Plant UDP-Arabinopyranose Mutase
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Teruko Konishi1,* and Tadashi Ishii2

1Faculty of Agriculture, University of the Ryukyus
(I, Senbaru, Nishihara, Okinawa 903–0213, Japan)
2Forestry and Forest Products Research Institute
(I, Matsunosato, Tsukuba 305–8687, Japan)

Abstract: Plant cell walls undergo dynamic changes during plant growth and development. Although the cell wall remodeling is an essential feature of plant growth and development, the biosynthesis mechanisms are poorly understood. Arabinan is a pectic polysaccharide which is linked by arabinofuranosyl (Araf) residues with α-(1,5) linkage. Arabinofuranosyl residues are a quantifiably important constituent of plant primary and secondary cell walls. Plants use UDP-arabinofuranose (UDP-Arap) to synthesize Araf regions of the polysaccharides containing Araf residues including proteoglycans and glycoproteins. However, it is unknown how UDP-Arapt is synthesized in plant cells. We succeeded to, for the first time, determine UDP-arabinopyranose mutase (UAM) activity and clone the gene of the enzyme from rice seedlings. UAM catalyzed the interconversion of UDP-arabinopyranose (UDP-Arap) to UDP-Araf. Microbial UDP-galactose mutases require reduced FAD for activity, however, the plant UAM do not require the cofactor. Thus, the plant mutase must have different catalytic mechanism. Three proteins were identified from partial amino acid sequence of UAM, which are encoded by Os03g40270, Os04g56520 and Os07g41360. These proteins have more than 80% sequence identity with reversibly glycosylated polypeptide. UAM genes are present in Chlamydomonas, Physcomitrella and pine, suggesting that UAM is widespread in green plants.

Key words: UDP-arabinofuranose, mutase, arabinan, reversibly glycosylated polypeptide, cell wall

Plant cell walls regulate cell growth and provide structural integrity and mechanical support for the plant. The plant cell wall is also important for renewable source of energy and as dietary fiber for humans. The structure of cell wall polysaccharides is mainly composed by cellulose, hemicelluloses and pectin. Pectin is a quantitatively major component in the primary walls and the middle lamella of dicots and nongraminaceous monocots. Pectin consists of 5 polysaccharides, homogalacturonan, rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), xylogalacturonan and apio galacturonan. RG-I contains arabinan, galactan and arabinogalactan side chains. Arabinan consists of linear (1,5)-linked α-L-arabinofuranosyl (Araf) residues that are substituted at O-3 or O-2 with Araf residues. However, the biosynthesis mechanism of arabinan has been unknown. Polysaccharides are synthesized by glycan synthases and glycosyltransferases from nucleotide sugar such like UDP-glucose for cellulose or xyloglucan, and UDP-xylene for xylan or xyloglucan. Thus, UDP-arabinopyranose (Arapt) which is only detectable nucleotide sugar containing arabinose in plant has been believed as a donor substrate for arabinosyltransferase. However, Nunan and Scheller9 showed that mung bean hypocotyls contain arabinosyltransferase activity that transferred Arap from UDP-Arap onto exogenous arabinoligosaccharides. Subsequently, Ishii et al.72 reported that the transferred Arap residue was β-linked to O-3 of the nonreducing terminal Araf residue. These results suggested that UDP-Arap is not the donor substrate for the biosynthesis of α-linked arabinan. Lately, we succeeded arabinan biosynthesis in vitro by using Golgi membranes from mung bean in the presence of UDP-AraA as a donor substrate and 2-aminobenzamide (2-AB)-labeled (1,5)-α-L-arabino-oligosaccharides as acceptors.7 The result in the experiment showed that the precursor of arabinan is UDP-Araf but not UDP-Arap.

There have been no reports regarding UDP-Araf, however, UDP-Arap is known to be synthesized from UDP-xylpyranose by UDP-xylpyranose 4-epimerase and from Arap-1-phosphate and uridine triphosphate by a UDP-Arap-1-phosphorylase. The interconversion of pyranose form to furanose form was carried by mutase.61 Indeed, Zhang and Liu60 showed that bacterial UDP-galactose mutase catalyzes the interconversion of UDP-Arap and UDP-Araf. This mutase is present in many prokaryotes, fungi and protozoa, but no plant homologues have been identified. We demonstrated that UDP-Arap mutase (UAM) is present in plant by the purification of UAM from rice.

