Biophysical Chemistry 136 (2008) 1–6

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Comparative study of electrokinetic potentials and binding affinity of lipopolysaccharides–chitosan complexes

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ARTICLE INFO

Article history:
Received 11 March 2008
Accepted 22 March 2008
Available online 8 April 2008

Keywords:
Lipopolysaccharide
Chitosan
Binding constant
ζ-potential

ABSTRACT

Electrokinetic properties of complexes of chitosan (Ch) with lipopolysaccharides (LPSs) from *Escherichia coli* O55:B5, *Yersinia pseudotuberculosis* 1B 598, and *Proteus vulgaris* O25 (48/57) and their size distribution were investigated using ζ-potential distribution assay and quasi-elastic light scattering. The interaction of LPS from different microorganisms with chitosan at the same w/w ratio of components (1:1) resulted in the formation of complexes in which the negative charge of LPS was neutralized (LPS from *E. coli*) or overcompensated (*Y. pseudotuberculosis* and *P. vulgaris*). The changing in size of the endotoxin aggregates during binding with chitosan was observed. The binding constants of chitosan with LPSs were determined by a method with using the anionic dye Orange II. The LPS from *E. coli* possess higher affinity to chitosan in comparison with the two others samples of endotoxin.

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1. Introduction

Bacterial endotoxins (lipopolysaccharides, LPSs) are the major biologically active amphiphilic components of the outer membrane of Gram-negative bacteria. LPSs from different microorganisms have a similar pattern of components. They are made up of a sugar portion: a core moiety and O-specific carbohydrate chain. The latter one has various lengths depending on the kind of bacterial strains and the conditions of cultivation. The polysaccharide part of LPS is covalently linked to the hydrophobic anchor, lipid A. Lipid A is a β-D-linked-glucosamine disaccharide phosphorylates and acylated by ester and amide-bound fatty acids [1].

The molecules of LPS exhibit a variety of biological activities in mammals that may be beneficial at low concentrations of LPS, but pathophysiological at higher endotoxin levels due to an overproduction of cytokine in immune cells, such as interleukins and tumor necrosis factor alpha (TNF-α). Endotoxins are one of the major agents that may cause septic shock, for which no satisfactory therapy exists. [2] Current antisepsis strategies use anti-LPS [3] or anti-TNF-α antibodies to bind and neutralize LPS or to capture the released TNF-α, respectively; however, these strategies have limited success [4]. An alternative strategy relies on the fact that naturally occurring positively charged proteins that interact with LPS, such as lactoferrin [5,6], CAP18 [7], NK-lysin [8], or shortened peptides derived from those molecules [9], are capable of inhibiting the LPS induced production of inflammatory cytokines by macrophages. A few years ago, David [10] reviewed several structural classes of cationic amphiphiles, including peptides and non-peptide small molecules (amphiphiles), in a broader context with the focus on developing novel antiendotoxin strategies. It is worth noting that lipopolyamines were shown to possess high affinity to bacterial LPSs and to neutralize their toxcity in *in vitro* as well in animal experimental models [11].

The molecular mechanism of the interaction of LPS with polycations in general is poorly understood. Interactions between negatively charged groups of the endotoxin and positive groups on the polymers that bind to it are generally thought to underlie this mechanism [12]. However, there are also data indicating that the predominant forces in the formation of stable endotoxin–polycations complexes are hydrophobic interactions [13]. Despite great interest in this problem, there are only limited data on the stoichiometry of the complexes and on the effect of the endotoxin structure on the complexation.

We showed earlier that LPSs from *Y. pseudotuberculosis* and *E. coli* interact with the natural polycation chitosan and produce stable complexes [14,15]. The interaction of chitosan with LPS was shown to modulate significantly the biological activity of LPS [15]. The binding of a cationic polymer, such as chitosan, with LPS undoubtedly has an electrostatic component involving ionic interactions between negatively charged groups of LPS and positive groups on chitosan. The phosphate and carboxyl substituents of LPS, which are located on the lipid A-core fragment, are important contributors to the total negative charge of LPS and appear to be major LPS binding sites for chitosan.

