Lipopolysaccharide-binding protein-mediated interaction of lipid A from different origin with phospholipid membranes.

Invited Lecture

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Investigations are reported into the interaction of lipid A, the ‘endotoxic principle’ of bacterial lipopolysaccharide (LPS), with phospholipid membranes in the absence and presence of an acute-phase lipid transport protein, lipopolysaccharide-binding protein (LBP) applying Fourier-transform infrared (FTIR) and fluorescence resonance energy transfer (FRET) spectroscopy. In the absence of LBP, intermixing of phospholipids with lipid A takes place on the time-scale of hours, while in the presence of LBP this process takes place in the order of minutes. A comparison of chemically different lipid A shows that a prerequisite for the intercalation of lipid A into the phospholipid membrane is a sufficiently high negative charge density of lipid A. Variations in the lipid A acyl chain fluidity may modulate the intercalation, whereas the type of lipid A aggregate structure has no influence on the intercalation. The intercalation is a necessary, but not sufficient prerequisite for cell activation. Only lipid A with a conical molecular shape and a tilt angle of more than 40° of the backbone with respect to the direction of the acyl chains induces cytokine induction in human mononuclear cells, while lipid A with a cylindrical shape and a small tilt angle does not exhibit this biological activity but may act antagonistically. This antagonistic effect may be explained by blocking of the binding-sites of the putative signal-transducing protein, possibly an ion channel, by the antagonist.

Introduction

Lipopolysaccharides (LPS) are major amphiphilic components of the outer leaflet of the outer membrane of Gram-negative bacteria. When released from the bacterial surface, LPS play an important role in the pathogenesis and manifestation of Gram-negative infection, in general, and of septic shock, in particular, and are thus called endotoxins. They are, however, also capable of producing beneficial effects in higher organisms depending upon amount and route of introduction. LPS consist of a sugar moiety (oligo- or polysaccharide) and a covalently linked lipid moiety termed lipid A, anchoring the molecule in the membrane. Since the lipid A moiety was found to harbour the main biological activities of LPS, it is called the ‘endotoxic principle’ of LPS. One of the biological activities of endotoxins is their capacity to induce the production of cytokines (tumor necrosis factor αTNFα and the interleukin family) by mononuclear cells (monocytes/macrophages).

The lipid A of Escherichia coli expresses the highest biological activity of all known lipid A. Chemically, this lipid A consists of a bisphosphorylated compound with 6 acyl chains ester- or amide-linked to a diglucosamine backbone in asymmetric distribution. Reduction of biological activity down to complete inactivity was observed for LPS from various non-enterobacterial sources with lipid A structures deviating from that of E. coli in the number and distribution of acyl chains linked to the sugar backbone, and/or different number and location of charges within the backbone region. Moreover, some of these lipid A variants exhibited strong antagonistic activity, i.e., they inhibited the agonistic action of biologically active LPS. These observations were interpreted as to indicate an influence of a variation in the primary structure of endotoxin molecules on their physicochemical behaviour. In previous studies we have shown that high biological activity is correlated to a conical shape of lipid A with a higher cross-section of the hydrophobic than that of the hydrophilic moiety, and antagonistic activity to a cylindrical shape with identical cross-sections. The conformational data were deduced from X-ray diffraction spectra of the supramolecular aggregates of lipid A.

The biological activity of endotoxins may be discussed in the context of the interaction of LPS molecules with the membranes of host cells, because the direct interaction of endotoxin molecules with host cell membranes mediated by transport proteins and/or hydrophobic interaction and binding to a signal protein is considered to be an important step in the initiation of the signalling cascade finally leading to the manifestation of the biological effects.