Here, we summarize that the biosynthesis mechanism of arabinan and UDP-Araf which is a precursor of polysaccharides containing Araf residues.

Identification of arabinofuranosyltransferase activity in Golgi fractions from mung bean hypocotyls.53 The assay for arabinosyltransferase activity was carried out in the presence of UDP-Arap as a donor substrate and 2AB-labeled arabinoligosaccharides as exogenous acceptors with Golgi membrane fraction isolated from etiolated mung bean hypocotyls. HPLC with fluorescence de-
Enzyme products analysis by incubation time.

(A) HPLC analysis of the products formed when Ara-2AB and UDP-Araf are reacted together in the presence of mung bean Golgi membrane fractions. The numbers indicated the DP and elution positions of the 2AB-labeled arabinino-oligosaccharides. (B) Amounts of product generated by arabinofuranosyltransferase with increasing incubation time.

Identification of UDP-arabinopyranose mutase in rice seedlings.

UDP-Arabinofuranose was used as the substrate to detect UAM activity in rice seedling extracts (Fig. 3). Only a small amount of UDP-Araf was generated when UDP-Arap was the substrate (Fig. 3 (C)). The data showed that the rice UAM has no activity of UDP-xylose 4-epimerase because no peak of UDP-xylose was detected in the enzyme products (Figs. 3 (B) and (C)). UAM was also detected in mung bean seedling extracts, indicating that mutase activity is present in dicots as well as monocots.

Fig. 1. Enzymic synthesis of Araf-containing oligosaccharides.

(A) HPLC analysis of the products formed when Ara-2AB and UDP-Araf are reacted together. (B) HPLC showing the products formed when UDP-Araf is reacted with the purified rice mutase. (C) HPLC showing the products formed when UDP-Arap is reacted with the purified rice mutase. The elution positions of standard Uridine monophosphate (UMP) (1), UDP-Arap (2), UDP-Araf (3) and UDP-Xyl (allow) are shown.

Fig. 2. Enzyme products analysis by α-L-arabinofuranosidase.

α-(1→5)-Arabinan synthesized using Golgi membrane from UDP-Araf was characterized by digestion with α-L-arabinofuranosidase which is specific for arabian consisting Araf residues. After treatment of enzyme products with the arabinofuranosidase, samples were detected by HPLC. Acceptor, Ara-2AB oligosaccharides; Products, enzyme products in the presence of UDP-Araf and Ara-2AB oligosaccharides; Ara+Araf(α), enzyme products in the presence of UDP-Arap and Ara-2AB oligosaccharides, which is a single Araf residue at a non-reducing end of Ara-2AB oligosaccharides. The amount of each product before the treatment was taken as 100%.

Identification of UDP-arabinopyranose mutase in rice seedlings. (A) UDP-Arap mutase catalyzes the interconversion of UDP-Arap (1) and UDP-Araf (2). The pyranose form is favored at equilibrium. (B) HPLC showing the products formed when UDP-Araf is reacted with the purified rice mutase. (C) HPLC showing the products formed when UDP-Arap is reacted with the purified rice mutase. The elution positions of standard Uridine monophosphate (UMP) (1), UDP-Arap (2), UDP-Araf (3) and UDP-Xyl (allow) are shown.

Fig. 3. The interconversion of UDP-L-Arap and UDP-L-Araf.

After treatment of enzyme products with the arabinofuranosidase, indicating that the transferred arabinosyl residues was in the furanose form with α-linkage.

Purification and characterization of UDP-arabinopyranose mutase. Approximately 80% of the UAM activity was detected in the cytosolic fraction of rice seedlings. Thus, UAM was purified from cytosolic fraction of rice seedling using a combination of hydrophobic interaction, size-exclusion, and anion-exchange chromatographies (Fig. 4). The UAM was purified up to 430-fold with 2.1% recovery of initial total activity. The purified UAM gave a single broad band (≈41 kDa) on SDS-PAGE, however, the molecular weight of UAM was estimated by size-exclusion chromatography to be ≈460 kDa (Fig. 4 (D)). The data suggests that UAM is likely to exist as a complex composed of numerous proteins.

