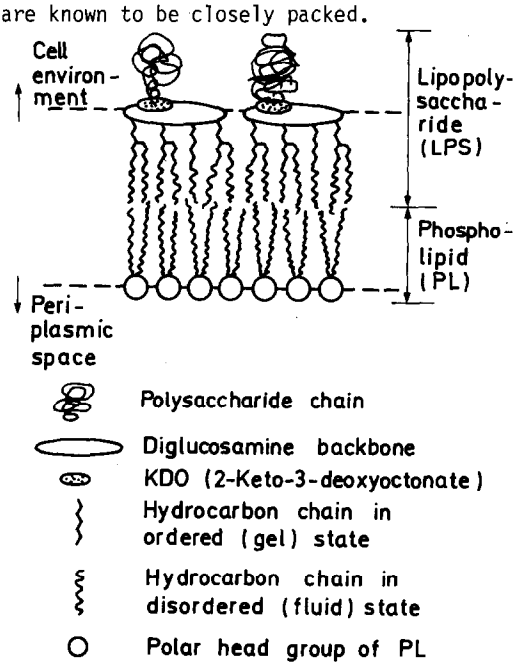


Fig. 3. DTA heating scans for 5.5 mg DMPE, (4) 1stscan, (3) 2ndscan, and 33.0 mg lipopolysaccharide Rz (1) as compared to 0.95 mg dodecanoic acid, respectively

In fig. 3 DTA scans are plotted for DMPE and LPS Rz and for a dodecanoic acid preparation. DMPE vesicles - prepared at room temperature - show in the 1st scan an endotherm at 59°C of as large as 75.8 kJ Mol^{-1} which is absent in all following scans. This endotherm originates from a hydration of the head groups as well as of a melting of the acyl chains. The remaining endotherm at app. 53°C corresponds to the melting of the hydrocarbon chains $L_\beta \rightarrow L_\alpha$ (ΔH_m = 23.2 kJ Mol^{-1}). The endotherm for LPS Rz is typical for the different LPS preparations with regard to the broadness of the transition and the enthalpy of app. 30 kJ Mol^{-1}. Regarding the fact that the hydrocarbon moiety of LPS consists of 6 to 7 acyl chains with - on

average - 14 carbon atoms each (ref. 5), ΔH_m should have values in the range 70 to 80 kJ Mol^{-1} assuming a "normal" $L_\beta \rightarrow L_\alpha$ transition as in DMPE and other PL preparations. Thus, it may be concluded that the hydrocarbon chains are already partially melted below T_c.

From monolayer measurements the quantity ϱ - the limiting area per molecule (area per molecule in the condensed state at $\pi \rightarrow \infty$) - was determined. The ϱ-value for free lipid A is 0.7 to 0.9 nm^2 per molecule - depending on the amount of phosphate and 2-keto-3-deoxyoctonate (KDO) - and increases to a limiting value of 1.25 nm^2 for the smooth mutant-LPS's (ref. 1). Compared to PE's (ϱ_{DMPE} = 0.23nm^2, ϱ_{DPPE}=0.30 nm^2) and PC's (ϱ_{DMPC}=0.30nm^2, ϱ_{DPPC}=0.50nm^2) the behaviour of free lipid A and the deep rough mutant-LPS's resembles that of PE's in which acyl chains are known to be closely packed.



The obtained data with the different techniques suggest, that the acyl chains of LPS are in a considerably higher order (gel state) near the disaccharide backbone and in a lower order near the methyl end groups than those of PL's in the inner leaflet of the outer membrane. From this, a model of the outer membrane under physiological conditions (at 37°C) can be derived (fig. 4). This model is backed by ESR-measurements of Janoff et al. (ref. 7) which gave evidence for the coexistence of a gel- and a liquid crystalline state of the outer membrane at different temperatures (12, 37 and 43°C).

Fig. 4. Model for the physical state of the outer membrane of Gram-negative bacteria

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