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ORIGINAL PAPER

Computational insight into novel molecular recognition mechanism of different bioactive GAs and the *Arabidopsis* receptor GID1A

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Abstract Gibberellin (GA) is an essential plant hormone and plays a significant role during the growth and development of the higher plants. The molecular recognition mode between GA and receptor *Arabidopsis thaliana* GIBBERELLIN INSENSITIVE DWARF1 A (AtGID1A) was investigated by molecular docking and dynamics simulations to clarify the selective perceived mechanism of different bioactive GA molecules to AtGID1A. The 6-COOH group of GA, especially its β configuration, was found to be an indispensable pharmacophore group for GA recognition and binding to AtGID1A. Not only does a strong salt bridge interaction between the 6β-COOH group of GA and Arg244 of AtGID1A play a very important role in the GA recognition of the receptor, but also an indirect water bridge interaction between the pharmacophore group 6β-COOH of GA and the residue Tyr322 of AtGID1A is essential for the GA binding to the receptor. The site-directed residues mutant modeling study on the receptor-binding pocket confirmed that the mutations of Arg244 and Tyr322 decreased the GA binding activity due to the disappearances of the salt bridge and the hydrogen bond interaction. The 3β-OH group of GA was well known to be necessary for the GA bioactivity due to its forming a unique hydrogen bond with Tyr127 of AtGID1A. In addition, the hydrophobic interaction between GA and AtGID1A was considered a necessary factor to lock the GA active conformation and stabilize the GA-GID1A complex structure. The novel molecular recognition mode will be beneficial in elucidating the GA regulation function on the growth and development of the higher plants.

Keywords AtGID1A · GA · Molecular recognition mechanism · Residue mutation · SAR

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Introduction

Gibberellin (GA), which is a well-known tetracyclic diterpenoid phytohormone, participates in control of higher plant throughout the life cycle process. GA may be responsible for a wide range of plant growth responses, including seed germination, stem elongation, leaf expansion, induction of flowering, and pollen maturation [1]. The famous agriculture 'Green Revolution' in the 1960s was reported to relate to the alterations in GA biosynthesis or its signaling transduction processes [2–4]. This was because GA induces the dwarfing traits in rice and wheat and further develops their high-yielding varieties. In addition, GA also results in the promotion of growth in a variety of fruit crops, an increase in the sugar yield from sugarcane, and the stimulation of the barley-malting process in the beer-brewing industry [5]. Therefore, GA exerts a great influence not only in agriculture production but also in commercial application.

Since the first discovery of GA_3 in the *Gibberella fujikuroi* fungus [6], 136 GAs have been identified from different plant, fungi and bacteria at present [7]. However, only a few, such as GA_3, GA_4, GA_1, and GA_7, function as bioactive plant hormones (Fig. 1) [8]. The others are either their precursors or degradation products in the GA biosynthesis and signaling transduction processes [9]. Currently, the empirical rule on the structure-activity relationship (SAR) of GA suggests that 6-COOH was important for the GA biological activity in the dwarf pea, rice, and barley [10]. The 3β-OH group and a lactone ring between C4 and C10 were confirmed to be necessary for the biological activity of GA in some dwarf

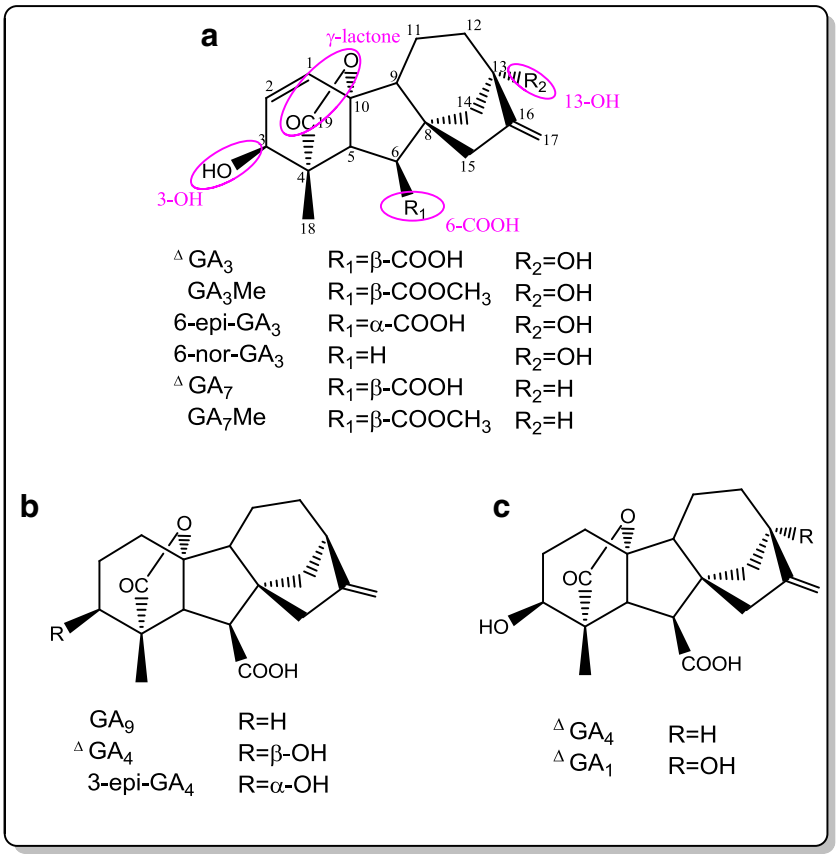

Fig. 1 Chemical structure of the bioactive and inactive GAs. a GAs of different functional groups on C6; b GAs of different functional groups at C3; c GAs of different functional groups at C13

plants [11, 12]. However, how to function for these pharmacophore groups of GA is not clear at a molecular level.