The rice UAM was characterized by enzyme assay at different temperature and pH value with varying amounts of either UDP-Arap or UDP-Araf. These results showed that the UAM was most active at 55°C, with a maximum...
activity for UDP-Araf forming between pH 7.0 and 7.5, for UDP-Arap between pH 5.5 and 6.0. The reaction is reversible because UAM converts either substrate into an equilibrium mixture of 90% of UDP-Arap and 10% of UDP-Araf.

The apparent Km and Vmax values for UDP-Araf and UDP-Arap interconversion confirm that the reaction favors pyranose formation. Rice UAM has a much higher affinity for UDP-Araf (Km = 55 µM) than a recombinant E. coli UDP-Galp mutase (Km = 600 µM).

Such a result is to be expected because UDP-Galp (Km = 147 µM) and UDP-Galp (Km = 600 µM) differ in their ability to form pyranose or furanose structures, with UDP-Galp favoring furanose formation and UDP-Galp favoring pyranose formation. The higher affinity of UAM for UDP-Araf suggests a preference for pyranose formation in this reaction, which is consistent with the observed pH dependence.
Identification of rice UDP-arabinopyranose mutase genes. 

The partial amino acid sequences of the mutase proteins were determined by high-performance liquid chromatography (HPLC)-MS/MS analysis. The results showed that three related protein corresponding to Os03g40270, Os04g56520 and Os07g41360, were identified. Interestingly, these proteins were annotated as reversibly glycosylated polypeptide (RGP), which are >80% sequence identity with rice UAM1 (Fig. 5) thereby extending the occurrence of UAMs to non-vascular land plants and to the chlorophyta. C. reinhardtii, P. patens and gymnosperms have all been shown to synthesize complex carbohydrates that contain Ara residues.

Recombinant rice UDP-arabinopyranose mutase and reversibly glycosylated polypeptide activity. 

To confirm that mutase and autoglycosylating activity are present in the same protein, we generated recombinant proteins of UAMs 1, 2 and 3 in E. coli. PCR fragments of Uam1, Uam2 and Uam3 were inserted into the pGEX-2KT vector (GE Healthcare Life Science). The constructs were designed to express the recombinant enzymes fused to glutathione S-transferase (GST) at the N-terminus. The resulting plasmids were introduced into E. coli strain BL 21 (DE3). These rUAMs 1 and 3 showed interconversion activity of UDP-Arap and UDP-Araf (Fig. 6 (A)). Recombinant rUAMs 1 and 3 (35.2 and 16.4 U mg protein) respectively, had mutase activity, although their specific activities are somewhat lower than that of the purified rice mutase (262 U mg protein). Such a result was not unexpected because the native mutase most likely exists as a complex composed of several different UAMs. No increase in mutase activity was obtained by mixing together different combinations of the recombinant UAMs nor were we able to demonstrate that rUAM2 alone had mutase activity (Fig. 6 (A)), suggesting that other plant factors might be required for optimal activity.

Recombinant UAMs 1 and 3 also showed the RGP activity which autoglycosylated when each protein was reacted separately with UDP-[14C]Glc (Fig. 6 (B)). Glycosylation of rice UAM is reversible because the radiolabeled was displaced using UDP-Glc, UDP-Xyl, UDP-Gal, UDP-Arap, UDP-Araf (Fig. 7). Neither mutase nor RGP activities was detected with rUAM2. These results suggested that reversible glycosylation and the interconversion of UDP-Arap and UDP-Araf are coupled reaction.

Rice UAM and the rUAMs contain no FAD, and they did not require this prosthetic group for activity. Mutase activity was not affected by oxidizing (K3FeCN6) or reducing reagents. These results were in direct contrast to microbial UDP-galactose mutase which requires reduced FAD for activity. A flavin-derived iminium ion has been reported to be necessary for the interconversion of the pyranose and furanose rings by these microbial enzymes. The Arg residue that is believed to be the site of autoglycosylation in RGP may have a catalytic function that is similar to FAD. Irrespective of ring interconversion
we predict that UAM will interconvert UDP-Galf and UDP-Galp although this enzyme is likely to have a much lower affinity for galactose than for arabinose. C. reinhardtii is the only plant has been reported to synthesize Galf-containing glycans. UDP-GlcP and UDP-XylP also form complexes with UAM but it is unlikely that ring contraction will occur because the interaction between C4 and C1 that is required for furanose ring formation is sterically hindered by an equatorial C-4 hydroxy group.

