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Silicon Forms in Soluble Pectin Substances Extracted by Hot Water from Plant Cell Wall

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Summary

We investigated the forms of silicon in soluble pectin substances (SPS) extracted by hot water from the cell wall of the roots of rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), soybean (*Glycine max* L.), eggplant (*Solanum melongena* L.), sunflower (*Helianthus annuum* L.), tomato (*Solanum lycopersicum* L.) and cucumber (*Cucumis sativus* L.) plants. The silicon content of SPS was not related to the amount of SPS extracted. However, the silicon content of SPS increased with increasing sugar content, except in soybean. When rice and eggplant SPSs were loaded onto Sephadex G-50, most silicon was co-eluted with sugar and uronic acid in the void volume (V_0). Digestion with pectinase remained most silicon with the sugar and uronic acid in the V_0, but part of the silicon was found in the lower molecular weight area with uronic acid. When loading onto the same column after hydrolyzing the eggplant SPS with hydrochloric acid, most silicon and sugar was eluted near the V_t, but the fraction numbers of both peaks were different, and a larger peak of silicon and a small peak of sugar remained in the V_0. Sugars in the SPS were mostly arabinose, galactose and glucose, but the silicon content of the SPS from some plants decreased with the lower glucose with *cis*-hydroxyl group in the 2, 4, 5-position. These results suggest that a part of the silicon in the SPS might form complexes with *cis*-hydroxyl groups in the glucose, and that if silicon content is high, as in rice, the remaining silicon might polymerize to glucose-silicon complexes.

Key words: cell wall, soluble pectin substance, silicon, glucose

Introduction

We previously reported that physiological disorders of the vegetative organs of plants as a result of silicon deficiency might be due to insufficient formation of the cell wall; that silicon accumulated in the soluble pectin substances extracted with hot water (SPS) of cell wall according to plant growth; and that silicon might exist in organo-silicate form but not as silica gel as results of infrared (IR) spectroscopy [1].

In this study, we investigated the form of silicon in the SPS, using the cell walls of a number of plant species.

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Materials and Methods

Plant culture and preparation of cell wall

Seventy-two rice (*Oryza sativa* L.) seedlings, 20 soybean (*Glycine max* L.) seedlings, 50 wheat (*Triticum aestivum* L.) seedlings, 50 maize (*Zea mays* L.) seedlings, 20 sunflower (*Helianthus annuum* L.) seedlings, 20 eggplant (*Solanum melongena* L.) seedlings, 10 cucumber (*Cucumis sativus* L.) seedlings and 10 tomato (*Solanum lycopersicum* L.) seedlings were transplanted to 20-L containers filled with culture solution adjusted by tap water to contain 33 mg L^{-1} silicon, on 14 May, 15 May, 15 May, 25 May, 22 May, 24 May, 21 Sept. and 21 Sept. 2007, respectively. The culture solution for rice was as described previously [2], and Hoagland's solution was used for the other plant species. The culture solution was changed once per week, and its pH adjusted to 5.5. The rice plants were harvested on 6 Sept., the soybean, wheat, maize, eggplant and sunflower plants on 29 June, and the cucumber and tomato plants on 6 Oct 20007. After cutting each root and freeze drying, the cell wall was adjusted by the previously described method [1]. The SPSs were extracted with hot water at 80℃ from the root cell wall of each plant, then freeze-dried and weighed.

Analysis of silicon, sugar and uronic acid.

Silicon was determined by the molybdenum blue method, sugar by the phenol-sulfate method and uronic acid by the Bitter-Mur method [3]. The silicon, sugar and uronic acid contents of each fraction separated by a molecular sieve were shown on the chromatogram as absorption intensity

Molecular sieve chromatograms of SPS

The concentrated SPS solution from rice or eggplant was loaded onto a Sephadex G-50 column (16 x 900 mm), and then eluted by water. After 3.5mL of elution was collected, sugar, uronic acid and silicon were determined and their absorption was shown on the chromatogram.

Pectinase action and hydrolysis treatment of SPS from eggplant

One milliliter of acetate buffer (pH 4.8) with 20mg pectinase (MACERASE; Yakult Corporation) was added to 1mL of the concentrated SPS solution from the eggplant. After 1 h, the mixture solution was treated in boiling water for 5 min, and centrifuged at 3,000 rpm for 10 min. The supernatant was loaded onto the Sephadex G-50 column (16 x 900 mm), and then eluted by water.

