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Functional analysis of synthetic DELLA domain peptides and bioactive gibberellin assay using surface plasmon resonance technology

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ABSTRACT

DELLA proteins and phytohormone gibberellin act together to control convergence point of plant development. A gibberellin-bound nuclear receptor that interacts with the N-terminal domain of DELLA proteins is required for gibberellin induced degradation of DELLA proteins. N-terminal DELLA domain includes two conserved motifs: DELLA and VHYNP. However, their respective functions remain unclear. Meanwhile, the identification and detection of several bioactive gibberellins from the more than 100 gibberellin metabolites are overwhelmingly difficult for their similar structures. Using in vitro biochemical approach, our work demonstrates for the first time that the synthetic GAI N-terminal DELLA domain peptides have similar bioactive function as the expressed protein to interact with AtGID1a receptor. Furthermore, our results reveal that DELLA motif is vitally important region and DELLA segment is essentially required region to recognize AtGID1a receptor. Finally, based on bioactive GA-dependent of the interaction between AtGID1a and DELLA protein, we generated a new method that could identify and detect bioactive GAs accurately and rapidly with surface plasmon resonance assays.

1. Introduction

DELLA protein as a key negative regulator in gibberellin (GA) signaling pathway represses GA-associated response [1–6]. In higher plants, especially in model plants *Arabidopsis thaliana* and rice (*Oryza sativa*), GA perception and signal transduction mechanisms have been widely studied and understood in-depth in recent years. Soluble GA receptors (GID1 in rice; three homologs AtGID1a, AtGID1b and AtGID1c in Arabidopsis) perceive and bind bioactive GAs, and then are transformed to interact with DELLA proteins which are subsequently recognized by a specific F-box protein (GID2 in rice and SLY in *Arabidopsis*) and degraded via the ubiquitin-26S proteasome system [7–9]. All DELLA proteins consist of an N-terminal DELLA domain and a C-terminal GRAS domain [2,10]. The N-terminal DELLA domain includes two conserved motifs (DELLA and VHYNP) [2,3,6,11,12]. In Arabidopsis, DELLA proteins comprise five homologs (GAI, RGA, RGL1, RGL2 and RGL3) [1,11,13–15]. A yeast two-hybrid system identified that both DELLA and VHYNP motifs of the DELLA protein RGA are necessary for AtGID1a receptor interactions [8], while another study demonstrated that the DELLA motif alone (amino acids 1–73) in the N-terminal domain of GAI is sufficient to mediate the interactions with AtGID1a [16]. Thus, there remains a gap in understanding the function of different motifs in the DELLA N-terminal domain.

Gibberellins (GAs) are a group of tetracyclic diterpenoid plant hormones. There are about 136 GAs with similar structure identified from plants, fungi and bacteria [17]. In plants, among the different forms of GAs, most of them are non-bioactive and exist as precursors and deactivated metabolites [18]. However, the bioactive GAs, such as GA_1, GA_3, and GA_4, play vital roles in promoting seed germination, vegetative growth, flower development, fruit expansion and response to biotic or abiotic stress [18–21]. The degradation of DELLA protein is GA dose-dependent and releases its suppression of GA signaling. Thus, the efficient and accurate detection of GAs is very important to understand the regulation role in plant growth and development. Up to now, many analytical methods have been established for the determination of plant hormone GAs, and almost all successful methods can be classified into two categories: immunological analyses and mass spectrometry (MS) detection. The former category is based on immunoassays, such as radioimmunoassay (RIA) and enzyme-linked

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immunosorbent assays (ELISAs), and the other category is the use of a mass spectrometry (MS) detector coupled with separation systems, such as high-performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE) [3,22–32]. These methods demand antibodies specific for GAs or require stable isotope-labeled GAs as internal standards; accordingly, the procedures for the preparation and detection of GAs are particularly laborious and time-consuming. Due to the restrictions of the former methods mentioned above, it is urgently required to establish a rapid, simple and effective detection method.