A number of GA binding proteins (GBPs) were identified as some GA receptor candidates in an early stage of the GA perception study [13]. These soluble GBPs in the pea and cucumber hypocotyls were used to evaluate the GA binding activity by the stem elongation assay experiment [14]. Until 2005, GIBBERELLIN INSENSITIVE DWARF1 (GID1) in rice was first determined successfully as a GA receptor by Ueguchi et al. [15]. Subsequently, three GID1 homologs, including AtGID1A, B, and C, were found in *Arabidopsis thaliana* [16–19]. It was very exciting that the crystal structures of GA_3/GA_4-AtGID1A-DELLA were successfully determined by Murase et al. in 2008 [20]. The complex structures of *Oryza sativa* GID1 (OsGID1) co-crystallized with GA_3 and GA_4 were reported almost at the same time [21]. The successful hunt for the GA receptor not only reveals a new insight into how GA was recognized by GID1A, but also enhances the molecular level understanding of the GA signaling transduction pathway. Therefore, a new model of the GA signal transduction pathway was proposed based on the reported crystal structure GA_3-AtGID1A-DELLA [20]. GA, as an 'allosteric inducer,' induces a GID1A conformational change in the N-terminal helical switch and then promotes a conformational transition of the N-terminal of the downstream DELLA protein to further enhance the binding interaction between GID1A and DELLA. Recently, this model was refined such that the GID1A-DELLA binding also leads to a conformational change in the C-terminal GRAS domain of DELLA to enhance its GRAS domain recognized by the F-box protein, which promotes polyubiquitination by SCFSLY1/GID2 [22]. Even though there are a number of studies on the interaction of GID1-DELLA to be reported, the studies focusing on the molecular interaction mode between active or inactive GAs and the receptor GID1 are still limited.

Although the crystal structures of two receptors AtGID1A and OsGID1 bound with GA_3 and GA_4 were both reported, respectively, there are only a few residue-mutant studies on OsGID1. The residue mutations at S127A, S123A, D250A, and V246A in OsGID1 were recently produced and their binding activities to GAs were examined in vitro. The obtained results showed that these four mutants only retained a low or moderate binding activity to GA_4 [21]. The other mutation studies on the variants F27L, I133L, I133V, and L330I in OsGID1 indicated to be their lower affinity and specificity for active and inactive GAs [23]. The latest mutant studies on S123A, Y134F, S198A, and Y329F in OsGID1 revealed that these residue mutations decreased the binding activity to GA_4 [24]. Therefore, these conserved residues in OsGID1 were indicated to be important for the GA binding

affinity. To the best of our knowledge, there are no studies on the residue mutation in AtGID1A and their influence on the binding affinity with GAs.

With the crystal structure determination on the GA receptor AtGID1A, therefore, the SAR of the GA molecule were investigated by molecular docking and dynamics simulations to clarify the key pharmacophore character of active and inactive GAs. Meanwhile, the site-directed mutant modeling study on some key residues in AtGID1A was performed with the aid of the molecular simulation technology to explore the importance of the conserved residues in AtGID1A to recognize the active GA. These studies will be favorable to elucidate the molecular mechanism of GAs perception to the receptor AtGID1A and further discover some novel GA-like active molecules based on the receptor AtGID1A structure.

Materials and methods

Materials

As shown in Fig. 1 [7], the active and inactive GA molecules GA_3/GA_3Me, GA_7/GA_7Me, GA_4/3-epi-GA_4/GA_9 and GA_1 were selected to investigate the SAR of the GA compounds and their molecular recognition mechanism based on the target AtGID1A. According to the pharmacophore group characteristics, these GA molecules were divided into three sample groups, namely, 6-COOH (a), 3-OH (b), and 13-OH (c). The IC_{50} of all of the selected GAs were shown in Table 1, which were determined by observing the competitive inhibition affinity between the tritiated 16,17-dihydro-GA_4 and various bioactive GAs binding to the receptor [25, 26]. The structure of AtGID1A was retrieved from the RCSB Protein Data Bank (PDB: 2ZSH) [20].

Methods

Molecular docking

Molecular docking calculations were carried out using a Surflex-dock algorithm in the Sybyl7.3 software package on the Linux platform [27]. The suitable putative pose of ligand called protomol was generated rapidly by means of the Hammerhead scoring function with a surface-based molecular similarity method [28–30]. In our study, the automatic mode was adopted to generate an ideal protomol in the active site of the receptor. All hydrogen atoms and MMFF94 charges were added to the receptor in our molecular simulation. The docked conformations of all GA molecules were generated and optimized based on the GA_3 conformation extracted from the reported crystal complex GA_3-AtGID1A-DELLA. A series of AtGID1A mutants were produced using the Biopolymer module and their energy minimization were performed by the MMFF94 force field and MMFF94 charges. All of the other parameters were defined as their default ones.

Molecular dynamic simulations

Molecular dynamics (MD) simulations on the complex GA_3-GID1A-DELLA and its mutants were performed using the GROMACS4.0.5 package [31]. The G43a1 force field was used to calculate the protein energy. The topology files and force field parameters of GA_3 were generated using the PRODRG program [32]. The whole complex was solvated with explicit solvent SPC water and was neutralized by adding 10 NA+ions to replace the corresponding water molecules. The steepest descent and conjugated gradient methods were used for the energy minimization of each system. The reference temperature was fixed at 300 K and all bonds were constrained with LINCS [33]. A long-range electrostatics

Table 1 Binding scores and bioactivity IC_{50} of different GAs for the receptor

GAs		Score	ΔG (kcal mol^{-1})	IC_{50} (M)	pIC_{50}	Ref.
6-COOH	GA_3	10.63	−14.44	5×10^{-6} (3×10^{-5})	5.30 (4.52)	a (b)
	GA_3Me	5.46	−7.42	$>5\times10^{-4}$	<3.30	a
	GA_7	10.92	−14.84	5×10^{-8}	7.30	a
	GA_7Me	5.99	−8.14	$>5\times10^{-4}$	<3.30	a
	6-epi-GA_3	8.14	−11.06	–	–	–
	6-nor-GA_3	8.21	−11.16	–	–	–
3-OH	GA_4	11.29	−15.34	3×10^{-7}(5×10^{-8})	6.52(7.30)	b(a)
	GA_9	9.21	−12.51	$>3\times10^{-4}$(5×10^{-5})	3.52(4.30)	b(a)
	3-epi-GA_4	8.85	−12.02	$>3\times10^{-4}$(5×10^{-4})	3.52(3.30)	b(a)
13-OH	GA_7	10.92	−14.84	5×10^{-8}	7.30	a
	GA_3	10.63	−14.44	3×10^{-5}(5×10^{-6})	4.52(5.30)	b(a)
	GA_4	11.29	−15.34	3×10^{-7}(5×10^{-8})	6.52(7.30)	b(a)
	GA_1	10.92	−14.84	3×10^{-5}(5×10^{-6})	4.52(5.30)	b(a)

a. IC_{50} of cucumber hypocotyls GBP for different GA molecules (Yalpani et al. [26])

b. IC_{50} of AtGID1A for different GA molecules in *Arabidopsis* (Nakajima et al. [16])

was handled using PME during the whole simulation process [34]. The energy minimization of the whole systems was first subjected to a position restrained simulations for 100 ps. Then, a full molecular dynamics simulation was performed for 10 ns on each system with the NPT canonical ensemble. The coordinates of the whole system were written to the trajectory file at 2 ps intervals.