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0301-4622/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.bpc.2008.03.006

But as is known, LPS, and lipid A in particular, can contain various amount of negative groups and therefore can have different mechanisms of interaction with polycations.

To clarify this aspect, a comparative study of electrokinetic aspects of the interaction of LPS from *Yersinia pseudotubersulois*, *Escherichia coli*, and *Proteus vulgaris* with chitosan was carried out.

2. Experimental

2.1. Bacterial strain, growth, and isolation of the lipopolysaccharide

P. vulgaris O25 (48/57) were from the Czech National Collection of Type Cultures (Institute of Epidemiology and Microbiology, Prague). The bacteria were cultivated under aerobic conditions in nutrient broth (BTL, Poland) under controlled conditions (37 °C, pH 7.4–7.6). Cells were harvested at the end of the logarithmic growth phase, centrifuged (5000 ×*g*, 30 min), washed with distilled water, and lyophilized. The LPS was isolated by the phenol-water procedure [16] and purified by treatment with DNase and RNase (Boehringer Mannheim, Germany). The LPS preparation thus obtained was practically free of nucleic acid and contained less than 2.5% proteins.

Cells of *Y. pseudotuberculosis* (serovar 1B, strain 598) isolated from a patient suffering from the far-eastern scarlatina-like fever (Institute of Epidemiology and Microbiology, Vladivostok, Russia) were grown at 4 °C in a previously described nutrient medium [17]. LPS from bacterial cells was isolated using a phenol-chloroform-petroleum ether procedure [18]. Nucleic acids were removed by ultracentrifugation at 105,000 ×*g*. The purified LPS (yield: 1.2%) contained less than 1% protein.

A preparation of LPS from *E. coli* 055:B5 was purchased from Sigma (Sigma Chemicals, St. Louis, MO, USA).

The monosaccharide composition and polymerization degree of the O-specific chain of LPS from *Y. pseudotuberculosis* and *E. coli* were established by GLC of monosaccharides prepared as polyol acetates [19], with xylose as an internal standard. According to the degree of polymerization of the O-specific chain of the LPS, the molecular mass of the LPS molecule was calculated.

The molecular mass of LPS from *P. vulgaris* O25 (48/57) was calculated according to the molecular mass of the polysaccharide part which was determined from the reaction of its reducing monosaccharide [20].

A chitosan (Ch) sample of molecular mass 130 kDa and a 4% degree of *N*-acetylation was obtained by alkaline treatment of crab chitin according to a published protocol [21]. The degree of *N*-acetylation of the chitosan sample was calculated according to IR-spectroscopy data [22]. The molecular weight of the chitosan was determined by the Archibald method at 12,000 rpm [23].

2.2. Preparation of LPS–Ch complexes

LPS (1 mg) and Ch (1 mg) were dissolved separately in 0.5 ml of 0.1 M sodium phosphate buffer (pH 5.0). The solutions were stored for 48 h at 37 °C, then mixed and incubated for 18 h at 37 °C for LPS from *Y. pseudotuberculosis* and *P. vulgaris* and at 25 °C for LPS from *E. coli*. The LPS and chitosan solutions were decontaminated of bacteria by filtration using "Millex GS" (Millipore, Ireland).

