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Induction of Expression of Genes Coding for Sporamin and β-Amylase by Polygalacturonic Acid in Leaf-Petiole Cuttings of Sweet Potato[1]

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ABSTRACT

Sporamin and β-amylase are two major proteins of tuberous storage root of sweet potato (*Ipomoea batatas*) and their accumulation can be induced concomitantly with the accumulation of starch in leaves and petioles by sucrose (K Nakamura, M Ohto, N Yoshida, K Nakamura [1991] Plant Physiol 96: 902–909). Although mechanical wounding of leaves of sweet potato only occasionally induced the expression of sporamin and β-amylase genes, their expression could be reproducibly induced in leaf-petiole cuttings when these explants were dipped in a solution of polygalacturonic acid or chitosan at their cut edges. Polygalacturonic acid seemed to induce expression of the same genes coding for sporamin and β-amylase that are induced by sucrose. Because polygalacturonic acid and chitosan are known to mediate the induction of wound-inducible defense reactions, these results raise an interesting possibility that β-amylase, in addition to sporamin, may have some role in the defense reaction. Expression of sporamin and β-amylase genes could also be induced by abscisic acid, and this induction by abscisic acid, as well as induction by polygalacturonic acid or sucrose, was repressed by gibberellic acid. By contrast, methyl jasmonate did not cause the significant induction of either sporamin or β-amylase mRNAs. Induction of expression of sporamin and β-amylase genes by polygalacturonic acid or sucrose was inhibited by cycloheximide, suggesting that *de novo* synthesis of proteins is required for both of the induction processes.

Reproductive storage organs of plants such as seeds or tubers usually contain large amounts of several proteins that are unique to these organs. In addition to storage proteins, inhibitors for proteinase or amylase are often found as one of the abundant proteins in storage organs. These inhibitor proteins are among the defensive proteins of plants that contribute to their resistance toward insect and pathogen attack (2, 23). One of the most well-characterized examples is Inhibitor I and II of tomato and potato (23, 24). They are inhibitors for serine proteinases, and they are highly abundant in fruits of wild tomato and tubers of potato. In addition to developmental regulation, expression of genes coding for Inhibitors I and II are induced in leaves by insect chewing or by severe mechanical wounding. Fragments of cell wall polysaccharides such as PGA[2] and chitosan are potent inducers of Inhibitor genes, and these oligosaccharide elicitors are probably released by the hydrolysis of cell wall polysaccharides upon wounding and mediate the wound induction. The molecular mechanisms of the oligosaccharide-mediated induction of defensive proteins have received extensive study and were reviewed recently (25). Presence in high amounts of these defensive proteins in storage organs probably represents a preformed guard against insect and pathogen attack.

In tuberous storage roots of sweet potato, 60 to 80% of their total soluble proteins is accounted for by sporamin that is encoded by a multigene family (9, 11). Sporamin probably plays primarily a role of a storage protein because it is not detectable or is present in only small amounts in organs other than the tuberous root in normal field-grown plants, and it is preferentially degraded during the growth of new sprouts from the tuberous root. Furthermore, the accumulation of large amounts of sporamin occurs in leaf-petiole cuttings excised from field-grown plants when they are supplied with high concentrations of sucrose or other metabolizable sugars (8, 18). This sucrose induction of sporamin, similar to sucrose induction of patatin in potato (22, 27), occurs concomitantly with the accumulation of starch and at least one of the other major proteins of the tuberous root, β-amylase. Like sporamins, β-amylase is not present, or is present in only small amounts, in organs other than the tuberous root in normal field-grown plants (18, 29). The accumulation of sporamin, β-amylase, and starch in leaf-petiole cuttings shows similar dependence on the concentration of sucrose (18). These results suggest that the expression of genes coding for sporamin and β-amylase occurs in close relation to the expression of cellular metabolite storage function.