Results and discussion

The SAR study of GA based on the receptor AtGID1A

All of the selected GA molecules were docked into the receptor AtGID1A to explore their molecular interaction mode and clarify the SAR of GAs. As shown in Fig. 2, the docked results on GA_3 indicated that the hydrogen bond interactions and the hydrophobic effect both cooperated for the signal molecule GA recognition of the binding pocket of AtGID1A. The docked conformation of GA_3 was overlapped well in the crystal structure GA_3-AtGID1A-DELLA with only a 0.52 Å root mean squared deviation (RMSD). To further confirm the docked system stability, MD simulations on the docked system with GA_3 and its crystal structure were performed in a water environment by the GROMACS program. Based on Fig. 3a, the simulation results indicated that the RMSD value of the C_α backbone in the docking system was almost the same to that of the crystal system with about 2.2 Å. It was clear that the docked system GA_3-AtGID1A-DELLA was very stable verified by the MD simulations. The docked results demonstrated that the 3-OH group of GA_3 formed a

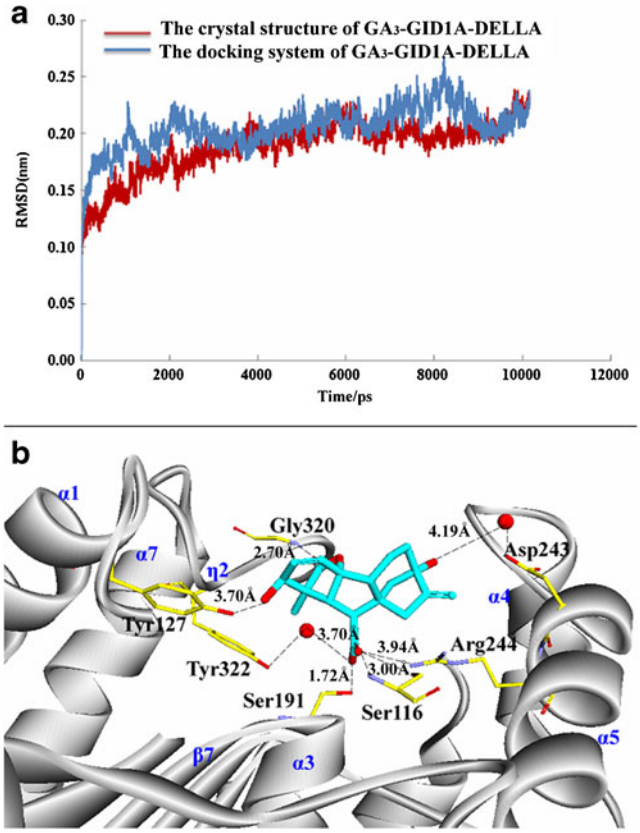

Fig. 3 The molecular dynamics results of GA_3-GID1A-DELLA complex. **a** The RMSD value of the docked (blue) and crystal complex (*red*) obtained during the 10 ns MD simulations. **b** The binding mode of GA_3 with AtGID1A after the MD simulations

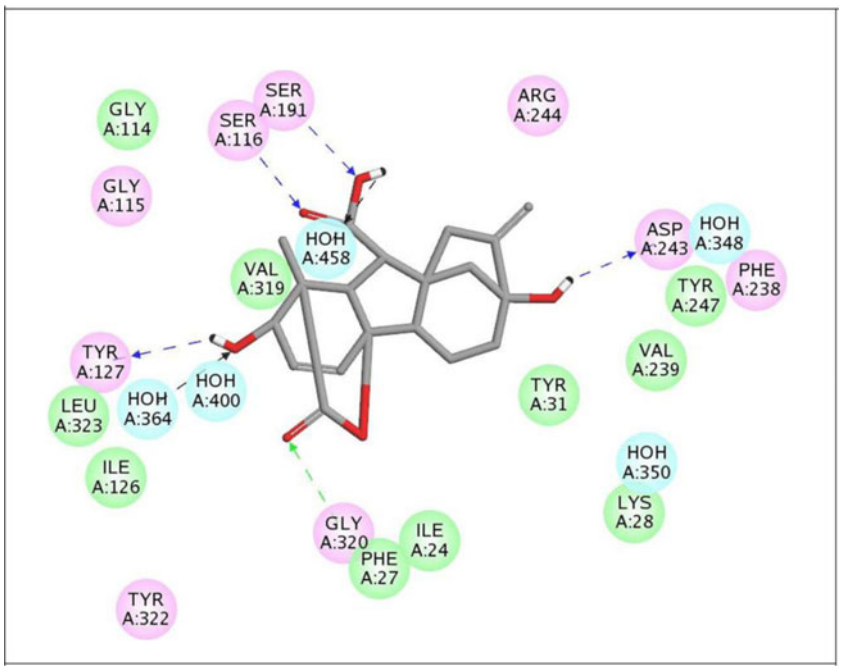

Fig. 2 The 2D diagram of the molecular interactions between GA_3 and the receptor AtGID1A. The *pink circles* represent residues involved in the hydrogen bond interactions. The *green circles* represent residues involved in the hydrophobic interactions. Water molecules are represented by the aquamarine circles. Hydrogen bond interactions with the water molecules, amino acid main-chains, and amino acid side-chains are represented by the *black*, *green*, and *blue dashed lines*, respectively, directed toward the electron donor

direct hydrogen bond to the phenolic hydroxyl group of Tyr127 with a distance of 2.95 Å (O/O) in Fig. 2. This corresponding distance became longer with 3.70 Å (O/O) after the simulations but was within the range of the hydrogen bond in Fig. 3b. The 6-COOH group of GA_3 not only formed a strong salt bridge interaction with a distance 2.61 Å to the guanidinium group of Arg244 in the receptor, but it was also involved in the multiple hydrogen bond network with residues Ser116, Ser191 and Tyr322 of AtGID1A in Fig. 2. It is important to note that these key interactions between the 6-COOH of GA_3 and AtGID1A were conserved even after 10 ns MD simulations in Fig. 3b. It was obvious that these interactions between the 3-OH, 6-COOH group of GA_3 and AtGID1A both contributed to the binding affinity between GA_3 and the receptor GID1A, which was in agreement with the previously reported results on the crystal structure [20]. However, as indicated in Fig. 2, the 13-OH group of GA_3 formed a direct hydrogen bond with a negatively charged residue Asp243 instead of Phe238 in the crystal structure due to the hydrogen atom orientation changed in the 13-OH group of GA_3 [20]. After the simulations, this hydrogen bond interaction still existed but was changed to an indirect water bridge via HOH350 in the Fig. 3b. In addition, some important hydrophobic interactions were found between the alkyl group and the carbocyclic ring of GA_3 and the surrounding residues Ile126, Leu323, Val319, Val239, Ile24, Phe27, and Tyr31 of AtGID1A in Fig. 2, which was in agreement with the results reported by Murase et al. [20].