The nonspecific interaction should consist of a direct interaction of small endotoxin aggregates down to monomers. This mechanism may be assumed to be responsible for cell activation at high endotoxin concentration. At low endotoxin concentration another mechanism of interaction is proposed that proceeds via specific coupling either directly to a membrane-bound receptor protein (mCD14, CD55) or indirectly to the
We have found that a rapid intercalation of lipid A into target membranes between two dyes in the labelled phospholipid membrane. FRET spectroscopy allows the detection of an intercalation of lipid A into phospholipid membranes independent of the respective fluidities, monitored by the resonance energy transfer (FRET) spectroscopy were applied. With FTIR spectroscopy, interactions of lipid A with phospholipid membranes can be monitored from changes of the peak position of the stretching vibrations of the methylene groups, e.g., \( \nu(CH_2) \), in cases in which the fluidities of the acyl chain moiety of lipid A and phospholipids differ significantly. The FRET spectroscopy allows the detection of an intercalation of lipid A into phospholipid membranes independent of the respective fluidities, monitored by the resonance energy transfer between two dyes in the labelled phospholipid membrane. We have found that a rapid intercalation of lipid A into target cell membranes is mediated by LBP in the range of seconds to minutes. The amount of intercalated lipid A may vary strongly in dependence on backbone and acylation pattern. A particular requirement for intercalation is a sufficiently high negative headgroup charge, whereas only a slight dependence on acyl chain fluidity and no dependence on aggregate structure can be observed.

We propose a model of cell activation, which comprises as the first step the intercalation of endotoxin molecules into the cytoplasmic membranes of immune cells. Intercalation is facilitated by the acute phase serum protein LBP. The intercalation is a necessary but not sufficient prerequisite for cell activation, since agonistic as well as antagonistic lipid A intercalate into membranes, and only completely inactive lipid A do not. As the second step, the endotoxin molecules then interact with the putative signal transducing protein. For agonistic action, a conical shape of lipid A is a prerequisite, which leads to mechanical stress at the site of the signal transducing protein thus triggering cell activation.

Materials and methods

Lipids and reagents

Deep rough mutant Re LPS from S. minnesota strain R595 was extracted from bacteria grown at 37 °C by the phenol/chloroform/petrol ether method, purified, and lyophilized. Bisphosphoryl lipid A was isolated by acetone buffer treatment of LPS Re LPS. After isolation, the resulting lipid A samples were purified and converted to the triethylamine salt form. Pentacycl lipid A was obtained from a bacterial mutant of Escherichia coli strain F515, which does not synthesize any hexacycl lipid A, and tetraacycl lipid A was a kind gift from U. Zähringer (Division of Immunochemistry, Research Center Borstel, Germany). The cultivation of the entero-bacterial strains Chromobacterium violaceum, Rhodobacter capsulatus, Campylobacter jejuni, Rhodospirillum fuscum, and Rhodomicrobium vannielli, the extraction of their LPS and isolation of lipid A was performed as described earlier. The known chemical structures of the lipid A were checked by the analysis of the amount of glucosamine, total and organic phosphate, and the distribution of the fatty acid residues applying standard procedures.

Hexacycl lipid A analogues 4-monophosphoryl lipid A (504), 1-monophosphoryl lipid A (505), bisphosphoryl lipid A (506), and 1-carboxymethyl 4-phosphoryl lipid A (CM-506) were synthesized as described earlier.

In Fig. 1, an overview of the various chemical structures of the used lipid A is given. As can be seen, all lipid A have only saturated acyl chains except that of Rhodobacter capsulatus with a cis-un saturated acyl chain at the 3' position of the diglucosamine. Lipid A from R. vannielli, which is completely unchanged, is not listed in Fig. 1 since the acylation pattern is not completely known.

The synthetic deuterated phospholipids dimyristoyl phosphatidylcholine (DMPC D54), dimyristoyl phosphatidylglycerol (14 : 0 PG D54), dimyristoyl phosphatidylserine (14 : 0 PS D54), and 3-sn-phosphatidylethanolamine from E. coli were purchased from Avanti Polar Lipids (Birmingham, AL, USA). Bovine brain 3-sn-phosphatidylserine, egg 3-sn-phosphatidylcholine, and sphingomyelin from bovine brain were obtained from Sigma (Deisenhofen, Germany). For preparation of liposomes from the phospholipid mixture resembling the composition of the cytoplasmic membrane of macrophages (PL360)—containing 3-sn-phosphatidylcholine, 3-sn-phosphatidylserine, 3-sn-phosphatidylethanolamine, and sphingomyelin in a molar ratio of 1 : 0.4 : 0.7 : 0.513—the lipids were solubilized in chloroform, the solvent was evaporated under a stream of nitrogen, and the lipids were resuspended in the appropriate volume of phosphate-buffered saline (PBS), and finally treated as described below. Lipopolysaccharide-binding protein (LBP) was a kind gift from S. Carroll (XOMA (US) LLC, Berkeley, CA, USA).