Two milliliters of SPS solution was hydrolyzed by addition of 0.2mL of 4 M hydrochloric acid for 12 h at 80℃. After neutralization by 0.2mL of 4 M sodium hydroxide, it was loaded onto a Sephadex G-50 column (16 x 900 mm).

Analysis of sugar component

One milliliter of 4M trifluoroacetic acid was added to 1mL of the concentrated SPS solution, and the mixture hydrolyzed at 120℃ for 1 h. The supernatant was concentrated after centrifugation and dried in an evaporator. After dissolving the dried residue in water, the sugars were determined by ion chromatography with PAD (Dionex Corporation).

Results

The weights of SPS from the root cell walls of the 8 plant species are shown in Table 1. Cucumber

Table 1. Weights of SPSs from root cell walls of some plants.

(g cell wall kg^{-1})

Rice	Wheat	Maize	Soybean	Sunflower	Eggplant	Cucumber	Tomato
17.0	64.5	44.0	63.0	75.0	64.2	144.2	107.9

Table 2. Sugar, uronic acid and silicon contents of SPSs from root cell walls of some plants.

(m mols SPS kg^{-1})

Plant	Sugar (as galactose)	Uronic acid (as galacturonic acid)	Silicon
Rice	2.76	0.47	7.00
Wheat	2.59	0.56	0.46
Maize	2.21	0.56	0.94
Soybean	1.90	0.16	0.85
Sunflower	2.36	0.50	0.20
Egg plant	2.55	0.25	0.80
Cucumber	2.71	0.16	0.85
Tomato	2.05	0.34	0.13

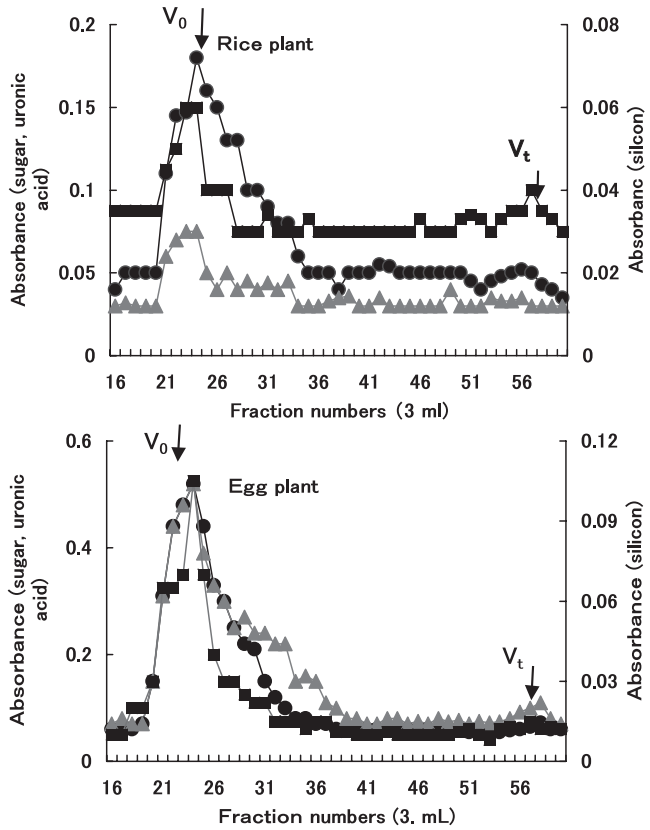

Figure 1. Molecular sieve chromatograms (Sephadex G-50) of SPS from cell walls of rice plant and eggplants.
v_0: void volum, vt: total volum
● : sugar (as galactose), ▲ : uronic acids (as galacturonic acid), ■ : silicon