The importance of the 6β-COOH group for the GA binding affinity

GA_3 and GA_7 were docked into the binding pocket of the receptor AtGID1A to explore the effect of the 6-COOH group on the GA binding ability to the receptor. The 6-COOH group of GA_7 was found to be similar to that of GA_3 and anchors the whole hormone molecule at the bottom of the binding pocket of AtGID1A by forming some multiple hydrogen bonds with residues Ser116 and Ser191 and with Tyr322 via two water molecules H_2O400 and H_2O458. More importantly, the negative charge on the 6-COOH group of GA_7 was neutralized by the formation of a salt bridge with residue Arg244 of AtGID1A. These docked results were consistent with the molecular interaction character described above between the 6-COOH group of GA_3 and the receptor AtGID1A. It was recently reported that the methylation of the 6-COOH group in GA would reduce the GA binding affinity to GID1A [16, 35]. To further clarify the influence of the 6-COOH methylation on the GA binding activity, GA_3Me and GA_7Me were also docked into the binding pocket of the protein AtGID1A. As indicated in Table 1, the calculated results revealed that the scores were significantly decreased from 10.63 for GA_3 to 5.46 for GA_3Me. A similar change was observed for GA_7 and GA_7Me with a score of 10.92 and 5.99, respectively. These molecular simulation scores were in good agreement with their IC_{50} values obtained from the GA-binding assay in vitro. As shown in Fig. 4a, this was because the indirect water bridge interaction surrounding the 6-COOH group in both GA_3Me and GA_7Me disappeared completely compared with GA_3 and GA_7. Meanwhile, the multiple hydrogen bond interactions were weakened because of the methylation of the

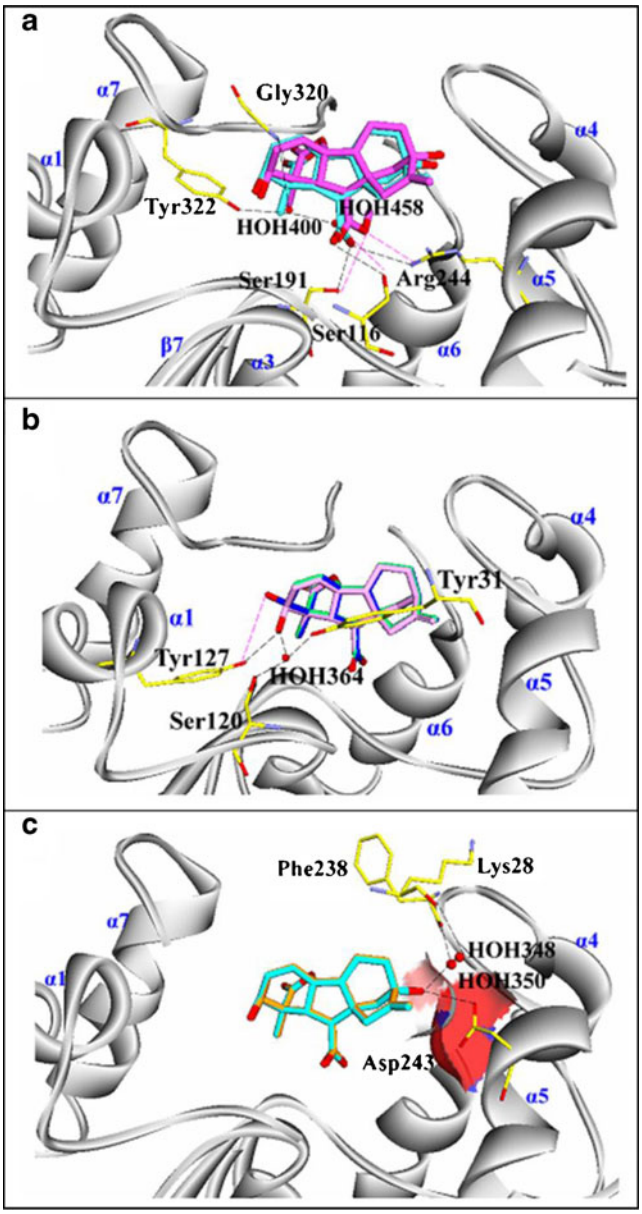

Fig. 4 The hydrogen bond interactions between GAs and AtGID1A. **a** The hydrogen bond interaction between the 6-COOH group of GA_3 (*cyan*), GA_3Me (*magenta*) and AtGID1A. The hydrogen bond between GA_3, GA_3Me and AtGID1A are represented by the *black* and *magenta broken lines*, respectively; **b** The hydrogen bond interactions between the 3-OH group of GA_4 (*pink*), GA_9 (green), 3-epi-GA_4 (*blue*) and the key residues of the binding pocket of AtGID1A; **c** The hydrogen bond and electrostatic interactions between 13-OH of GA_3 (cyan), GA_7 (orange) and AtGID1A

6-COOH group in GA. The distance of the hydrogen bond between Ser191 of AtGID1A and 6-COOH of GA was increased from 1.84 Å for GA_3 to 2.57 Å for GA_3Me. At the same time, the distance between the side-chain of Ser116 in AtGID1A and 6-COOH of GA_3 was also increased from 2.58 to 3.01 Å. More importantly, the strong salt bridge binding interaction was replaced by a weak hydrogen bond interaction between 6-COOH of GA and the guanidinium group of Arg244 in AtGID1A with a distances of 2.53 Å and 2.54 Å for GA_3Me and GA_7Me, respectively. It was clear that the 6-COOH group in GA was indicated to be absolutely necessary to recognize and binding to its receptor AtGID1A.

