

ELISA-based protein-PAMP interaction assay

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Method Article

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Abstract

Introduction

The interaction between HMC or metHb with LPS or LTA are examined by ELISA using LPS- or LTA-immobilized plates and probed with anti-hemocyanin or anti-hemoglobin antibody. To test the specificity of the ELISA, we further examined whether or not pre-incubation of HMC or Hb with LPS or LTA could reduce the ELISA-readout in a dose-dependent manner.

Procedure

1. Incubate aliquots of 100 μ l LPS or LTA which is resuspended in 0.01 M pH 7.4 PBS in a 96-well microtitre plate (Maxisorp®, NUNC, Denmark) at room temperature overnight.
2. Rinse the wells three times with 300 μ l of PBS each.
3. Block the wells with 150 μ l of PBS with 2% (w/v) BSA at 37 °C for 2 h.
4. Rinse the wells three times with 300 μ l of PBS each.
5. Add to each well, 100 μ l of the serially diluted protein (HMC or Hb in 2% BSA) in PBST (PBS containing 0.05% v/v Tween 20) and incubate at 37°C for 1 h.
6. Rinse the wells three times with 300 μ l of PBST each.
7. Add to each well, 100 μ l of primary antibody (rabbit-anti HMC or rabbit-anti Hb), which is diluted to 1:500 with 2% BSA in PBST and further incubate at 37 °C for 30 min.
8. Rinse the wells three times with 300 μ l of PBST each.
9. Add to each well, 100 μ l of the horseradish peroxidase-conjugated goat-anti-rabbit antibody (DAKO, Japan), which is diluted to 1:1000 in 2% BSA in PBST, and incubate at 37 °C for 30 min.
10. Rinse the wells three times with 300 μ l of PBST each.
11. Immediately before using, dissolve peroxidase substrate tablet (ABTS, 2,2'-azino-bis[3-ethylbenzthiazoline-6-sulfonic acid], Roche) in substrate solution (Roche) as indicated by the manufacturer's manual.
12. Add to each well, 50 μ l of the peroxidase substrate and incubate for 10 min at room temperature.
13. Read the OD at 405 nm.
14. To verify the specificity of the protein-PAMP interaction observed in the ELISA, further examine whether or not pre-incubation of HMC or Hb with LPS or LTA could reduce the ELISA-readout in a dose-dependent manner.
 - a) Choose a concentration of either HMC or Hb that shows an ELISA-readout (A_{405}) at the linear range.
 - b) Incubate the HMC or Hb with 0-200 μ g/ml LPS or LTA in 2% BSA in PBST at 37 °C for 1 h.
 - c) Then transfer the reaction mixture to the LPS- or LTA-immobilized microtitre plate.
 - d) Conduct the ELISA as described in steps 5-13.
 - e) The reduction of the readout of HMC or Hb pre-incubated with LPS or LTA respectively, demonstrates the specificity of the protein-PAMP interaction.

Anticipated Results

As shown in Figure 8, the typical ELISA readout of HMC-LPS interaction is $A_{405} = 0.1-0.4$; and that of Hb-LPS interaction is $A_{405}=0.4-1.6$

Figures

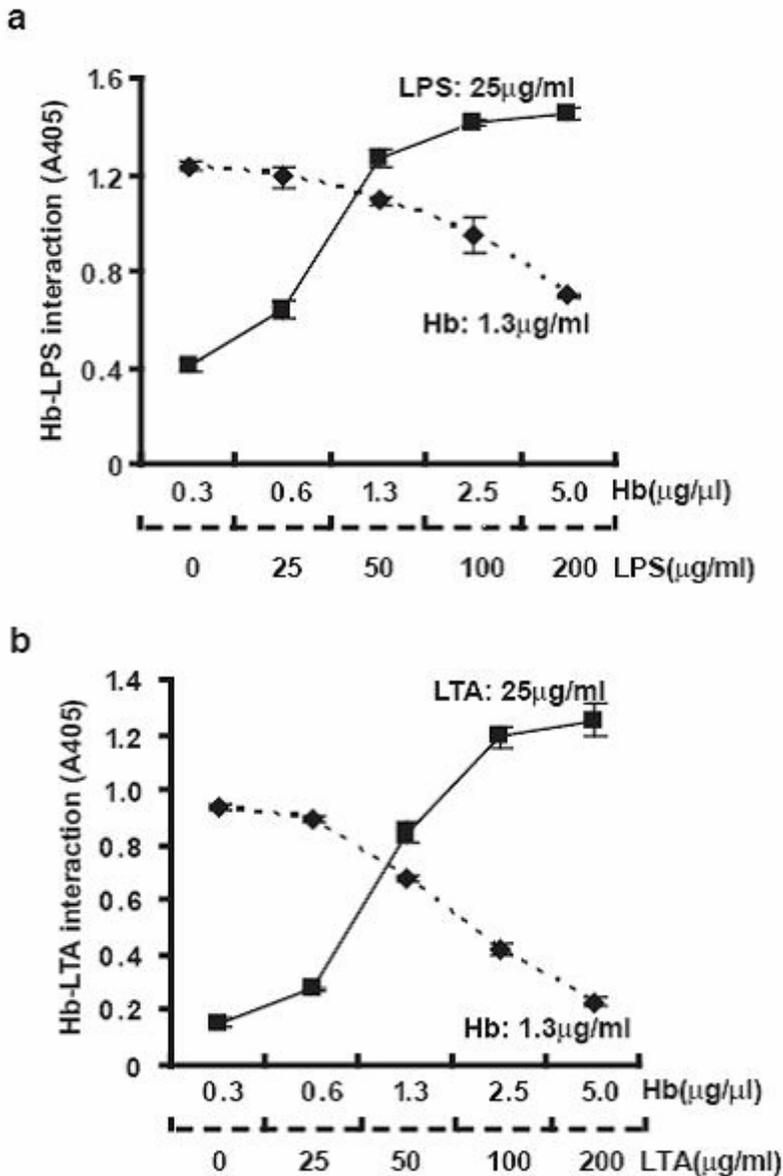


Figure 1

ELISA-based assay of the interactions between: (a) HMC-LPS and (b) Hb-LPS. (a) Dose-dependency between the amount of HMC applied and the ELISA readout (A405) suggests a specific interaction between HMC and LPS (solid line); pre-incubation of HMC with LPS dose-dependently reduces the ELISA readout, confirming the specificity of the HMC-LPS interaction. (b) Using Hb and LPS, similar observations were obtained as that in (a), suggesting the specific interaction between Hb and LPS.