Research Square Preprint: Please note that this article has not completed peer review.

Measurement of phenoloxidase activity

CURRENT STATUS: POSTED

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

DOI:
10.1038/nprot.2007.468

SUBJECT AREAS
Biochemistry *Computational biology and bioinformatics*

KEYWORDS
prophenoloxidase (PPO), phenoloxidase (PO), 4-methylcatechol

Introduction

In the chelicerate, hemocyanin is the functional substitution of prophenoloxidase (PPO). Upon activation, hemocyanin is converted to phenoloxidase (PO), which oxidizes the phenolic substrate such as 4-methylcatechol to produce quinone product, which has a maximum absorbance at 405 nm.

Procedure

PO activity was measured as described[1], with modifications.

1. Incubate purified hemocyanin with proteases in 50 mM Tris-HCl, pH 8.3, containing 0.05 M NaCl, at 20 ˚C for 10 min.
2. Add PAMPs (pathogen-associated molecular patterns eg: LPS or LTA) dissolved in the same buffer.
3. Then add 1 mM 4ME in 0.1 M potassium phosphate, pH 6.0.
4. Immediately after the addition of the substrate, continuously monitor the absorbance at 405 nm using a microplate reader (Molecular Devices, USA).
5. The PO activity is presented as A405 at 5 min after the addition of substrate.

Anticipated Results

The PO activity varies depending on the type of microbial proteases and PAMPs which are used for the activation. The typical range of PO activity at A405 is 0.1-0.8.

References

1. Jiang, H., Wang, Y. & Kanost, M. R. Pro-phenol oxidase activating proteinase from an insect, *Manduca sexta*: a bacteria-inducible protein similar to Drosophila easter. *Proc Natl Acad Sci U S A.* **95**, 12220-12225 (1998).

Figures

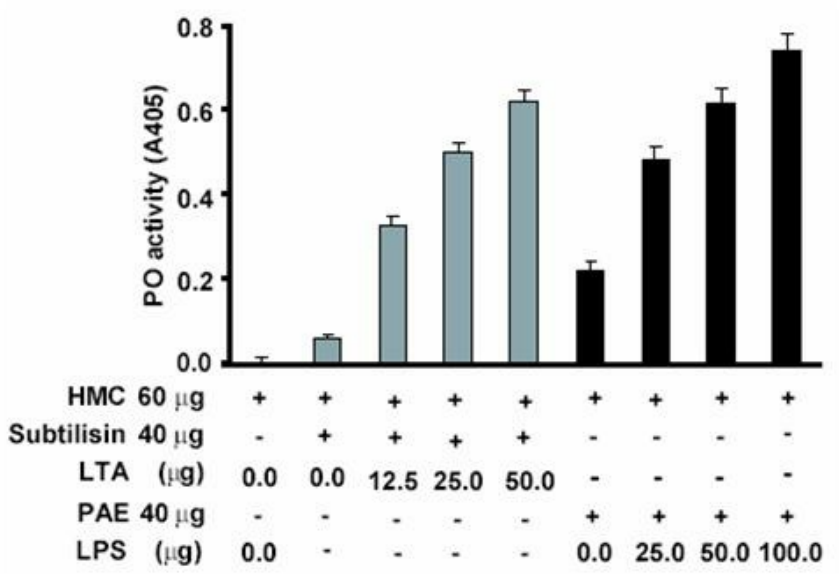

Figure 1

Activation of HMC/PPO to PO by microbial proteases and PAMPs PO activity triggered from hemocyanin by microbial proteases such as subtilisin (column 2) and Pseudomonas elastase (column 6). PAMP molecules such as lipoteichoic acid (LTA) and lipopolysaccharide (LPS) further enhances the PPO activation induced by microbial proteases, in a dose-dependent manner.

Respiratory protein–generated reactive oxygen species as an antimicrobial strategy

by Naxin Jiang, Nguan Soon Tan, Bow Ho & Jeak Ling Ding
Nature Immunology (13 June, 2007)