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Interaction of Porin from *Yersinia pseudotuberculosis* with Different Structural Forms of Endogenous Lipopolysaccharide

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Abstract—The interaction of *Yersinia pseudotuberculosis* porin solubilized in deoxycholate with the S- and R-forms of endogenous lipopolysaccharide (LPS) was studied by the quenching of intrinsic protein fluorescence. The samples of S-LPS differed both in the length of O-specific polysaccharide (n = 1 and 4) and in the acylation degree of the 3-hydroxytetradecanoic acid residues of the lipid A moiety (12-66%). R-LPS (12%) binding to porin was found to occur with positive cooperativity on two integrated structural regions of the R-LPS macromolecule, namely, core oligosaccharide and lipid A. The mode of porin interaction with low-acylated S-LPSs (15 or 20%) coincided with a model involving three types of binding sites. The shape of Scatchard curves of binding indicates that a complex formation between porin and low-acylated S-LPS is cooperative at low and moderate ligand concentration, whereas at near-saturating LPS concentrations porin binds to LPS independently on two types of binding sites. The O-specific polysaccharide chain in the S-LPS macromolecule increases the affinity of its interaction with porin in comparison with R-LPS–porin binding. A significant increase (to 66%) in the degree of S-LPS acylation substantially changed its porin-binding character: the process becomes anti-cooperative with lowered affinity. Thus, the features of LPS–porin interaction significantly depend on the conformational changes in the LPS molecule due to expanding of its hydrophobic region.

Key words: *Yersinia pseudotuberculosis*, outer membrane, lipopolysaccharide, porin, lipopolysaccharide–protein interaction, protein fluorescence

Lipopolysaccharide (LPS) and trimeric pore-forming proteins (porins) are the essential components of the outer membranes of gram-negative bacteria [1]. LPS is a peculiar amphiphilic biopolymer. Three molecular fragments, namely, the core oligosaccharide, lipid A, and O-specific polysaccharide chain compose the S-LPS molecule (S stands for smooth, characterizing the form of bacteria). Mutant and some wild bacteria strains produce an LPS molecule lacking polysaccharide chain, called R-LPS. Porins are β-structured transmembrane proteins consisting of three identical subunits. Porins provide an important transport function in microbial cells: they form hydrophilic pores in the native membrane that low-molecular-weight substances, nutrients, and cellular metabolites diffuse through.

A specific interaction between LPSs and porins in the native membrane determines the outer membrane properties and is crucial for the life of the bacterial cell [1-3]. Among the LPS–protein complexes (LPPC) containing LPSs and porins are phage receptors, colicins, and conjugation factors. LPPCs also play an important role in the membrane assembly: only interacting to LPS, porin oligomerizes and reconstitutes as a "mature" protein to the outer membrane. It has been found experimentally that both the LPS structure and porin conformation largely determine the properties of the individual complex, as well as the molecular organization and stability of the entire outer membrane [4]. Nonetheless, the LPPC stoichiometry and the effect of LPS structure on the character and efficiency of complex formation are still poorly investigated [5-7]. The mechanism of complex formation and quantitative characteristics of this process remain to be defined and adjusted.

Despite increasingly wide application of molecular dynamics methods in the evaluation of biologically active macromolecule interaction mechanisms, the use of these approaches to study an interaction of bacterial membrane structural components is still limited. On the other hand,

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the application of existing theoretical models for the description of specific interaction of complex multifunctional macromolecules, such as LPS and porins, with their ligands is also complicated. The progress in our knowledge about spatial organization and interaction of these compounds demands detailed investigation of the influence of their structural elements on physical and chemical properties and correlation of the results obtained to the data acquired from theoretical models. In this connection, the approaches remain valid which can be used to analyze the effect of structural variations on the changes in biological activity or interaction character of macromolecules.

The present study is a part of the regular research program [8-10] concerning the interaction of monomeric and trimeric forms of the pore-forming protein from the outer membrane of *Yersinia pseudotuberculosis*, which we have named yersinin, with endogenous LPS and its structural fragments. This study applies the method of quenching of intrinsic protein fluorescence to the interaction of porin trimer with endogenous LPSs. The goal of the study was to evaluate the effect of LPS structure, namely, the length of O-specific polysaccharide chain and acylation degree of the 3-hydroxytetradecanoic acid residues of lipid A on the character and efficiency of complex formation.

