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Short Communication

Phosphorylation of rice sucrose synthase isoforms promotes the activity of sucrose degradation

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Abstract Sucrose is utilized as an initial material for production of the storage substances. Sucrose synthase reversibly catalyzes reactions of the sucrose degradation and its synthesis between sucrose with UDP and UDP-glucose with fructose. They also had the activity of the reactions for sucrose degradation of sucrose with ADP, and sucrose synthesis from ADP-glucose and fructose. Rice has three representative isoforms of sucrose synthase, Rsus1, Rsus2, and Rsus3, in which Rsus1 and Rsus3 are highly expressed in developing seeds. These three isoforms were phosphorylated by SPK, a calcium-dependent protein kinase. By phosphorylation, they showed increase of their reactivity for sucrose degradation on both reactions using UDP and ADP. In contrast, the synthetic activity of these isoforms was not altered by phosphorylation in any cases of the reactions with UDP-glucose and ADP-glucose. These results indicated that phosphorylation of sucrose synthase isoforms selectively led to enhance the reactivity for sucrose degradation.

Key words: phosphorylation, protein kinase, rice, sucrose synthesis and degradation.

Sucrose synthase reversibly catalyzes the sucrose degradation and its biosynthesis. UDP-glucose, the main product of the sucrose degradation, is important as the starting material for the biosynthesis of cellulose and storage starch (Amor et al. 1995). Sucrose synthase genes compose a small multigene family in plant genomes, such in those of pea, potato, maize and *Arabidopsis* (Barratt et al. 2001; Baud et al., 2004; Chourey et al. 1998; Weckwerth et al. 2004; Zrenner et al. 1995). In *Arabidopsis*, more than one isoform or pair of isoforms must be able to provide UDP-glucose to cellulose synthase, and it is likely that more than one isoform can provide substrates for energy production for phloem loading and carbon for lipid synthesis in developing embryos (Bieniawska et al. 2007).

In the rice genome, six genes for the isozymes of sucrose synthase, *Rsus1* to *OsSus6*, have been reported (Hirose et al. 2008; Su 1995). Among them, *Rsus1*, *Rsus2*, and *Rsus3* are classified to SUS–I clade, and these isoforms are highly conserved with structural similarity (Supplementary Figure 1). In the developing seeds, *Rsus1* and *Rsus3* are specifically expressed in endosperm, whereas *Rsus2* is broadly expressed in immature seeds, suggesting that *Rsus1* and *Rsus3* is highly involved in storage starch biosynthesis, and *Rsus2* functions as the sucrose transport into endosperm from source organ (Wang et al. 1999).

Rice endosperm accumulates a massive amount of storage starch, which is synthesized from ADP-glucose by the synthetic reaction of starch synthase in amyloplast. The ability of ADP-glucose supply is considered to determine the strength of storage starch production. The main pathway supplying ADP-glucose is shown to be a reaction from glucose-1-phosphate and ATP to ADP-glucose by ADP-glucose pyrophosphorylase (Bahaji et al. 2016). Although UDP is generally considered to be the preferred nucleoside diphosphate for sucrose synthase, ADP also serves as an effective substrate to produce ADP-glucose, which may facilitate the production of storage starch by the enzymes such as starch synthase (Bahaji et al. 2016; Su 1995).

Sucrose synthase is phosphorylated by a calcium-dependent protein kinase (CDPK), and is controlled its enzymatic activity for sucrose degradation (Huber et al.

Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; UDP, uridine diphosphate; NADP, nicotinamide adenine dinucleotide phosphate; SDS, sodium dodecyl sulfate.

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1996). Phosphorylation of maize Sus2 could be involved in its ability to associate with membranes (Duncan et al. 2006). We have found a CDPK, termed SPK, which is specifically expressed in developing seeds, may phosphorylate rice Rsus2 (formerly called as Susy1). We have also shown that lacking the SPK activity results in deficient storage starch accumulation in developing seeds, suggesting that SPK is tightly involved in the reaction of sucrose synthase in endosperm (Asano et al. 2002). However, it is unclear what effect occurs on the enzyme activity by phosphorylation. In this study, we focused on the SUS–I clade sucrose synthase isoforms, *Rsus1*, *Rsus2*, and *Rsus3*, and determined their characters in detail. We analyzed the reaction kinetics of the isoforms of rice sucrose synthase for the reaction of sucrose synthesis and sucrose degradation using UDP or ADP. Here, we report the effect of phosphorylation on the enzyme activities.

The full-length cDNAs corresponding to the genes for sucrose synthase isoforms, Rsus1, Rsus2, and Rsus3 (AK100546, AK100334, and AK100006, respectively), were obtained from the Rice Genome Resource Center of the National Institute of Agrobiological Sciences, Japan. Each of proteins of sucrose synthase isoforms was prepared as a fusion protein with glutathione-S-transferase (GST) using an *E. coli* expression system. The portion of the GST tag in the fusion proteins were removed using Factor Xa Cleavage Capture Kit (Novagen Madison, WI, USA). SPK (D13436) was used as the protein kinase of sucrose synthase. The kinase reaction was carried out as described previously (Asano et al. 2002).

We have determined that a protein kinase SPK may phosphorylate the lysine residue of the Rsus2, which was located at the conservative phosphorylation site (Asano et al. 2002). To identify whether similar modification may occur on the other isoforms, Rsus1 and Rsus3 (formerly called as Susy2 and Susy3, respectively) were prepared as the GST-fusion proteins, and examined their phosphorylation by SPK. Similarly to Rsus2, phosphorylation of the GST-fusion proteins of Rsus1 and Rsus3 was detected (Figure 1), whereas we detected self-phosphorylation of GST-SPK and no phosphorylation of GST. These results suggested that these isoforms of sucrose synthase were phosphorylated by SPK.

