We identified a rapid and novel system to effectively metabolize a large amount of H$_2$O$_2$ in the suspension cells of *Scutellaria baicalensis* Georgi. In response to an elicitor, the cells immediately initiate the hydrolysis of baicalein 7-O-β-D-glucuronide by β-glucuronidase, and the released baicalein is then quickly oxidized to 6,7-dehydrobaicalein by peroxidases. Hydrogen peroxide is effectively consumed during the peroxidase reaction. The β-glucuronidase inhibitor, saccharic acid 1,4-lactone, significantly reduced the H$_2$O$_2$-metabolizing ability of the *Scutellaria* cells, indicating that β-glucuronidase, which does not catalyze the H$_2$O$_2$ degradation, plays an important role in the H$_2$O$_2$ metabolism. As H$_2$O$_2$-metabolizing enzymes, we purified two peroxidases using ammonium sulfate precipitation followed by sequential chromatography on CM-cellulose and hydroxyapatite. Both peroxidases show high H$_2$O$_2$-metabolizing activity using baicalein, whereas other endogenous flavones are not substrates of the peroxidase reaction. Therefore, baicalein predominantly contributed to H$_2$O$_2$ metabolism. Because β-glucuronidase, cell wall peroxidases, and baicalein pre-exist in *Scutellaria* cells, their constitutive presence enables the cells to rapidly induce the H$_2$O$_2$-metabolizing system.

Plants display a broad range of defense responses to protect themselves against mechanical damage or pathogen attack. One of the earliest responses is the production of large amounts of reactive oxygen species (ROS), which is called the oxidative burst (1–4). The physiological roles of the oxidative burst have been well examined to date. For example, several studies demonstrated that ROS directly reduce pathogen viability (5) and lipid peroxidation, as well as by-products of redox reactions under normal conditions, but they are maintained at a low level by ROS-metabolizing enzymes such as superoxide dismutase (10, 11). However, the detoxification mechanism of ROS produced by the oxidative burst has not been clearly understood. In this work, we establish that the suspension cells of *Scutellaria baicalensis* Georgi (skullcap plants) have a rapid and novel system to detoxify a large amount of H$_2$O$_2$. *S. baicalensis* contains numerous flavones, and their pharmacological properties have been extensively investigated. Among them, baicalein (BA) has attracted considerable attention, as it has a variety of interesting activities such as antibacterial (12), antiviral (13), anticancer (14), and lipoxygenase-inhibitory effects (15). In addition, this plant has long been known to possess a β-glucuronidase, called baicalinase (16). Previously, we purified β-glucuronidase from the callus culture and demonstrated that this enzyme displays high activity for baicalein 7-O-β-D-glucuronide (BAG), the main flavonoid of this plant (17). To establish the physiological roles of β-glucuronidase and BAG, we attempted further studies. Consequently, we found that the suspension cells of *S. baicalensis* effectively metabolize H$_2$O$_2$ by a sequential reaction, including the hydrolysis of BAG to BA by β-glucuronidase and then the oxidation of BA by cell wall peroxidases. In this paper, we describe the detoxification mechanism of H$_2$O$_2$ in *Scutellaria* cells. We also report on the purification of the peroxidases involved in H$_2$O$_2$ degradation and their kinetic properties.

**Experimental Procedures**

**Plant Materials**—The calluses were induced from the shoot stem segments of *S. baicalensis*, as described previously (17). The suspension cells were obtained by incubating the 4-week-old calluses in liquid Murashige-Skoog medium (18) containing 2,4-dichlorophenoxyacetic acid (0.5 mg/liter) and N6-benzyladenine (0.5 mg/liter) at 25 ± 1°C under 16-h light conditions. The suspension cells were subcultured every 4 weeks in liquid Murashige-Skoog medium under the same culture conditions.