MATERIALS AND METHODS

Isolation and purification of porin. Trimer of porin was prepared as described previously [11] followed by gel filtration on the TSK gel (ToyoSoda, Japan) [12]. The degree of porin purification from admixed proteins was determined using SDS-PAGE following Laemmli [13]. The protein banding was visualized in the gel with Coomassie blue G-250 in 3.5% perchloric acid [14]. Protein was determined by a modified method of Lowry in the presence of 2% sodium dodecyl sulfate (SDS) [15], and monosaccharides were determined by a standard method [16]. The lyophilized porin prepared contained 75-80% of protein and no more than 5% of monosaccharides.

To remove LPS the protein sample was incubated for 16 h at 37°C in 20 mM Tris-HCl, pH 7.5, containing 1 mM EDTA (buffer A) and 0.5% sodium deoxycholate (DOC) and then separated on a column filled with Sephadex G-200 in buffer A containing 0.25% DOC. LPS content in fractions was monitored using the test with thiobarbituric acid [17] and SDS-PAGE [13] with the staining of the gel with silver [18].

Isolation and purification of LPS. Two isogenic strains of *Y. pseudotuberculosis* serovar O:1b constructed in the Gamaleya Institute of Epideomology and Microbiology (Moscow) were used in the research: the plasmid-free (82^-) one and that containing the pVM82 plasmid (82^+). The bacteria were grown under aerobic conditions in the medium described previously [19] at 8°C (cold variant) and 37°C (warm variant) and pH 7.0. LPS-1 and LPS-3 were isolated from the plasmid-free (82^-) strain by extraction of the warm and cold variant cells respectively with hot aqueous phenol by the method of Westphal [20]. LPS-2 and LPS-4 were isolated from strain 82^+ by treatment of the cells of warm and cold variants respectively with phenol–chloroform–petroleum ether mixture (2 : 5 : 8 v/v) [21]. The total carbohydrate, protein, and nucleic acid contents were determined as described previously [19]. The quantitative assay of monosaccharides in the polyol acetates form was performed by gas–liquid chromatography (GLC) using xylose as an internal standard. The LPS polymerization degree was calculated from the molar ratio of mannose (the monosaccharide included in the O-specific polysaccharide of the *Y. pseudotuberculosis* LPS [22]) to the sum of L-glycero-D-manno- and D-glycero-D-manno-heptose components of the core monosaccharide [23]) in LPS hydrolyzed with 1 M trifluoroacetic acid (100°C, 2 h). Fatty acids were prepared by the hydrolysis of LPS with 4 M NaOH (100°C, 4 h), and their methyl esters obtained by treatment with diazomethane were analyzed by GLC and GLC–MS using tetradecanoic acid as a standard [24]. The degree of acylation of 3-hydroxytetradecanoic acid was calculated as the molar ratio of dodecanoic and 3-hydroxytetradecanoic (100%) acids [25].

Fluorescence. Spectra of protein fluorescence were recorded on a Hitachi 850 spectrofluorometer (Japan) at 25°C in a quartz cuvette (optical length 1 cm). The fluorescence was excited at 280 nm. The fluorescence spectra corrected using rhodamine B (Wako Pure Chemical Industries, Japan) were recorded subtracting the Raman band of the buffer. The slit width of both excitation and emission monochromators was 5 nm.

The experimental conditions were as follows: the aliquots (20 μl) of corresponding LPS were added to one milliliter of the porin ($5.4 \cdot 10^{-7}$ M) solution in 0.25% SDC, pH 7.0, with vigorous agitation, and when the equilibrium was achieved (after 20-30 min), the protein fluorescence spectra were recorded. The LPPC formation was accompanied by a decrease in porin fluorescence intensity to the limit, which was determined at λ_{max} = 327 nm. The protein fluorescence value not further diminished by ligand addition was taken as F_∞. The degree of saturation θ (ratio of number of binding sites on porin molecule occupied by ligand to their total number) was calculated from the change in the protein fluorescence level:

$$\theta = (F_o - F)/(F_o - F_\infty) = \Delta F/\Delta F_{max},$$

where F_o and F are, respectively, the initial protein fluorescence intensity at 327 nm and the fluorescence inten-

sity (at the same wavelength) in the protein solution after LPS addition and ΔF_{max} is the maximum decrease in fluorescence. All of the fluorescence values (F) were corrected for both the dilution of the porin sample because of LPS addition and the internal filter effect [26].