2.3. Titration of the Orange II–chitosan complex with LPS solution

To 80 μl of 0.005 M phosphate buffer (pH 5.0), 40 μl of Orange II (4-(2-hydroxy-1-naphthylazo) benzenesulfonic acid sodium salt) solution (0.08 mg/ml) and 20 μl of chitosan solution (100 μg/ml) were added. The mixture was incubated for 20 min at 25 °C. Then the resulting complex was titrated with LPS solution (200 μg/ml), preincubated at 37 °C for 48 h, by adding different portions of LPS. The mixtures were incubated at 37 °C until a constant mean optical density of the solutions during 2 h (LPS *Y. pseudotuberculosis*) or 6 h (LPS *P. vulgaris*), or at 25 °C for 2 h (LPS *E. coli*). The absorption was determined with a μQuant Bio-TEK Instruments spectrophotometer (USA) at 483 nm in three parallel samples, and the mean arithmetic value was calculated. The value of $\Delta D = D_{exp} - D_0$ was calculated, where D_0 and D_{exp} were absorptions of the solutions before and after the addition of LPS, respectively. The values of ΔD_{max} and K_b were determined from the Scatchard plot in $\Delta D/C_{LPS}$ versus ΔD coordinates. The chitosan saturation (Q) with LPS molecules was determined from the ratio $\Delta D/\Delta D_{max}$. The degree of cooperatively (h) was determined from the Hill equation in logarithmic form [24]:

$$\log\ (Q/1-Q) = h\ \log\ [C_{LPS}] - \log\ [K_b].$$

The number of binding sites on the endotoxin molecule per glucosamine unit of chitosan was assessed from the saturation curve by plotting a tangent to the point that corresponded to the maximal change in the reaction mixture's absorption. Then the LPS concentration corresponding to the chitosan saturation with the endotoxin was determined.

Table 1
The characteristics of lipopolysaccharides

LPS	n[a]	Average molecular mass, kDa	Structure of O-specific chain	References
Y. pseudotuberculosis 1B	2.4	6.2	α-Par$_f$-1 ↓ 3 →3)-α-D-Gal$_p$-(1→3)-α-D-Man$_p$-(1→4)-α-D-Man$_p$-(1→3)-α-L-Fuc$_p$-(1→	[26]
E. coli 055	25	24	→3)-α-D-Gal$_p$-(1→3)-β-D-Gal$_p$NAc-(1→6)-α-D-Glc$_p$NAc-(1→ 3 ↑ 1-Gal$_p$-D-β-(2←1)-Col$_p$-α	[27]
P. vulgaris 025 (48/57)	2	5.0	α-D-Glc$_p$-3-(R-Lac) 1 ↓ →2)-α-L-Rha$_p$-(1→2)-β-D-Rib$_f$-(1→4)-β-D-Gal$_p$NAc-(1→3)-β-D-Glc$_p$NAc-(1→	[20]

[a] Average polymerization degree of O-specific chain.