Sample preparation for IR spectroscopy

The lipid samples were usually prepared as aqueous dispersions at a buffer content of 95% using 20 mM HEPES (pH 7). For this, the lipids were suspended directly in buffer and were temperature-cycled 3 times between 5 and 70 °C and then stored at 5 °C for at least 12 h before measurement.

Determination of phase transition and unspecific interaction of lipid A with phospholipid membranes

The \( \beta \rightarrow \alpha \) to liquid crystalline phase transition of the hydrocarbon chains of lipid A preparations and the unspecific interaction of lipid A from Salmonella minnesota with phospholipid liposomes was studied with Fourier-transform infrared spectroscopy on a FTIR spectrometer IFS-55 (Bruker, Karlsruhe, Germany). The lipid samples were placed in a CaF2 cuvette separated by a 12.5 mm thick teflon spacer.

For the determination of phase transition temperatures \( T_c \), temperature-scans were performed automatically in the range from 10 to 70 °C with a heating-rate of 0.6 °C min\(^{-1}\). For determination of the unspecific interaction, phospholipid liposomes were incubated at 37 °C and lipid A was added. As measuring parameter, the peak position of the symmetric stretching vibration of the methylene groups \( \nu(CH_2) \) of the acyl chains was taken. To differentiate between the two lipid systems, perdeuterated phospholipids were utilized. The applicability of this method implies that the states of order at 37 °C of the two lipids differ to a sufficient degree. This is given for the system lipid A (\( T_c = 45 °C \)) and dimyristoyl lecithin and glycerol DMPC and DMPG (\( T_c \approx 23–25 °C \)). Thus, the peak position of \( \nu(CH_2) \) lies—according to the fluidity of the...
Fig. 1 Chemical structures of various lipid A from enterobacterial and nonenterobacterial origin.

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<td><em>Campylobacter jejuni</em></td>
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<td><em>Chromobacterium violaceum</em></td>
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<td><em>Rhodospirillum fulvum</em></td>
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For these measurements, deuterated lipids were put into a IR cuvette heated at 37 °C; afterwards, lipid A preparations pre-heated to 37 °C were added, and spectra were taken every 10 min up to a time of 70 h.

Incorporation of lipid A and Re LPS into phospholipid membranes

The ability of lipid A or LPS to incorporate into target cell membranes on its own or mediated by lipopolysaccharide-binding protein (LBP) was determined from fluorescence resonance energy transfer (FRET) spectroscopic measurements as described elsewhere. Briefly, phospholipid liposomes made from a phospholipid mixture corresponding to the composition of the macrophage membrane PLsph (phosphatidylycholine, phosphatidylserine, phosphatidylethanolamine and sphingomyelin in a molar ratio of 1 : 0.4 : 0.7 : 0.5) were double-labeled with the fluorescent dyes N-(7-nitro-2,1,3-benzoxadiazol-4-yl)-PE (NBD-PE) and N-(Rhodamine B sulfonyl)-PE (Rh-PE), which were purchased from Molecular Probes (Eugene, OR, USA). For this, NBD-PE and Rh-PE were dissolved in chloroform and added respective lipid chains—at 2850 to 2853 cm⁻¹ for lipid A and at 2090 to 2095 cm⁻¹ for the deuterated lipids.¹⁸
to the lipids in the chloroformic phase to a final molar ratio [PL]: [NBD-PE]: [Rh-PE] = 100:1:1. The emission wavelength of one dye, the donor (NBD-PE), is in the range of the excitation wavelength of the second dye, the acceptor (Rh-PE). The energy transfer between these two dyes is sensitive to spatial separation. Intercalation of unlabeled molecules into the double-labeled liposomes leads to probe dilution and with that to a decrease in the efficiency of RET: the emission intensity of the donor increases and that of the acceptor decreases (for the sake of clarity, here we only show the donor emission intensity). A preparation of 900 µl of the double-labeled PL400 liposomes (0.01 mM) at 37 °C was excited at 470 nm (excitation wavelength of NBD-PE), and the fluorescences emissions of NBD-PE (531 nm) and Rh-PE (593 nm) were adjusted to yield identical intensities and recorded for 50 s under continuous stirring to determine the base line. After 50 s, unlabeled lipid A (100 µl, 0.1 mM) was added, after a further 50 s the appropriate amount of LBP was added and the emission signals were recorded for at least 5 min.