The experimental data were plotted in Scatchard's coordinates: dependence of $\theta/[LPS]_{tot}$ on θ, where θ is the saturation extent and $[LPS]_{tot}$ is the concentration of total LPS added in the reaction volume. The association constants were calculated as described in [10] using software designed by A. V. Kukarskikh on the basis of analytical methods proposed in [27, 28]. The degree of cooperativity (the Hill coefficient) was determined as the tangent of the slope angle of the straight line describing the dependence of $\log[\theta/(1-\theta)]$ on $\log[LPS]_{tot}$ [29].

RESULTS AND DISCUSSION

Isolation and purification of LPS and porin. Lipopolysaccharides of *Y. pseudotuberculosis* are a heterogeneous population of molecules varying in molecular weight and degree of hydrophobicity, which is determined by the ratio of hydrophilic and hydrophobic molecule parts. The measure of LPS hydrophilicity is served by the lengths of their O-specific polysaccharides, which are in turn determined by the number of repeating units in the latter molecules. Lipid A, the hydrophobic part of the *Y. pseudotuberculosis* LPS molecule, consists of β-1,6-linked glucosamine disaccharide modified by phosphate, 3-hydroxytetradecanoic (3OH14:0), and/or 3-dodecanoyl-hydroxytetradecanoic acid residues [30-32]. The hydrophobicity of lipid A and LPS as a whole is defined by the acylation degree of the 3-hydroxytetradecanoic acid by the dodecanoic acid residues. LPS synthesis in *Y. pseudotuberculosis* cells is controlled by growth temperature. At lowered temperature (8-10°C) LPSs with longer polysaccharide chains, on average up to five repeating parts, prevail [19]. At 37°C, the portion of LPS molecules with shortened O-polysaccharide chains and O-polysaccharide-lacking ones (R-LPS) and with low content of 3-acyloxyalkanoic acids increases in the pseudotuberculosis cells [19]. Lipid A from the pseudotuberculosis bacteria grown in the cold is characterized by high content of the 3-dodecanoyloxytetradecanoic acid (acylation degree of 3OH14:0 in the "cold" variants of bacteria reaches 50%), that results in substantial hydrophobicity increase of lipid A and LPS as a whole [19].

The hydrophobicity of LPS is determined by lipid A in its composition; the latter consists of two β-1,6-linked glucosamine residues modified by phosphate, 3-hydroxytetradecanoic (3OH14:0), and/or 3-dodecanoyl-hydroxytetradecanoic acid moieties [30-32]. The pseudotuberculosis bacterium can also produce two S-LPS types, which have close degrees of O-specific polysaccharide polymerization, but differ in the degree of 3OH14:0 acylation [19].

We grew the bacteria at 8 and 37°C and used two extraction methods to obtain LPSs differing in the length of polysaccharide chains and acylation degree of lipid A. The analytical data for these LPS samples are given in Table 1.

According to the amino acid sequence, the monomer of yersinin contains 3 tryptophan residues and 25 tyrosine residues [33]. Circular dichroism and fluorescence data showed that yersinin is a typical β-structured protein with dynamic tertiary structure [34, 35]. Its conformation depends on the purification procedure used [12] and on the nature of the solubilizing detergent [35].

The trimeric form of yersinin was isolated from the peptidoglycan–protein complex by cleavage of the latter with lysozyme [11]. The purified porin contained up to 5% of tightly attached LPS. The LPS-free protein can be obtained by the treatment of porin with 30% SDS [36]. However, as we reported previously [37], high concentration of this ionic detergent causes conformational changes in both secondary and tertiary structures of this protein. So, in this study we used the relatively mild nonionic detergent DOC and gel chromatography on Sephadex G-200 for the porin purification of LPS [38].

