AN INVESTIGATION OF A LIPOPOLYSACCHARIDE-PROTEIN COMPLEX FROM *Yersinia pseudotuberculosis* BY THE LIGHT-SCATTERING METHOD

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A characteristic component of the outer membrane of Gram-negative bacteria, the lipopolysaccharide-protein complex (LPPC), possesses a broad spectrum of action on the macroorganism. The size of this complex plays an important role in the manifestation of immunogenic properties [1]. Attempts to evaluate the molecular-weight parameters of the endotoxins of *Serratia marcescens* 2 and some species of Salmonella have been described previously [3, 4]. However, the information on the structure of the LPPC and on the nature of the bond between its components is extremely self-contradictory, and therefore it is of interest not only to determine the molecular weights of the endotoxins but also to study the processes of aggregation of the LPPC. We have determined the weight-average molecular weight of the particles of the LPPC from *Yersinia pseudotuberculosis*, strain 341, in water by the light-scattering method [5] and have also investigated the influence of urea, guanidine hydrochloride, sodium dodecyl sulfate (SDS), and an increase in the ionic strength of the solution on the process of aggregation.

We studied preparations of LPPC obtained by extraction with the butanol – water system [6] and purified by gel filtration on Sepharose 2B. The purified LPPC possessed antigenic activity and toxicity. Below we give the analytical figures for the crude (C) and purified (C1) LPPC from *Y. pseudotuberculosis*:

Fraction	Content, %			
	MS	Protein	KDO*	NA
C	31	36	3.5	37.7
C1	35.9	27	13.1	1.1

The results of gel filtration through Sepharose 2B show that the molecular weights of the particles of the LPPC in water are greater than $1.5 \cdot 10^6$. In determining the weight-average molecular weight ($\overline{M}_W$) by light scattering and by the characteristic viscosity ($[\eta]$), values for $\overline{M}_W$ of the order of $5 \cdot 10^8$ and $[\eta]$ of ~366 ml/g were obtained.

The prolonged keeping of the LPPC in solutions of sodium chloride (2 M) and urea (8 M) leads to a decrease in $\overline{M}_W$ to values of $1\text{-}3 \cdot 10^7$ and in $[\eta]$ to 160 and 121 ml/g, respectively. In solutions of guanidine hydrochloride (5 M) and SDS (1%) still more considerable breakdown of the LPPC particles takes place to particles with $\overline{M}_W$ of the order of $6\text{-}7 \cdot 10^6$ and particles with $\overline{M}_W$ of the order of $3\text{-}5 \cdot 10^6$ and $[\eta]$ ~23 ml/g, respectively.

It is obvious that in water the LPPC exists in the form of aggregates produced by various types of bonds between the molecules of the complex. The tendency to aggregation of the lipopolysaccharide has been shown previously [7].

The influence of the ionic strength of the solution on the size of the LPPC particles is shown in the replacement of aqueous solutions by 2.5% NaCl solutions at all stages of the isolation and purification of the LPPC. Such a modification led to the production of an LPPC fraction with a lower molecular weight (Fig. 1,b). When this compound was dialyzed against distilled water, larger particles with M_W of the order of 10^8 were formed (Fig. 1a).

Apparently, the formation of highly aggregated particles of LPPC takes place in the isolation process, when certain conditions of its existence in the outer membrane of bacteria are disturbed.

*Unidentifiable Russian acronym – Publisher.

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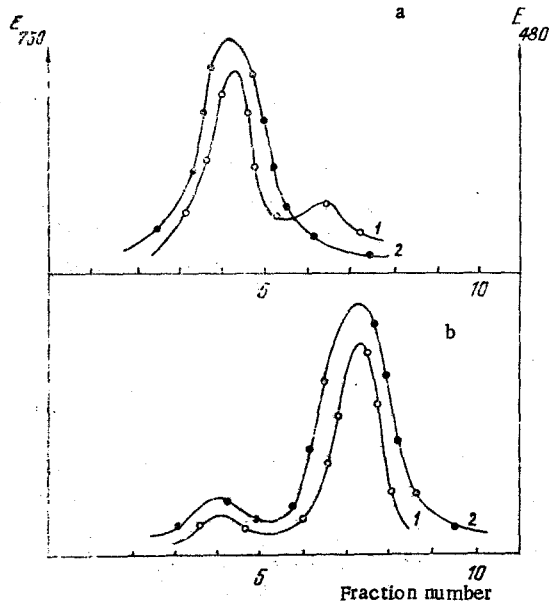

Fig. 1. Gel filtration through Sepharose 2B of fractions of LPPC from Y. pseudotuberculosis. Isolation of the LPPC and elution on gel filtration were performed with Tris-HCl buffer, 0.03 M, pH 7.8 (a) and Tris-HCl buffer, 0.03 M, pH 7.8, in 2.5% NaCl (b): 1) monosaccharides by the phenol–sulfuric acid method; 2) protein at E_{750}.

EXPERIMENTAL

The scattering of light was measured in a FPS-3 photoelectric light-scattering instrument. Highly dilute solutions of LPPC with concentrations of $0.01\text{-}0.8 \cdot 10^{-3}$ g/ml were used for analysis. The solutions were freed from dust by passage through membrane filters with dimensions of 0.3-1.5 μ in a filter holder under pressure. The light-scattering measurements were interpreted by the double extrapolation method.

The viscosities were measured in an Ubbelohde viscometer with a capillary having a diameter of 0.54 mm at 25 ± 0.1°C.

Gel filtration was performed on a column (2.5 × 40 cm) of Sepharose 2B, equilibrated with: 1) 0.03 M Tris-HCl buffer, pH 7.8; and 2) 0.3 M Tris-HCl buffer, pH 7.8 in physiological salt solution.

Solutions of LPPC in the presence of disaggregating agents were kept in the cold for a week or at 37°C for four days after preliminary boiling for five minutes [6].

SUMMARY

An estimate has been made of the molecular weights and characteristic viscosities of aggregates of the LPPC from Yersinia pseudotuberculosis in water, and also in aqueous solutions of sodium chloride, urea, guanidine hydrochloride, and sodium dodecyl sulfate. It has been shown that a high ionic strength of the solution and the presence of disaggregating agents lead to a considerable decrease in the molecular weight of the LPPC.

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