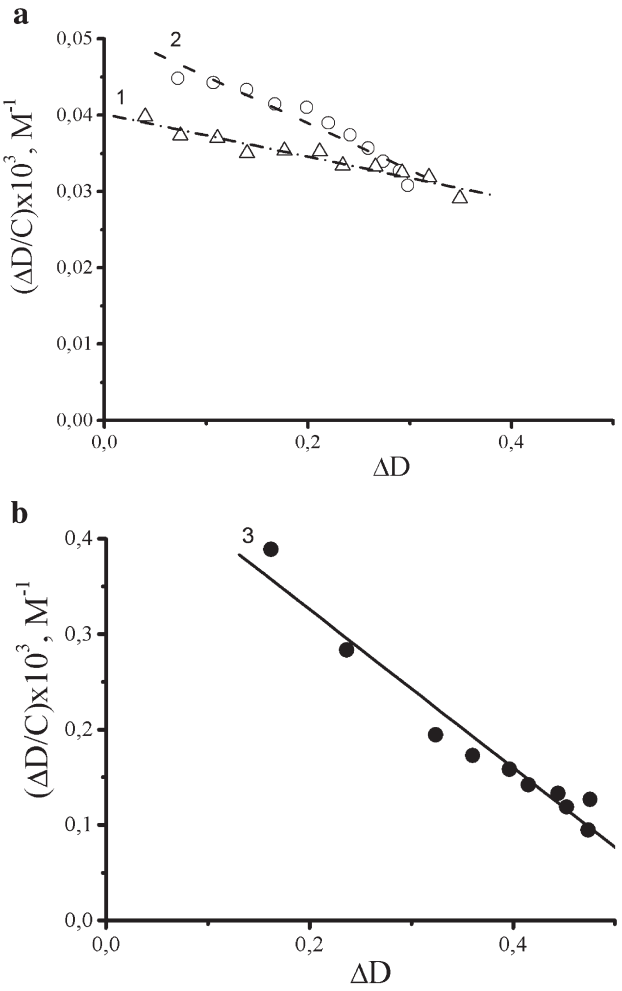


Fig. 1. Scatchard plots for the binding of LPS with chitosan a) *P. vulgaris* LPS (1), *Y. pseudotuberculosis* LPS (2); b) *E. coli* LPS (3). The chitosan–Orange II complex (100 μl) was titrated with LPS solution (200 μg/ml) by successive addition of 5–10 μl portions. The absorption of the solution was determined at 483 nm in three parallel samples, and the arithmetic mean was calculated. *C* is the concentration of the added LPS, Δ*D* is the difference between the optical absorption of the chitosan–Orange II solution before and after the addition of LPS.

2.4. Size and electrophoretic properties of the LPS–chitosan complexes

The sizes and ζ-potentials of the LPS aggregates and LPS–chitosan complexes in solution were determined by means of a ZetaSizer Nano ZS (Malvern, UK) operating at a wavelength 633 nm. Prior to measurement the samples were left for 1 h to allow the large aggregates to settle, as they can interfere with the measurements even if their content does not exceed a few percent. The measurements were performed at 25 °C for LPS from *E. coli* and at 37 °C for LPS from *Y. pseudotuberculosis* and *P. vulgaris*. The hydrodynamic diameters of the particles were automatically calculated with the instrument's software based on analysis of the autocorrelation function. ζ-potentials were calculated from experimentally determined electrophoretic mobility using the Henry equation [25].

3. Results

3.1. The characteristics of lipopolysaccharides and chitosan

LPSs were isolated from the *Y. pseudotuberculosis* 1B 598 and from *P. vulgaris* O25 (48/57) strains; a commercial sample of LPS from *E. coli* O 55:B5 (Sigma) was also used. The average degree of polymerization of the repeating units of the O-specific polysaccharides and the molecular masses of the corresponding LPSs as well as the structures of the corresponding O-specific polysaccharides are presented in Table 1.

The degree of *N*-acetylation of the glucosamine residues of chitosan was 4%. The average molecular weight of chitosan was estimated as 130 kDa. The chitosan sample was shown by analytical centrifugation to have a rather narrow distribution of molecular weigh [28].

LPS and chitosan interact with the involvement of charged groups (amino groups of chitosan on the one hand and carboxyl and phosphate groups of LPS on the other). As a result, the pH value of the medium, which determines the ionization degree of these groups, could significantly influence complex formation. Indeed we found that the LPS–chitosan complex was produced at the pH range from 4.0 to 7.0. Therefore, to study the formation of the complexes, a pH of 5.0 was chosen.

3.2. The determination of LPS–chitosan binding constants

The binding constants of the lipopolysaccharides with the chitosan were determined using the displacement of the anionic dye Orange II (4-(2-hydroxy-1-naphthylazo) benzenesulfonic acid sodium salt) by lipopolysaccharide in its complex with chitosan. The dye has been previously shown [29] to bind to every ionized amino group in the polycation molecule, resulting in decreased the dye absorption (to 70%) at 483 nm. On addition of LPS solution to the tropaeolin–chitosan complex, the absorption of the reaction mixture at 483 nm increased to the value corresponding to that of the free dye. This indicated that the dye was displaced by lipopolysaccharide from its complex with chitosan and the endotoxin–polycation complex was formed [30]. No effect of LPS on the absorption of Orange II was recorded.

It should be noted that LPS changes the optical absorption of the Orange II–polycation solution only after preincubation of the parent LPS at 37 °C, whereas the endotoxin failed to displace the dye from its complex with chitosan at 25 °C.