In addition to the role as a storage protein, sporamin may have additional functions because the amino acid sequence of sporamin is homologous to Kunitz-type inhibitors for trypsin, chymotrypsin, subtilisin, or α-amylase of legume and cereal seeds (11; K. Matsuoka, unpublished results). It is also homologous to the wound-inducible *Win3* gene product of poplar (3) and cathepsin D inhibitor of potato tuber which is also wound inducible (26; A. Ishikawa and K. Nakamura, unpublished results). Although the proteinase, whose activity is inhibited by sporamin, has not been identified so far, these

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[2] Abbreviation: PGA, polygalacturonic acid.

structural resemblances of sporamin to proteins that may have a role in defense-related reactions prompted us to examine whether sporamin may also be a wound-inducible protein having some kind of defense-related function. Although mechanical wounding of sweet potato leaves only occasionally induced sporamin, we found that PGA or chitosan reproducibly induced sporamin in both leaf and petiole portions of the leaf-petiole cuttings. Interestingly, this PGA induction of sporamin accompanied the induction of β-amylase as well. The PGA induction of β-amylase raises an interesting question concerning the physiological role of this enzyme in plants.

MATERIALS AND METHODS

Plant Materials, Explants, and Chemicals

Sweet potato (*Ipomoea batatas* Lam. var Kokei No. 14) was grown at Nagoya University Experimental Farm. Petioles from plants with the intact leaves still attached were cut with a sharp razor blade. Five to 10 of these leaf-petiole cuttings were combined and dipped in a solution of sucrose, PGA, chitosan, or ABA at the cut edge of the petioles, and they were treated in such a way in darkness at 25°C unless otherwise indicated. Petiole portions of these cuttings, after removing the dipped parts, were used immediately or frozen in liquid nitrogen and stored at −80°C.

For the treatment of leaf-petiole cuttings, sucrose was dissolved in distilled water. PGA from Sigma was dissolved in 10 mM NaOH and dialyzed against distilled water for 18 h at 4°C to remove low mol wt components. Chitosan of crab shells obtained from Sigma was dissolved in 1% acetic acid and treated with sodium nitrite (1%). After the solution was neutralized with NaOH, it was extensively dialyzed against distilled water. (+)ABA was a generous gift from Dr. M. Ito of Shin-Nihon Chemical Industries, Ltd. (Anjo, Japan). GA_3, cycloheximide, and chloramphenicol were obtained from Sigma. Methyl jasmonate, which was a generous gift from Dr. T. Yoshihara, School of Agriculture, Hokkaido University, was dissolved in ethanol and diluted with water at a final concentration of 30 μM and used to treat leaf-petiole cuttings in the same manner as the treatment with sucrose or PGA. Alternatively, methyl jasmonate was applied to leaf-petiole cuttings that were dipped in water in a sealed container as a vapor.

Extraction of Proteins and Analysis of Sporamin and β-Amylase

Proteins were extracted from tissues and the protein content of each extract was determined as described previously (10). SDS-PAGE, two-dimensional PAGE, and immunoblotting with sporamin-specific antisera were carried out as described previously (10). Proteins in the extract were separated by polyacrylamide gel containing soluble starch and stained for β-amylase activity as described previously (29).

Determination of Starch

Determination of starch was carried out as described previously (18).

Northern Blot Hybridization Analysis of RNAs

Total RNA was prepared from tissues, and northern blot hybridization of sporamin and β-amylase mRNAs was carried out as described previously (18). As a control, hybridization with ^{32}P-labeled cDNA insert of $pF_1\gamma GE$-1 coding for the γ-subunit of sweet potato mitochondrial F_1 ATPase (A. Morikami, G. Ehara, T. Asahi, and K. Nakamura, unpublished data) was also carried out. After the membranes were washed, either they were exposed to X-ray film (Kodak, X-Omat) for autoradiography or bands corresponding to sporamin mRNA and β-amylase mRNA were subjected to the determination of radioactivities by a radioanalytical imaging system (Ambis, San Diego, CA).

RESULTS

Induction of Accumulation of Sporamin and β-Amylase by PGA or Chitosan in Leaf-Petiole Cuttings

Leaves of field-grown sweet potato plants were mechanically wounded, and extracts prepared from various parts of the plants were examined for the accumulation of sporamin by immunoblotting of SDS-polyacrylamide gels. Occasionally, we detected significant amounts of sporamin in extracts not only from wounded leaves but also from petioles attached to the wounded leaf and leaves remote from the wounded leaf (data not shown). However, no significant induction of sporamin was observed in several other experiments.