We analyzed the activity of each enzyme by measurement of the amount of fructose that was generated as a product of the sucrose degradation. When the reaction was performed using each isoform of the sucrose synthase with UDP, the reaction condition was settled in a reaction cocktail containing a fixed amount of enzyme solution (5 μg protein), UDP (5 mM), and a series of different amount of sucrose (0 to 1000 mM). In this condition, Rsus2 showed good reactivity. Production of fructose rapidly leached to a certain constant value

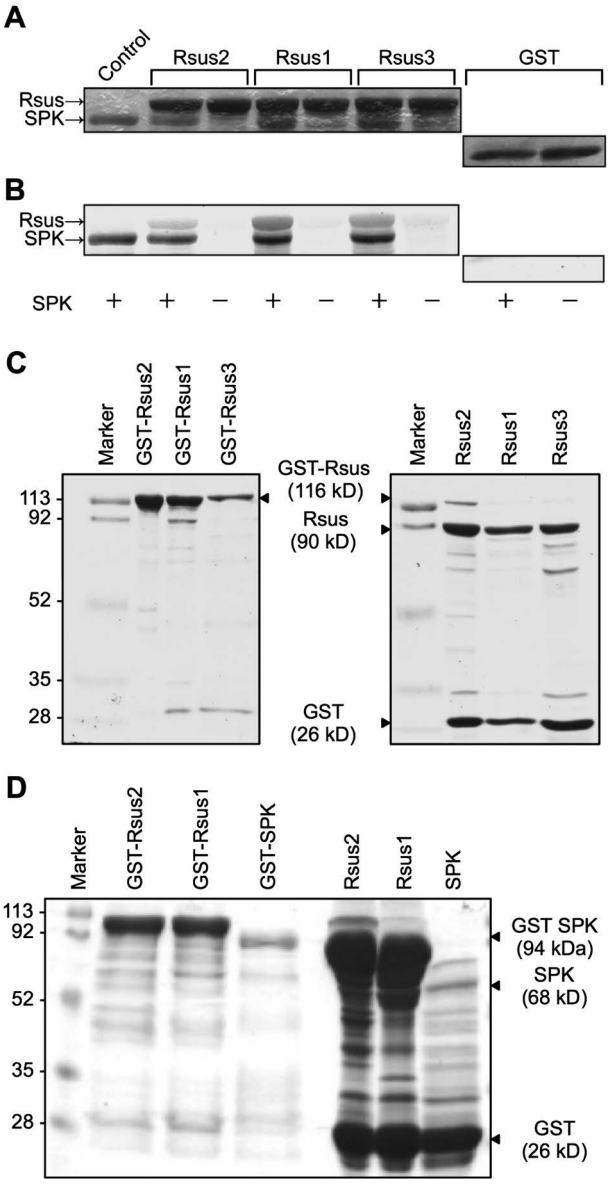

Figure 1. *In vitro* phosphorylation of GST–Sucrose synthase isoforms (Rsus) in the presence or absence of GST–SPK (SPK). (A) Proteins on a SDS-polyacrylamide gel were detected by Coomassie blue–stain. A GST-sucrose synthase fusion protein was purified using a Glutathione Sepharose 4B column (GE Healthcare, Japan) as described previously (Asano et al. 2002). (B) Detection of as phosphorylated sucrose synthase isoforms. SPK (D13436) was used as the protein kinase of sucrose synthase (Asano et al. 2002). After 20-min incubation with each isoform of sucrose synthase and GST-fusion SPK at 30°C, the ATP remaining in the reaction mixture was removed by means of a Sephadex G-50 spin column (GE Healthcare). Phosphorylated proteins were separated on a 10% SDS-polyacrylamide gel and stained with Coomassie blue, and detected using Pro-Q Diamond staining (Promega WI, USA). Lanes for Control, Rsus2, Rsus1, Rsus3, and GST indicate the results of the reactions with GST-fused SPK, Rsus2 with/without GST-fused SPK, Rsus1 with/without GST-fused SPK, Rsus3 with/without GST-fused SPK, and GST, respectively. The presence and absence of SPK in the reactions is indicated below by (+) and (−). Arrows indicate the position of the corresponding proteins on the gel. (C) and (D) GST–Sucrose synthase isoforms (lanes 2–4 in the left panel of (C)), GST–removed sucrose synthase isoforms (lanes 2–4 in the right panel of (C)), GST–SPK (lane 4 of (D)), and GST–removed SPK (lane 7 of (D)) on a SDS-polyacrylamide gel were detected by Coomassie blue stain. The portion of the GST tag in the fusion proteins were removed using Factor Xa Cleavage Capture Kit.

in maximum that was 600 mM sucrose (Figure 2B). Rsus1 and Rsus3 showed similar patterns of the enzyme reactivity, and amounts of the generated fructose linearly increased depending on the amount of sucrose (Figure 2A, C). Lineweaver–Burk plots, by which kinetic parameters were evaluated, indicated a linear correlation between values of $1/v$ and 1/[sucrose content] in the reaction mixture (Supplementary Figure 2).

Phosphorylation of the isoforms showed larger amount of fructose production with similar patterns of enzymatic kinetics of the corresponding isoforms without phosphorylation. Among three isoforms, the reactivity of Rsus2 significantly increased by phosphorylation. The other isoforms also showed higher reactivity by phosphorylation, but ratio of increase was relatively small as compared with that of Rsus2 (Figure 2A–C). The *Km* values to the amount of sucrose were lowered by phosphorylation of isoforms