To reach the equilibrium state of the reaction mixture, a certain time was required. To achieve this, the components of the complex were mixed and maintained 37 °C for 2 h and 6 h for *Y. pseudotuberculosis* 1B LPS and *P. vulgaris* O25 (48/57) LPS, respectively, for to obtain a constant value of the optical absorption of the solution at 483 nm. In the case of *E. coli* O55 LPS, the characteristic saturation curve was obtained only at 25 °C (2 h).

Data on LPS binding with chitosan, presented as linear Scatchard plots (Fig. 1), suggest that the interaction occurs at independent sites of the same type and that cooperativity during the interaction is absent [31]. The values of the cooperativity coefficients determined from the Hill plot [24] are close to unity, confirming our suggestion (Table 2).

The binding constants for the chitosan–LPS complexes were determined from the Scatchard and Hill plots assuming the independence of the binding sites on chitosan. Their values are presented in Table 2. The LPS concentration corresponding to the point of chitosan saturation by endotoxin was determined from the saturation curves and the number of binding sites was calculated (Table 2).

Table 2
Parameters of the LPS binding with chitosan

LPS	$K_b \times 10^5$, M (Scatchard)	$K_b \times 10^5$, M (Hill)	$K_b \times 10^5$, M (mean value)	*h**	Number of LPS moles per glucosamine unit of chitosan
P. vulgaris O25	0.264	0.280	0.272	0.986	0.58
Y. pseudotuberculosis 1B	0.617	0.594	0.606	0.997	0.13
E. coli O55	7.930	7.970	7.950	1.034	0.06

*h** — Hill's coefficient calculated according to materials and methods.

Table 3
ζ-potential and particle size distribution for LPS and LPS–chitosan

	Zeta potential	Size, nm
LPS *P. vulgaris*	−32.7±2.4	350.3±31.9
	−8.0±2.2	96.7±9.8
Complex	27.9±2.5	300.5±37.5
	2.4±2.7	56.05±4.31
		>1 μm
LPS *Y. pseudotuberculosis*	−31.4 мВ	32.2±2.5
	−6.04 мВ	147.0±22.6
Complex	21.2±0.6	137±11.9
	1.6±2.4	38.9±4.7
		453±5.8
LPS *E. coli*	−14.5±2.0	107.8±7.3
Complex	0.83±0.09	887.0±75.3
		229.6±20.6

3.3. Surface charge compensation of LPS aggregates by chitosan

The results of measurements of the ζ-potentials and particle size distributions of LPS and LPS–chitosan are presented in Table 3. The measurements of the ζ-potentials of the LPS solutions showed that under the experimental conditions (0.05 M Na-phosphate, pH=5.0) LPS particles were negatively charged. In accordance with the size distributions, two populations of particles varying in ζ-potential were detected for LPS from *Y. pseudotuberculosis* 1B and *P. vulgaris* O25, while a monomodal ζ-potential distribution was observed for *E. coli* O55 LPS. Electrostatically driven interactions of LPSs from different sources with chitosan at the same w/w ratio of components (1:1) resulted in the formation of complexes in which the negative charge of LPS was neutralized (*E. coli* LPS) or overcompensated (*Y. pseudotuberculosis* and *P. vulgaris* LPS) (Table 3).

The particle size distribution in LPS solutions, characterized by dynamic light scattering (DLS), revealed a monomodal distribution only for *E. coli* O55LPS, while the presence of two particle fractions was clearly detected in solutions of LPS isolated from *P. vulgaris* O25 and *Y. pseudotuberculosis* 1B (Fig. 2a, Table 3).

The DLS data indicate the formation of LPS–chitosan complexes with sizes different from those of free LPS for all the studied systems. It should also be noted that the complex of LPS from *P. vulgaris* O25 with chitosan has a very broad particle size distribution with high polydispersity.

