Konvensionele Aleksa 488-hidrasied en die kwantumdot-etikettering van Conjugaten vir Lipopolisakkariede bindingstudies in *Arabidopsis thaliana* protoplaste

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How to cite this article:
Mgcina, L. & Piater, L.A., 2014, 'Konvensionele Aleksa 488-hidrasied en die kwantumdot-etikettering van Conjugaten vir Lipopolisakkariede bindingstudies in *Arabidopsis thaliana* protoplaste', *Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie* 33(1), Art. #1226, 1 page. http://dx.doi.org/10.4102/satnt.v33i1.1226

Note:
This paper was initially delivered at the School of Environmental Sciences and Development of the North-West University, Potchefstroom Campus, South Africa on 05 October 2012.

Conventional Alexa 488 Hydrazide in Quantum dot labelling of conjugates for lipopolysaccharide binding studies in *Arabidopsis thaliana* protoplast. Lipopolysaccharide (LPS) is a complex lipoglycan that is found in the outer membrane of Gram-negative bacteria and composed of three regions namely the fatty acid Lipid A, a core region of short oligosaccharide chains and an O-antigen region of polysaccharide. When LPS is used as a MAMP/PAMP, it not only induces an innate immune response in plants but also stimulates the development of defense responses such as the immediate release of ROS/ROI, PR gene expression and activation of the hypersensitive response (HR), resulting in stronger subsequent pathogen interactions. The identification and characterization of the elusive LPS receptor or receptor complex in plants is thus of importance, since understanding the mechanism of perception and specific signal transduction pathways will clarify, and lead to the advancement of, basal resistance in plants in order to decrease plant crop losses due to pathogen attack. In mammals, LPS binds to LBP (LPS binding protein) to form a LPS-LBP complex which is translocated to MD2 with the presence or absence of its co-receptor, a glycosylphosphatidylinositol (GPI)-linked protein, CD14. The interaction occurs on the host membrane and triggers an inflammatory defense response through the signaling cascade activated by the interaction with Toll-like receptor 4 (TLR4). A similar LPS-receptor interaction is however, unknown in plants. To address this, biological binding studies with regard to concentration, incubation time and temperature, affinity, specificity and saturation were conducted using LPS labeled with Alexa 488 hydrazide. Although such labeling does not affect the biological activity in mammalian studies, the same cannot necessarily be said for plant systems. Thus, quantum dots, which allow non-covalent hydrophobic labelling of LPS, were further employed in binding studies. The conjugation to LPS was confirmed by transmission electron microscopy and results illustrated higher fluorescence values as compared to Alexa-LPS fluorescence analysis. Furthermore, inhibition of the process is also reported using Wortmannin and Brefeldin A as suitable endocytosis inhibitors.

Lipopolisakkariede (LPS) is 'n komplekse lipoglikaankomponent wat gevind word in die buitenste membraan van Gram-negatiewe bakterieë en bestaan uit drie dele, naamlik die vetsuur lipid A, 'n kerngebied van kort oligosakkariedkettings en 'n O-antigeen van die polisakkaried. Wanneer LPS as 'n mikrob of patogeen-geassosieerde molekulêre patroon (M/PAMP) gebruik word, induseer dit nie net die inherente immuunsisteem in plante nie, maar ook die verdediging deur die onmiddellike vrylating van reaktiewe suurstofspesies (ROS), patogenieen-verwante (PR) proteïene en aktivering van die hipersensitiewe-reaksie (HR). Toekomigste stimulasie lei dan tot 'n sterker respons. Die identifisering en karakterisering van die ontwykende LPS-reseptor of reseptor kompleks in plante is dus nodig, aangesien 'n beter begrip van die meganismes van waarneming en spesifieke seintransduksie kan lei tot die bevordering van basale weerstandbiedendheid in plante. Die uiteindelike doel hiervan is daarop gemik om plantverliese as gevolg van siekte te bekamp.

In soogdiere, bind LPS aan 'n LPS bindingsproteïen (LBP) om 'n LPS-LBP-kompleks te vorm wat dan na MD2 translokeer word met die teenwoordigheid of afwesigheid van sy mede-reseptor met, 'n glikosilfosfatidilinositaal (GPI)-gekoppelde proteïen, CD14. Die interaksie vind plaas op die gasheer membraan en aktiveer 'n inflammatoriese reaksie deur met 'Toll-tipe reseptor-4' (TLR4). 'n Soortgelyke LPS-reseptorinteraksie is egter onbekend in plante. Om dit aan te spreek, is biologiese bindingstudies met betrekking tot konsentrasie, inkubasie tyd en temperatuur, affiniteit, spesifisiteit en versadigbaarheid uitgevoer deur gebruik te maak van LPS gemerk met Alexa 488-hidrasied. Hoewel sodanige etikettering nie die biologiese aktiwiteit in soogdierstudies beïnvloed nie, kan dieselfde nie noodwendig gesê word vir plante nie. Dus, is kwantumdots, wat nie-kovalente hidrofobiese interaksie met LPS toelaat, in bindingstudies uitgevoer. Die binding aan LPS is bevestig deur transmissie-elektronmikroskopie. Resultate illustreer hoër fluoressensiewaardes in vergelyking met Alexa-LPS fluoressensie-analise. Verder word inhibisie van die proses ook met behulp van Wortmannin en Brefeldin A getoets aangesien hierdie chemiese stowwe endositose inhibeer.