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Unveiling the molecular mechanism of brassinosteroids: Insights from structure-based molecular modeling studies

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ABSTRACT

Brassinosteroid (BR) phytohormones play indispensable roles in plant growth and development. Brassinolide (BL) and 24-epibrassinolide (24-epiBL) are the most active ones among the BRs reported thus far. Unfortunately, the extremely low natural content and intricate synthesis process limit their popularization in agricultural production. Earlier reports to discover alternative compounds have resulted in molecules with nearly same scaffold structure and without diversity in chemical space.

In the present study, receptors structure based BRs regulation mechanism was analyzed. First, we examined the detailed binding interactions and their dynamic stability between BL and its receptor BRI1 and co-receptor BAK1. Then, the binding modes and binding free energies for 24-epiBL and a series of representative BRs binding with BRI1 and BRI1–BAK1 were carried out by molecular docking, energy minimization and MM–PBSA free energy calculation. The obtained binding structures and energetic results provided vital insights into the structural factors affecting the activity from both receptors and BRs aspects. Subsequently, the obtained knowledge will serve as valuable guidance to build pharmacophore models for rational screening of new scaffold alternative BRs.

1. Introduction

Brassinosteroids (BRs), the sixth class of plant hormones, are essential for plant growth, reproduction and responses to a wide range of abiotic and biotic stressess, such as drought, salinity, heat, cold, virus infection, and pathogen attack [1,2]. BRs are recognized by extracellular leucine-rich repeat (LRR) domain of brassinosteroid-insensitive 1 (BRI1) [3]. BRI1–BRs binding induces heteromerization of BRI1 with a family of somatic embryogenesis receptor kinases (SERKs), then the BRI1 and SERKs kinase domains transphosphorylate each other. Thus, the BRs-induced BRI1–BRs–SERKs stable association leads to the activation of the cytoplasmic signaling cascade, triggering plant growth and differentiation.

BRs have been found in an extremely wide range of plant species [4]. Among the BRs, brassinolide (BL) (Fig. 1) is the most active one. Unfortunately, the natural content is extremely low; and both the isolation from plant material and synthesis at reasonable cost are difficult. Therefore, scientists found 24-epibrassinolide (24-epiBL, Fig. 1), the stereoisomer of BL, and it is the most widely used brassinosteroid till now. However, 24-epiBL is also expensive, which limit its popularization and practical applications. Hence, it is of great practical significance to develop novel molecules with good activity and low cost.

Due to the lack of the knowledge about the three-dimensional structures of the brassinosteroids receptors and brassinosteroids regulation mechanism at molecular level till 2013 [5–8], earlier efforts to explore new brassinosteroids depended only upon the reported structures and activities of BL analogues. As a result, the structures of the designed new alternative molecules fall into a very small range of chemical space, and no brand-new compound with novel scaffold was found [4].

To effectively and rationally design novel BRs, the most important point should be the BRs-receptors interactions and corresponding critical structural features determining the activity of BRs. The recently reported BRs receptors crystal structures [5–8] make the molecular level analysis feasible now. As illustrated above, BRs binding at the cell surface activates the brassinosteroids signaling by two steps: first, BRs bind to a hydrophobicity-dominating surface groove on BRI1 LRR domain; then SERKs LRR domain, as a co-receptor, heteromerized to the BRI1–BRs complex.

Representative brassinosteroids were selected to explore the BRs-receptors recognition mechanism, and structure–activity

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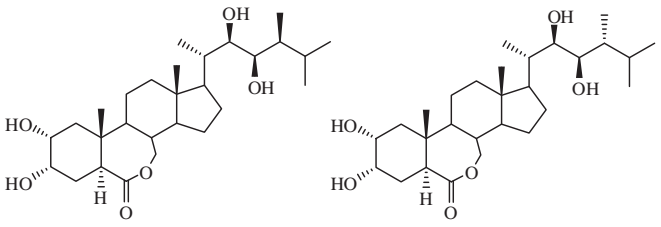


Fig. 1. The structures of brassinolide (BL) and 24-epibrassinolide (24-epiBL).

relationship, and finally to summarize the key residues on receptors and structural features of the BRs influencing the activity by investigating the binding modes and binding affinity of the BRs with BRI1 and BRI1–BAK1 (BRI1-associated kinase 1, also known as SERK3) employing molecular docking, molecular dynamics simulations and free energy calculations. Following the knowledge obtained in this work, our final aim is to carry out pharmacophore-based virtual screening and experimental test to gain novel BRs. This may provide potential lead scaffolds to develop BRs plant growth regulators with low cost and high activity for agricultural production.