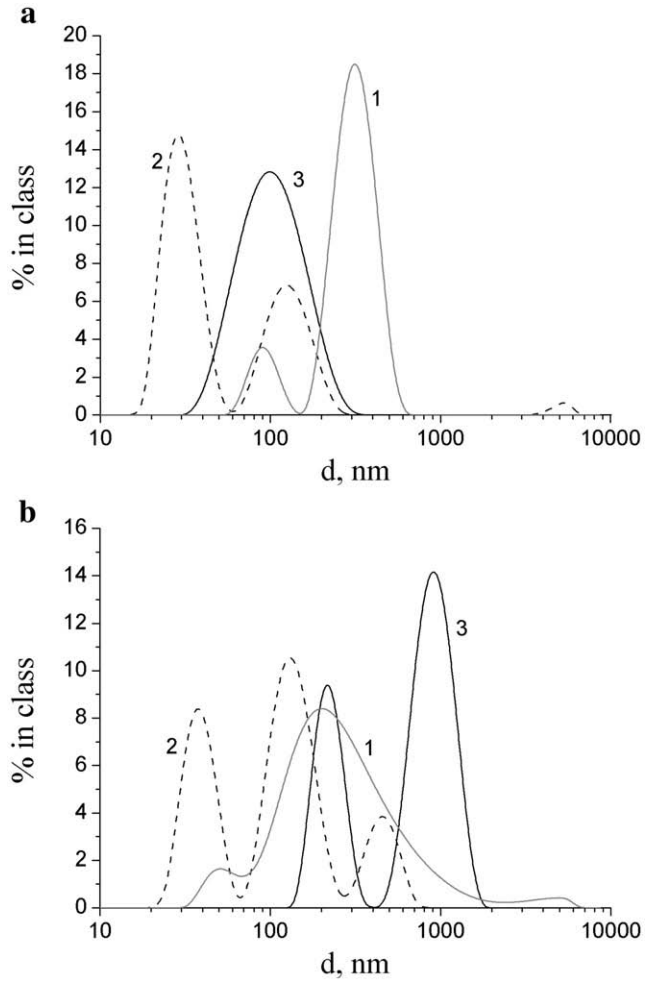
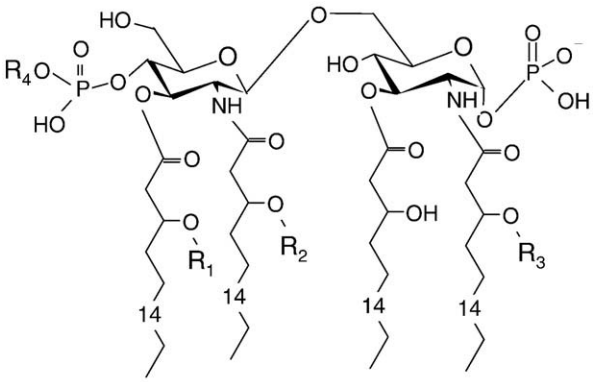


Fig. 2. Particle size distributions (by intensity) for LPS (a) and LPS–chitosan complexes (b): 1— *P. vulgaris* O25 LPS (gray solid lines), 2 — *Y. pseudotuberculosis* LPS (dashed lines), 3 — *E. coli* LPS (solid lines).

4. Discussion

Now it is conventional fact that cation binding to LPS can be interpreted, at least initially, to be electrostatic and to result from the attraction between the positive charges (lysine and arginine side-chains) of the peptides and the negative charges (phosphates and carboxylates) of LPS [32]. Similar investigations have been carried out mainly on LPS isolated from *Salmonella minnesota* and *E. coli*. However, LPSs isolated from various microorganisms have different amounts of negatively charged groups, which can be an important factor in their binding with polycations. Furthermore, LPSs are amphiphylic compounds and can form aggregates with various parking types depending on their primary chemical structure and ambient conditions. These properties in their turn can affect the varied availability of the negatively charged groups to bind with chitosan.

In order to study the influence of these aspects on the process of endotoxin interactions with chitosan, LPSs from three different microorganisms, *Y. pseudotuberculosis*, *E. coli*, and *P. vulgaris*, were chosen. These LPSs possess different chemical structures: the number of negatively charged groups in the lipid A-core moiety, the degree of acylation of lipid A (Fig. 3), and the various lengths of the O-specific chains (Table 1), that may influence the sizes [33] and packing densities of the acyl chains in aggregates of LPS [34]. In addition, lipid A of *P. vulgaris* LPS has an aminoarabinose group bound to phosphate on non-reducing end of molecule and only single free phosphate groups.