Because PGA and chitosan are known to induce wound-inducible proteinase inhibitors in tomato and potato (23, 25), leaves with petioles still attached were excised from the field-grown plants and dipped in a solution of PGA or chitosan at their cut edges. When the extract from the petiole portions of the cuttings that had been treated with 0.2% PGA for 4 d in darkness was examined, the accumulation of sporamin that was significantly higher than petioles treated with water was detected (Fig. 1). The accumulation of sporamin was observed with 0.05% solution of PGA, and 0.1% solution of PGA gave the level of sporamin almost identical with that obtained with 0.2% solution (data not shown). The amount of sporamin accumulated in petioles treated with 0.2% PGA was similar to that accumulated in petioles treated with 6% sucrose (Fig. 1). Similar results were obtained with extracts prepared from the leaf portions of the cuttings (data not shown). Chitosan also induced sporamin. However, the induction by chitosan was less profound compared to treatments with PGA (Fig. 1).

Treatment of leaf-petiole cuttings with PGA or chitosan also induced the accumulation of β-amylase both in the petiole portions (Fig. 1) and in the leaf portions (data not shown) of the cuttings. In addition to the activity staining of gels shown in Figure 1, induction of β-amylase was confirmed by immunoblotting of the gel (data not shown). The level of β-amylase induced by a 0.2% PGA or a 0.2% chitosan was less than that induced by 6% sucrose.

In contrast to the induction of sporamin by sucrose, treatment of the cuttings with PGA or chitosan did not induce significant accumulation of starch (Fig. 1). Addition of sucrose to a final concentration of 0.5%, the concentration that does not induce the accumulation of sporamin (8, 18), to a 0.2% solution of PGA did not stimulate the accumulation of starch

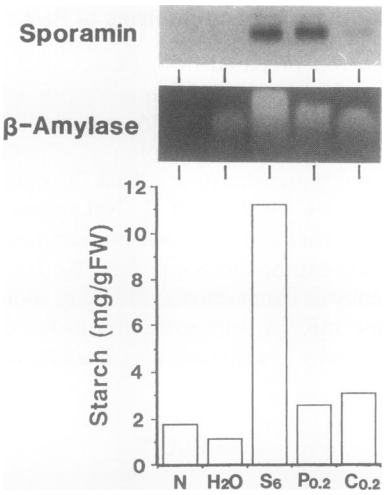

Figure 1. Induction of sporamin and β-amylase in the petiole portions of leaf-petiole cuttings of sweet potato by PGA and chitosan. Leaf-petiole cuttings were treated with water, a 6% solution of sucrose (S_6), a 0.2% solution of PGA ($P_{0.2}$), or a 0.2% solution of chitosan ($C_{0.2}$) for 4 d in darkness. N, Nontreated petioles; FW, fresh weight. The amount of proteins used for immunoblotting of sporamin and activity staining of β-amylase were 8 and 3 μg, respectively. The amount of starch was determined with a portion of the same petioles as were used for the extraction of proteins.

significantly (data not shown). These results suggest that PGA or chitosan activates the expression of sporamin and β-amylase genes independently from the starch accumulation.

Kinetics of Accumulation of Sporamin and β-Amylase mRNAs in Petioles after Treatment of Leaf-Petiole Cuttings with PGA or Sucrose

Total RNAs were prepared from the petiole portions of the cuttings that had been treated with a solution of 6% sucrose or 0.2% PGA for 0, 6, 12, 24, and 48 h, and they were analyzed for the level of sporamin and β-amylase mRNAs by northern blot hybridization. Although the response of leaf-petiole cuttings to 0.2% PGA or 6% sucrose fluctuated slightly from experiment to experiment, probably reflecting differences in the age and physiological condition of the explants, treatment of leaf-petiole cuttings with 0.2% PGA caused dramatic increases in both of the sporamin mRNA and β-amylase mRNA similarly to the treatment with sucrose. Figure 2 shows autoradiographs of filters from one such experiment. Neither electrophoretic patterns of RNAs after staining with ethidium bromide (data not shown) nor the level of mRNA for the nuclear-encoded γ-subunit of mitochondrial F_1 ATPase (A. Morikami, G. Ehara, T. Asahi, and K. Nakamura, unpublished data) differed significantly among these preparations of RNA.