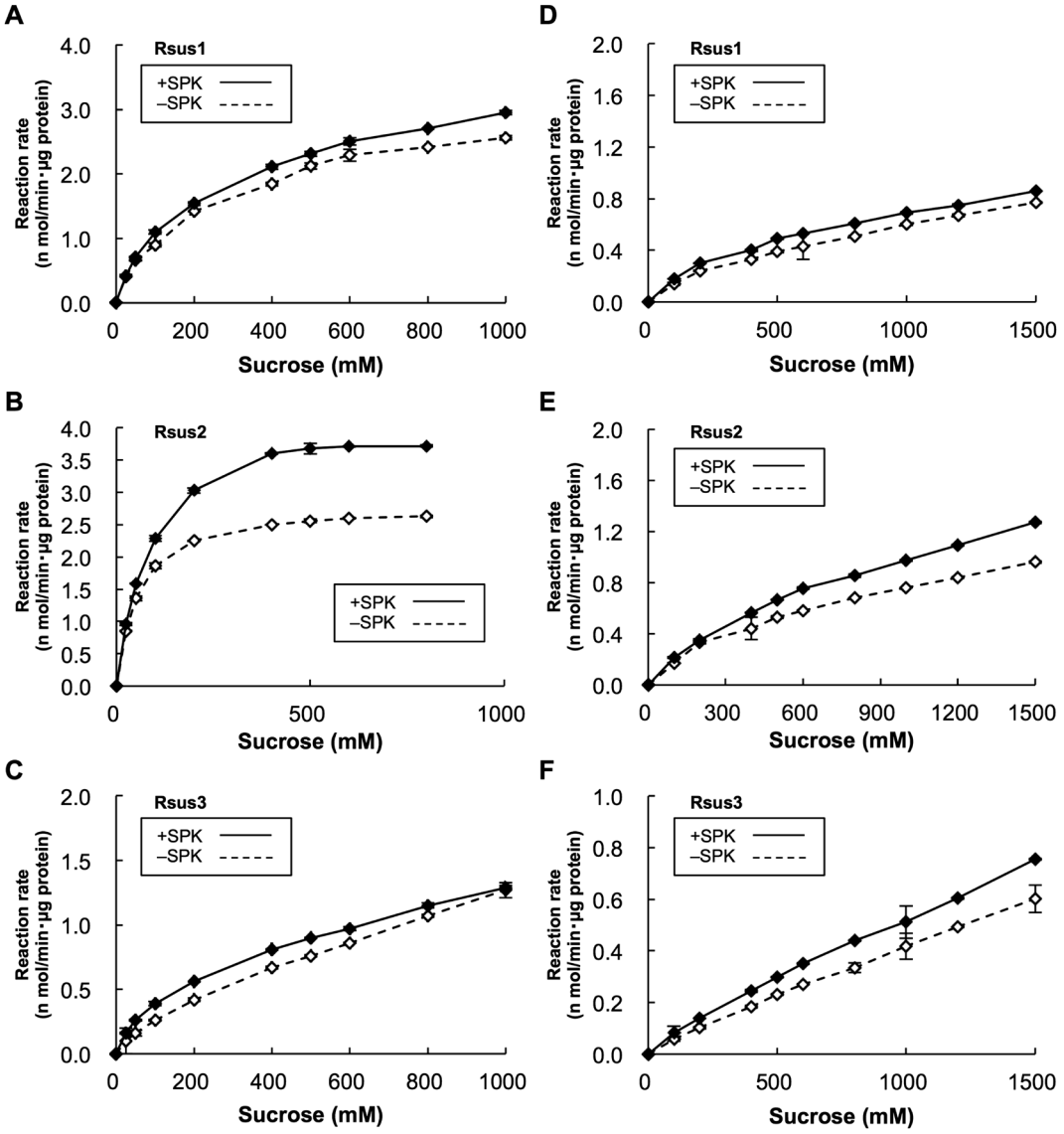

Figure 2. Reactions of the isoforms of rice sucrose synthase for the sucrose degradation using UDP or ADP. (A)–(C). The activities of Rsus1, Rsus2, and Rsus3 were measured in the direction of sucrose degradation using UDP as a substrate. Each of Rsus1, Rsus2 and Rsus3 solutions corresponding to 5 μg protein was used as the enzyme in 50 mM Tris-HCl (pH 7.5). For the reaction with the phosphorylated sucrose synthase, 5 μg of sucrose synthase was combined with 5 μg of GST-SPK solution in 50 mM Tris-HCl (pH 7.5) and incubated at 30°C for 10 min. The cleavage reactions of sucrose were performed at 30°C for 15 min in 400 μl of the reaction mixture containing the enzyme solution supplemented to 20 mM Tris-HCl (pH 7.5), 10 mM ATP, 25 μM $CaCl_2$, 2 mM $MgCl_2$, 5 mM UDP and an appropriate amount of sucrose (0–1000 mM). The reaction terminated by heating at 95°C. (D)–(F). The activities of Rsus1, Rsus2, and Rsus3 were measured in the direction of sucrose degradation using ADP as a substrate. The reaction was performed by the reaction condition with the fixed amount of enzyme solution (5 μg protein), ADP (5 mM), and an appropriate amount of sucrose (0–1500 mM). Solid lines and broken lines indicate the results of reactions using the enzyme with phosphorylated (+SPK) or without phosphorylation (–SPK), respectively. Generated fructose by the cleavage reaction was measured using F-kit (J-K International, Tokyo, Japan), by which coupled assays containing Hexokinase, Glucose-6-phosphate dehydrogenase, and phosphoglucose isomerase was performed, and increase of NADP was monitored at 340 nm. Error bars represent standard deviations of these measurements. $n=3$.

of sucrose synthase, and shown as smaller values than those without phosphorylation on Rsus1, Rsus2, and Rsus3, respectively (Table 1). The values of the catalytic efficiency significantly increased on their phosphorylated isoforms (Table 1). These results showed that phosphorylation of all isoforms of sucrose synthase led to increase the affinity to the substrates as well as their reactivity, suggesting that the reaction of sucrose degradation using UDP was greatly promoted by phosphorylation.

Sucrose synthase also has an activity for degradation of sucrose with ADP to ADP-glucose and fructose (Bahaji et al. 2016). We analyzed the kinetics on the reaction of sucrose degradation with ADP as a substrate by each of isoforms with/without phosphorylation. In this case, the reaction condition was settled in a reaction cocktail containing a fixed amount of enzyme solution (5 μg protein), ADP (5 mM), and a series of different amount of sucrose (0 to 1500 mM). When they were determined by the similar reaction condition as those with UDP-glucose and fructose, smaller values of reactivity were observed on all isoforms as compared with those with UDP. Likely to those of the sucrose degradation reactions with UDP, Rsus1, Rsus2, and Rsus3 showed similar patterns of the reactivity with/without phosphorylation. Amounts of the generated fructose linearly increased depending on the amount of sucrose in the reaction cocktails (Figure 2D–F). Lineweaver–Burk plots showed a linear correlation between values of 1/*v* and 1/[sucrose content] in the reaction mixture (Supplementary Figure 2).

By phosphorylation of the isoforms of sucrose synthase, *Km* values to the amount of sucrose was greatly smaller than those of Rsus1, Rsus2, and Rsus3, respectively (Table 1), and the catalytic efficiency showed larger values than those of isoforms without phosphorylation (Table 1). These results indicated that their activities on the sucrose degradation with ADP were also enhanced by phosphorylation on all of isoforms.

Next, we analyzed the reaction of sucrose synthesis by each of sucrose synthase isoforms without phosphorylation. We evaluated the reactivity by the similar reaction condition used on the sucrose degradation. When a reaction was performed in the reaction cocktail composed of a fixed amount of enzyme (5 μg protein), fructose (10 mM), and a series of different amount of UDP-glucose (5 to 20 mM), no clear correlation was detected between reactivity and the UDP generation. When we analyzed the enzyme activity in the reaction mixture containing smaller amount of UDP-glucose ranging between 0.125 to 1.0 mM, Rsus1 and Rsus2 showed a linear correlation between the amount of generated UDP and the reaction time (Figure 3A, B). However, Rsus3 did not give a clear correlation between them because the amount of produced UDP even by this reaction condition. For the reaction of Rsus3, when amount of enzyme was changed to 10 μg (protein) in the reaction mixture, a linearity of the dose-dependent increase of UDP generation was observed (Figure 3C). The amounts of the generated UDP by Rsus1 and Rsus3 were smaller than those by Rsus2 (Figure 3A–C). Lineweaver-Burk Plot analysis showed a linear correlation between values of 1/*v* and 1/[UDP-glucose content] in the reaction mixture (Supplementary Figure 3).