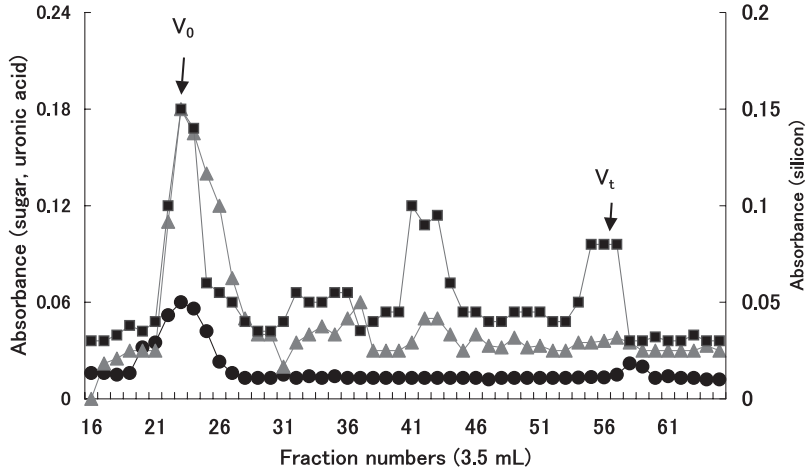

Figure 2. Molecular sieve chromatograms (Sephadex G-50) after acted pectinase to SPS from cell wall of eggplant.
V_0: void volume, V_t:total volume
●: sugars (as galactose), ▲: uronic acids (as galacturonic acid), ■: silicon

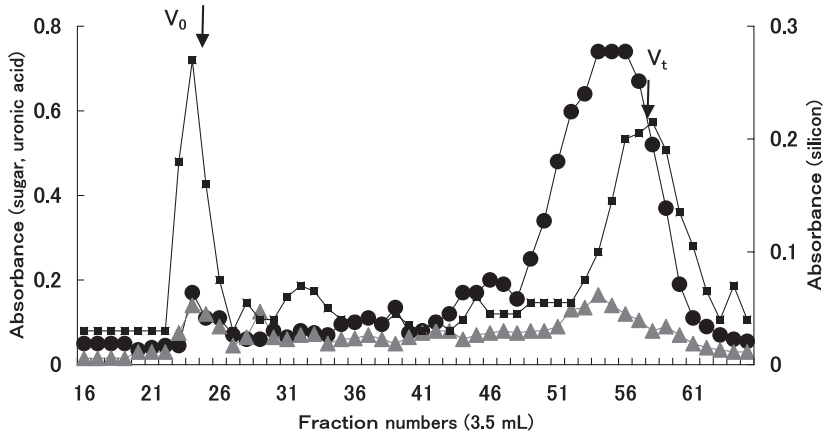

Figure 3. Molecular sieve chromatograms (Sephadex G-50) after hydrolysis of SPS from root of egg plant.
V_0: void volume, V_t: total volume
●: sugars (as galactose), ▲: uronic acids (as galacturonic acid), ■: silicon

contained the highest weight of SPS, followed by tomato; rice had the lowest weight of SPS among the plants.

Table 2 shows the SPS contents of sugar, uronic acid and silicon from root cell walls. In all plants, SPS contained higher contents of sugar than uronic acid. The sugar content was higher in rice and cucumber than in the other plants. Uronic acid content was much lower in rice and soybean than in other plants. Silicon content of SPS was highest in rice, followed by cucumber and soybean. Tomato had the lowest content.

The chromatograms of the sugar, uronic acid and silicon of the rice and eggplant are shown in figure 1. The silicon in the SPSs of both plants eluted with sugar and uronic in the void volume (V_0). When SPSs from both plants were loaded onto Sephadex G-75, their peaks were found in the total

Table 3. Ratio of sugar component in SPSs from rice, soybean, cucumber and tomato plants.

(%)

	Rice	Soybean	Cucumber	Tomato
Galacturonic acid	0.39	5.8	6.3	12.8
Fucose	1.50	2.5	0.96	1.8
Rhamnose	trace	10.9	5.0	10.3
Arabinose	22.0	33.4	35.2	34.7
Mannose	trace	1.4	0.7	2.7
Xylose	18.0	5.8	4.5	7.6
Galactose	20.0	9.5	29.8	22.3
Glucose	38.0	16.9	17.5	8.4

volume (V_t) (not shown in figure 1).

When the SPS solution of the eggplant digested with pectinase was loaded onto Sephadex G-50, silicon peaks were observed in the V_0, near fraction numbers (Fr.) 41-44 with uronic acid and in the V_t with sugar (figure 2). After loading SPS from eggplant hydrolyzed with hydrochloric acid onto Sephadex G-50, a larger amount of silicon and smaller amounts of sugar and uronic acid remained in the V_0 (figure 3). Silicon peaks outside the V_0 were found at Fr. 32 and 54, while the sugar or uronic acid peak was found in V_t or at Fr. 54, respectively.

Table 3 shows the SPS sugar components of the root cell wall of rice, soybean, cucumber and tomato. The main sugars found were arabinose, galactose and glucose, but their percentage contents differed among the four plants, especially, for glucose (33% for rice, 8% for tomato). Galactose content was 20% to 30% except for soybean.