Lipid A	R_1	R_2	R_3	R_4
P. vulgaris	14:0	14:0	16:0	AraN
Y. pseudotuberculosis	12:0	H	H	H
E. coli	14:0	12:0	H	H

Fig. 3. Schematic structure of lipid A of LPS [35,36,2].

DSL data and ξ-potential measurements were used to study the influence of these features of LPS structure to forming of LPS–chitosan complexes. Several populations of particles were registered after complex formation, and notable differences in the size distributions of the LPS and LPS–chitosan complexes were observed. According to the DLS data (Table 3), the complex of *P. vulgaris* LPS with chitosan has a multimodal particle size distribution with very high polydispersity (Fig. 2b). It is interesting to note that DLS and also sucrose gradient sedimentation (unpublished data's) indicated the formation of complexes with high polydispersity in size for LPS *P. vulgaris*. Similar polydispersity in size was observed for complexes of LPS *Y. pseutuberculosis* with chitosan by DSL method (Fig. 2b). Earlier formation of a number of LPS–chitosan complexes of different molecular weight was registered for *Y. pseutuberculosis* LPS having short O-specific chains by sucrose gradient sedimentation method [14]. At first sight it is possible to expect that the formation of a number of complexes LPS–chitosan in these cases is caused by the heterogeneity of initial LPS. However, the experiments carried out don't confirm this, because after fractionating of LPS and the subsequent binding with the chitosan a number of complexes of different composition were also obtained [14].

The measurements of ζ-potential in LPS solutions showed that under the experimental conditions (pH=5.0), LPS particles were negatively charged. Showing the same trend as the size distributions, a monomodal ζ-potential distribution was observed only for the *E. coli* LPS, while two populations of particles varying in ζ-potential were detected for the LPS from *Y. pseudotuberculosis* and *P. vulgaris*. Electrostatically driven interactions of LPS from different microorganisms with chitosan at the same w/w ratio of components (1:1) resulted in the formation of complexes in which the negative charges of LPS were neutralized (*E. coli* LPS) or overcompensated (*Y. pseudotuberculosis* and *P. vulgaris*) (Table 3). Taking into account that the chitosan content in all the complexes was much higher than required for charge neutralization of LPS, the difference observed in the electrokinetic properties of the complexes suggests that the availability of negatively charged groups on LPS molecules is completely different and depends on the aggregate structure of the endotoxins. Hexoacylated *E. coli* LPS with a long O-specific chain formed monodispersed aggregates with the lowest ζ-potential among the studied LPSs. Thus at the fixed mass ratio (1:1) one can expect the highest overcharging for the chitosan–*E. coli* LPS complex. However, in this case the complex with the lowest surface charge was formed. This can be connected with a macromolecular organization of LPS aggregates. It is known that the structure of LPS is highly ordered, and the degree of order increases with lengthening O-specific chain [37]. It was also established that the package of LPS aggregates could be stabilized by carbohydrate–carbohydrate interactions between long O-polysaccharide chains [38]. It has to be taken into account that sugar part of LPS is directed towards the outside and represent a barrier for the chitosan due to steric reasons. In this context O-chains of *E. coli* LPS screens anion sites of endotoxin and its accessibility for binding may be difficult. On the other hand chitosan as enough hydrophilic polymer may penetrate to anionic sites of most hydrophilic LPS but its excessive positive charge can be screened by neutral O-specific chains.

This agrees with the data of the dye displacement assay (Table 2): 1 the chitosan glucosamine unit is bound with a small portion of mole of *E. coli* LPS, confirming existence steric hindrances to interaction with the polycation. (Table 2).