Table 1. Enzymatic kinetics for the sucrose degradation by the isoforms of rice sucrose synthase.

Sucrose+UDP					
Isoform	Phosphor-ylation	*Km* (mM)	*Vmax* (nmol min^{-1} (μg protein)$^{-1}$)	*kcat* (min^{-1})	catalytic efficiency (min^{-1} mM^{-1})
Rsus1	−	219±50.6	3.23±0.25	295±33.7	1.33±0.15
	+	160±49.0	3.37±0.21	308±14.7	1.80±0.26
Rsus2	−	82.7±26.5	2.73±0.06	255±5.13	3.80±1.32
	+	74.7±8.62	3.83±0.47	355±44.5	4.77±0.67
Rsus3	−	525±54.2	1.63±0.06	149±8.39	0.29±0.01
	+	384±93.0	1.67±0.20	151±18.0	0.41±0.06
Sucrose+ADP					
Isoform	Phosphor-ylation	*Km* (mM)	*Vmax* (nmol min^{-1} (μg protein)$^{-1}$)	*kcat* (min^{-1})	catalytic efficiency (min^{-1} mM^{-1})
Rsus1	−	1799±358	1.47±0.15	129±14.2	0.08±0.01
	+	1571±92.1	1.93±0.21	170±21.1	0.12±0.03
Rsus2	−	896±165	1.77±0.38	163±40.9	0.19±0.09
	+	781±94.8	2.03±0.51	185±47.6	0.24±0.09
Rsus3	−	2085±89.6	1.40±0.17	128±19.2	0.06±0.01
	+	1467±92.6	1.55±0.35	131±31.8	0.10±0.02

Kinetic parameters, *Km* and *Vmax*, were evaluated by Lineweaver–Burk plots (Supplementary Figure 2), which were determined on the reactions for sucrose degradation (see Figure 2). Values were determined on the correlation between amounts of fructose generated and sucrose with UDP or ADP as substrates. *kcat* was estimated as *Vmax*/enzyme content. Catalytic efficiency was evaluated as the value of *kcat*/*Km*. Each value±SD is shown (n=5).

We analyzed the effect of phosphorylation on the synthetic reactions of these isoforms. The reactivity of Rsus2 increased by their phosphorylation, but the ratio of enhancement was relatively small compared with those on the reaction of sucrose degradation (Figure 3B). No significant difference was detected on the reactions by phosphorylation on Rsus1 and Rsus3 (Figure 3A, C). The phosphorylated Rsus1 showed about 1.5 times larger *Km* value than that without phosphorylation. That of phosphorylated Rsus2 showed no significant