Discussion

We previously reported IR spectroscopy results that showed that silicon in the SPS from the cell wall might exist in organosilicate form, not as silica gel [1]. As shown in Table 1, SPS weight was lowest in rice, which accumulated more silicon than the other plants, indicating that silicon accumulation in the SPS was not related to the amount extracted.

Christopher et al., using NMR spectroscopy, showed that common carbohydrates including many aliphatic pyranosic and furanosic sugars such as gluconic acid, lyxose and ribose, form a stable organosilicate in aqueous solution [4]. As shown in Table 2, the silicon content of SPS was lower in tomato and sunflower, which were low in sugar, compared with the other plants, except soybean. When the SPSs from rice and eggplant were loaded onto Sephadex G-50 (figure 1) or G-75 (data not shown), in the former, a peak of silicon in both plants was observed for sugar and uronic acid in the V_0 but not in other Frs., while in the latter, both sugar and uronic acid were eluted with silicon in the V_t, indicating that the molecular weights of SPSs range from 30,000 to 50,000. Furthermore, when the eggplant SP digested with pectinase was loaded onto Sephadex G-50, larger peaks of silicon were observed with sugar and uronic acid in the V_0, and smaller peaks of silicon were observed from Fr. 31 to Fr. 36 and near Fr. 43 with uronic acid (figure 2). In eggplant SPS hydrolysed with hydrochloride (Fig. 3), a larger amount of silicon was eluted with sugar near V_t, but both peaks were in different Frs., while a higher peak of silicon and a lower peak of sugar remained in the V_0.

In diatoms, most silicon is polymerized on silicon associated with hydroxyl amino acids [5]. As previously stated, results of IR showed that silicon did not exist in silica gel form [1]. These results

suggest that, in the SPS extracted from the cell wall, part of the silicon might form complexes with sugars and that when cell wall silicon content is high, as in rice, the remaining silicon might polymerize to silicon-sugar complexes but not silica gel. However, the polymerizing forms of silicon are unknown.

Silicon forms complexes with many sugars with double hydroxyl groups in the cis-position [4]. The main sugars in the SPS were arabinose with a *cis*-hydroxyl group in the 3, 4-position, galactose with a *cis*-hydroxyl group in the 2, 5 position and glucose with a cis-hydroxyl group in the 2, 4, 5-position (Table 3). As shown in Table 2, the silicon content of the SPS was highest in rice, which had the highest percentage of glucose of the 4 plant species studied, while in contrast, tomato with the lowest silicon content among the 8 plant species had the lowest percentage of glucose, suggesting that part of the silicon in the SPS may be associated with glucose. Although the SPS of soybean was low in sugar, its higher silicon content may depend on the high proportion of glucose in the sugars.

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植物の細胞壁から抽出された熱水可溶性ペクチン物質中のケイ素の存在形態

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要　約

植物細胞壁から抽出される熱水可溶性ペクチン物質（SPS）に蓄積されるケイ素の存在形態を明らかにするため，水稲，コムギ，トウモロコシ，ダイズ，ヒマワリ，ナスビ，キュウリ，トマトの根細胞壁からのSPS中の糖，ウロン酸，ケイ素含量を比較検討した。

SPS中のウロン酸含量は糖含量に比べて著しく低く，またケイ素含量は糖含量の増加と共に高くなった。水稲，ナスビからのSPSをSephadex G-50カラムでゲルロ過すると，両者ともvoid volume（V_0）に，またSephadex G-75ではtotal volume（V_t）にケイ素は糖，ウロン酸とともに溶出した。ナスビのSPSをペクチナーゼ処理後G-50カラムでゲルロ過すると，糖はV_0に残ったが，ケイ素のピークはフラクションNo41，V_tに観察された。一方塩酸で加水分解したSPSは糖とケイ素の一部はV_0に，大部分の糖はV_tnの前のフラクションに，ケイ素はV_tに溶出した。

水稲，キュウリ，ダイズ，トマトのSPS中の糖組成を調べると，2, 4, 5の位置にシス型OH基も持つグルコースの割は，ケイ素含量の多い水稲で最も高く，逆にケイ素含量が最も少ないトマトで最も低かった。

これらの結果は，SPS中のケイ素の一部はグルコースとOH基に配位し，大部分呉ケイ素はそれらにポリマーとして結合していることが推定される。しかし結合ポリマーの形態は不明である。

キーワード：細胞壁，可溶性ペクチン物質，ケイ素，グルコース

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