To probe the configuration of the 6-COOH group effect on the GA binding affinity, 6-epi-GA_3 with a α-configuration 6-COOH group and 6-nor-GA_3 without 6-COOH were also docked into the receptor AtGID1A. As shown in Table 1, the calculated results showed that the scores of 6-epi-GA_3 and 6-nor-GA_3 were remarkably decreased to 8.14 and 8.21, respectively, compared with 10.63 for GA_3 with a β-configuration 6-COOH group. It was because even the configuration change of 6-COOH in GA also led to the disappearances of its salt bridge interaction with the guanidinium group of Arg244 and its hydrogen bond interaction of Ser116 in AtGID1A. Therefore, 6-epi-GA_3 was found to be similar to 6-nor-GA_3 with almost the same binding score to the receptor AtGID1A. Moreover, the MD simulations further indicated that the β-configuration of 6-COOH in GA_3 was stable in the dynamic binding process to the receptor AtGID1A, which was in good agreement with the previous study [10]. Therefore, not only the 6-COOH group but also its β-configuration of GA was indicated to be vital for GA binding to the receptor AtGID1A. These multiple hydrogen bond networks between 6β-COOH of GA and AtGID1A were also found in the GA binding to the other receptor OsGID1 [21]. It was proposed that the 6β-COOH group of GA should be conserved to recognize not only AtGID1A but also OsGID1.

The 3β-OH group was necessary for the GA bioactivity

It was well known that the 3-OH group of GA was necessary for its bioactivity [8], but there was no molecular mechanism explanation from the point of view of the receptor AtGID1A structure. Therefore, GA_4, GA_9, and 3-epi-GA_4 were docked into the receptor AtGID1A. As shown in Table 1, the binding order of GA was GA_4>GA_9>3-epi-GA_4 with a score of 11.29, 9.21 and 8.85, respectively. The different hydrogen bond action modes between the 3-OH group of GA_4, GA_9, 3-epi-GA_4 and AtGID1A were shown in Fig. 4b. The obtained results indicated that the hydrogen bond networks surrounding GA_4 were similar to those of the previously reported crystal structure of GA_4-AtGID1A [20]. Our MD studies on the docked system GA_4-AtGID1A further verified that these hydrogen bonds were conserved even after the 10 ns simulations. The 3β-OH group of GA_4 was found to shape a hydrogen bond to the phenolic hydroxyl group of Tyr127 of AtGID1A with a distance of 2.95 Å (O/O) in our study, which was very close to the corresponding distance of 2.75 Å reported in the crystal structure [20]. In addition, the 3β-OH group in GA_4 was also found to form two hydrogen bonds via H_2O364 with the hydroxyl group of the Ser120 and Tyr31 of AtGID1A. In contrast to GA_4 with the 3β-OH group, this distance between the 3α-OH group of 3-epi-GA_4 and the phenolic hydroxyl group of Tyr127 in AtGID1A was obviously increased to 3.24 Å, which led to a lower binding score with 8.85 to the receptor. GA_9 without 3-OH group was indicated as almost the same lower score with 9.21 to AtGID1A because of the disappearance of these hydrogen bond interactions. The reported GA-binding assay in vitro also confirmed that the IC_{50} value of GA_4 with the 3β-OH group was the highest one among these three molecules mentioned above [16]. Therefore, the 3-OH group and especially its β-orientation were necessary for the GA high activity due to its multiple hydrogen bond interactions with the important residues Tyr127, Ser120, and Tyr31 in the binding pocket of AtGID1A.

The 13-OH group also modulated the GA binding affinity

It was well known that GA_3 and GA_7 were considered as the high activity of GA widely applied in modern agriculture. However, the GA_7 binding affinity to GBP was reported to be higher with two orders of magnitude than that of GA_3 [16]. To clarify the reason from the point of view of the receptor, GA_3 and GA_1 with the 13-OH group were docked into the receptor At-GID1A to investigate the influence of the 13-OH group on the GA bioactivity compared with GA_7 and GA_4 without the 13-OH group. As shown in Fig. 4c, the 13-OH group of GA_3 formed a hydrogen bond with Asp243 (O/O) with a distance of 2.95 Å in the molecular docking model, but not with Phe238 reported in the crystal complex. It was obvious that the orientation of the 13-OH group of GA_3 was free to change and interact with the adjacent other residues of the binding pocket of AtGID1A. The new hydrogen bond interaction between the 13-OH group of GA_3 and the carboxyl group of Asp243 was confirmed to still exist after MD simulations and was only turned into an indirect water bridge interaction via HOH350 in Fig. 3b. As shown in Table 1, the calculated score of 10.63 for GA_3 was almost the same as that of GA_7 with 10.92 in our study. However, the IC_{50} value of GA_3 was previously measured to be 100 times lower than GA_7 in the GA-binding assay in vitro. Previous studies explained that there was a strong repulsive interaction between the electronegative 13-OH group of GA_3 and the surrounding negatively charged residue Asp243 of GID1A, which led to a weaker binding affinity of GA_3 [20]. As shown in Fig. 4c, besides this repulsive effect found in our docking simulations, GA_3 with the 13-OH group was also indicated to form an additional hydrogen bond with Asp243 of AtGID1A.

Therefore, these two positive and negative interactions counteracted each other to show the same binding score to the receptor between GA_3 and GA_7. It was concluded that the 13-OH group of GA_3 was not essential to the binding affinity to the receptor AtGID1A.

The determination of the key residues significance of AtGID1A using the residue mutant modeling

The key residues involving Ser123, Ser198, Tyr134, Tyr329, Ser127, Phe27, Ile133 and Leu330 of OsGID1 were reported to be important for the GA binding based on the site-directed residue mutation experiment [21, 24]. However, there were no reports on some residue mutation studies of AtGID1A. To explore the importance of some homology residues in AtGID1A, a series of residue mutants in the AtGID1A binding cavity were modeled [21, 36] using the Biopolymer module of the Sybyl 7.3 program.