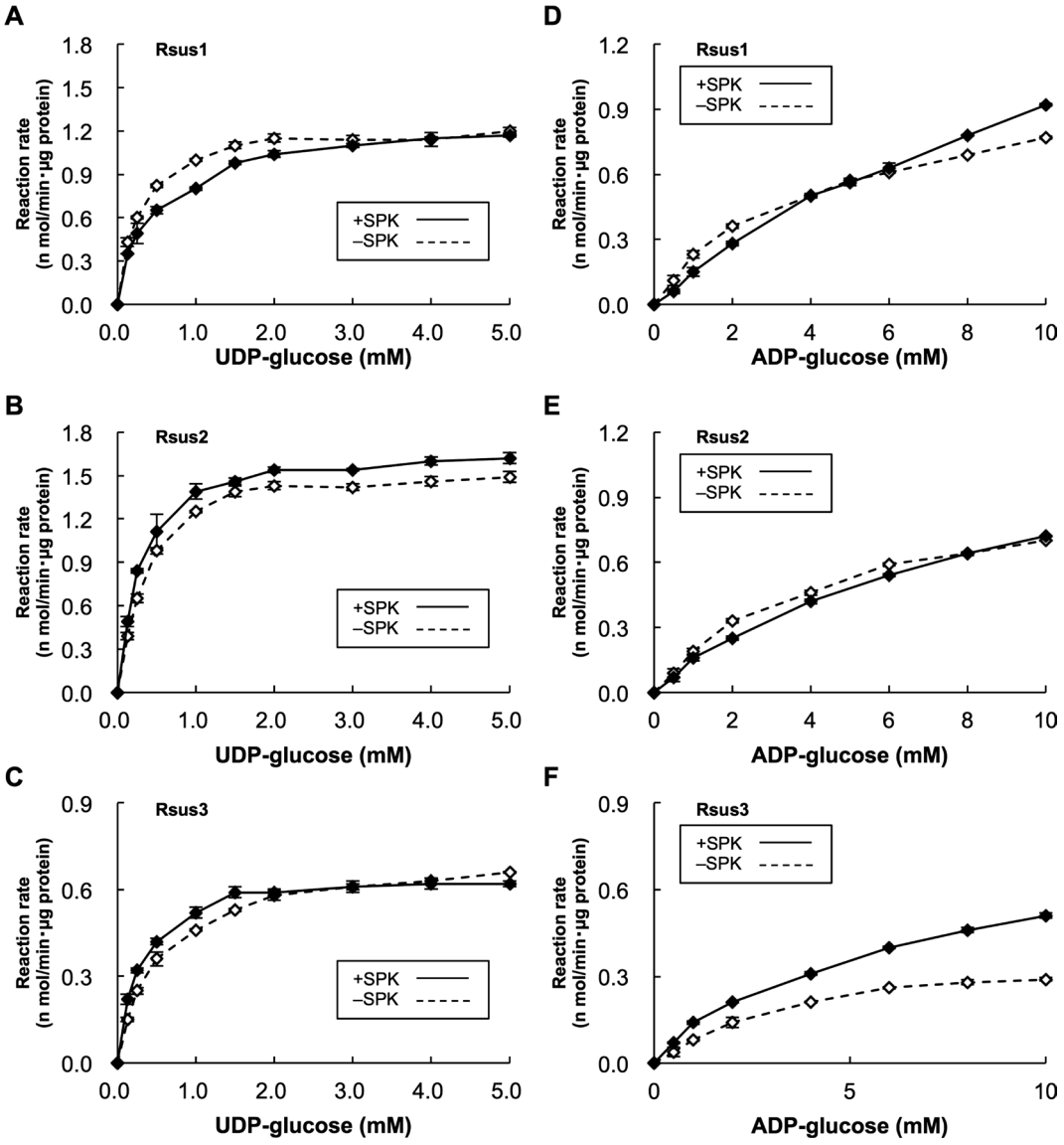

Figure 3. Reactions of the isoforms of rice sucrose synthase for the sucrose synthesis using UDP-glucose or ADP-glucose. (A)–(C). The activities of Rsus1, Rsus2, and Rsus3 were measured in the direction of sucrose synthesis using UDP-glucose and fructose as substrates. Each of Rsus1, Rsus2, and Rsus3 solutions corresponding to 5 μg protein was used as the enzyme in 50 mM Tris-HCl (pH 7.5). For the reaction with the phosphorylated sucrose synthase, 5 μg of sucrose synthase was combined with 5 μg of GST-SPK solution in 50 mM Tris-HCl (pH 7.5), and incubated at 30°C for 10 min. The synthetic reaction were performed at 30°C in 400 μl of the reaction mixture containing the enzyme solution supplemented to 20 mM Tris-HCl (pH 7.5), 10 mM ATP, 25 μM $CaCl_2$, 2 mM $MgCl_2$, 10 mM fructose, and appropriate amount of UDP-glucose (0–5.0 mM). For the enzyme solutions, each of sucrose synthase with/without phosphorylation was used. The synthetic reaction terminated by heating at 95°C. Generated UDP was analyzed by the coupled assays using pyruvate kinase and NADH. 80 μl of reaction mixture was combined with 10 μl of 30 mM phosphoenolpyruvate, 10 μl of 6 mM NADP, 180 μl of 50 mM Tris-HCl (pH 7.5), and 5 μl of 50 U/ml pyruvate kinase (Sigma-Aldrich, St. Louis, USA), and incubated at room temperature for 10 min. Then 10 μl of 40 U/ml lactate dehydrogenase (Sigma-Aldrich) was added to the mixture and incubated at room temperature for 30 min. The amount of UDP was determined by measurement of decrease of NADP monitored at 340 nm. (D)–(F). The activities of Rsus1, Rsus2, and Rsus3 were measured in the direction of sucrose synthesis using ADP-glucose and fructose as substrates. The reaction was performed by the reaction condition with the fixed amount of enzyme solution (5 μg protein for Rsus1 and Rsus2, and 10 μg protein for Rsus3), fructose (10 mM), and an appropriate amount of ADP-glucose (0–10 mM). The amount of generated ADP was measured using ADP-colorimetric/Fluorometric Assay kit (Sigma-Aldrich). Solid lines and broken lines indicate the results of reactions using the enzyme with phosphorylated (+SPK) or without phosphorylation (−SPK), respectively. Error bars represent standard deviations of these measurements. $n=3$.

difference. Reduction by one-third was shown on that of the phosphorylated Rsus3 (Table 2). As for the catalytic efficiency, a large reduction was detected on Rsus1, and slight reduction or no effective changes were detected on Rsus2 and Rsus3 (Table 2). These results indicated that effect of phosphorylation of these isoforms was negatively or not so large on their activities of the sucrose synthesis with UDP.

It has been reported that sucrose synthase may utilize not only UDP but also ADP in sucrose synthesis (Baroja-Fernández et al. 2003). We examined the activity of sucrose synthesis using ADP-glucose (0 to 10 mM) and fructose under the similar reaction condition. However, in this reaction, a dose-dependency was not clearly shown on the ADP production. When ADP-glucose in the reaction cocktail was enriched to the larger amount ranging between 0.5 to 4.0 mM, Rsus1 and Rsus2 showed a linear correlation between amount of ADP generated and the ADP-glucose added in the reaction mixture (Figure 3D, E). However, Rsus3 still showed very low reactivity. Sufficient production of ADP was detected reproductively when 10 μg of the enzyme was applied to a reaction (Figure 3F). Lineweaver-Burk Plot analysis showed a linear correlation between values of $1/v$ and 1/[ADP-glucose content] in the reaction mixture (Supplementary Figure 3).

Effect of phosphorylation on the reaction of sucrose synthesis from ADP-glucose and fructose was analyzed. Although Rsus2 showed no significant change on the reaction between with/without its phosphorylation, reactivity of Rsus1 and Rsus3 was greatly enhanced by their phosphorylation (Figure 3D–F). The phosphorylated Rsus3 showed significantly increase of ADP production as compared with those without phosphorylation, although the productivity of ADP on this reaction was still low (Figure 3F). Alteration of *Km* values to the amount of sucrose significantly was increased (~100%) on all isoforms by phosphorylation (Table 2). The values of catalytic efficiency were significantly reduced (~30%) (Table 2). These results indicated that phosphorylation of these isoforms was influenced for repression of the activity of sucrose synthesis with ADP.

In vitro reactions indicated that all of the isoforms catalyzed the reversible reaction of sucrose synthesis not only from UDP-glucose but also from ADP-glucose and fructose, by which sucrose degradation with ADP to generate fructose and ADP-glucose. We analyzed the effect of phosphorylation on the activity of each isoforms of sucrose synthase. The activity of sucrose degradation was enhanced by phosphorylation on all isoforms when either UDP or ADP was used as a substrate. Phosphorylation of the isoforms of sucrose synthase resulted in reduction of the *Km* values for the reactions of sucrose degradation, suggesting that increase of reactivity and catalytic efficiency may occur for the reactions both with UDP and ADP (Figure 2, Table 1). In contrast, very small effect was detected when they catalyzed the reaction of the sucrose synthesis by the phosphorylated isoforms of sucrose synthase using either UDP-glucose or ADP-glucose (Figure 3, Table 2).

Sucrose synthase determines the intracellular levels of UDP-glucose, ADP-glucose and starch, and total yield in potato tubers. Enhancement of sucrose synthase activity results in increased levels of the total yield (Baroja-Fernández et al. 2009). Our findings suggest that

Table 2. Enzymatic kinetics for the sucrose synthesis by the isoforms of rice sucrose synthase.