**Flavones**—Apigenin and luteolin were purchased from Wako Pure Chemical Industries (Osaka). Oroxylin A was donated by Dr. Yamamoto (Hokuriku University, Japan). BAG, BA, and wogonin were isolated from the dried roots of *S. baicalensis* (Uchida Wakanaku, Tokyo) as described previously (17).

**Structural Determination of 6,7-Dehydrobaicalein**—Because the amount of the unknown compound produced from the elicited cells by oxidative burst was not enough to analyze its structure, we enzymatically synthesized this compound from BA using CM-52 eluate as a crude peroxidase preparation. The CM-52 eluate (50 ml), which was prepared from 4-week-old cells (300 g) as described below, was added to 50 mM citrate buffer (pH 4.0, 200 ml) containing 2 mM H$_2$O$_2$. BA (30 mg) dissolved in dimethyl sulfoxide (1 ml) was slowly added to the enzyme solution and stirred at room temperature for 5 min. We confirmed by HPLC analysis that the reaction product had the same retention time as the authentic 6,7-dehydrobaicalein. This paper is available on line at http://www.jbc.org

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**Note**

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† The abbreviations used are: ROS, reactive oxygen species; BA, baicalein; BAG, baicalein 7-O-β-D-glucuronide; DBA, 6,7-dehydrobaicalein; SAL, saccharic acid 1,4-lactone; HPLC, high performance liquid chromatography; PAGE, polyacrylamide gel electrophoresis.

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as the unknown compound produced by the oxidative burst. The reaction mixture was applied to the reversed-phase polystryrene gel, MCI-gel CHP 20P (Mitsubishi Chemical Co., Tokyo, 2.0 × 20.0 cm) previously equilibrated with distilled water. After the column was washed with 100 ml of distilled water, fractions containing the unknown compound were eluted with 100 mM sodium citrate buffer (pH 4.0), known to contain flavonoid-specific β-glucuronidase. The enzyme activity was determined by incubating an assay mixture containing the enzyme solution, together with 200 μM BA, 200 μM H2O2, 0.3% (v/v) Triton X-100, and 100 mM sodium citrate buffer (pH 4.0) at 30 °C for 10 min in a final volume of 200 μl. The amount of H2O2 in the reaction mixture was measured as described above. The enzyme activity (katal) was defined as the amount (mole) of H2O2 consumed per second.

Preparation of Protoplasts—The suspension cells (4.0 g) were soaked in 0.6 M mannitol (50 ml) containing 1.0% (w/v) Cellulase Onozuka R-10 (Yakult, Tokyo) and 0.1% (w/v) Macerozyme R-10 (Yakult). After incubation at 30 °C for 120 min, the reaction mixture was centrifuged at 100 × g for 10 min. The resulting protoplasts were washed twice with 0.6 M mannitol and centrifuged as described before.

Purification of Cell Wall Peroxidases—All procedures were carried out at 4 °C, unless otherwise indicated. Four-week-old suspension cells (300 g) were shaken in 1 l NaCl (1,000 ml) at 100 rpm for 60 min and then filtered with Nuclepore. After the filtrate was centrifuged at 20,000 × g for 15 min, the supernatant was fractionated by addition of ammonium sulfate. Proteins precipitating between 10 and 75% saturation were collected by centrifugation at 20,000 × g for 30 min and then redissolved in 0.1 M sodium phosphate buffer (pH 7.0). The dialyzed sample was applied to a Whatman CM-cellulose column (2.5 × 35.0 cm) equilibrated with the same buffer. After the column was washed with the above buffer (200 ml), bound proteins were eluted with a 1,000-ml linear gradient of NaCl (0–0.4 M) at a flow rate of 1.0 ml/min. The eluent was collected in 20-ml fractions. The most active fractions (fractions 23–25) were concentrated by ultrafiltration (Advantec, Tokyo) and dialyzed against 10 mM sodium phosphate buffer (pH 7.0). Peroxidase 1 was eluted from the same column with a 400-ml linear gradient of ionic strength sodium phosphate buffer (pH 7.0, 10–100 mM). The most active fractions (fractions 12–14) were pooled, concentrated by ultrafiltration, and dialyzed against 10 mM sodium phosphate buffer.