It should be noted here that due to bleaching of the dyes only intercalation processes in a maximum range of 0.5 to 1 h can be monitored.

Small-angle X-ray diffraction

Small-angle X-ray diffraction measurements of lipid A were performed using the double focusing monochromator-mirror camera X33. Diffraction patterns in the range of the scattering vector 0.07 < s < 1 nm⁻¹ (s = 2π sin θ/λ, 2θ = scattering angle, λ = wavelength = 0.15 nm) were recorded with an exposure time of 2 min using a linear detector with delay line readout. From the spacing ratios of the diffraction maxima an assignment to defined three-dimensional aggregate structures is possible, i.e., to micellar (e.g., H₃), uni- or multilamellar (L), cubic (Q) and hexagonal II (H₂) structures. From this, the conformation (shape) of the individual molecules can be inferred: conical/convex for micellar structures (the cross-section of the hydrophilic part is larger than that of the hydrophobic part), cylindrical in the case of lamellar structures (the cross-sections of the hydrophilic and hydrophobic moieties are identical), and conical/concave in the case of inverted cubic and HII structures (the cross-section of the hydrophobic portion is higher than that of the hydrophilic one).

Results

Phase transition of lipid A preparations

For different E. coli-type lipid A with a bisphosphorylated diglucosamine backbone, the β→α gel to liquid crystalline phase transition of the hydrocarbon chains was monitored via evaluation of the symmetric stretching vibration of the methylene groups ν(CH₂) of lipid A. In Fig. 2, the results are shown for bisphosphoryl tetraacyl, pentaacyl, and bisphosphoryl hexaacyl lipid A, and for synthetic 1-carboxymethyl 4-phosphoryl hexaacyl lipid A (CM-506), in which the 1-phosphate group of natural lipid A has been exchanged by a carboxymethyl group. Clearly, a systematic dependence of the phase transition temperature Tc on the acylation patterns can be observed with the lowest value of Tc for bisphosphoryl tetraacyl and the highest for 1-carboxymethyl hexaacyl lipid A. The state of order—inversely proportional to the ‘fluidity’—at 37 °C may be an important quantity with respect to an interaction with target cells. The vertical line in Fig. 2 indicates that tetraacyl lipid A and also pentaacyl lipid A have a very high fluidity (low state of order). In contrast, the bisphosphoryl hexaacyl and even more the 1-carboxymethyl hexaacyl lipid A have a low fluidity, i.e., their hydrocarbon chains are still well ordered at 37 °C.

Interaction of deuterated phospholipids with lipid A

To study the unspecific interaction of a phospholipid membrane with endotoxins, the shift of the peak positions of ν(CH₂) from lipid A (at 2850 cm⁻¹) and ν(CD₂) from the deuterated phospholipids (at 2092 cm⁻¹) were monitored at 37 °C after incubation of the phospholipids or a mixture of them and addition of lipid A. Clearly, the peak position of ν(CH₂) is slowly shifted to higher wavenumber values (Fig. 3A) and reaches a nearly constant value after more than 10 h (the wavenumber ‘jump’ at the beginning results from temperature equilibration in the cuvette), which is indicative of a decrease in lipid A acyl chain order (increase of fluidity). Analogously, the peak position of
\(v_1(\text{CD}_3)\) is shifted to lower wavenumber values (Fig. 3B) indicating an ordering of the phospholipid acyl chains. Similar experiments with pure DMPC_{deut} and DMPC_{deut–DMPG_{deut}} mixtures showed a similar, but quantitatively less pronounced effect corresponding to the content of zwitterionic lecithin.