**Conclusions.**

We demonstrated that arabinan synthesis in vitro requires UDP-Araf as a donor substrate. Furthermore, we determined, for the first time, the UAM activity in plant that catalyzes the interconversion of UDP-Arap to UDP-Araf. The peptide sequence analysis of rice UAM revealed that UAM is the same protein encoded by RGP that its biological function in the biosynthesis of plant polysaccharides is poorly understood.

Identification and characterization of a UAM in rice seedlings and the production of enzymatically active recombinant proteins provides some of the tools required to generate UDP-Araf more effectively than by chemical synthesis. The identification of plant enzymes that catalyze the interconversion of UDP-Arap and UDP-Araf and the demonstration that genes encoding these proteins are likely to be present in all land plants and in many green algae provides new opportunities to study the synthesis of Araf-containing polysaccharides and glycoproteins in plants at the molecular, biochemical, and evolutionary levels.

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植物由来 UDP-アラビノピラコースムターゼ
小西照子 1, 石井 忠 2
1 琉球大学農学部生物資源科学科
(903-0213 沖縄県西原町千原 1 番地)
2 独立行政法人森林総合研究所
(305-8687 つくば市松の里 1)

植物細胞壁多糖であるアラビノは、高等植物においては細胞接着や栄養に関与し、植物の伸長成長にも関わる多糖として、多くの研究者の注目を集めている。アラビノはアラビノピラコース残基が α-1,5 結合した多糖で、植物細胞壁中ではベクチン塩基多糖のラムノガラクタンと同様に、アラビノピラコース膜を基質として合成される。この UDP-アラビノピラコースはアラビノ以外にも、アラビノキシンやアラビノガラクタンプロテインなどアラビノフランノサ基を含む多糖の合成に利用されていると考えられている。しかし、アラビノおよび UDP-アラビノフランノスの生合成機構については未だ不明な点が多い、近年、我々は UDP-アラビノフランノス合成に関わる酵素、UDP-アラビノフランノスムターゼ (UAM) を発見した。UAM は UDP-アラビノフランノスから UDP-アラビノフラコースの生成を触媒する酵素である。我々はイネ芽生えより UAM を精製し、遺伝子の単離・同定に成功した。遺伝子単離の結果、UAM は reversibly glycosylated polypeptides (RGP) と一致することがわかった。また、微生物では UDP-ガラクトフランノスの合成に関与する酵素、UDP-ガラクトフランノスムターゼが既に単離され、UDP-アラビノフランノスも基質として認識し、UDP-アラビノフランノスの生成も行うことが報告されていたが、単離した UAM は微生物の UDP-ガラクトフランノスムターゼとは異なる特徴を示した。それゆえ、植物由来 UAM は微生物の UDP-ガラクトフランノスムターゼの触媒反応とは異なる機構が有することが示された。

【質問】
農研機構・食総研 徳安
1) UAM は UDP-Glc, Gal, Xyl に対してもムターゼ活性をもつのか。
2) イネでも UAM はアラビノースを基質としているのか。
【答】
1) フランノス型とピラコース型の変換には C4 と C1 の立体構造が関与していると考えられます。グルコースとピラコースでは C4 のアデノシン基がエントリアルに配向しており、このため環の変換が行われるということから、UAM はこれらのヌクレオチド基に対してムターゼ活性を持つものと考えます。一方 Gal は C4 のピラコース基はアクシャル位であり環の変換が行われることから、UAM は UDP-フラクトースに対してもムターゼ活性を示すと考えられます。
2) UAM の基質は UDP-アラビノフランノスおよび UDP-アラビノフランノスであることから、単糖のアラビノースは UAM の基質にはなり得ないと考えます。

【質問】
岩手生物工学研究センター 竹田
アラビノ合成におけるアクセプターの特異性について、キシンへの転移反応は行うか。
【答】
アラビノ合成酵素は α-1,5 結合でアラビノースを転移します。単糖基に存在するアラビノキシンのアラビノース残基は α-1,2 結合であることから、アラビノ合成酵素はアラビノキシン合成には関与していないものと考えられます。

【質問】
鶴見島大・農 藤田
天然にわずかに存在する arabino-pyranose も β-1,3 結合の可能性が高いか？
【答】
可能性は高いと思われます。実際、カラメツ由来のアラビノガラクトタンに含まれるアラビノピラコースは β-1,3 結合であることが確認されています。