Table 1. Properties of LPS from *Y. pseudotuberculosis*

LPS type	*T*, °C	Molecular mass, kD	*n*	*a*, %
LPS-1 (R)	37	3.6	0.0	12.4
LPS-2 (S)	37	4.7	1.3	20.1
LPS-3 (S)	8	6.8	4.0	15.3
LPS-4 (S)	8	7.0	3.8	66.0

Note: *T*, °C, temperature of cultivation *Y. pseudotuberculosis; n*, degree of polymerization of O-specific polysaccharide; *a*, degree of acylation of 3-hydroxytetradecanoic acid.

The data of circular dichroism and fluorescence show that both the secondary and tertiary structures of porin remain unchanged upon the treatment with DOC [38]. The test for LPS with thiobarbituric acid was negative with the porin isolated. The silver staining of the protein band after SDS-PAGE did not reveal LPS as well.

Complex formation between LPS and porin was studied by protein fluorescence quenching. From our preliminary studies, the fluorescence spectrum of yersinin in DOC solution (λ_{ex} = 280 nm) has a maximum at 327 nm. The excitation at 296 nm only inducing the emission of tryptophan residues results in a shift of the spectral maximum to longer wavelength by as few as 3 nm. The quantum yields in both cases are comparable. It is reasonably safe to conclude that the excitation of porin in DOC at 280 nm results in the preferable emission of tryptophan residues despite the abundance of tyrosine residues [39].

The binding of LPS to porin decreases the protein fluorescence intensity with no effect on the spectral maximum position. This fact suggests that the microenvironment of tryptophan residues is not changed and the quenching of their emission is due to intra- and intermolecular interactions. An example is given in Fig. 1 demonstrating the quenching of the porin fluorescence by LPS-1. Nonlinear decrease in the protein fluorescence in the course of complex formation implies the complicated character of the interaction between porin and LPS.

The saturation curves constructed in $\theta = f([LPS]_{tot})$ coordinates (Fig. 2) have a sigmoid shape for all the ligands examined, suggesting a complex and specific interaction between porin and LPS, as well as the presence of several non-identical binding sites interdependent to each other [40].

The data of our experiments on the porin binding with the examined LPSs given in Scatchard's coordinates (Fig. 3) suggest that the difference in character of the interaction of the protein with ligands probably depends on the peculiarities of the LPS molecular structures.

The parameters of porin binding with the examined LPSs differing in length of O-specific polysaccharide chain and degree of lipid A acylation are given in Table 2.

The Scatchard plot for LPS-1 (that is substantially R-LPS with low acylation degree of 12.4%) forms a concave-downwards curve (Fig. 3, curve *1*). This curve type suggests cooperative interaction between receptor and ligand [40]. We suppose on the basis of known structure of LPS-1 that it binds to porin on sites of two types, namely the core oligosaccharide and lipid A. Note that the same shape (concave-downwards) of the Scatchard plot might result from heterogeneity of ligand [41]. However, in our opinion is namely the structure of the LPS-1 that determines to a greater extent the character of the LPS–poring binding than the possible heterogeneity of the sample. This supposition stems from the fact that LPS-1 was prepared from the warm variant of *Yersinia pseudotuberculosis* producing preferably R-LPS and S-LPS with short-