**LBP-mediated incorporation into phospholipid liposomes**

For the LBP-mediated interaction of lipid A with phospholipid liposomes, FTIR spectroscopy could not be applied since the amounts of LBP available requires a technique much less material-consuming. For this, FRET spectroscopy is suitable since lipid concentrations in the range \(10^{-3}\) M can be applied rather than concentrations of mM or higher for FTIR spectroscopy.

Thus, we have investigated, whether lipid A of different origin are able to incorporate in the presence of LBP into a phospholipid membrane resembling the composition of the macrophage cytoplasmic membrane (PL_{mac}) on a time-scale of minutes.

For this, we have compared different natural and synthetic lipid A preparations and lipid A part structures with variations in the backbone and the acyl chain moiety, and different lipid A preparations from various bacterial strains (chemical structures see Fig. 1).

In Fig. 4, the results of the FRET experiments with different lipid A analogues and part structures are shown: The increase in NBD-fluorescence intensity for all lipid A samples after addition of LBP indicate a rapid intercalation of the lipid A into the PL_{mac}-membrane on the time-scale of minutes but no intercalation in the absence of the transport protein on the same time-scale. For the different lipid A, no clear correlation with the acylation pattern is observed: the signal for pentacyl lipid A is higher than that of tetraacyl lipid A. A change in the backbone charge leads to significant changes in the intensity of the fluorescence signal for the lipid A samples. The signal for tetraacyl lipid A is higher than that of hexaacyl lipid A.

In some experiments the LBP-concentration was increased without changing the other experimental conditions. It turned out that saturation of the amount of incorporated lipid A took place at a two- to three-fold increase of the LBP-concentration (data not shown).

The results for the FRET measurements with lipid A from different bacterial species are shown in Fig. 5. Again, the detailed acylation pattern does not play the decisive role: Interaction of the lipid A with a symmetric 3/3 configuration of the hydrocarbon chains (from *C. violaceum*) leads to the highest signal, and the pentaacyl lipid A from *R. capsulatus* and the hexaacyl lipid A from *C. jejuni* with, on the average, longer acyl chains cause a lower increase in the donor signal than lipid A from *S. minnesota*. In the case of lipid A from *R. vannielii*, the backbone of which carries no negative charges, no increase in intensity is observed.

**Small-angle X-ray diffraction and molecular shape**

Small-angle X-ray diffraction measurements of a variety of chemically different lipid A were performed to determine their molecular shape. In Fig. 6, diffraction patterns at 85% water content and 40 °C are presented for hexaacyl and pentaacyl lipid A from *E. coli* and for lipid A from *R. fulicium* and *C. violaceum*. For the hexaacyl lipid A from *E. coli*, the positions of the reflections are indicative of the presence of an inverted cubic structure, whereas the other lipid A exhibit characteristics typical for lamellar structures deduced from reflections at equidistant ratios. Thus, the molecular shape of the former lipid A is conical–concave with a higher cross-section of the hydrophobic than the hydrophilic moiety, while for the other lipid A the molecular shape is cylindrical due to identical cross-sections of the two parts.

These data were correlated with the ability of the lipid A samples to induce interleukin-6 (IL-6) in human mononuclear cells (agonistic activity) as well as the ability of those lipid A, which exhibit no agonistic activity to act antagonistically against agonistically highly active wild-type (S-form) LPS from *S. minnesota*. As given in Fig. 7, agonistic activity is found only for the conically-shaped (concave) hexaacylated—monophosphoryl as well as bisphosphoryl—lipid A from *E. coli*, deduced from the cubic inverted aggregate structure in the X-ray diffraction experiment. In contrast, all endotoxins exhibiting a cylindrical molecular conformation are found to have strongly reduced or even vanishing biological activity. The tetraacylated compound 406 adopts a lamellar aggregate structure and, with that, the individual molecules have a cylindrical conformation. This compound exhibits no agonistic but strong antagonistic activity. From the evidence of a superimposed micellar H\(_4\) aggregate structure it can be deduced that 406 has a slight tendency to adopt a conical shape, which is reversed (convex) as compared to that of biologically active lipid A with the cross-section of the hydrophobic part of the molecule being smaller than that of the disaccharide backbone.

