Biochimica et Biophysica Acta, 558 (1979) 320—324
© Elsevier/North-Holland Biomedical Press

BBA Report

BBA 71411

CHANGES IN SURFACE CHARGE DENSITY ON LIPOSOMES INDUCED BY *ESCHERICHIA COLI* ENDOTOXIN

TOYOSHI ONJI and MAW-SHUNG LIU

Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103 (U.S.A.)

(Received August 9th, 1979)

Key words: Endotoxin; Liposome; Phospholipid; Surface charge; Ca^{2+}; (E. coli)

Summary

Changes in surface charge density of liposomes induced by *E. coli* endotoxin were studied by microelectrophoresis. Endotoxin altered the surface charge of phosphatidylcholine liposomes from neutral to negative. The negative charge of the endotoxin-phosphatidylcholine complex was neutralized electrostatically by binding with Ca^{2+} (2 mM). Phosphatidylcholine liposomes were made positive by addition of the positively charged detergent, hexadecyltrimethylammonium chloride. Endotoxin made the positively charged liposomes less charged. On the other hand, phosphatidylserine liposomes which were negatively charged became less charged in the presence of high concentration of endotoxin (8 mg/ml). The endotoxin effect on phosphatidylserine liposomes was abolished by EDTA (1 mM) but potentiated by $CaCl_2$ (0.1—2 mM). These results indicate that endotoxin interacts with liposomes both hydrophobically and electrostatically.

The interaction between endotoxin (lipopolysaccharide) and phospholipid vesicles (liposomes) is of considerable importance because the biochemical and physiological responses in endotoxin shock have been associated with the binding of endotoxin to the cell surface [1—3]. It has been suggested that endotoxin could initiate various biological responses by acting as a messenger on a specific receptor site [4—6] or, alternatively, by incorporating into the membrane lipid bilayer [1,3]. The present report describes the endotoxin-induced alterations in surface charge density of various phospholipid liposomes.

Abbreviation: HDTMAC, hexadecyltrimethylammonium chloride.

Three mg of L-α-phosphatidylcholine (Sigma Chemical Co.) in hexane solution or L-α-phosphatidylserine (Sigma Chemical Co.) in chloroform/methanol (95 :5, v/v) solution were dried under a nitrogen gas stream. The dried phospholipids were suspended in 1 ml of a solution containing 0.25 M sucrose and 20 mM Tris-HCl (pH 7.4) and sonicated in an Ultrasonics Model 13 sonifier at 22°C. The duration of sonication was 5 s for phosphatidylcholine and 20 s for phosphatidylserine. Positively charged liposomes were prepared by sonicating phosphatidylcholine in the presence of 0.5 mM hexadecyltrimethylammonium chloride (HDTMAC) (Eastman Kodak Co.).

The electrophoretic mobility of liposomes (approx. 1 μm in diameter) was measured as described by Bangham et al. [7] on a Rank Mark I microelectrophoretic apparatus (Rank Brothers, Cambridge, U.K.) at 37°C. The electrophoretic mobility was used as a measure of the surface charge density of the liposomes [8].

Calcium content of endotoxin preparation was determined by atomic absorption spectrometer after heating 8 mg of *E. coli* endotoxin (Difco Laboratories, Detroit) in 0.5 ml of 70% perchloric acid solution at 170°C for 10 h.

Fig. 1 shows the effect of *E. coli* endotoxin on the electrophoretic mobilities of neutrally charged phosphatidylcholine and positively charged (phosphatidylcholine + HDTMAC) liposomes. Phosphatidylcholine liposomes had zero net charge (neutral) in the absence of endotoxin, while the charge became negative in the presence of various amounts of endotoxin (1—8 mg/ml). This change was most likely caused by the incorporation of endotoxin molecules into liposomes since endotoxin has been shown to possess negatively charged groups such as phosphate, pyrophosphate, and carboxylate [9]. It is conceivable that the interaction between liposomes and endotoxin is a result of the hydrophobic insertion of lipid A subunits into the hydrocarbon region of the liposomes. Shands [1] has observed a stacking arrangement and vesiculation of phosphatidylcholine liposomes following incubation