Protein Assay—Protein concentrations were measured according to Bradford (20) using bovine serum albumin as the standard.

Determination of Molecular Mass and Isoelectric Point—SDS-PAGE analysis was carried out with the system of Laemmli (21) in a 12.5% acrylamide gel of 0.75-mm thickness (Bio-Rad). The subunit molecular mass of the enzyme was determined by comparison with low molecular mass protein standards (Sigma). Isoelectric focusing was conducted according to O’Farrell (22) using 7.5-mm glass tubes (Atto, Tokyo). The pH of the purified enzyme was determined by comparison with marker proteins (Sigma). The native molecular masses of peroxidases 1 and 2 were estimated by gel filtration chromatography on a 1.5 × 75.0-cm column of Sephacryl S-200 HR (Amersham Pharmacia Biotech) equilibrated with 10 mM sodium phosphate buffer (pH 7.0) at a flow rate of 0.3 ml/min. Fractions of 15 ml were collected. Molecular mass markers from 29 to 700 kDa were resolved under the same conditions prior to running the fractions containing peroxidase activity.

RESULTS

Elicitor Treatment of Cell Suspension of S. baicalensis in the Presence of β-Glucuronidase Inhibitor—S. baicalensis is known to contain flavonoid-specific β-glucuronidase, although its physiological importance is not understood (16). To reveal the roles of this enzyme in S. baicalensis, we first investigated effects on the cells induced by inhibition of β-glucuronidase. In this study, SAL was used as the β-glucuronidase inhibitor.

A significant effect of β-glucuronidase inhibition was observed in the elicited cells but not in the nonelicited cells. When Scutellaria cells were treated with the elicitor yeast extract in the presence of SAL, numerous components that should exist within the cells were released to the medium (Fig. 1A). In addition, α-mannosidase (0.60 nanokatal/g fresh cells), which is...
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Fig. 1. HPLC analysis of the extracellular fluid after elicitation. The cells were incubated with 0.1% yeast extract at 25 °C for 1 h in the presence (A) or absence (B) of 1 mM SAL. After removal of the cells by filtration, the media were analyzed using an HPLC system as described under “Experimental Procedures.” The column was initially equilibrated with 5% aqueous acetonitrile. After injection of the samples, the concentration of acetonitrile was linearly increased to 70% in 30 min at a flow rate of 1 ml/min.

We measured the amount of $\text{H}_2\text{O}_2$ produced by the elicitation. As shown in Fig. 2, the SAL-treated cells immediately started to produce $\text{H}_2\text{O}_2$ after addition of the elicitor. The amount of $\text{H}_2\text{O}_2$ was maximum after 10 min of incubation and thereafter was slowly degraded. The SAL-untreated cells also produced the maximum level of $\text{H}_2\text{O}_2$ at 10 min after elicitation, although the amount was lower, as compared with SAL-treated cells. After 30 min of incubation, the $\text{H}_2\text{O}_2$ amount decreased to the same level as that before elicitation, showing that the SAL-untreated cells effectively metabolize $\text{H}_2\text{O}_2$. Based on these results, we concluded that the rapid degradation of $\text{H}_2\text{O}_2$ depends largely on a hydrolytic reaction by $\beta$-glucuronidase. Because $S$. baicalensis possesses a large amount of BAG along with BAG-specific $\beta$-glucuronidase (17, 24), it was suggested that the aglycone BA contributes to the $\text{H}_2\text{O}_2$ metabolism.

Production of 6,7-Dehydrobaicalein in Elicited Cells—HPLC analysis showed that the elicited cells in the absence of SAL released an unknown compound (retention time, ~8 min) to the medium (Fig. 1B). In contrast, this unknown peak was much smaller on the chromatogram of the SAL-treated cells (Fig. 1A). As this compound was also assumed to be involved in the $\text{H}_2\text{O}_2$ metabolism, we attempted a structural elucidation. After several unsuccessful attempts, its structure was determined as DBA by the method as described under “Experimental Procedures.” DBA is the first flavone with an ortho-diketone moiety. Judging from its structure, this flavone was considered to be derived from the oxidation of BA.