Arg244 and Tyr322 were important for the stability of the complex GA_3-AtGID1A

A series of variant experiments on Arg244 in AtGID1A were performed by the molecular docking and dynamics simulations to investigate the important role of the described above salt bridge interactions [20, 37]. As shown in Table 2, the GA docking scores were decreased by 2~3 orders of magnitude compared with wild-type (WT) AtGID1A when Arg244 were exchanged with other neutral and electronegative residues, such as Ala, Asp, and Glu. It was because some hydrogen bonds between the 6-COOH group of GA_3 and its surrounding other residues Ser116 and Ser191 became different when Arg244 was mutated to different electrical property residues. As shown in Fig. 5a, when Arg244 was mutated to Ala, the H atom of the main-chain hydroxyl group of Ser116 was found to become longer ~4.26 Å from the O of the carbonyl group of GA_3 and beyond the range of the hydrogen bond distance. At the same time, the other hydrogen bond interaction between the 6-COOH group of GA_3 and the side chain of Ser191 also becomes weaker with a distance increased from 2.82 Å to 3.41 Å (O/O). The MD simulations further validated that these two hydrogen bond interactions both became weaker accompanied with the residue Arg244 mutation. As indicated in Fig. 5a, the corresponding mutated residue in the mutant R244D and R244E formed a new hydrogen bond with its surrounding residue Trp248, which resulted in the disappearance of the above-mentioned salt bridge interaction and thus lowered the binding score with GA. However, a mutation of Arg244 by the same positively charged residue Lys resulted in almost the same docking score to the bioactive GAs except for GA_3 in Table 2. From Fig. 5a, the hydrogen bond interaction of the mutant R244K with GA_4, GA_7, GA_1 was found to be consistent with the WT. However, the mutant R244K caused an H orientation change in the 3-OH group of GA_3 and then broke its hydrogen bond with Tyr127, thus lowering the GA_3 binding score to AtGID1A. Therefore, the positively charged residue Arg244 in AtGID1A played an important role in the stability of the complex GA-AtGID1A due to the formation of a key salt bridge interaction with the 6-COOH group of GAs.

Based on our docking simulations, another residue mutation for Y322A was indicated to remarkably decrease the GA binding score by 3~4 orders of magnitude compared with that of WT AtGID1A, which was consistent with the previous reports that Y322A showed very little or no bioactivity for GAs [21]. As shown in Fig. 5b, the 6-COOH group of GAs showed some nonbeneficial orientations binding to the Y322A mutant model, which resulted in the lower binding scores to the receptor. The distance between the O in H_2O458 and the 6-COOH of GA_3 was beyond the range of the hydrogen bond interaction in the Y322A mutation, which was verified by the MD simulations. These results further confirmed that the hydroxyl group of the phenolic side-chain of Tyr322 played an important role in the stability of the complex GA-AtGID1A by an indirect hydrogen bond [24]. It was believed that this residue Tyr322 of AtGID1A was also necessary for perceiving the 6-COOH group of GA.

In a previous report, it was argued that the residue Ser116 of AtGID1A may not be necessary for binding GA, but it may be involved in another interaction between AtGID1A and the protein DELLA [24, 36]. As shown in Table 2, Ser116 of AtGID1A was mutated to Ala without a significant loss of the GA binding activity to be found. It was speculated that the residue Ser116 of AtGID1A might not be important for the GA binding because the adjacent residue Ser191 also exhibited a similar function by forming a hydrogen bond with the 6-COOH group of GA.

Tyr127 was necessary for the GA binding to AtGID1A

The residue Tyr134 in OsGID1 was found to be necessary for the GA binding due to its interaction with the 3β-OH group of GA [21, 24]. As discussed above in the GA SAR, the 3β-OH group of GA was found to be essential for the binding activity due to the formation of a hydrogen bond with the homology residue Tyr127 in AtGID1A. Therefore, two residue mutants were modeled to investigate the importance of the residue Tyr127 in AtGID1A for recognizing the 3β-OH group of GA. Ala and Phe mutations of Tyr127 resulted in the GA binding score decreased with 2~3 orders of magnitude compared with the WT. As shown in Fig. 5c, the hydrogen bond interaction between the 3β-OH group of GA_3 and the phenolic hydroxyl group of Tyr127 completely disappeared in the Y127 mutants of AtGID1A. However, the 3β-OH group of GA_3 was found to be very close to the carbonyl group of the other bottom residue Asp190 with ~2.00 Å and form a new hydrogen bond in these two mutants. It was believed that the ring fragment of GA_3 moved down and led to a much weaker