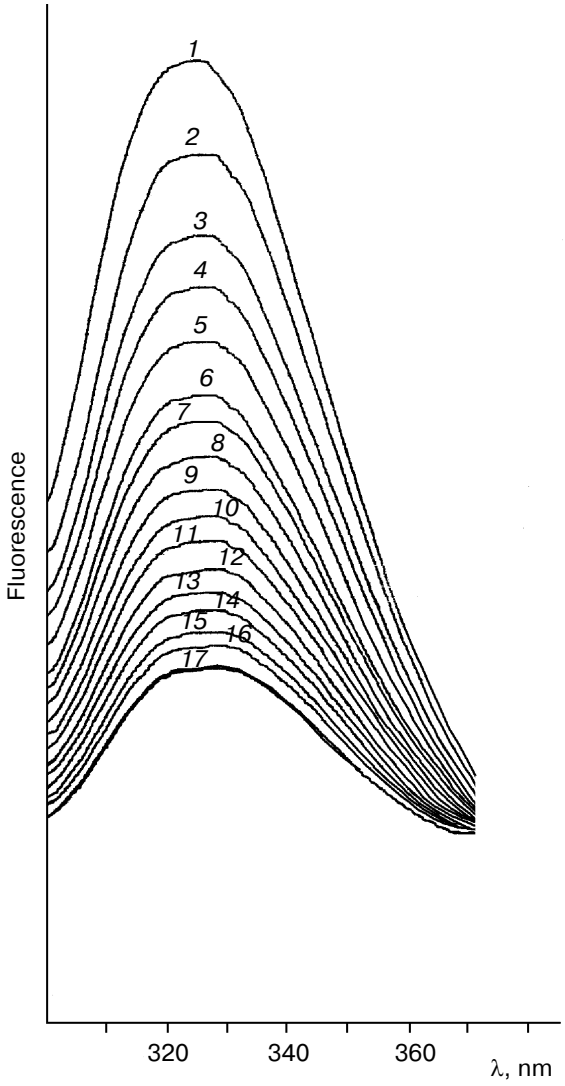

Fig. 1. Fluorescence spectra of porin in the absence (*1*) or presence of LPS-1 (all other) in 0.25% DOC, pH 7.0. The initial protein concentration is 0.54 μM. The spectra were recorded 30 min after a sequential addition of LPS-1 (8.4 μM) aliquot to 1 ml of vigorously agitated porin solution; λ_{ex} = 280 nm.

ened O-specific chain. [42]. Besides, as was earlier shown [42] the use of selective extraction promotes obtaining the narrow fraction of one or the other variant of LPS.

If the LPS molecule is complicated by the attachment of a single O-specific polysaccharide unit (LPS-2, Table 1), the Scatchard dome-shaped curve turns into a concave-upwards line at high degree of porin binding site saturation with the ligand (Fig. 3, curve *2*). The Scatchard plot for LPS-3 (Fig. 3, curve *3*) possessing four O-specific polysaccharide units resembles that for LPS-2. These results indicate that the interaction of S-LPS with porin at low and moderate ligand concentrations is a highly cooperative process. However, in this case, as compared to LPS-2, the saturation of the binding sites on the porin

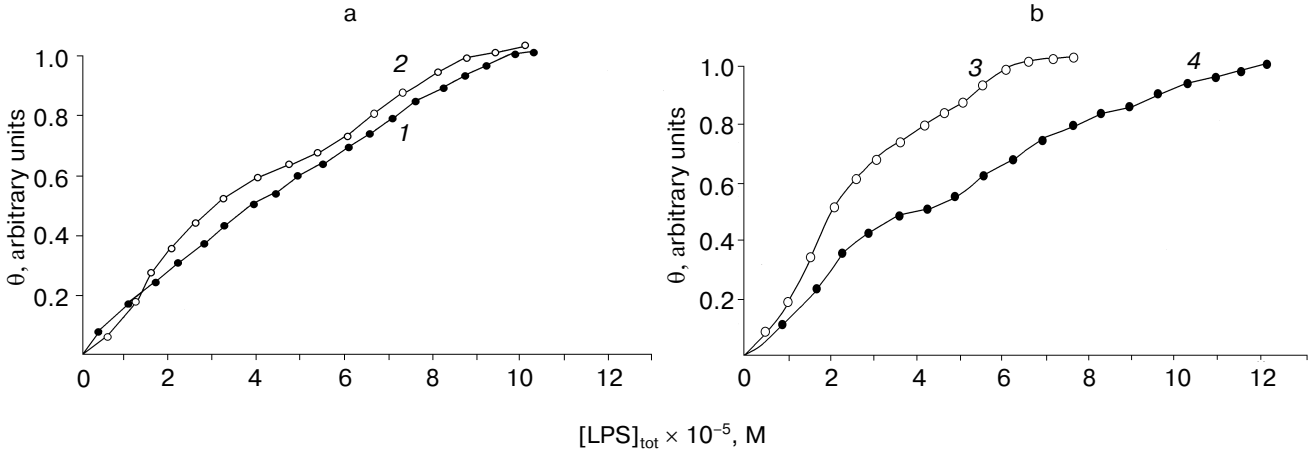

Fig. 2. Effect of LPS concentration on the saturation degree θ of binding sites on the porin molecule. *1*) LPS-1; *2*) LPS-2; *3*) LPS-3; *4*) LPS-4. The fluorescence intensity was measured at 327 nm. $[LPS]_{tot}$ is the concentration of total LPS in the reaction volume.

molecule occurs at lower ligand concentration. This possibly results from the increased hydrophilicity of the LPS-3 molecule because of its longer O-specific polysaccharide chain (Table 1) and, hence, from higher solubility of this sample [43].

