ELISA-based protein-PAMP interaction assay

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Naxin Jiang
National University of Singapore

Nguan Soon Tan
National University of Singapore

Bow Ho
National University of Singapore

Jeak Ling Ding
National University of Singapore

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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 µl 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 µl of PBS each.
3. Block the wells with 150 µl of PBS with 2% (w/v) BSA at 37 °C for 2 h.
4. Rinse the wells three times with 300 µl of PBS each.
5. Add to each well, 100 µl 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 µl of PBST each.
7. Add to each well, 100 µl 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 µl of PBST each.
9. Add to each well, 100 µl 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 µl 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 µl 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(A405)at the linear range.

b) Incubate the HMC or Hb with 0-200 µg/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.

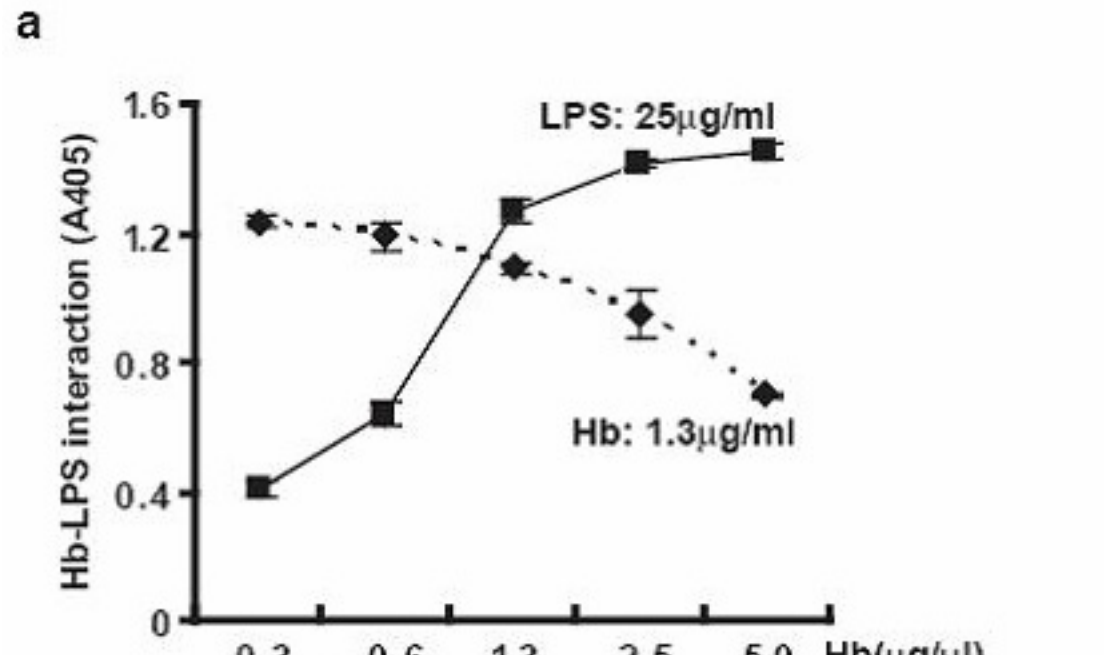
d) Conduct the ELISA as described in steps 5-13.

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 A405 = 0.1-0.4; and that of Hb-LPS interaction is A405=0.4-1.6

Figures



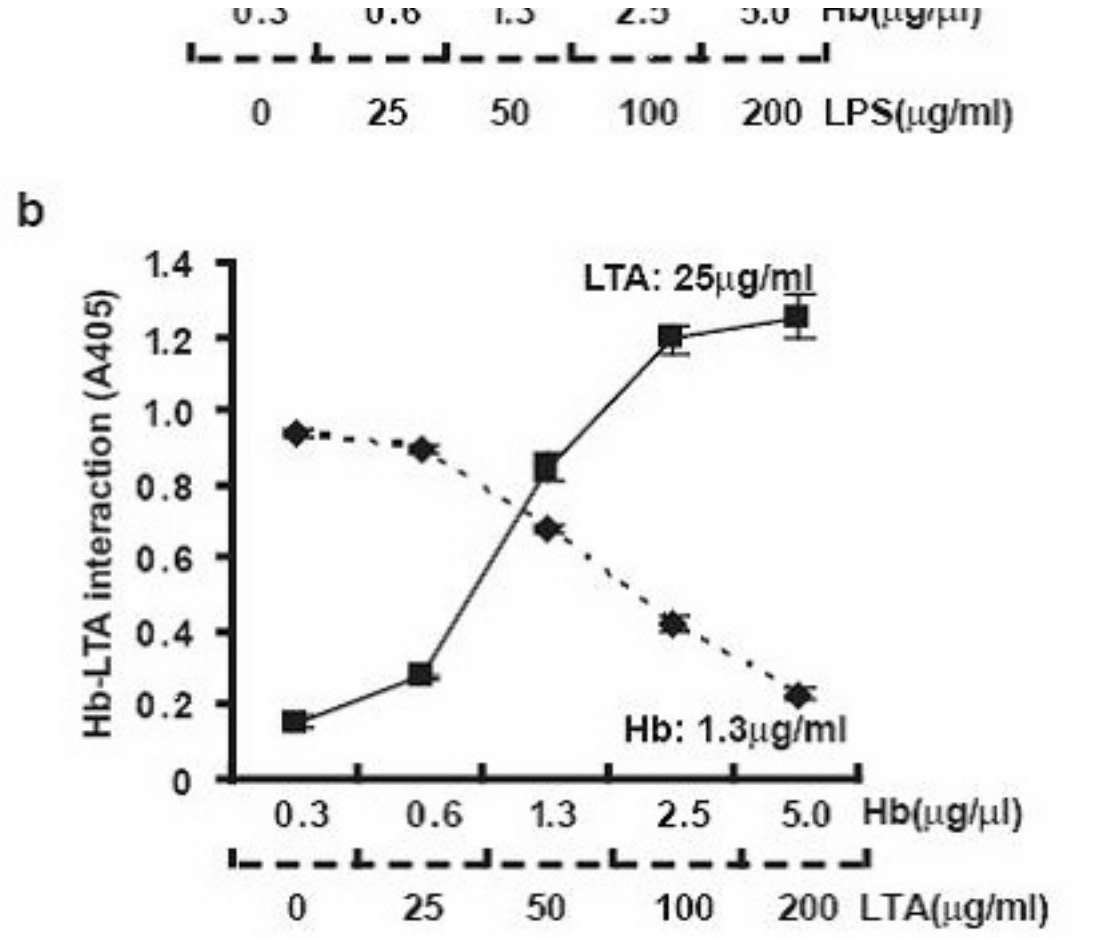


Figure 1

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.

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