Detection of lipopolysaccharide (LPS) and identification of its serotype by an enzyme-linked immunosorbent assay (ELISA) using poly-L-lysine

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A new solid-phase enzyme-linked immunosorbent assay (ELISA) was developed for detection of LPS and identification of its serotype with antisera. Since LPS binds poorly to polystyrene microplates, precoating with poly-L-lysine was used before coating LPS on the surface of microplates. The small amount of LPS in complex mixtures (i.e., less than 1 μg/ml) could be detectable in ELISA. Use of poly-L-lysine with high molecular weight (MW) provided a higher sensitivity than poly-L-lysine with low MW. Precoating with polymyxin B, or poly-L-histidine was less effective in the sensitivity than precoating with poly-L-lysine, but it was still better than no precoating. The newly developed ELISA technique could be also applied for detection of anti-LPS antibodies in sera or for screening of monoclonal anti-LPS antibody.

Key words: Lipopolysaccharide; ELISA; Poly-L-lysine; Polymyxin B

Introduction

The adequate quantification of LPS and identification of its serotype has been done by passive hemagglutination and passive hemolysis. But more recently ELISA have been described for the measurement of antibodies against different parts of LPS. However, LPS and free lipid A do not coat plastic surfaces well in this form. Galanos et al. (1972) showed that core antigens of LPS, complexed to bovine serum albumin (BSA) are biologically fully active. Appelmelk et al. (1985) applied the core region of LPS-BSA complexes for solid-phase coating in ELISA since BSA binds to plastic surfaces. There are several other reports on ELISA for detection of LPS (Scott et al., 1987; Peborde et al. 1989). This paper reports a simple and easy ELISA for detection of LPS and identification of its serotype in a single experimental set up using poly-L-lysine before coating of LPS.

Materials and methods

Reagents

Poly-L-lysine with high MW ranging from 150,000 to 300,000, and with low MW ranging
from 1000 to 4000, polymyxin B, poly-L-glutamic acid (MW, 15,000–50,000), and poly-L-histidine (MW, 15,000–50,000) were purchased from Sigma Chemicals, St. Louis, MO, USA.

LPS

*Klebsiella* O3 LPS was prepared from *Klebsiella pneumoniae* LEN-1 (O3 : K1-) by the phenol water method (Westphal et al., 1965; Yokochi et al., 1989). The LPS preparations of *Escherichia coli* O55, O111, and O128, and *Salmonella enteritidis* were obtained from Difco Laboratories, Detroit, MI. The free lipid A fraction was isolated from *Klebsiella* O3 LPS by heating at 100°C for 1 h in 1% acetic acid (Kato et al., 1985).

Antibody

Rabbit antiserum to O antigen of *Klebsiella* O3 LPS was prepared according to the procedure described by Edwards and Ewing (1962). Rabbit anti-lipid A serum was also prepared according to the procedure described by Galanos et al. (1971) using the lipid A fraction extracted from *Klebsiella* O3 LPS. A panel of antisera to O antigens of *Klebsiella* LPS were supplied by Dr. N. Kato at Nagoya University.

ELISA

The technique described by Engvall and Perlmann (1972) was used as the basis for the assay. Below are the steps used in our modified ELISA.

**Precoating of poly-L-lysine.** A solution (100 μl) of poly-L-lysine in 0.01 M phosphate-buffered saline at pH 7.2 (PBS) was placed in disposable polystyrene microplates (Nunc Immunoplate, InterMed, Denmark). The solution was incubated overnight at room temperature. A solution of polymyxin B, poly-L-histidine, or poly-L-glutamic acid (10 μg/ml) was also used for precoating. The negative control well was treated with PBS alone.

**Binding of LPS.** Aliquots (100 μl) of various concentrations of LPS suspended in PBS were placed in poly-L-lysine-precoated plates, and then incubated for 1 h at 37°C. The plates were washed three times with PBS containing 0.05% Tween 20 (T-PBS).

**Antiserum.** An optimum dilution (1/250) of rabbit immune serum (100 μl) was added to each well, incubated for 1 h at 37°C, and washed three times with T-PBS.

**Conjugate.** Aliquots (100 μl) of a 1/500 dilution of peroxidase-conjugated protein A (EY Labs, San Mateo, USA) were added to each well, incubated for 1 h at 37°C, and washed three times with T-PBS.