Fructose + UDP-glucose					
Isoform	Phosphor-ylation	*Km* (mM)	*Vmax* (nmol min^{-1} (μg protein)$^{-1}$)	*kcat* (min^{-1})	catalytic efficiency (min^{-1} mM^{-1})
Rsus1	−	0.27±0.04	1.3±0.20	104±16	389±9.4
	+	0.39±0.13	1.2±0.06	99±4.6	271±69
Rsus2	−	0.33±0.02	1.4±0.35	108±28	336±109
	+	0.32±0.01	1.6±0.21	130±17	409±64
Rsus3	−	0.33±0.15	0.58±0.19	20±7.7	66±32
	+	0.24±0.04	0.70±0.06	28±2.3	119±31
Fructose + ADP-glucose					
Isoform	Phosphor-ylation	*Km* (mM)	*Vmax* (nmol min^{-1} (μg protein)$^{-1}$)	*kcat* (min^{-1})	catalytic efficiency (min^{-1} mM^{-1})
Rsus1	−	2.25±1.06	0.70±0.32	56±25	25±0.38
	+	7.80±3.54	1.65±0.2	132±17	18±6.1
Rsus2	−	3.05±1.48	0.79±0.25	63±20	22±3.9
	+	5.30±0.71	1.00±0.14	80±11	15±0.12
Rsus3	−	4.40±0.42	0.50±0.07	20±2.8	4.6±1.1
	+	5.65±0.21	0.86±0.09	34±4.0	6.1±1.0

Kinetic parameters, *Km* and *Vmax*, were evaluated by Lineweaver–Burk plots (Supplementary Figure 3), which were determined on the reactions for sucrose synthesis (see Figure 3). Values were determined on the correlation between amounts of UDP generated and fructose with UDP-glucose, or ADP generated and fructose with ADP-glucose, respectively. *kcat* was estimated as *Vmax*/enzyme content. Catalytic efficiency was evaluated by *kcat*/*Km*. Each value±SD is shown (n=5).

phosphorylation of sucrose synthase isoforms may have an effect to enhance the reaction of sucrose degradation and repress the synthetic activity when ADP is used as a substrate. Interestingly, the catalytic efficiency of the sucrose degradation with ADP was greatly enhanced by phosphorylation of sucrose synthase isoforms, while those with UDP gave a little enhancement (Figure 2). This suggests that phosphorylation of the sucrose synthase isoforms may change the equilibrium between sucrose synthesis and degradation, to shift it to be preferable to the sucrose degradation direction.

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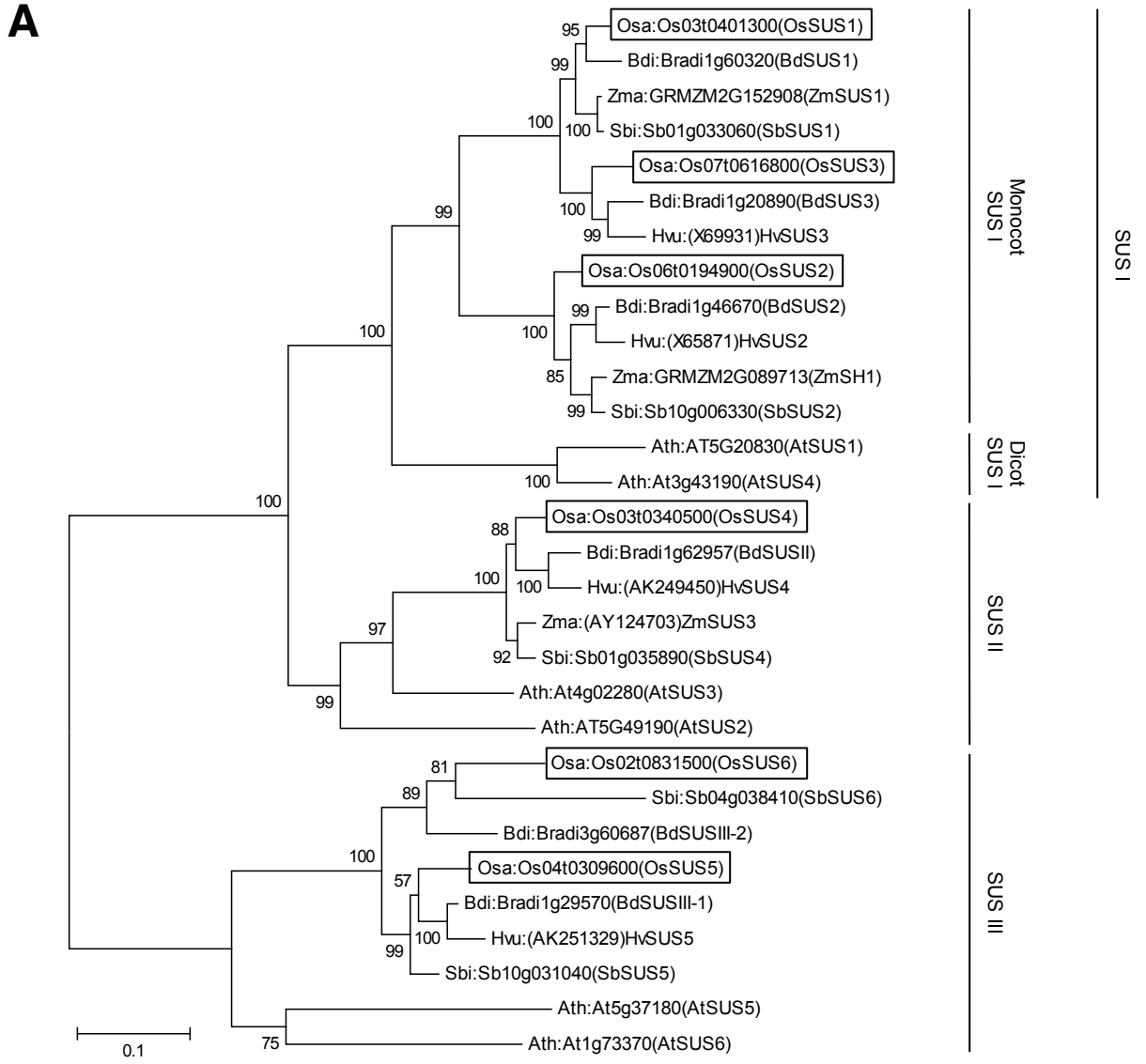
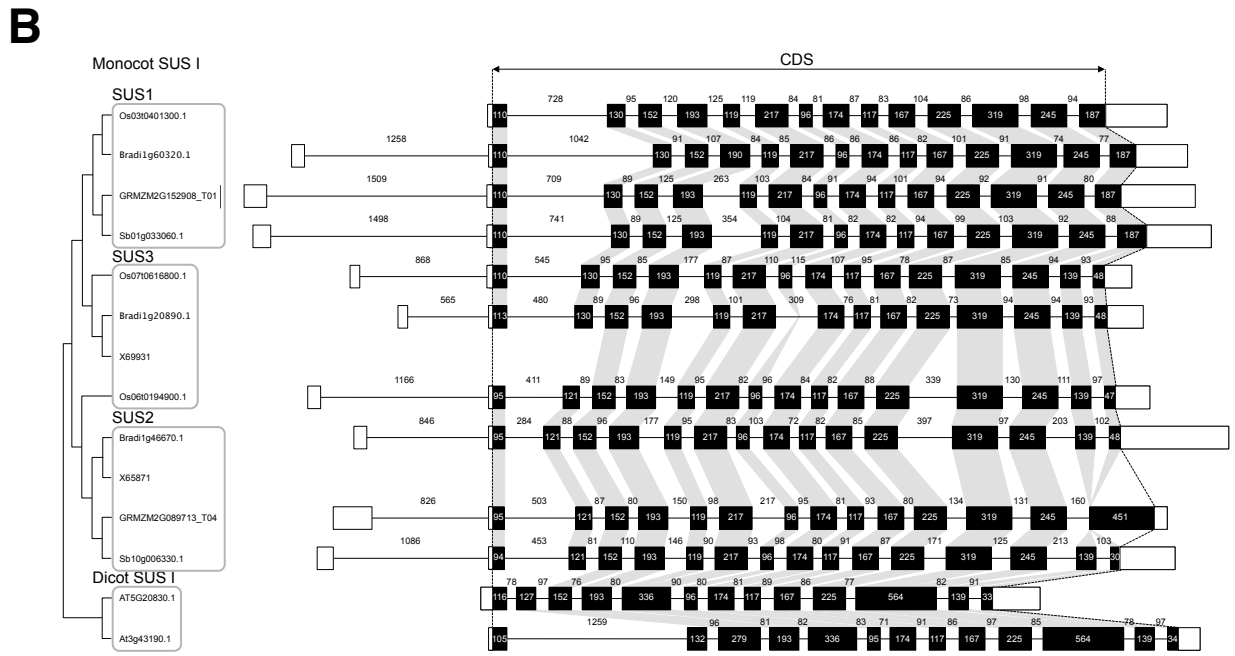