It should be noted that the narrow size distribution (Fig. 2b) as well as the low surface charge of this complex exclude bridging the LPS aggregates with chitosan as a possible mechanism of complexation. The presence of the second population of particles with the same ζ-potential but larger dimensions most likely reflects aggregation of weakly charged particles of the complex rather than the formation of structures of another type or the presence of the initial components of the complex.

In contrast to *E. coli* LPS with a long O-specific chain, the LPSs from *Y. pseudotuberculosis* and *P. vulgaris* have short O-chains and penta- and hepta-acylated lipid A, respectively (Fig. 3). According to the chemical structures of the LPS it is possible to expect that it will form densely packed aggregates of larger size [34,33]. In fact, according to DLS data (Table 3), LPS from *P. vulgaris* forms aggregates with the maximal size. Due to the short length of the O-chains, the negative charges of LPS are not screened and the aggregates show more negative electrokinetic potential than aggregates of *E. coli* LPS.

According to our earlier data [28], chitosan is a sufficiently rigid linear polymer. Its penetration into the more densely packed aggregates of LPS was difficult. In this regard, we can probably consider the complex formation as binding on the surface of LPS aggregate. Thus, in case of the *Y. pseudotuberculosis* and *P. vulgaris*, LPSs we obtained complexes with high positive ζ-potentials. In this case the interaction of the densely packed LPS aggregates with chitosan can be very schematically presented as the interaction between a highly charged polyelectrolyte and a charged sphere, which is known to occur with surface overcharging upon polyelectrolyte adsorption [39].

It is necessary to note that the O-specific polysaccharide of *P. vulgaris* LPS has a negative charged residue of lactic acid (Table 1). Complexation may be a result of the interaction of the amino groups of chitosan with the two possible types of binding sites of *P. vulgaris* LPS: the negatively charged groups in the core-lipid A part and/or the carboxylic groups in the O-specific polysaccharide. Analysis of Scatchard's plot (Fig. 2) point to an interaction with a single type of binding site, but its affinity for binding with chitosan is notably less than those of the two other investigated LPSs (Table 2). This is perhaps connected with the different natures of the binding sites of the LPSs. In the case of *P. vulgaris* LPS, chitosan is perhaps bound with carboxylic residues in the O-specific chains of the endotoxin and the binding of *P. vulgaris* LPS with chitosan takes place on the surfaces of LPS aggregates. The highest amount of mole of *P. vulgaris* LPS bound with 1 chitosan aminogroups (Table 2) confirmed this opinion.

E. coli LPS has the highest affinity to chitosan ($K_b = 7.95 * 10^5$ M), which is ten times more than the chitosan binding affinities of *P. vulgaris* and *Y. pseudotuberculosis* LPSs. This fact is well in accord with our earlier data which showed that the affinity of LPS–chitosan binding and the number of binding sites are determined by the length of the O-specific polysaccharide of *Y. pseudotuberculosis* LPS [30]. At the same time in contrast to the *E. coli* LPS, *Y. pseudotuberculosis* LPS with the same length of O-specific chains has the $K_b = 2.8 * 10^5$ M [30]. It is a reflection of features of lipid component of these LPS namely fatty acid arrangement of lipid A or/and features of structure of core region studied LPS and would be objects of future investigations.

5. Conclusion

The results submitted in the present study showed that the process of interaction of LPS and chitosan is complicated and is supplemented by changing supramolecular organization of LPS. Interaction depends on at least on three factors: the surface charge of the LPS particles, the lengths of the O-specific polysaccharide, as well as the fatty acid content of lipid A. So for correct interpretation all aspects of endotoxin binding with polycation it is necessary to consider both lipid and polysaccharide parts of LPS molecule in complex.

Studying the influence of these factors on the supramolecular structure of LPS and its change during interaction with chitosan and also following modifications in its biological activity is important for choosing strategies of antisepsis therapy in bacterial infection.

Acknowledgements

This work was supported financially by the programs for basis research of the Russian Academy of Sciences: “Fundamental Sciences to Medicine” and “Molecular and cell biology” Scientific Scholl academician Ovodov Y.S. — 5796.2006.4.

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