Changes in the Amounts of BAG, BA, and DBA during Elicitation—We assessed changes in the amounts of BAG, BA, and DBA during elicitation. In the absence of SAL, the cells immediately initiated the hydrolysis of BAG after addition of the elicitor, and BAG continued to decrease until 30 min after incubation (Fig. 3A). The decrease of BAG correlated well with the $\text{H}_2\text{O}_2$ degradation such that $\text{H}_2\text{O}_2$ induced by the oxidative burst also decreased until 30 min after incubation, as shown in Fig. 2. On the other hand, BA increased rapidly with BAG hydrolysis, and the BA amount at 30 min was about two times higher than that before elicitation (Fig. 3B). Although at least ~2.5 μmol of BAG should be hydrolyzed after elicitation, only a slight increase (~0.06 μmol) in the BA amount was observed even at the maximum level, suggesting that most of the BA is immediately metabolized. SAL-untreated cells produced more DBA than BA, and its amount reached a maximum level after 60 min of incubation (Fig. 3C). Thus, the production of DBA also occurred rapidly in response to the elicitor. In contrast, addition of SAL extensively inhibited the hydrolysis of BAG (Fig. 3A) and the production of DBA (Fig. 3C), indicating that the hydrolysis of BAG is essential for the production of DBA. Therefore, we concluded that DBA is produced by the hydrolysis of BAG to BA, followed by the oxidation of BA. Hydrogen peroxide may be consumed during the conversion of BA to DBA because this step is an oxidative reaction. DBA was also assumed to undergo further metabolism, based on the facts that the decrease in the DBA amount was observed on incubation over 120 min and that only ~15% of BAG hydrolyzed was recovered as DBA (0.38 μmol at the maximum level). However, the mechanism of DBA metabolism was not established in this study.

Identification of the $\text{H}_2\text{O}_2$-metabolizing Enzyme—We investigated whether the oxidation of BA to DBA required $\text{H}_2\text{O}_2$. Consequently, crude enzyme extract from $S$. baicalaria cells was shown to catalyze the formation of DBA from BA by consuming...
H$_2$O$_2$, indicating that H$_2$O$_2$ is metabolized by peroxidase. Furthermore, we confirmed that BA metabolism by enzymes except for peroxidase is ineffective because the crude enzyme extract did not metabolize BA in the absence of H$_2$O$_2$. To extract this peroxidase effectively, extraction conditions were optimized. Crude enzyme extracts were prepared using various solvents, and then the peroxidase activity in each extract was measured by quantifying the amount of H$_2$O$_2$ consumed by the oxidation of BA. The higher activity was observed in the extract prepared with 1 M NaCl, which is often used for solubilization of proteins ionically bound to the cell walls. Moreover, the protoplasts prepared by digestion of the cell walls displayed much lower metabolizing activity for H$_2$O$_2$ (0.5 nanokatal/g cells) as compared with the intact _Scutellaria_ cells (11.5 nanokatal/g cells), and most of the activity was recovered in the digestion medium. These results apparently indicate that the H$_2$O$_2$-metabolizing enzymes exist in the cell walls.

The Activity of β-Glucuronidase and the Cell Wall Peroxidase after Elicitation—Because it was confirmed that β-glucuronidase and the cell wall peroxidase contribute to the detoxification of H$_2$O$_2$, we measured the activity of both enzymes after elicitation. As shown in Fig. 4, neither activity increased during the incubation period (180 min) tested, and the peroxidase activity decreased more than the β-glucuronidase activity. Hence, we concluded that the metabolism of H$_2$O$_2$ produced by the oxidative burst is catalyzed by these enzymes that are present constitutively in _Scutellaria_ cells.