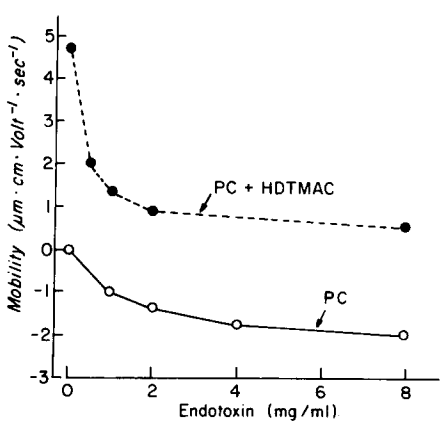

Fig. 1. Effect of various concentrations of *E. coli* endotoxin on the electrophoretic mobilities of phosphatidylcholine and phosphatidylcholine plus hexadecyltrimethylammonium chloride liposomes. Each value represents the average of two separate experiments. Twenty liposomes were observed in each experiment. ○——○, phosphatidylcholine (PC); ●- - -●, PC plus hexadecyltrimethylammonium chloride (HDTMAC).

with endotoxin, and has concluded that the observed phenomenon was a result of endotoxin incorporation into liposomes. Addition of HDTMAC changed the surface charge of phosphatidylcholine liposomes from neutral to positive (Fig. 1). The positive surface charge was reduced markedly by endotoxin at the concentrations of 0.5 to 2.0 mg/ml. The reduction in surface charge density can be explained by the electrical neutralization between the two oppositely charged groups in HDTMAC and endotoxin molecules after the adsorption of endotoxin to the liposomes. Thus, it is possible that the formation of positively charged liposome-endotoxin complexes involves not only a hydrophobic interaction, but also an electrostatic attraction.

The charge-mediated interaction between endotoxin and negatively charged liposomes is illustrated in Fig. 2. Phosphatidylserine liposomes had a negative electrophoretic mobility of 6.4 $\mu m \cdot cm \cdot V^{-1} \cdot s^{-1}$ at zero endotoxin concentration. The negative mobility was decreased slightly (by 6.2 — 12.5%) by endotoxin at the concentrations of 1—4 mg/ml. This finding suggests that because of an electrostatic repulsion endotoxin and phosphatidylserine liposomes may not interact or form endotoxin-liposome complexes at low concentrations (4 mg/ml) of endotoxin. Alternatively, the apparent lack of interaction at low endotoxin levels may relate to the very high negative charge of phosphatidylserine. The extent of negativity of phosphatidylserine liposomes, however, was markedly reduced (by 59.4%) by endotoxin at a higher concentration (8 mg/ml). The observed effect of endotoxin was abolished by 1 mM EDTA (Fig. 2). These findings suggest that there is an interaction between negatively charged liposomes and endotoxin at high concentrations and that this interaction probably requires trace amounts of multivalent cations (such as Ca^{2+}, Mg^{2+}, Fe^{2+}, or Al^{3+}) which were present in endotoxin preparations. The view that the interaction between negatively charged liposomes and endotoxin requires multivalent cations is further supported by the finding that the negative mobility of phosphatidylserine liposomes was

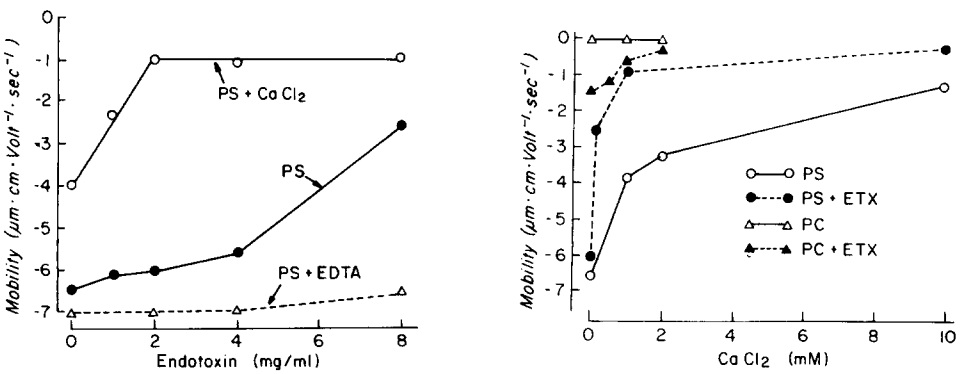

Fig. 2. Effect of *E. coli* endotoxin on the surface charge of phosphatidylserine liposomes in the absence or presence of $CaCl_2$ or EDTA. Number of experiments is the same as Fig. 1. ●——●, phosphatidylserine (PS); ○——○, phosphatidylserine in the presence of 1 mM $CaCl_2$; △- - -△, phosphatidylserine in the presence of 1 mM EDTA.