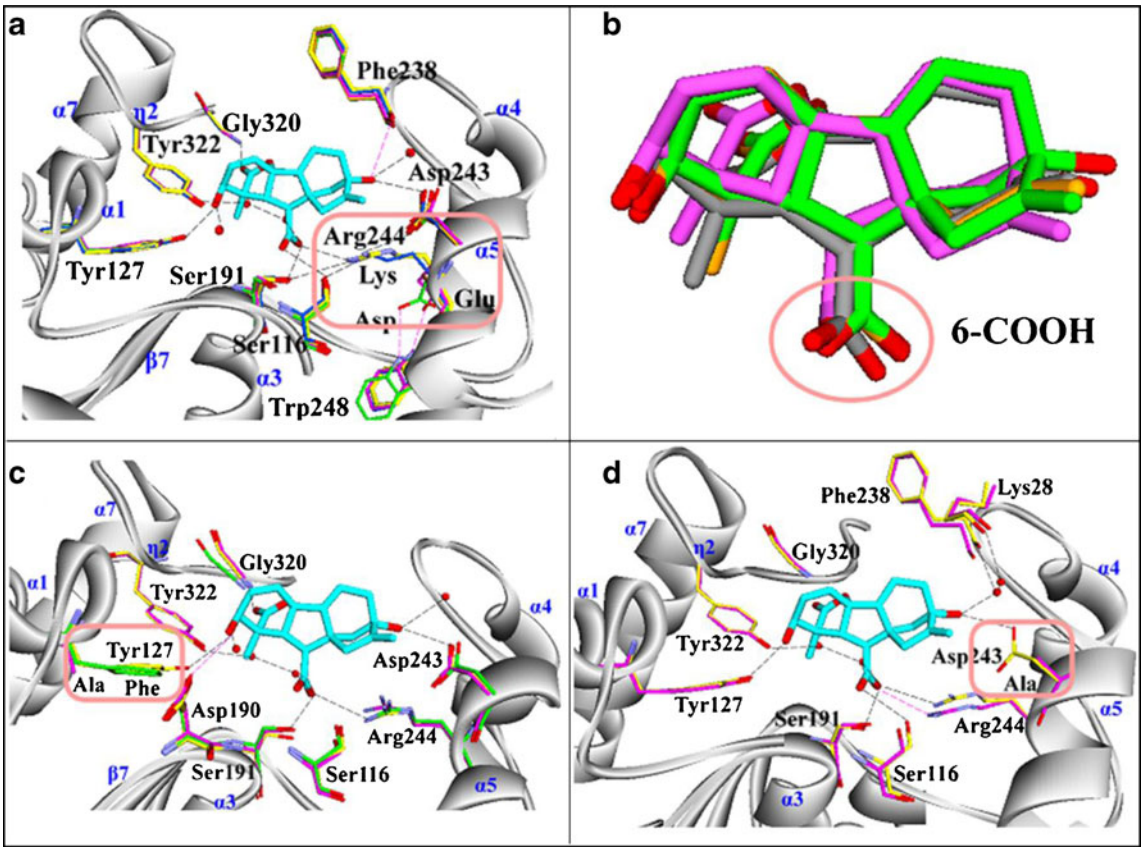

Fig. 5 The hydrogen bond interactions of GA_3 with WT and mutants of AtGID1A. **a** The hydrogen bond networks of GA_3 with WT (*yellow*) and the mutants R244A (*orange*), R244D (green), R244E (*magenta*), and R244K (*blue*) of AtGID1A; **b** Superposition of different GAs docked with the Y322A mutants (GA_3, GA_4, GA_7, and GA_1 are shown in gray, pink, orange, and green, respectively); **c** Superposition of the key residues of the mutants Y127A (*pink*), Y127F (*green*) and WT (*yellow*) AtGID1A; **d** The hydrogen bond interactions of GA_3 with WT (*yellow*) and mutant D243A (pink) of AtGID1A

Table 2 Binding scores and bioactivity of GAs to AtGID1A and mutants of the hydrogen bond residues

Mutants		Docking scores				Bioactivity (GA_4)	
		GA_4	GA_3	GA_7	GA_1	K_d (D)[c] of OsGID1/M	K_b(B)[d] of OsGID1/M^{-1}
WT		11.29	10.63	10.92	10.92	5×10^{-7}	$(5.2\pm0.3)\times10^{6}$
6-COOH	R244A	8.84	7.53	8.40	8.38	–	–
	R244D	8.79	7.96	8.35	8.27	–	–
	R244E	8.65	8.04	8.23	8.50	–	–
	R244K	11.39	8.82	10.81	11.08	–	–
	Y322A	7.64	6.52	7.51	7.07	–	–
	S116A	10.95	9.79	10.61	10.17	–	$(1.8\pm0.2)\times10^{6}$
3-OH	Y127A	7.87	7.82	7.94	7.63	–	–
	Y127F	8.34	8.20	8.40	8.25	–	–
	Y31A	10.80	10.24	10.75	10.47	–	–
	S120A	11.25	10.50	10.92	10.74	2×10^{-6}	–
13-OH	D243A	10.13	10.42	9.69	10.91	–	–

K_d: Dissociation constants; K_b: binding constants

[c] The K_d value was obtained by GA-binding assays in vitro (Shimada et al. [21])

[d] The K_b value was measured by isothermal titration calorimetry (Xiang et al. [24])

hydrophobic interaction between the whole GA molecule and the top residues in the binding pocket compared with the WT. Therefore, the score for GA_3 was calculated to be much lower in the Y127 mutants than that of the WT. In a word, all of the results revealed that residue Tyr127 of AtGID1A was necessary for the GA binding ability due to the formation of a unique and strong hydrogen bond interaction with the key 3β-OH group of GA.

The other two residues Tyr31 and Ser120 of AtGID1A both interacted indirectly with the 3β-OH group of GA_3 via H_2O364. There has been some debate regarding the importance of these two residues for the GAs recognition and binding to the receptor in rice. Shimada et al. suggested that the replacement of Tyr31 with Ala did not significantly decrease the GA binding activity, whereas the replacement of Ser127 with Ala resulted in very little activity to GA in rice [21]. However, Ueguchi-Tanaka determined that the residue Ser127 was not necessary for the GA binding using the Ala scanning experiment in rice [36]. Therefore, some mutations of these two homologous residues Tyr31 and Ser120 in *Arabidopsis thaliana* were performed to explore the role of Tyr31 and Ser120 in the recognition of GA. The results indicated that the docking scores of GA for Y31A and S120A variants were similar to the ones obtained for WT AtGID1A. The hydrogen bond indirectly formed via H_2O364 between Tyr31 and Ser120 of AtGID1A and the 3β-OH group of GA_3 disappeared because of these two residue mutations. The two residues Tyr31 and Ser120 were speculated to be insignificant for the GA-GID1A interaction in *Arabidopsis thaliana*, which was different from the corresponding conserved homologous residues of OsGID1.

D243A manifested no effect on the GA_3 binding activity

The 13-OH group of GA_3 formed a hydrogen bond with Asp243 of AtGID1A, which was mentioned above in our docking simulation, but did not form a hydrogen bond with the other residue Phe238, which was considered a key residue for recognizing the 13-OH group of GA in the crystal structure GA_3-AtGID1A [20]. To explore the significance of the residue Asp243 for the GA binding, mutation of Asp243 with Ala was performed in our study. As shown in Table 2, the mutation of Asp243 resulted in very similar docking scores for the 13-hydroxylated GA_1 and GA_3 but decreased by an order of magnitude for the non-13-hydroxylated GA_4 and GA_7 compared with the WT. As shown in Fig. 5d, the negatively charged residue Asp243 was mutated to a neutral Ala, which decreased the repulsive effect between the electronegative 6-COOH group in GA_3 and the negatively charged residue Asp243 in AtGID1A. More importantly, the 13-OH group of GA_3 formed a strong hydrogen bond to H_2O350 with a distance of 2.87 Å (O/O) in the mutation compared with the crystal complex. Therefore, the 13-hydroxylated GA_1 and GA_3 exhibited a similar binding score between the D243A mutation and the WT of AtGID1A. However, in the GA_4 binding model, the mutation of Asp243 influenced the salt bridge between its adjacent residue Arg244 and the 6-COOH group of GA_4 and thus led this interaction to become weaker with a distance increase from 3.38 Å to 3.63 Å (O/N). Therefore, the D243A model exhibited a ten times lower affinity for the non-13- hydroxylated GA_4 and GA_7 compared with the WT. In a word, the D243A mutant of AtGID1A demonstrated a different change of the binding score for GA_3 with the 13-OH group and for GA_4 without the 13-OH group. These results indicated that the residue D243 of AtGID1A might not be a necessary residue for the GA binding to the receptor.