In most of the experiments, the level of sporamin mRNAs accumulated after treatment with 0.2% PGA was similar to those accumulated after treatment with 6% sucrose. By contrast, the level of β-amylase mRNA accumulated after treatment with 0.2% PGA was less than that induced by 6% sucrose. The decline in the level of β-amylase mRNA after 24 h of treatment with PGA (Fig. 2) was also observed in several other experiments.

Comparison of Members of Sporamin Multigene Family Induced by PGA and Sucrose

Sporamin is encoded by a multigene family (11, 17), and a hexaploid genome of the sweet potato contains a total of about 60 copies of sporamin genes (Y. Kowyama, T. Hattori, T. Kumashiro, and K. Nakamura, unpublished data). By contrast, β-amylase is encoded by a gene that is present as a single-copy per haploid genome (28). We previously showed that genes for sporamin induced by exogeneously supplied sucrose in leaf-petiole cuttings were essentially identical with genes for sporamin expressed in the tuberous roots of field-grown plants (8, 10).

To examine the members of the sporamin multigene family whose expression is induced by PGA, we analyzed sporamin polypeptides extracted from petiole portions of the leaf-petiole cuttings that had been treated with 6% sucrose or 0.2% PGA by two-dimensional PAGE. Sporamin consists of isoproteins with different isoelectric points, and subclasses of sporamin show specific and different electrophoretic mobilities on SDS-polyacrylamide gels under certain conditions (17). Immunoblotting of proteins after separation by two-dimensional gel electrophoresis with antiserum against sporamin (Fig. 3) showed that isoproteins of sporamin could be resolved into about 10 different spots. Although some spots showed quantitative differences between the two sources, it was evident from a mixing experiment that sporamin isoproteins induced by sucrose and by PGA were qualitatively similar to one another. These results suggest that sporamin and β-amylase genes expressed in the tuberous roots can be activated in the petioles by exogeneously supplied sucrose or PGA.

Induction of Sporamin and β-Amylase mRNAs by ABA and Its Repression by GA_3

Although the wound induction of potato and tomato Inhibitor gene families occurs not only locally but also systemically

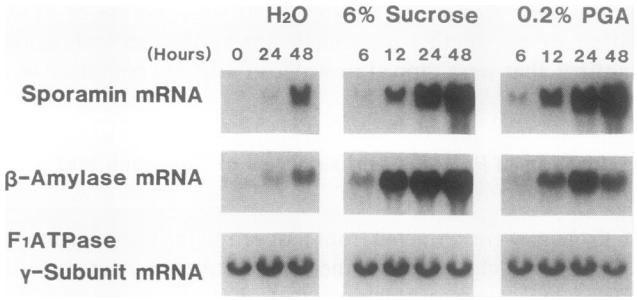

Figure 2. Northern blot hybridization analysis of mRNAs for sporamin and β-amylase in the petiole portions of leaf-petiole cuttings. The gels were loaded with 20 μg each of total RNA from the petiole portions of leaf-petiole cuttings that had been treated with water, a 6% solution of sucrose, or a 0.2% solution of PGA for indicated periods under continuous light. Filters were hybridized with cDNA probes specific for sporamin, β-amylase, and F_1 ATPase γ-subunit. Exposure of filters was continued for 6 h for sporamin mRNA, 17 h for β-amylase mRNA, and 120 h for F_1 ATPase γ-subunit mRNA.