Fig. 3. Effect of $CaCl_2$ on the surface charge of phosphatidylserine and phosphatidylcholine liposomes in the absence or presence of endotoxin. The number of experiments is identical to that of Fig. 1. ○——○, phosphatidylserine (PS); ●- - -●, phosphatidylserine in the presence of endotoxin (ETX) (2 mg/ml); △——△, phosphatidylcholine (PC); ▲- - -▲, phosphatidylcholine in the presence of ETX (2 mg/ml).

reduced by 75% by endotoxin (2 mg/ml) in the presence of 1 mM $CaCl_2$ (Fig. 2). The mechanism of interaction can be explained by the formation of liposome-Ca^{2+}-endotoxin complexes through cation-mediated linkages between the head group of the liposome and the endotoxin molecule.

If the decreasing effect of endotoxin on the negativity of phosphatidylserine liposomes were a result of Ca^{2+} contamination of the endotoxin preparation, one would expect that the addition of the same amount of endotoxin to phosphatidylserine liposomes (closed circles and dotted line of Fig. 3) would shift the curve to the same extent as in the absence of endotoxin (open circles and solid line of Fig. 3) at various concentrations of $CaCl_2$. However, the results shown here indicate that this was not the case. Furthermore, the total calcium content (bound and free) in the endotoxin preparation was found to be 10 μg per mg of endotoxin. Therefore, the concentration of endogenous free calcium in the endotoxin preparation available for interaction with liposomes was even lower than 0.5 mM (Fig. 3). Hence, the observed endotoxin action on the surface charge of phosphatidylserine liposomes cannot be ascribed solely to the effect of contaminating Ca^{2+}.

The surface charge density of phosphatidylcholine liposomes was not affected by calcium chloride (open triangles and solid line of Fig. 3). The charge density of phosphatidylcholine liposomes was changed from neutral to negative (closed triangles and dotted line of Fig. 3; see also Fig. 1) following adsorption of endotoxin to the liposomes in the absence of Ca^{2+}. However, the negative charge of the phosphatidylcholine-endotoxin complexes was decreased by increasing concentraction of Ca^{2+} (closed triangles and dotted line of Fig. 3). This observation suggests that the negative charge of phosphatidylcholine-endotoxin complexes is neutralized electrostatically by binding with Ca^{2+}.

Fried and Rothfield [10] reported that the phosphatidylethanolamine and endotoxin molecules formed a complex at air-water interfaces in molecular monolayers. Although the phenomenon was observed in the absence of added cations, the authors suggested the possible involvement of Mg^{2+} in the interaction between the two molecules.

Our findings, that Ca^{2+} modified the surface charge of the phospholipid-endotoxin complex, reveal the role of this ion on the action of endotoxin in altering membrane properties. This contention is supported by the earlier findings that reassembly of the extracted endotoxin [11] and its binding to leukocytes [12] depends upon the presence of divalent cations.

The results presented here demonstrate that endotoxin changes the surface charge of phospholipid liposomes in the absence (in the case of neutrally and positively charged liposomes) or presence (in the case of negatively charged liposomes) of Ca^{2+}. Changes in the surface charge of the phospholipid bilayer may alter the properties of biological membranes.

This work was supported by United States Public Health Service Grants HL 23618 (formerly HL 21223) and AM 25270 (formerly AM 21052). We are grateful to Drs. B.M. Waite and C.C. Cunningham for their valuable advice and for making their laboratory facilities available to us.

References

1 Shands, J.W., Jr. (1973) J. Infect. Dis. 128 (Suppl.), S197—S201
2 Walker, R.I. (1976). Mil. Med. 141, 97—99
3 Davies, M., Stewart-Tull, D.E.S. and Jackson, D.M. (1978) Biochim. Biophys. Acta 508, 260—276
4 Hawiger, J., Hawiger, A., Steckley, S., Timmons, S. and Cheng, C. (1977) Br. J. Haematol. 35, 285—299
5 Hawiger, J., Hawiger, A. and Timmons, S. (1975) Nature 256, 125—127
6 Springer, G.F., Adye, J.C., Bezkorovainy, A. and Murthy, J.R. (1973) J. Infect. Dis. 128 (Suppl.), S202—S211
7 Bangham, A.D., Heard, D.H., Flemans, R. and Seaman, G.V.F. (1958) Nature 182, 642—644
8 Brinton, C.C., Jr. and Lauffer, M.A. (1959) in Electrophoresis (Bier, M., ed.), Vol. 1, pp. 427—492, Academic Press, New York
9 Nikaido, H. (1973) in Bacterial Membranes and Walls (Leive, L., ed.), pp. 131—207, Marcel Dekker, New York
10 Fried, V.A. and Rothfield, L. (1978). Biochim. Biophys. Acta 514, 69—82
11 DePamphilis, M.L. (1971) J. Bacteriol. 105, 1184—1199
12 Gimber, P.E. and Rafter, G.W. (1969) Arch. Biochem. Biophys. 135, 14—20