LPS-4 possessing a polysaccharide chain of the same length as in LPS-3 was used as a ligand with high degree of acylation (66%). As one can see from Fig. 3 (curve *4*), increased hydrophobicity results in dramatic change in the binding character between LPS and porin. This is evident from the concave-upwards Scatchard curve with two segments corresponding to two binding sites. This kind of plot shape implies either the binding of ligand to two independent site types or the anti-cooperative interaction process between ligand and receptor, when the ligand preferably binds to the sites with higher affinity. It is known [40] that discrimination between those two models of receptor binding is a complex problem even for the simplest molecules. Nonetheless, combination of the sigmoid shape of saturation curve and the above shape of Scatchard curve obtained for LPS-4 is characteristic of an anti-cooperative process of interaction [40].

General features of the interaction between the protein and LPS were evaluated earlier [10] from experiments on the binding of radioactively labeled S- and R-LPS with porin trimer immobilized on Sepharose. The molecule of porin was shown to possess different site types specific to structural fragments of the LPS molecule. The structure of ligand determines the character of its interaction with the protein, and lipid A plays the main role in this interaction. The sites specific to lipid A and to the core oligosaccharide are two independent site types interacting with porin [10, 44], and O-specific chains, when they appear in the ligand structure, result in a cooperative binding process [10]. The best suited to the observed cooperativity is the commonly accepted explanation that relatively high local concentration of LPS accumulates on adjacent sites of the protein molecule, thus providing the complex stability due to the carbohydrate–carbohydrate interactions described in LPS aggregates [45].

Our data are generally in agreement with abovementioned peculiarities of the protein–LPS interaction. However, both the processing of the data with regard to experimental conditions and their comparison with the previously reported conclusions allow introducing some refinements into the mechanism proposed for the interaction between porin and LPS.

The approach we have chosen, namely the monitoring of variations in the intrinsic fluorescence of porin, enables detection of the overall effect observed due to the changes in the protein conformation in the course of LPS binding. Previously, we reported [12, 35] that the local tertiary structure of porin from the pseudotuberculosis microbe is sensitive to the nature of the detergent used for the protein solubilization. The spatial structure of integral proteins in solutions of nonionic detergents is known to coincide very closely to that observed in a native membrane [46]. Unlike ionic detergents, such as SDS, which being dissolved together with a protein form spherical or ellipsoidal micelles (mixed protein–detergent micelles) completely protecting the protein globule surface from water, the nonionic detergents form so-called quasi-micellar structures. In this case, the detergent molecules only enclose the hydrophobic surface of a protein, whereas its hydrophilic areas remain exposed to water. It is pre-