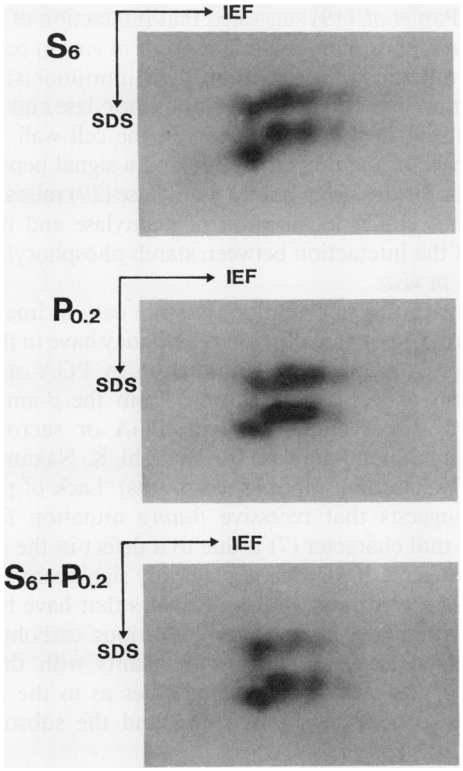

Figure 3. Two-dimensional gel electrophoretic analysis of sporamin polypeptides accumulated in leaf-petiole cuttings after treatment with sucrose or PGA. Total proteins (20 μg) extracted from the petiole portions of leaf-petiole cuttings that had been treated with a 6% solution of sucrose (S_6) or a 0.2% solution of PGA ($P_{0.2}$) for 4 d in darkness or a mixture of 10 μg proteins each of two extracts (S_6 + $P_{0.2}$) were separated by two-dimensional gel electrophoresis, and sporamin-related polypeptides were analyzed by immunoblotting with antiserum against sporamin. IEF, Isoelectric focusing.

in leaves remote from the wounded leaf (24, 25), polyuronide oligosaccharides have been reported not to travel long distances in plants (1). ABA (21), methyl jasmonate (6), and a polypeptide systemin (20) have been shown to induce Inhibitor genes and they are postulated to mediate the wound response in potato and tomato.

Leaf-petiole cuttings of sweet potato were treated with ABA, and sporamin and β-amylase mRNAs were examined after 12 h of treatment by northern blot hybridization. As shown in Figure 4, both of the sporamin mRNA and β-amylase mRNA were induced by 100 μM of ABA. The induction by ABA was greatly repressed by 50 μM GA_3, although the inhibition was not complete (Fig. 4). GA_3 also repressed the induction of sporamin and β-amylase mRNAs by PGA, suggesting that ABA might be involved, at least in part, in the mechanism of PGA induction of sporamin and β-amylase genes. Interestingly, sucrose-induced accumulation of sporamin and β-amylase mRNAs was also inhibited by GA_3.

In contrast to ABA, application of methyl jasmonate to leaf-petiole cutting by a solution did not induce the accumulation of significant levels of sporamin and β-amylase mRNAs (data not shown). In leaves of these methyl jasmonate-treated cuttings, large amounts of a new polypeptide with an apparent mol wt of 18,000 are induced (data not shown), indicating that methyl jasmonate induced changes in the pattern of gene expression in sweet potato leaves.

Effects of Inhibitors for Protein Synthesis on the Induction of Sporamin and β-Amylase mRNAs by PGA and Sucrose

To examine whether the induction of accumulation of sporamin and β-amylase mRNAs by PGA or sucrose depends on *de novo* synthesis of proteins, leaf-petiole cuttings were pretreated for 2 h with cycloheximide, an inhibitor of protein synthesis in the cytosol, or chloramphenicol, an inhibitor of protein synthesis in organelles, before treatment with 6% sucrose or 0.2% PGA. After treatment in darkness for 24 h, total RNAs were prepared from the petiole portions and analyzed for sporamin and β-amylase mRNAs by northern blot hybridyzation. As shown in Figure 5, both the induction of sporamin mRNA and of β-amylase mRNA by sucrose and by PGA were completely inhibited by cycloheximide. By contrast, chloramphenicol did not affect the induction severely. Although chloramphenicol slightly affected the PGA-induced accumulation of β-amylase mRNA in the experiment shown in Figure 5, no significant inhibition was observed in other experiments (data not shown). These results suggest that both the induction by PGA and the induction by sucrose of sporamin and β-amylase mRNAs require *de novo* synthesis of proteins in the cytosol.

DISCUSSION

Two major proteins of tuberous roots of sweet potato, sporamin and β-amylase, are usually not detectable in leaves and petioles. We previously reported that treatment of leaf-petiole cuttings with high concentrations of sucrose induces the expression of genes coding for sporamin and β-amylase concomitantly with the accumulation of starch (8, 18). In this paper, we found that oligo- or polysaccharide, such as PGA and chitosan, can also induce the expression of these genes.