Mutant of residues involved in the hydrophobic interaction

In our binding model between GA and AtGID1A, the hydrophilic carboxyl group of GA pointed toward the bottom of the receptor and its hydrophobic aliphatic rings were located at

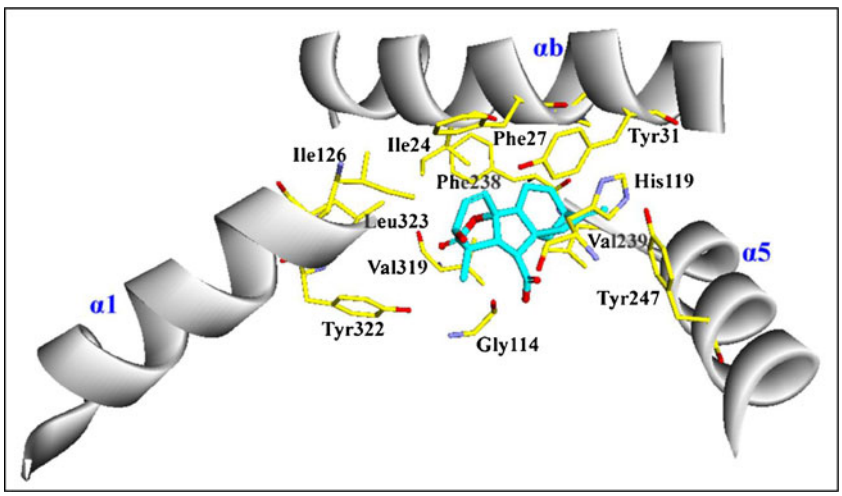

Fig. 6 The hydrophobic effect map of GA_3 and AtGID1A

the entrance of the binding pocket of the receptor, which was in agreement with the reported crystal structure GA_3-AtGID1A-DELLA [20]. As shown in Fig. 6, a 'hat' hydrophobic region surrounded GA_3 by some important hydrophobic residues of AtGID1A. The N-terminal extension helices of AtGID1A were projected by some residues, i.e., Ile24, Phe27, Lys28, and Tyr31 and recognized the fragment of the GA_3-fused rings by forming some unique 'sandwich' type T-π or π-π interactions. The other residues, i.e., Ile126, Leu323, and Val319 of AtGID1A were found to contact C2, C3, and the γ-lactone ring between C4 and C10 of GA_3, respectively. In addition, some conserved residues Val239 and Tyr247 were located in the other helix of AtGID1A and held the five-membered ring between C13 and C16 of GA_3. It was obvious that the hydrophobic interactions were very important for the location and orientation of the whole GA molecule in the binding pocket of the receptor AtGID1A [20].

Except for the active GA_3, inactive GA_{37} with the 20-C ring was also docked into the WT and the mutants of AtGID1A to explore the role of these hydrophobic residues in the binding of GA-AtGID1A. As shown in Table 3, the mutant V319A exhibited a higher binding score for GA_{37} with the 20-C ring and GA_3 with the 19-C ring. It was because GA_{37} had a larger γ-lactone ring and better contacted the short side chain residue Ala in the mutant V319A of AtGID1A via some hydrophobic interactions. Val in the GID1 proteins was evolved from His in the homology HSL catalytic triad and this His residue was confirmed previously to be better adapted to the binding of active GA [38]. Therefore, residue Val319 of AtGID1A was predicted to be important as a hydrophobic residue for recognizing the surface of the γ-lactone ring of GA, as reported in the crystal structure GA_3-AtGID1A [21]. As indicated in Table 3, the replacements of residues F27 and Y31 in the N-terminal lid of AtGID1A were not accompanied by a significant decline in the GA binding score, which was in agreement with the results reported by Shimada et al. [20]. These mutation results revealed that the two residues F27 and Y31 were not necessary in the binding of GA, but probably involved in the interaction with the DELLA protein [23]. In addition, other mutations, i.e., V239A, Y247A and L323I also had no effect on the score of GA in Table 3 and might not be important for binding the GA molecule. In a word, the hydrophobic interaction between GA and AtGID1A was found to be necessary to locate and recognize the GA molecule in the binding site of AtGID1A, but almost had no influence on the GA binding affinity to the receptor.

Table 3 Docking scores and bioactivity of GAs to AtGID1A and the mutants of the hydrophobic residues

Mutants	Docking scores			K_d (D)[c] of OsGID1/M (GA_4)
	GA_3	GA_4	GA_{37}	
WT	10.63	11.29	10.95	5×10^{-7}
V319A	10.19	11.07	11.09	–
F27L	10.82	11.64	11.60	5×10^{-7}
Y31A	10.24	10.80	10.31	–
L323I	10.37	11.31	11.20	5×10^{-7}
V239A	10.58	11.25	11.13	–
Y247A	9.11	11.17	11.19	–

[c] The K_d value was obtained by in vitro GA-binding assays (Shimada et al. 2008)

Conclusions

The molecular recognition mode between GA and its receptor AtGID1A was studied by molecular docking and dynamics simulations. The obtained results indicated that the 6-COOH group of GA and its β-configuration should play a crucial role in the binding process of the receptor due to its formation of a strong salt bridge interaction with the guanidinium group of Arg244 in AtGID1A. Moreover, the multiple hydrogen bonding networks between the 6β-COOH group in GA and some key binding residues of AtGID1A were also indicated to be important for the GA recognition and binding to the receptor. These included the formation of several direct hydrogen bond interactions of the 6β-COOH group in GA with the hydroxyl group of Ser191 and Ser116 in AtGID1A and some indirect water bridge interactions with the phenolic hydroxyl group of Tyr322 in AtGID1A. More importantly, the salt bridge and multiple hydrogen bond interactions between 6β-COOH of GA_3 and GID1A were both found to be stable during the 10 ns MD simulations. In addition, a unique hydrogen bond interaction between the 3β-OH group of GA and a key residue Tyr127 of the binding pocket of the receptor was found to be the most influential factor in the GA binding to AtGID1A. The 13-OH group of GA was shown to be not important to the GA binding to AtGID1A because the electrostatic repulsion and an alternative hydrogen bond between the 13-OH group of GA_3 and the surrounding residue Asp243 of GID1A counteracted each other. The AtGID1A site-directed mutant modeling study demonstrated that the mutations of some key residues, i.e., Arg244, Tyr322 and Tyr127 resulted in the reduction of the bioactive GA binding score. In addition, the hydrophobic interactions between GA and AtGID1A were found to be important for the bioactive GA location and orientation in the receptor-binding pocket. Some hydrophobic residue mutation results further confirmed that Val319 influenced the receptor binding to the γ-lactone ring of GA. In summary, a novel molecular recognition mechanism was determined in which the signaling molecule GA interacted with the receptor AtGID1A via an important salt bridge and multiple hydrogen bond interactions and hydrophobic interactions. This study provides significant guidance for the GA

structural modification and the GA-GID1A signal pathway recognition.

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