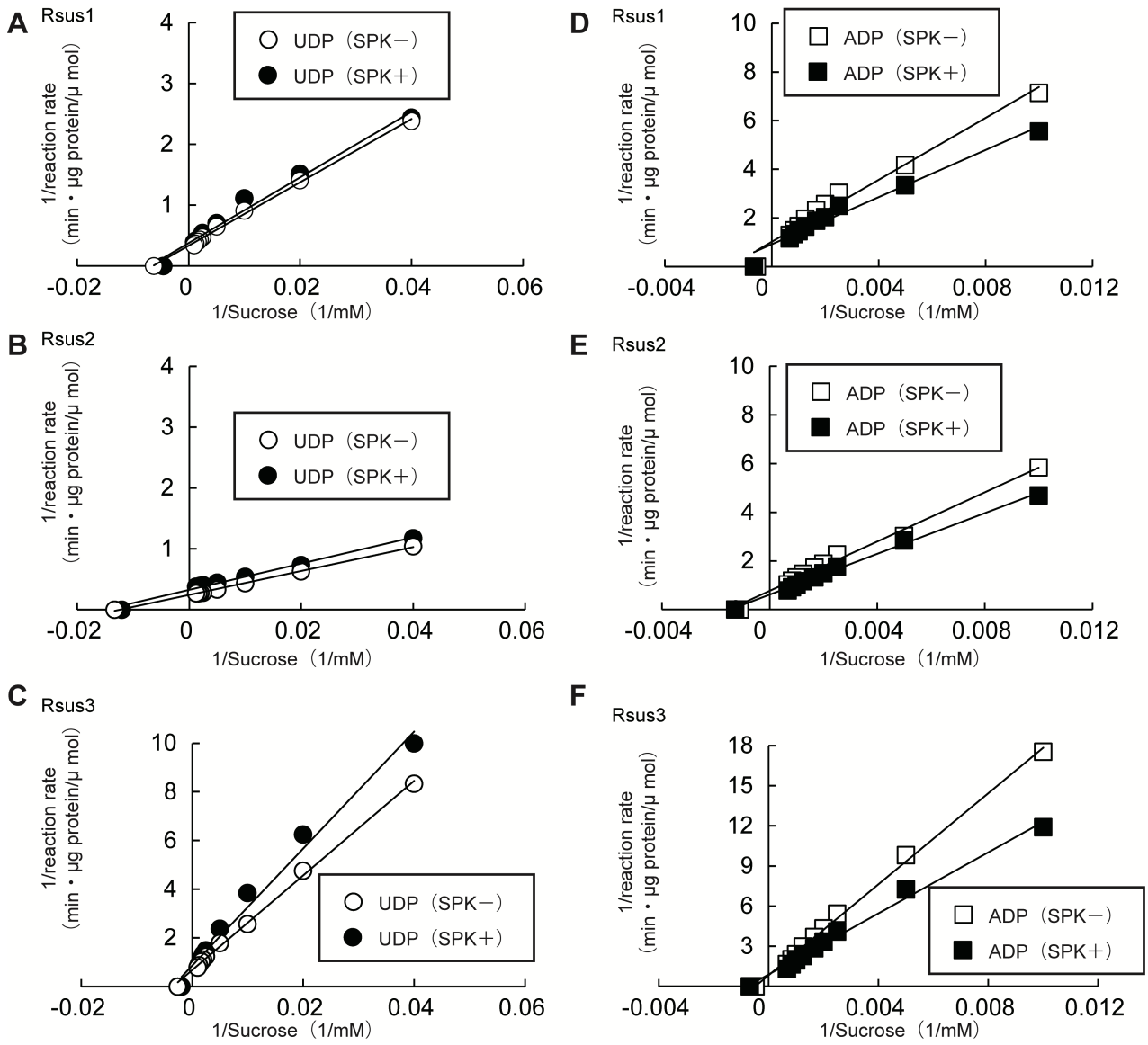
Supplementary Figure 1. Molecular phylogenetic relationship of the members of sucrose synthase isoforms, and exon/intron structure of genes in SUS–I clade.

(A) Phylogenetic relationship of sequences within the sucrose synthase genes family of *Arabidopsis thaliana* (Ath), *Brachypodium distachyon* (Bdi), *Hordeum vulgare* (Hvu), *Oryza sativa* (Osa), *Sorghum bicolor* (Sbi), and *Zea mays* (Zma). BLAST search was carried out using EnsemblPlants (http://plants.ensembl.org/index.html) with the default runtime options with deduced amino acid sequence of Rsus1 (AK100546) as a query sequence. Phylogenetic analyses were carried out using MEGA5 (Tamura *et al.* (2005) *Mol. Biol Evol* 28: 2731–2739). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei (1987) *Mol Biol Evol* 4: 406–425). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The tree is drawn to scale, with branch length in the sane units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutional distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. (B) Structure of the genes in the SUS–I clade. Exons in the coding regions (CDS) and untranslated regions (UTR) are indicated by the filled and white boxes, respectively. Numbers indicate the length of exons and introns.