Lipid A from *C. jejuni* has a very slight tendency for an
inverted structure, i.e., for a conical shape. This fact correlates with its non-zero agonistic activity, which again seems to be connected with the lack in antagonistic activity (negative values!).

Discussion

Our data presented here clearly indicate a strong interaction of lipid A with phospholipid membranes. The time-scale of this interaction is in the range of hours in the absence of lipid transport proteins such as LBP (Fig. 3). Regarding the mechanism of this interaction, it should be noted that lipid aggregates are always in dynamic equilibrium with their monomers. In the case of two different lipid systems, the monomers of one lipid then may readily incorporate also into the other lipid system due to pure hydrophobic interaction. Since the number of lipid A monomers can be assumed to be very low (the critical micellar concentration should be very low \( < 10^{-7} \text{M} \)), which again is connected with very high aggregate stability, it becomes understandable that the lipid

![Fig. 6](image1)

**Fig. 6** Synchrotron radiation X-ray small-angle diffraction patterns of hexa- and pentaacyl lipid A from *Escherichia coli* and lipid A from *Rhodospirillum fulvum* and *Chromobacterium violaceum* at a water content of 85%, 5 mM Mg\(^{2+}\) and 40 °C.

![Fig. 7](image2)

**Fig. 7** Agonistic and antagonistic activities and molecular shapes of various entero- and nonenterobacterial lipids. The antagonistic activity of agonistically inactive lipid A are expressed as percentage inhibition of IL-6 induction due to preincubated lipid A (100 ng ml\(^{-1}\)) 30 min prior to stimulation by LPS (S-form LPS from *S. enterica* sv. Minnesota) with 1 ng ml\(^{-1}\). The inhibition value obtained for lipid A from *R. capsulatus* (LPS-induced IL-6 production was reduced to 2.3 ± 1%) was set 100% and the data obtained with the other preparations related to this standard. Error bars are standard deviations resulting from three independent experiments (adapted from ref. 6, with permission of Blackwell Science Press, Oxford, UK).
intermixing observed here is a very slow process. A direct interaction of lipid aggregates with each other leading to their fusion does not take place in the absence of high amounts of cations such as \( \text{Ca}^{2+} \), as has been demonstrated by us for the system DMPC and lipid A.\(^{23}\)

It should be noted here, that the concentrations in the mM range used for these measurements are far above physiological conditions, but had to be applied to get an evaluable signal. Therefore, it is unclear whether this completely unspecific interaction plays a role at all in biological systems also regarding the slow intercalation kinetics. It has been observed that stimulation induced by endotoxins leads to an immediate reaction in the range of minutes on the level of messenger ribonucleic acid (mRNA).\(^{24}\) Furthermore, although it is known that cell activation may occur in the absence of serum as well as of membrane-bound mCD14,\(^{25}\) probably in any cellular system other membrane proteins are present, which act as enhancers of the biological reaction, such as CD55.\(^{8}\)

In the presence of LBP, the time-scale of lipid A intercalation into PL(340) is in the range of minutes (Figs. 4 and 5), a time which is typical for the first appearance of biological reactions.\(^{24}\) From the results presented in these figures it may be concluded that different lipid A acyl chain fluidities—for samples with identical aggregate structures—modulate the intercalation. As shown in Fig. 2, tetraacyl and pentaacyl lipid A forming lamellar aggregates\(^{14}\) both have high acyl chain fluidity, which is even higher for the former. Fig. 4 shows, that both samples are readily incorporated into the liposomal membrane, but tetraacyl lipid A has the lower efficiency. These results would imply that the lipid A with a lower acyl chain fluidity is incorporated more efficiently. Similarly, the results for the hexaacyl lipid A 506 and its carboxymethyl derivative CM-506 can be understood: Both compounds adopt identical (inverted cubic)\(^{14}\) aggregate structures, but CM-506, having the lower acyl chain fluidity (Fig. 2) incorporates more readily into PL(340) (see Fig. 4).