Purification of Cell Wall Peroxidases—Previously, we purified β-glucuronidase from the _Scutellaria_ calluses and characterized its properties (17). At present, there is no information on the cell wall peroxidases in this plant. Therefore, to characterize precisely the cell wall peroxidases, we attempted to purify them. The 4-week-old cells of _S. baicalensis_ were shaken in 1 M NaCl and then filtered. The filtrate was fractionated by ammonium sulfate saturation. More than 70% of the peroxidase activity was precipitated between 10 and 75% saturation of ammonium sulfate, resulting in a 3-fold purification. As a first chromatographic step, the solubilized ammonium sulfate fraction was applied to a CM-cellulose (CM-52) column, where a peroxidase was eluted with a linearly increasing gradient of NaCl (0–0.4 M). A lower level of peroxidase activity was recovered in the void volume, whereas most of the peroxidase activity eluted at −0.2 M NaCl. This step increased the specific activity in the latter fractions by a factor of 23-fold with a recovery of 43%. Because the total enzyme activity in the latter fractions was about 50 times higher than that in the former fraction, further purification of the former fractions was not carried out. As a final purification step, the latter CM-52 eluate was applied to a hydroxylapatite column. A peroxidase activity was eluted with 10 mM phosphate buffer (pH 7.0) containing 10 mM mercaptoethanol and 2 M NaCl, respectively. After centrifugation at 20,000 × g for 5 min, the activities of β-glucuronidase and peroxidases in the supernatants were measured as described under “Experimental Procedures.” Data are means of three replicated assays.
Both peroxidases are the first flavone-metabolizing enzymes to be purified.

**Molecular Mass and Isoelectric Point of Peroxidases 1 and 2**—SDS-PAGE of peroxidases 1 and 2 showed subunit molecular masses of 38 and 34 kDa, respectively (Fig. 5). The native molecular masses were estimated from the elution volume of both peroxidases on Sephacryl S-200 HR chromatography, where peroxidases 1 and 2 eluted as a single molecular species with a molecular mass of about 35 kDa in each case. These results suggested that each peroxidase exists as a monomeric enzyme. The pI values for peroxidase 1 and 2 were determined to be 8.7 and 8.9, respectively by comparison with marker proteins of known pI on isoelectric focusing gels.

**Standard Assay Conditions of Peroxidases 1 and 2**—We determined the optimum pH of each peroxidase using BA. The activity of peroxidase 1 was maximum between pH 4.0 and 4.5, with half-maximal activities at pH values around 3.0 and 6.0. Peroxidase 2 also showed the maximum activity between pH 4.0 and 4.5, although its activity was slightly lower than that of peroxidase 1. Based on these results, standard assays were carried out with citrate buffer (pH 4.0).

**Effects of Various Flavones on H2O2-metabolizing Activity of Peroxidases 1 and 2**—In *Scutellaria* cells, wogonin and oroxylin A are also biosynthesized as minor flavones (24). To reveal whether these endogenous flavones are involved in the detoxification of H2O2, the substrate specificity was examined with BA peroxidases 1 and 2 showed the high H2O2-metabolizing activity using BA with peroxidase 1 showing somewhat higher activity than peroxidase 2. In contrast, BAG does not undergo oxidation by either peroxidase. *Scutella* cells contain a much lower level of BA than BAG (9.5 μmol/g fresh cells) than BA (9.5 μmol/g fresh cells), indicating that the cells to hydrolyze BAG to effectively degrade H2O2. In addition, peroxidases 1 and 2 could not oxidize any of the endogenous flavones except for BA. In contrast to BA, these flavones possess a methoxyl or a glucuronyl group in their molecule; therefore, it is assumed that both enzymes can only oxidize flavones with hydroxyl groups. Moreover, we evaluated the peroxidase activity using apigenin (5,7,4′-trihydroxyflavone) and luteolin (5,7,3′,4′-tetrahydroxyflavone), which have not been identified in *S. baicalensis*. Both peroxidases showed a high activity with luteolin, whereas apigenin was not oxidized by either peroxidase. Like BA, luteolin has an *ortho*-dihydroxyl moiety in its molecule, thus suggesting that an *ortho*-dihydroxyl group is required for the peroxidase reaction.