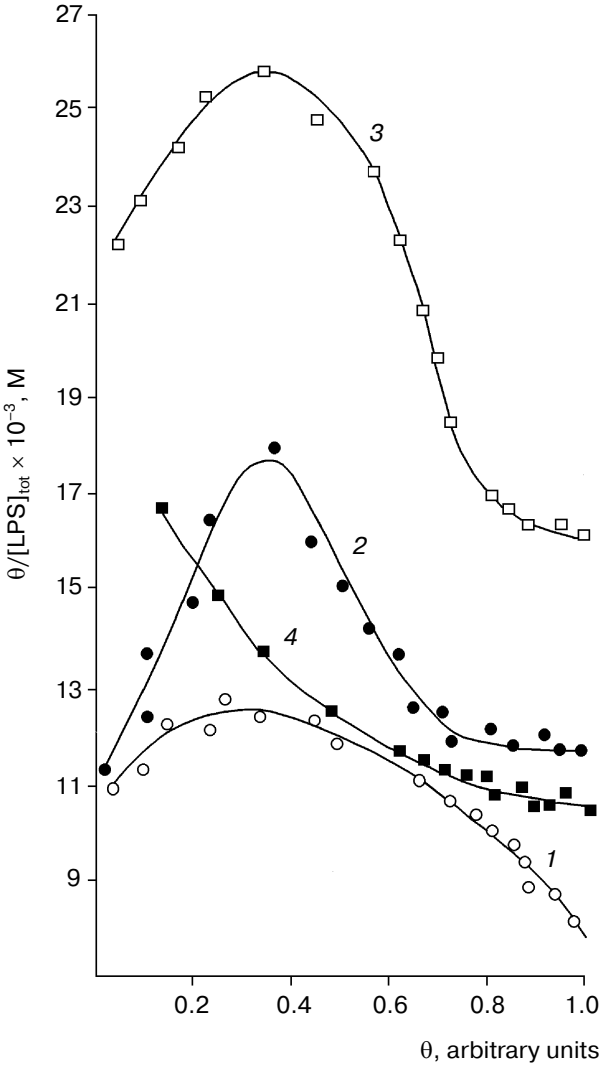

Fig. 3. Scatchard plots for the interaction of porin with R-LPS (*1*), LPS-2 (*2*), LPS-3 (*3*), and LPS-4 (*4*).

cisely this peculiarity of protein conformation status in the presence of DOC that might determine the "equiprobable" interaction of LPS with hydrophobic and hydrophilic sites. This hypothesis is also supported by similar values of K_1 and K_2 for the interaction of R-LPS with immobilized porin in SDS solution [10] or solubilized in DOC. In other words, the binding sites specific to the core and lipid A have similar affinities when porin is interacting with R-LPS. However, these sites can be either "independent" or "interacting" depending on experimental conditions (whether SDS or DOC is used) to determine the binding features. In particular, the curve descriptive of the interaction of the immobilized porin with R-LPS in the presence of SDS [10] is typical of binding with independent sites of different type. Alternatively, when the protein is solubilized in DOC and retains its "free" conformational flexibility in the binding process, we observed a weak cooperative interaction between the same binding sites, as evidenced by the Hill coefficient of 1.8.

According to the spatial model of porin structure [47], both disordered and α-helical segments of polypeptide chain lie along the area of so-called loops connecting the β-strands of the protein. So we can speculate that, unlike the lipid A-specific sites located possibly on the protein surface submerged in membrane [47], the sites responsible for the porin binding with O-specific core polysaccharide are located on the polypeptide area exposed on the membrane surface. It is likely the loop area enriched with hydrophilic amino acid residues and capable, due to its nature, to interact with ionic groups of the core oligosaccharide.

The interaction of S-LPSs (LPS-2 and LPS-3) with the protein is drawn by dome-shaped curves ending with concave-upward lines (Fig. 3). This curve type on the Scatchard plot is observed already for LPS-2, whose molecule differs from the R-LPS molecule by a single unit of O-specific polysaccharide chain. The same curve type is characteristic of the protein interaction with LPS-3, whose molecule contains four polysaccharide repeating units. Approximation of the curves descriptive of porin binding to S-LPS with different interaction models has shown the model involving three binding site types to be best fitted. This probably suggests three areas on the protein molecule, which differ in their specificity and correspond to three fragments of the S-LPS molecule. Since data were reported that each fragment of the S-LPS mol-

Table 2. Parameters of complex formation between porin and LPS

LPS	Association constant, M^{-1}			Hill coefficient
	K_1	K_2	K_3	
LPS-1	$1.0 \cdot 10^4$	$1.8 \cdot 10^4$	—	1.8
LPS-2	$3.3 \cdot 10^5$	$1.1 \cdot 10^6$	$2.0 \cdot 10^4$	2.8
LPS-3	$1.3 \cdot 10^5$	$4.4 \cdot 10^5$	$2.7 \cdot 10^4$	2.6
LPS-4	$4.2 \cdot 10^4$	$9.0 \cdot 10^3$	—	0.75

ecule inhibits the interaction with porin to nearly equal extent [10], one can suppose that O-polysaccharide chains are directly involved in the interaction with porin. Nevertheless, one should guard against making any definite inference about the number of binding sites or their interaction in the case of auto-associating ligands, such as LPS [48].