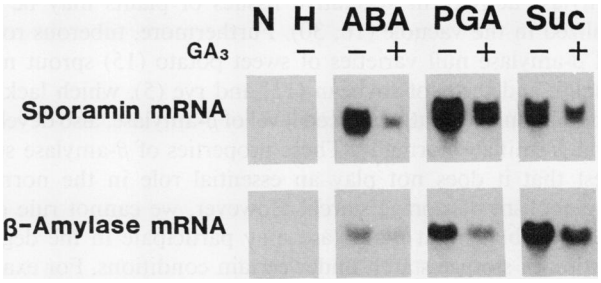

Figure 4. Induction of sporamin and β-amylase mRNAs in leaf-petiole cuttings by ABA and its inhibition by GA_3. Leaf-petiole cuttings were treated with water (H), a 100 μM solution of ABA, a 0.1% solution of PGA, or a 6% solution of sucrose (Suc) in the absence (−) or the presence (+) of 50 μM GA_3 for 12 h in darkness, and total RNAs were isolated from the petiole portions. Leaf-petiole cuttings treated in the presence of GA_3 were pretreated with 50 μM GA_3 for 2 h, and other cuttings were pretreated with water for 2 h. The gels were loaded with 25 μg each of total RNA, and filters were hybridized with cDNA probes specific for sporamin and β-amylase. N, Nontreated petioles.

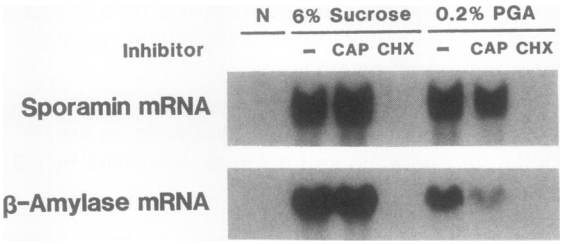

Figure 5. Effects of cycloheximide and chloramphenicol on the induction of sporamin and β-amylase mRNAs by sucrose or PGA. Leaf-petiole cutting were treated with 300 μM solutions of chloramphenicol (CAP) or cycloheximide (CHX) for 2 h before the treatment with a 6% solution of sucrose or a 0.2% solution of PGA for 24 h in darkness. Solutions of sucrose and PGA also contained appropriate inhibitors. Controls (−) did not contain inhibitor. The gels were loaded with 20 μg each of total RNA from the tissue, and filters were hybridized with cDNA probes specific for sporamin and β-amylase. N, Nontreated petioles.

Oligo-galacturonide fragments and chitosan are known to induce various defense-related proteins such as proteinase inhibitors, β-glucanases, and chitinases (2, 25). Although mechanical wounding of leaves of field-grown sweet potato only occasionally induced sporamin and β-amylase genes, induction by PGA and chitosan suggests that sporamin and β-amylase may play some defense-related function in sweet potato. Because sporamin is homologous to various inhibitor proteins (see "Introduction"), it may have inhibitor activity against an unidentified proteinase or amylase and play a role in the defense reaction against insect or microbial attack in addition to a role as a storage protein.

The physiological role of β-amylase in plants is not precisely known at present in spite of extensive enzymological studies of its amylolytic activity *in vitro*. β-Amylase in plants is often found in large abundance in storage organs such as seeds and tuberous roots where a large amount of starch is stored. However, the precursor for sweet potato β-amylase (29) does not contain the N-terminal transit peptide sequence that directs proteins into plastids, and most of the cellular β-amylase activity in vegetative tissues of plants may be localized in the vacuole (16, 30). Furthermore, tuberous roots of β-amylase null varieties of sweet potato (15) sprout normally, and seeds of soybean (12) and rye (5), which lack or contain only a greatly reduced level of β-amylase, also develop and germinate normally. These properties of β-amylase suggest that it does not play an essential role in the normal metabolism of storage starch. However, we cannot rule out the possibility that β-amylase may participate in the degradation of storage starch under certain conditions. For example, autophagic uptake of starch granules into the vacuole may occur under certain conditions.

Sucrose induction of β-amylase occurs concomitantly with the induction of sporamin and starch in sweet potato (18), and β-amylase may play a role as a storage protein as previously suggested by Giese and Hejgaard (7) for β-amylase in barley seeds.

β-Amylase may have an activity other than exoamylase. Sweet potato β-amylase has been shown to inhibit the activity of starch phosphorylase in the direction of glucan synthesis (4, 19). Pan *et al.* (19) suggested that interaction of β-amylase with starch phosphorylase might occur *in vivo* in parenchyma cells of tuberous roots based on their immunohistochemical observation that both the starch phosphorylase and β-amylase are localized in the amyloplast and the cell wall. However, the absence of the transit peptide and a signal peptide in the precursor for the sweet potato β-amylase (29) raises questions about the cellular localization of β-amylase and the significance of the interaction between starch phosphorylase and β-amylase *in vivo*.