**Substrate.** The peroxidase substrate solution containing 0.01% o-phenylene diamine and 0.03% H2O2 (100 μl) was added to each well. Reactions were stopped about 30 min later by 1 N H2SO4. The optical density (OD) unit was read relative to a LPS blank or irrelevant LPS at 492 nm on an automatic microplate reader (MTP-32, Corona Electric Co., Japan). Less than 0.02 OD unit was judged as the non-specific background.

Results

**Optimization of precoating with poly-L-lysine**

The optimization of precoating with poly-L-lysine was examined (Fig. 1). Microplates were precoated with varying concentrations of poly-L-lysine with MW ranging from 150,000 to 300,000, and then coated with *Klebsiella* O3 LPS at 10 μg/ml. Definite reactions were detectable at the poly-L-lysine concentration more than 1 μg/ml.
The maximum response was obtained with a poly-L-lysine concentration of more than 10 µg/ml. Therefore, precoating of microplates with poly-L-lysine at 10 µg/ml was used for further experiments. No significant reactions were detectable on the wells that LPS was directly coated up to 100 µg/ml and on the LPS blank well in precoated microplates. Irrelevant negative control antisera did not show significant non-specific backgrounds, either. Their OD values were less than 0.01. Next, precoating with poly-L-lysine with high MW (150,000–300,000) or low MW (1000–4000) were compared for binding of LPS. Poly-L-lysine with high MW gave higher OD values than that with lower MW. The differences were statistically significant (data not shown).

Detection of LPS and identification of the serotype in ELISA

Quantitative detection of LPS was examined when poly-L-lysine was precoated at the given concentration (10 µg/ml) (Fig. 2). Precoating with poly-L-lysine at 10 µg/ml could detect less than 1 µg/ml of LPS. Subsequently, we tried to identify the serotypes of various kinds of LPS using the ELISA technique. Antisera to the O antigen of Klebsiella O3 LPS specifically reacted with Klebsiella O3 LPS, whereas the antisera did not detect the other LPS, such as E. coli O55, O111, O128 and S. enteritidis. Antisera to various O antigens of Klebsiella LPS could not crossreact with Klebsiella O3 LPS (data not shown).

Comparison of various reagents for precoating in ELISA

Precoating with polymyxin B, poly-L-histidine, poly-L-glutamic acid, and bovine serum albumin were tested for binding of LPS in ELISA. The results are shown in Fig. 3. Poly-L-lysine gave the highest OD values in ELISA. Precoating with...
poly-\(L\)-histidine, or polymyxin B was less efficient in detection of LPS than precoating with poly-\(L\)-lysine with low MW. Reactions were not detectable in precoating at any concentrations of poly-\(L\)-glutamic acid and bovine serum albumin (data not shown).

**Detection of the free lipid A in ELISA**

It was tested whether or not our ELISA method could detect the free lipid A fraction prepared by acid treatment (Fig. 4) The free lipid A obtained from *Klebsiella* O3 could be detected by anti-lipid A antibody in ELISA, although precoating with polymyxin B gave higher OD units than that with poly-\(L\)-lysine.

**Discussion**

The present study demonstrates that ELISA using poly-\(L\)-lysine as a precoating reagent before coating of LPS was useful for detection of LPS and anti-LPS antibody. This technique provided much higher sensitivity on detection of LPS because ELISA with no precoating could not detect LPS. There are several reports on ELISA improved for detection of LPS or anti-LPS antibody, since LPS does not coat the surfaces of plastic microplate wells. Appelmelk et al. (1985) reported that LPS-BSA complexes were used for the coating of ELISA plates. Further, Pebode et al. (1989) developed an inhibition ELISA technique using LPS-BSA complexes. Our ELISA technique does not require glutaraldehyde activation for preparing LPS-BSA complexes. The use of LPS-polymyxin B complexes was also reported by Scott and Barclay (1987). They prepared LPS-polymyxin B complexes, dialyzed, and then coat the complexes on microplates. But we showed that it was unnecessary to make LPS-polymyxin B complexes before coating. Moreover, our ELISA technique using poly-\(L\)-lysine was more sensitive than that using polymyxin B. The ELISA using 

**References**