Judging from the shape of Scatchard curves and constant values, the binding character changes during the complex formation between porin and either LPS-2 or LPS-3: it is highly cooperative at low and moderate ligand concentration (Table 2) and becomes independent at LPS concentrations that are close to saturating the binding sites on the protein molecule. It is worth noting that even the small difference between LPS-2 and LPS-3 in the length of their O-specific polysaccharide leads to significant difference in the K_2 values. We consider this effect of O-specific chains, which at first glance seems to be undirected, to be of apparent importance. It is likely evident that the entirety of LPS structure is the principal condition for the high-affinity, that is, specificity of the interaction between LPS and porin. To explain this effect one should consider that a biological system (in this case it is LPPC, a fragment of outer bacterial membrane), belongs to the systems with so-called "intellectual regularity" [49]. This means that while such system type is forming, the "quality" of the information transferred to its individual components, in other words, "recognition" of the structure, becomes to be the determining factor along with fulfillment of necessary thermodynamic requirements.

Similar examples have been reported in the literature. In particular, it was found from studies on the molecular mechanisms of bacterial endotoxin activity [50] that the characteristic properties of lipid A structure determine the interaction of LPS with receptor proteins. Different values of thermodynamic parameters of binding obtained for a number of model amino acid sequences proposed as the interaction sites suggest the specific recognition of the lipid A molecular structure by the protein receptors.

As for the influence of O-specific polysaccharide chain on the interaction process between porin and LPS, the higher affinity to the protein was shown for S-LPS as compared to R-LPS [6, 51]. Moreover, the preference in *E. coli* porin interaction with S-LPS is observed even in the presence of R-LPS [5]. The K_1 and K_2 values we have determined in the present study for LPS-2 and LPS-3 are higher than that for LPS-1, which is consistent with the literature.

As reported previously [49, 52], lipid A plays the dominant role in the interaction of LPS with multiple LPS-binding proteins. The data on maximum inhibiting activity of lipid A in studies of LPS–porin interaction have led to a similar conclusion [9]. This fact is indirect evidence that it is these lipid A-specific sites whose interaction exerts the determining effect on the resultant character of LPS–porin binding. In our experiment, we found a drastic change in the character and decrease in affinity of binding with highly acylated S-LPS. Taking into consideration the fact that LPS-2 and LPS-3 with low acylation degrees display significantly higher affinity at interaction with porin than LPS-4 (66%, Table 2) we supposed that the anomalously high hydrophobicity of lipid A in LPS-4 may be a cause of this phenomenon. The acyloxyalkanoic acid residues bound to lipid A obviously make a significant steric obstruction to the extent that they change the character of porin–S-LPS binding. Unlike the S-LPS with low degree of acylation (LPS-2 and LPS-3), this character becomes anti-cooperative. Recent reports on the structure of lipid A as the governing factor in endotoxic activity suggest this explanation [50, 53, 54]. It is the acyl moiety of lipid A (i.e., the number and length of β-hydroxyalkanoic acid residues) that plays an important role in regulation of the hydrophilic molecule skeleton conformation [50]. A direct correlation was observed between the number and distribution of acyl residues in glucosamine disaccharide and the conformation of the lipid A molecule [53]. The conic shape of the lipid A molecule with cross-section of the hydrophobic part exceeding the section of its hydrophilic frame is characteristic of hexa- and pentaacyl derivatives of glucosamine disaccharide carrying two phosphate residues [53]. The hydrophobic interaction between acyl chains can keep the disaccharide frame in this special conformation recognized by receptors. Some alterations in acylation degree and acyl residue distribution lead to the change of lipid A conformation determining, in turn, the character of interaction between the LPS molecule and membrane of host cell [53].

Thus, our data on the character of interaction between LPS and porin, the two major components of outer bacterial membrane, are evidence that the molecular/spatial structures of both components determine the process of their binding. The changes in the LPS conformation induced by increased hydrophobic part of the molecule are the most significant. The conformational state of porin is determined by the protein solubilization conditions in the given experiment.

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