Even with this information, it is not easy to imagine what kind of role β-amylase of sweet potato may have in the defense reaction as suggested by its induction by PGA or chitosan. Treatment of leaf-petiole cuttings from the β-amylase null variety of sweet potato (7) with PGA or sucrose induce sporamin but not β-amylase (K. Hayashi, K. Nakamura-Kito, and K. Nakamura, unpublished results). Lack of pleiotropic effects suggests that recessive *β-amy* mutation for the β-amylase null character (7) is due to a defect in the β-amylase structural gene. If β-amylase plays any significant role in the metabolism of carbohydrates in tissues that have been stimulated with PGA, comparison of various carbohydrates in PGA-treated leaves of the *β-amy* plants with that of the normal plants may give us some clues as to the role of β-amylase in the defensive reaction and the substrate of β-amylase *in vivo*.

Large abundance in the storage organ and the induction by PGA in leaves are properties of sporamin and β-amylase that are shared with Inhibitors I and II of potato and tomato (24). In addition to PGA and chitosan, ABA (21), methyl jasmonate (6), and a polypeptide systemin (20) also induce the expression of Inhibitor II genes and these substances are suggested to be involved in the transfer of signals from the wound site. Although we dip leaf-petiole cuttings into a solution of PGA only at their cut edges, induction of sporamin and β-amylase occurs not only in petiole portions of the cuttings but also in leaf portions of the cuttings. If PGA molecules also do not travel through the vascular system (1) in sweet potato, some kind of secondary movable signal may also participate in the PGA induction of sporamin and β-amylase genes. Methyl jasmonate did not induce sporamin and β-amylase mRNAs significantly (data not shown). On the other hand, ABA induced both the sporamin and β-amylase mRNAs, although the levels of induction were weaker compared to the induction by PGA (Fig. 3). Similar to the induction of Inhibitor II mRNAs in potato (21), induction of sporamin and β-amylase mRNAs by PGA was repressed by GA_3, suggesting that ABA may participate, at least in part, in the transfer of PGA-induced signal in sweet potato as well.

Genes coding for sporamin and β-amylase are also different from Inhibitor II genes of potato in their response to sucrose. Sucrose enhances the wound-induced expression of Inhibitor II genes in detached leaves of potato (14). However, application of sucrose to detached leaves of potato that had not been wounded only slightly induced the expression of Inhibitor II genes. In this regard, sporamin and β-amylase genes are more similar to class I patatin genes of potato (22, 27) than to Inhibitor II genes. The concentration of sucrose that is required to maximally stimulate the wound-induced expression of Inhibitor II genes (14) is lower than the concentration of

sucrose required to induce the maximum level of expression of patatin genes in potato explants (27) and that required for the induction of sporamin and β-amylase genes in sweet potato explants (8, 18). However, patatin genes do not show significant response to wounding and ABA (our unpublished results). Methyl jasmonate also does not induce patatin genes (our unpublished results). It is interesting that the same genes coding for sporamin and β-amylase seem to show responses that are played by different genes in potato.

Expression of the members of the sporamin multigene family and the β-amylase gene is coupled under various conditions analyzed so far, *i.e.* high-level expressions in tuberous roots of field-grown plants and in stems of plantlets cultured *in vitro* (10; our unpublished results) and induction in leaf-petiole cuttings by sucrose (18) and by PGA (this paper). Genes coding for sporamin and β-amylase seem to share the regulatory mechanisms for their expression, at least in part. Sequence blocks conserved in the 5′-upstream regions of two genes for sporamin and of β-amylase gene (9, 28) and a nuclear protein that binds to these genes (13) are likely to be involved in the coordinated regulation of expression of these genes. Induction of expression of sporamin and β-amylase genes by PGA and by sucrose both require *de novo* synthesis of proteins (Fig. 5), and GA_3, which repressed the induction of sporamin and β-amylase genes by ABA or PGA, also repressed the induction by sucrose (Fig. 4). However, it is not known at present whether the PGA induction and sucrose induction of these genes are mediated by independent pathways or whether they share some common components in their signal-transduction pathways.

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