2. Materials and methods

2.1. Data set

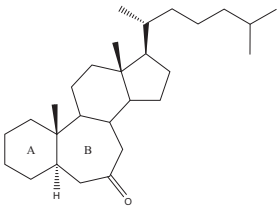
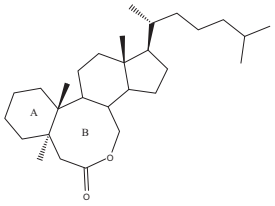
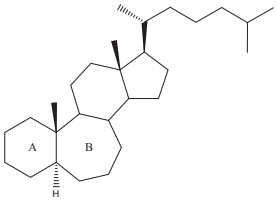
The brassinosteroids examined in the present study include brassinolide, 24-epibrassinolide, a series of 5α-cholestanes derivatives with 2α,3α-dihydroxy, 3α,4α-dihydroxy substitutes on ring-A and 7-oxo, 8-oxalactone, 7-deoxo as ring-B [9]. The structures and activities of the BRs are summarized in Table 1. The activity is characterized by the maximal prolongation of the second internode in mm in the bean second internode bioassay [9].

2.2. Molecular docking

Molecular docking was carried out by using GOLD 5.2 Suite [10,11]. All of the BRs were constructed in SYBYL 6.9 molecular modeling package [12] based on the active conformation of brassinolide in crystal structure. Energy minimizations were performed using the Tripos force field [13] with a distance-dependent dielectric and Powell method [14] with a convergence criterion of 0.05 kcal/mol. Partial atomic charges were calculated using Gasteiger-Hückel method [15].

BRs were docked into BRI1 and BRI1–BAK1 binding pockets separately to analyze the key features influencing the activity of BRs in the two-steps signaling activation process. The crystal structures of BRI1 with BL in the binding site and BRI1–BAK1 with BL at the interface of BRI1 and BAK1 are available from the RCSB protein data bank [16] with PDB codes as 3RGZ and 4M7E, respectively. Before docking, the proteins were prepared by adding hydrogen atoms, assigning protonation states and carrying out energy minimization with a small number of steps to relax amino-acid residue side chains and BL to relative appropriate positions. The protonation state of histidine residues was assigned according to the structure of residues surrounding the histidine side chain. The binding site was defined as all atoms of the protein within 10 Å of the bound-BL in BRI1 and BRI1–BAK1. Subsequently, the docking was performed with GOLD software using the genetic algorithm (GA) search strategy. The GA parameters included 200,000 genetic operations on an initial population of 100 divided into five subpopulations. The number of generated poses was set to 10 for each compound and early termination was turned off. Atom types

Table 1
The structures and activities (maximal prolongation of the second internode in mm by applied amount 10^{-9} M in the bean second internode bioassay (for compound 2: 0.481 μg/plant; 3, 4: 0.432 μg/plant; 5, 6: 0.449 μg/plant; 7, 8: 0419 μg/plant)) of studied BRs.

Compound	Structure		Activity/mm
1	Brassinolide (Fig. 1)		–
2	24-epibrassinolide (Fig. 1)		38.5
5α-cholestanes derivatives			
3		2α,3α-dihydroxy	2.8
4		3α,4α-dihydroxy	22.5
	7-oxo for ring-B		
5		2α,3α-dihydroxy	9.0
6		3α,4α-dihydroxy	20.5
	8-oxalactone for ring-B		
7		2α,3α-dihydroxy	2.5
8		3α,4α-dihydroxy	11.9
	7-deoxo for ring-B		

for BRs and receptor were set automatically by the GOLD. GoldScore was selected as the scoring function. For each BR, the agonist-receptor structure similar to BL with the best-scoring pose was selected as the initial conformation for the energy minimization as described below.

2.3. Energy minimization and molecular dynamics

Energy minimization and MD simulation of the docked complexes were performed in Amber (version 12) with Amber ff99SB force filed [17]. In the simulations for BRI1 complexes, three waters bridging hydrogen bond interactions between BL and Tyr 599, Tyr 597, His 645, and Ser 647, located in the binding site in the crystal structures were maintained according to the structural descriptions in two references [7,8]. The partial atomic charges for the BRs atoms were calculated using the RESP protocol [18] after electrostatic potential calculations at with the HF/6-31G* level using Gaussian (03 version) [19]. Each BR-receptor binding complex was neutralized by adding suitable counter-ions and was solvated in a truncated octahedron box of TIP3P water molecules [20] with a minimum solute wall distance of 10 Å. The solvated systems were carefully energy-minimized by minimizing hydrogen, solvent, side chains and all atoms.

The BRI1–BL and BRI1–BL–BAK1 systems were gradually heated from $T = 10$ K to $T = 298.15$ K in 60 ps before a production MD simulation run for 20 ns, making sure that we obtained a stable MD trajectory for the simulated systems. The time step used for the MD simulations was 2 fs. Periodic boundary conditions in the NPT ensemble at $T = 298.15$ K with Berendsen temperature coupling [21] and $P = 1$ atm with isotropic molecule-based scaling