As reported previously,\(^{14}\) clearly the type and number of charges are the main determinants of intercalation. Uncharged lipid A such as that from \( \text{R. vannielli} \) (Fig. 5) or with a strongly reduced number of headgroup changes (lipid A from \( \text{R. fulvum} \)) are not incorporated to a significant degree. Lipid A with a reduced number of negative charges in the polar backbone, such as the monophosphoryl hexaacyl lipid A compounds 504 and 505 (Fig. 4), are incorporated to a significantly lower degree. From the preference of LBP to negatively charged lipid A a positive binding domain of LBP might be concluded to exist. Actually, although LBP has 47 negative and 43 positive single charges leading to a net negative charge, a positive charged domain exists at the N-terminal region which is assumed to be the LPS-binding domain.\(^{26}\) A possible influence of different aggregate structures on LBP-mediated incorporation may be ruled out: Lipid A aggregates from \( \text{C. violaceum} \), from \( \text{R. capsulatus} \), and from \( \text{R. vannielli} \) are lamellar but lead to either a higher or a lower incorporation (Fig. 5) than that of \( \text{S. minnesota} \) lipid A, which has an inverted cubic aggregate structure.\(^{6}\) These differences can also not be explained by differences in the acyl chain fluidity, which increases in the order lipid A from \( \text{C. jejuni} \), over that from \( \text{C. violaceum} \) to that from \( \text{R. capsulatus} \).\(^{5,6}\)

The mechanism of interaction of LBP with lipid A and the subsequent transfer to phospholipid membranes is by far not fully understood. It has been proposed that LBP acts as a shuttle between lipid A/LPS and the target cell membranes and leads to the disaggregation of the endotoxin aggregates,\(^{27}\) however, direct proof for the existence of such process has never been presented. From the considerations above a significant contribution of the interaction of LBP with lipid A monomers can be excluded. Also, the observed saturation of lipid A intercalation at a 2–3 fold increase of the LBP concentration makes a shuttle process unlikely.

At this point, it should be noted that besides the ability of LBP to transport endotoxins, the protein by itself tends to intercalate into negatively charged membranes. More detailed investigations about this phenomenon will be published elsewhere.\(^{28}\)

In the model of cell activation, proposed by our group,\(^{6}\) for agonistically as well as antagonistically acting lipid A a prerequisite to exert bioactivity is its incorporation into the target cell membrane, mainly mediated by LBP, and possibly also by the pathway via CD14, to which LBP/endotoxin complexes may be bound.\(^{29}\)

All lipid A with a particular ‘endotoxic conformation’, which is a conical shape and a tilt angle of the backbone of more than 45° with respect to the direction of the acyl chains,\(^{30}\) then induce a conformational change at the site of a transmembrane signal transduction protein, possibly an ion channel, due to sterical stress.

Cylindrically-shaped lipid A, for which this tilt angle is very low (<15°)\(^{30}\) do not induce a sterical stress, but may occupy the binding sites for biologically active endotoxins, thus acting as an antagonist.

The lack of antagonism for the lipid A without negative backbone charge can be explained by the lack of incorporation into target cells, although with their cylindrical molecular shape they fulfill the prerequisite for antagonism.\(^{6}\)

Summarizing these data, a model of the initial steps in endotoxin signalling can be established according to Fig. 8. The schematic of this model, of course, does not quantify the single steps of cell activation. The ‘endotoxic conformation’ of highly active hexacyl lipid A must undergo a very specific interaction with the signalling protein to induce the conformational change necessary for cell activation. So far, however, no details of this interaction process are known.

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References


**Fig. 8** Schematic model of endotoxin-induced activation of macrophages.