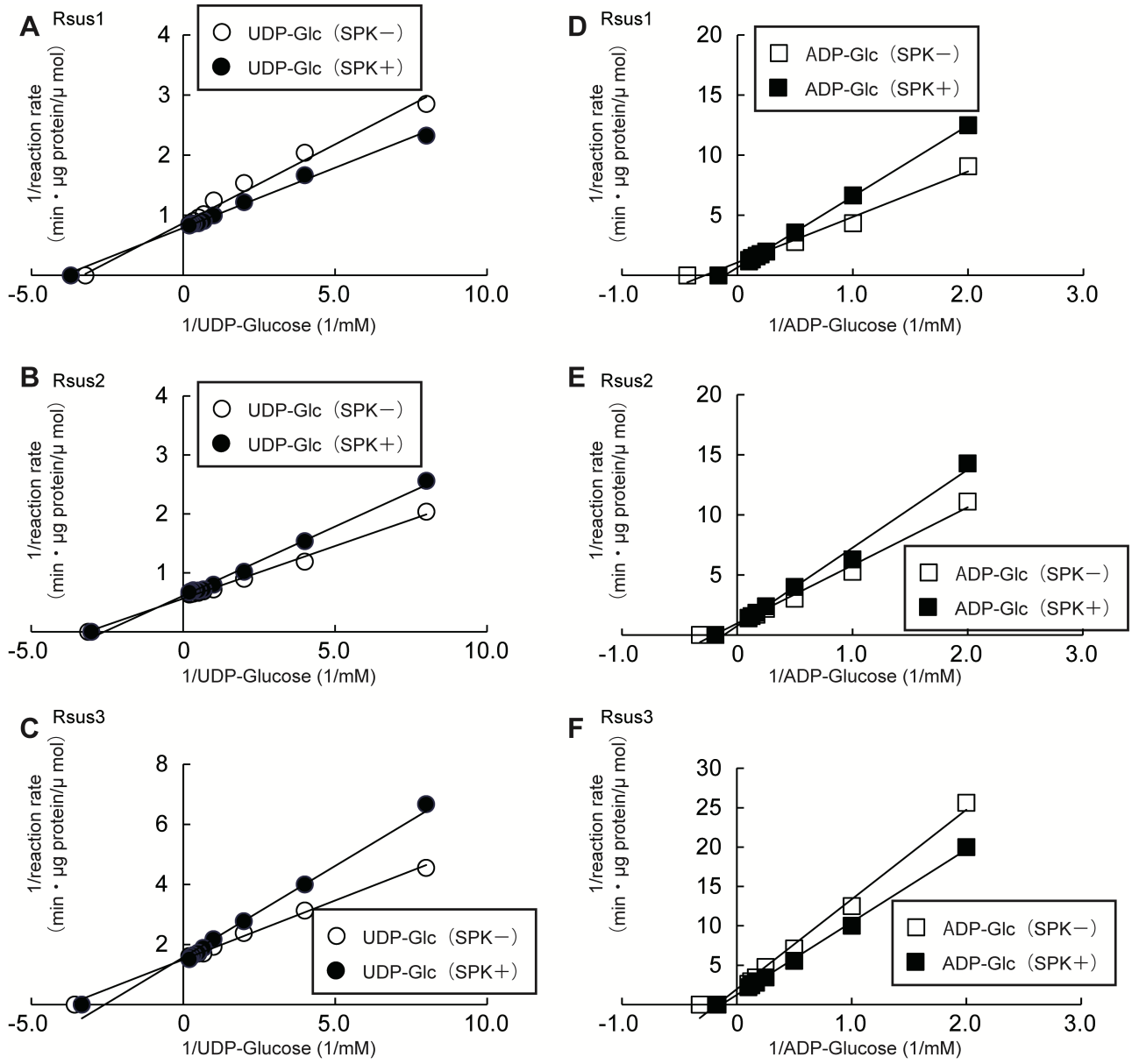
Supplementary Figure 1 (continued). Molecular phylogenetic relationship of the members of sucrose synthase isoforms, and exon/intron structure of genes in SUS–I clade.

(A) Phylogenetic relationship of sequences within the sucrose synthase genes family of *Arabidopsis thaliana* (Ath), *Brachypodium distachyon* (Bdi), *Hordeum vulgare* (Hvu), *Oryza sativa* (Osa), *Sorghum bicolor* (Sbi), and *Zea mays* (Zma). BLAST search was carried out using EnsemblPlants (http://plants.ensembl.org/index.html) with the default runtime options with deduced amino acid sequence of Rsus1 (AK100546) as a query sequence. Phylogenetic analyses were carried out using MEGA5 (Tamura *et al.* (2005) *Mol. Biol Evol* 28: 2731–2739). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei (1987) *Mol Biol Evol* 4: 406–425). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The tree is drawn to scale, with branch length in the sane units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutional distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. (B) Structure of the genes in the SUS–I clade. Exons in the coding regions (CDS) and untranslated regions (UTR) are indicated by the filled and white boxes, respectively. Numbers indicate the length of exons and introns.



Supplementary Figure 2. Kinetic studies of the reaction rate in the reaction for sucrose degradation by the isoforms of sucrose synthase.

(A)–(C). Lineweaver-Burk plots of 1/*v* versus 1/[sucrose] using UDP by the isoforms of Rsus1, Rsus2, and Rsus3 with/without phosphorylation. (D)–(F). Lineweaver-Burk plots of 1/*v* versus 1/[sucrose] using ADP by the isoforms of Rsus1, Rsus2, and Rsus3 with/without phosphorylation. Error bars represent standard deviations of these measurements.



Supplementary Figure 3. Kinetic studies of the reaction rate in the reaction for sucrose synthesis by the isoforms of sucrose synthase.

Lineweaver-Burk plots of 1/*v* versus 1/[UDP-glucose] by the isoforms of Rsus1, Rsus2, and Rsus3 with/without phosphorylation. (D)–(F). Lineweaver-Burk plots of 1/*v* versus 1/[ADP-glucose] by the isoforms of Rsus1, Rsus2, and Rsus3 with/without phosphorylation. Error bars represent standard deviations of these measurements.