

## IMMUNOSENSOR ASSAY: A NOVEL METHOD TO ANALYZE PHYTOHORMONES

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### ABSTRACT

Phytohormones play very important roles at almost all developmental stages of plants. Because of their extremely low concentrations in plant tissues and they are highly sensitive to environmental factors such as light, heat and oxygen, sensitive assay is a limiting factor for phytohormonal research. All traditional phytohormonal assays including bioassay, GC, HPLC, ELISA and RIA have their disadvantages. Mainly based on radioimmunoassay and biosensor technology, immunosensor assay is a new technology for phytohormonal analysis proposed by Hunan Provincial Key Laboratory of Phytohormones and Growth Development and State Key Laboratory of Chemo/Biosensing and Chemometrics at Hunan University in 2002. After construction of the first indole acetic acid (IAA) immunosensor, several kinds of immunosensors for phytohormones were successfully developed.

IAA immunisensors: Currently we developed the two types of immunosensors for IAA. (1) Piezoelectric immunosensor. The detection was based on competitive immunoreactions between IAA and an bound to the anti-IAA antibody immobilized on a quartz microbalance, the frequency change of sensor caused by antigen was linearly related to logarithm of the concentration of IAA in the range from 0.5ng / mL to 5 $\mu$ g / mL. (2) Amperometric immunosensor. The determination was based on an enzyme-linked competitive immunoreaction between free IAA and IAA labeled with HRP to bind on the anti-IAA antibody immobilized on the sol-gel-alginate-carbon composite electrode(SACE) surface. The response signal expressed as percentage current reduction (CR%) was linearly related to the logarithm of the concentration of IAA in range of 5 $\mu$ g / mL to 500 $\mu$ g / mL with a regression equation of the form  $y = 37.80x - 22.47$  and correlation coefficient of 0.9922.

Cytokinin immunosensor: The immunosensor based on a multilayer-coated glassy carbon electrode was designed to determine isopentenyl adenosine (iPA) in plants. The multilayer consists of polypyrrole and poly(m-phenylenediamine) with  $K_4Fe(CN)_6$  and horseradish peroxidase (HRP) entrapped during electropolymerization. The ferrocyanide doped in polypyrrole functions as the mediator. The glucose oxidase bound on the immunosensor by the competitive immunoreaction involving iPA catalyzed the oxidation of the added glucose with the formation of  $H_2O_2$ , which was in turn reduced in the presence of HRP entrapped in poly(m-phenylenediamine).

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The current of the oxidized production of ferrocyanide reduced at -50mV was inversely proportional to the concentration of iPA in the competitive immunoreaction. The percentage of current response reduction (CR%) was linearly related to the logarithm of the concentration of iPA in the 5 $\mu$ g / mL to 300  $\mu$ g / mL range, with a regression equation of the form  $y = 42.13x - 27.79$  and a correlation coefficient of 0.9861.

Immunosensors for other phytohormones: Similar work to develop immunosensors for other phytohormones such as gibberellins and abscisic acid is also in progress. Further study is needed to improve the sensitivity and reuse time of immunosensors. Immunosensor assay has been used to analyze hybrid rice samples and the results were in satisfactory agreement to those obtained by high-performance liquid chromatography method, indicating its high application potential.

