Structural Basis for Gibberellin Recognition by Its Receptor GID1

Gibberellins are a large family of tetracyclic diterpenoid plant hormones that induce a wide range of plant growth responses including seed germination, stem elongation, leaf expansion, pollen maturation and induction of flowering. Gibberellins were first discovered by a Japanese plant pathologist, Eiichi Kurosawa, from the pathogenic fungus *Gibberella fujikuroi* in 1926 [1]. Kurosawa was working on rice plant diseases caused by this fungus, bakanae (foolish seedling), and found that some metabolite of this fungus might be responsible for the stimulated seedling growth. In 1935, an agricultural chemist, Teijiro Yabuta, isolated a crystalline active material that he named gibberellin [2]. In the 1950s, it was identified as natural components of noninfected plants and recognized as a plant hormone. Since then, some 136 different kinds of structurally similar gibberellins have been identified, although not all of them are biologically active as hormones in plants. Only a few gibberellins, such as GA_1, GA_3, and GA_4 (Fig. 1), are bioactive hormones in plant [3]. In 2005, a research group led by Makoto Matsuoka discovered a nuclear receptor of gibberellins, gibberellin-insensitive dwarf1 (GID1), from rice [4]. Unexpectedly, GID1 has sequence similarity to hormone-sensitive lipases (HSLs), which are enzymes involved in lipid metabolism. This fact raises the following two questions. (i) How different are their tertiary structures? (ii) How does GID1 manage to specifically interact with bioactive gibberellins while maintaining the conserved structure of the HSL family?

We investigated the structural basis of gibberellin recognition in the rice, *Oryza sativa* GID1 (OsGID1) and revealed, on the basis of the structure, how GID1 has acquired the gibberellin reception ability that is lacking in HSLs [5]. The crystal structures of OsGID1 complexed with GA_4 or GA_3 have been solved by the Hg-SAD method and refined at 1.9 Å resolution. X-ray diffraction data for phasing and refinement were collected at beamline **BL41XU**. The structures revealed an α/β-hydrolase fold resembling that of HSLs (Fig. 2(a)). The gibberellin-binding cavity extends above Ser198 that corresponds to the catalytic residue of HSLs (Fig. 2(b)). Aside from having this catalytic Ser, the residues corresponding to the catalytic triad of HSLs, namely, Ser, His, Asp, are similarly arranged except for the replacement of His with Val in GID1 (Fig. 2(b)). The most notable difference between GID1 and HSL structures appears in the function of an amino-terminal lid (Fig. 2(a)). In HSLs, the lid covers the substrate binding site and opens upon substrate binding. In contrast, in GID1, the lid is open in the absence of the substrate

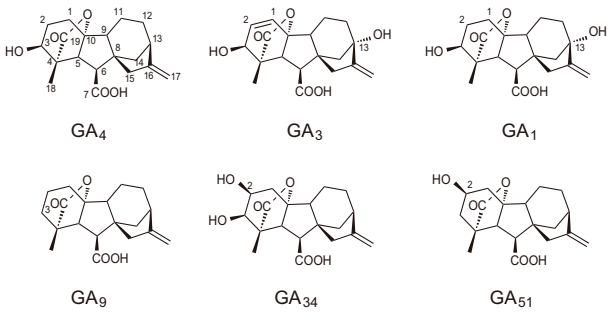

Fig. 1. Chemical structures of the typical gibberellins. GA_4, GA_3, and GA_1 are biologically active gibberellins in higher plants. The subscript numbers for GA are the chronological order of their identification.

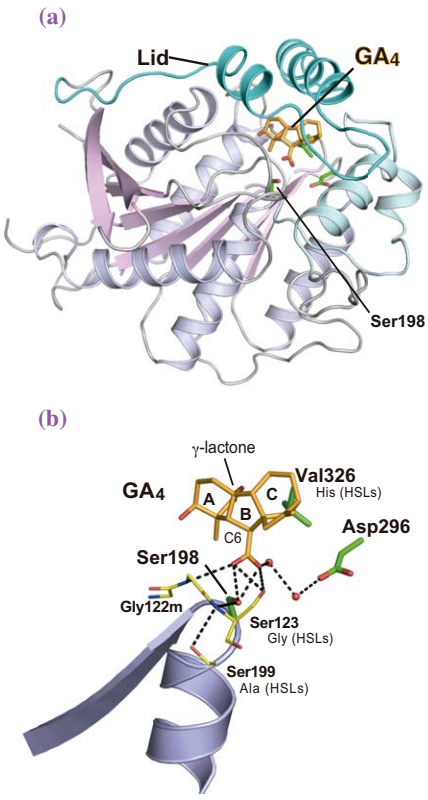

Fig. 2. Crystal structures of rice GID1 complexed with GA_4. **(a)** GA_4—GID1 complex structure shown as a ribbon diagram. **(b)** GID1 structure around the GA binding site including corresponding residues for the catalytic triad of HSLs, Ser198, Asp296, and Val326. The residues of GID1 are indicated and the corresponding residues of an HSL(AeCXE1) are in parentheses. Water molecules are shown as red spheres. Reprinted from Ref. [5] with some modifications.

and closes upon gibberellin-binding. The bound gibberellin is held in place by a network of hydrogen bonds as well as many nonpolar interactions that are considered to facilitate the closing of the lid over the binding pocket, in which the molecular shape of the gibberellin structure is firmly recognized.