Surface plasmon resonance (SPR) is an optical technique that measures changes in the refractive index on the sensor surface based on the evanescent wave phenomenon [33–36]. Since it was reported in 1983 [37], SPR has been increasingly popular in fundamental biological studies and subsequently been used to enable the rapid detection, characterization and quantification of specific compounds in the environment in real time without any labeling requirements [33,38–42]. In recent years, the application of SPR in the field of phytohormone study has attracted researchers' interests. Most of the studies focused on understanding the mechanisms of hormone metabolic pathways and signal transductions. Glutathione S-transgerase (GST)-fused SLR1 (the unique DELLA protein in rice) N-terminal fragment ($SLR1^{4-125}$) and maltose-binding protein (MBP)-tagged RGL1 N-terminal fragment ($RGL1^{1-137}$) were expressed in *Escherichia coli* and purified as ligands, which were respectively immobilized on the sensor chip surface using anti-GST and anti-BMP antibodies, and the SPR biosensor assay demonstrated the binding of $SLR1^{4-125}$ and $RGL1^{1-137}$ to GID1s in the presence of bioactive GAs [12,43,44]. However, the recombinant expression and purification of N-terminal fragment of the DELLA protein with high quality is a laborious and costly process, and the protein is prone to lose activity during the in vitro assay. The sensitivity of SPR technique depends on the thickness and spatial organization of interfacial surfaces, so stable SPR transducer and sensor chips have also been considered [45]. Thereby, the chemical synthesis of functional DELLA domain peptides may be an optimal selection to build a stable, high efficiency SPR assay platform. SPR technology has also been applied in detection of phytohormones. An SPR-based detection methodology of identification and quantification of ^{1}H-indole-3-acetic acid (IAA) in plants was proposed by Wei et al. in 2011 [46]. They combined SPR sensing and a molecularly imprinted monolayer (MIM) to establish this detecting method, and this method showed good recoveries and precision (over 95%). However, the application of SPR in quantifying bioactive GAs has not been reported so far. Our aim is to develop a new method using SPR to determine bioactive GAs; taking into consideration that the regulation functions of GAs during plant growth and development are initially dependent on the binding of bioactive GA to its receptor. The specific bioactive GA binding properties with GA receptors provide the possibility to detect GAs through SPR technology, which requires the immobilization of biomolecules to the sensor surface. However, the low-molecular-weight GAs (~346 Da) are difficult to be directly detected as analyte on the SPR sensor chip with immobilized GA receptors. On the other hand, bioactive GA as ligand immobilized on the sensor chip may cause it to lose binding ability to GA receptors, since the immobilized GAs hardly enter the specific pocket of the GA receptor followed by the closure of its N-terminal extension lid. Whereas GA receptor AtGID1s interact with DELLA protein in a bioactive GA-dependent manner [8,16,47–49], the DELLA protein may be a preferred candidate as ligand immobilized on an SPR sensor chip for GA analysis.

In this study, we present the possibility to apply the synthetic peptide instead of the expressed protein used in SPR technology to develop a new method for exploring the function of the DELLA domain, and detecting bioactive GAs. The results reported in this paper explicitly verify that the DELLA motif is the key region and the DELLA segment is the determinative region to recognize the AtGID1a receptor. Furthermore, our preliminary results showed the possibility to go further in the direction of biosensing-based methods for the detection of bioactive GAs and their possible application in the field of hormone study.

2. Materials and methods

2.1. Synthesis of DELLA domain peptides

Based on the amino acid sequences of AtGAI protein, the biotin-labeled DELLA domain peptides were synthesized (Bootech), the sequences of peptides are shown in Fig. 1. The 76-mer GAI^{19-94} peptide was synthesized with GVL (amino acids 55–57) instead of VMM, to avoid being oxidized. VL had been found in AtRGL1, AtRGL2, and AtRGL3; and GVL had appeared in DELLA homologs in *Helianthus petiolaris*, *Helianthus tuberosus* (Supplementary Material Fig. S1). GAI^{19-94} peptide contains two conserved motifs: DELLA motif (amino acids 28–54) and VHYNP motif (amino acids 73–94) [12,50]. GAI^{19-55} peptide only contains the DELLA motif. $GAI^{19-55,\Delta DELLA28-32}$ peptide was synthesized with the deletion of the five DELLA amino acids on the basis of the amino acid sequence of GAI^{19-55}. The other three peptides were synthesized with the replacement of opposite hydrophilic and hydrophobic amino acids of the DELLA amino acids (i.e. $GAI^{19-55,D28A}$: D→A, $GAI^{19-55,LL30-31DD}$: LL→DD, and $GAI^{19-55,A32D}$: A→D).

2.2. Recombinant AtGID1a protein expression, purification and identification

AtGID1a (At3g05120) open reading frame was amplified through polymerase chain reaction (PCR) from wild-type *A. thaliana* Columbia cDNA. For expression and purification, *AtGID1a* fragment (residues 1–345) was cloned into the *Eco*RI/*Sac*I site of pGEX-KG (Amersham) to generate pGEX-KG-*AtGID1a*. Sequences of primers used in this study were GID1aEcoFW: 5′ ATT*GAATTC*AAATGGCTGCGAGCGATGAAGTTAATC 3′, GID1aSacRV: 5′ CCA*GAGCTC*ACATTCCGCGTTTACAAACGCCGAAATCT 3′. All PCR fragments were sequenced to confirm that no mutations were introduced. The pGEX-KG-*AtGID1a* was transformed into *E. coli* strain Rosetta (DE3). Protein was induced at 16 °C with 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 12 h in Luria-Bertani medium. Cells were collected, resuspended in ice-cold PBST buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 0.005% (v/v) Tween 20, pH 7.4), and lysed by sonication. After ultracentrifugation at 10,000*g* for 40 min, the supernatant was filtered through a 45 μm filter immediately before it was applied to a Glutathione Sepharose (GSTrap FF column, GE Healthcare), and was further purified by gel filtration chromatography (Superdex200 column, GE Healthcare) according to the instructions of the columns. The quality of the protein was checked by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Protein concentration was measured by the Bradford method using bovine serum albumin (BSA) as the standard. A western blot assay was performed to identify GST–GID1a protein using a GST-tag monoclonal antibody (Novagen), 5 μL aliquots of the purified protein were separated by SDS-PAGE, electro-transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore) for 3 h by using a Mini Trans-Blot electrophoretic transfer cell (Bio-Rad). The blot was blocked with TBST buffer (0.1% (v/v) Tween 20 in 50 mM Tris–HCl, pH 7.5, and 150 mM NaCl) containing 5% (w/v) nonfat dry milk powder overnight at 4 °C and subsequently washed three times with TBST buffer. The blot was then incubated