To verify the structural assignment, we produced 17 OsGID1 mutants in which residues assigned to be involved in gibberellin-binding were replaced with Ala, and examined their gibberellin-binding activity *in vitro* (Fig. 3). The mutants showed little or no activity, confirming their critical role in gibberellin recognition. Most of the residues important for gibberellin-binding are conserved within plant GID1s but not in HSLs. Interestingly, GID1 proteins in the lycophyte, *Selaginella moellendorffii* (SmGID1s), contain nonconserved residues (Fig. 3) and as a result have lower affinity and specificity for certain gibberellins. We hypothesized that such amino acid replacements in SmGID1s may have led to their lower affinity and specificity for certain gibberellins. To examine this hypothesis, we exchanged the amino acid residues of OsGID1 with the corresponding residues of SmGID1s. We found that some mutated proteins had lower affinity for the biologically active gibberellin, GA_4, but were more accommodating of its 2-hydroxy derivative, such as GA_{34} (Fig. 1). These observations indicated that GID1 evolved from HSL through the loss of its catalytic function and alteration of the substrate binding pocket to increase the affinity and specificity for bioactive gibberellins.

The structure determination of GID1 allows us to design more effective and useful gibberellin agonists and antagonists for agriculture. These compounds may contribute to solving the global issues related to food and biofuel productions.

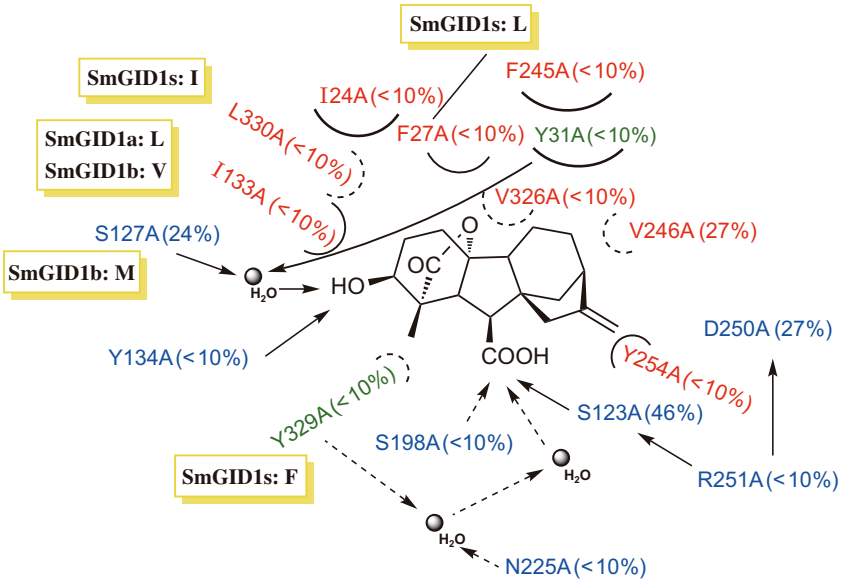

Fig. 3. GA binding activity of mutagenized rice GID1(OsGID1). Binding activities of mutants and their interaction sites with GA_4, relative to the native OsGID1. Polar and nonpolar interactions are indicated by arrows and circles, respectively. The interactions occurring behind GA are shown as broken lines. Mutants related with polar, nonpolar, and both interactions are shown in blue, red, and green, respectively. The amino acid residues corresponding to those in lycophyte GID1s (SmGID1s: both SmGID1a and SmGID1b) are in yellow boxes. Reprinted from Ref. [5] with some modifications.

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References

[1] E. Kurosawa: Nat. Hist. Soc. Formosa **16** (1926) 213.
[2] T. Yabuta: Nogyo-oyobi-Engei **10** (1935) 17.
[3] P.J. Davis: Ed. "Plant Hormones: Biosynthesis, Signal Transduction, Action!" - Kluwer, Dordrecht, 2004.
[4] M. Ueguchi-Tanaka *et al.*: Nature **437** (2005) 693.
[5] *A. Shimada, M. Ueguchi-Tanaka, T. Nakatsu, M. Nakajima, Y. Naoe, H. Ohmiya, H. Kato and M. Matsuoka: Nature **456** (2008) 520.*