### Table I

**Effects of various flavones on H2O2-metabolizing activity**

| Flavone Substitution | Activity μg/mg protein |
|----------------------|-----------------------|
| BA 5,6,7-triOH       | 1.38 ± 0.09           |
| BAG 5,6-diOH, 7-O-GluA| 1.02 ± 0.15           |
| Oxyrin A 5,7-diOH, 6-OMe | —                    |
| Wogonin 5,7-diOH, 6-OMe | —                    |
| Apigenin 5,7,4′-triOH| —                     |
| Luteolin 5,7,3′,4′-tetraOH | 1.26 ± 0.21       |

a The numbering of a flavone skeleton was described in Fig. 3A.

b GluA, β-D-glucuronyl.

c —, no activity was detected.

### Discussion

ROS play important roles in plant defense such as the pathogen growth inhibition, cell strengthening, and the hypersensitive reaction. However, they are also thought to inflict serious damage on the host plant cells. In particular, huge amounts of ROS are quickly produced by the oxidative burst, although their detoxification mechanism is not fully understood. As results of our studies on flavonoid metabolism, we identified in *Scutellaria* cells a novel H2O2-metabolizing system that is closely linked with the metabolism of the flavone BAG. Such a H2O2-metabolizing system has not been hitherto reported. Like *Scutellaria* cells, rye leaves (23), *Perilla lobata* cells (25, 26), apple leaves (27), and garbanzo beans (28) also possess endogenous-flavonoid-specific glycosidase in addition to flavonoid glucosidase. Interestingly, their glycones are assumed to be oxidized by a peroxidase reaction or to have a potent antioxidant activity, suggesting that these plants may have ROS-detoxifying systems similar to that in *Scutellaria* cells.

The first step in the metabolic pathway of H2O2 is the hydrolysis of BAG by β-glucuronidase. Previously, we reported that β-glucuronidase in *S. baicalensis* shows a high activity for the endogenous flavone BAG, but its roles have remained unclear (17). In the present study, we found that β-glucuronidase catalyzes the production of an antioxidant flavone BA. Keppler and Novacky reported that exogenous addition of antioxidants reduces death of hypersensitively responding cells (29), but surprisingly, *Scutellaria* cells can produce the antioxidant flavone BA in response to an elicitor or H2O2. To our knowledge, hydrolases involved in H2O2 metabolism have not been reported so far. Under normal conditions, BAG and β-glucuronidase are thought to exist in the different cellular compartments, because despite the presence of high β-glucuronidase activity, the amount of BA is much lower than that of BAG. We assumed that the oxidative burst inflicted serious damage on the compartmentation, resulting in the hydrolysis of BAG, based on the fact that after addition of an elicitor or H2O2, the cells released components that should exist within the cells into the extra-cellular medium. Flavonoid hydrolysis initiated by stress has also been reported for other plants. In *P. lobata* the hydrolysis of isoflavone glucosides is initiated by the elicitor treatment (25, 26), whereas the infection of pathogens to apple leaves causes hydrolysis of chalcone glucoside (27). As a second step, released BA was rapidly converted to DBA by cell wall peroxidases, and H2O2 was confirmed to be detoxified at this step. To characterize the properties of the enzymes in pure forms, we attempted to purify them from *Scutellaria* cells. After a combination of ammonium-sulfate precipitation and two chromatographic steps, two ionic forms of peroxidases (peroxidases 1 and 2) were resolved. The structural properties of peroxidases 1 and 2 are similar to each other. The molecular mass (38 and 34 kDa) of each peroxidase resembles those of...
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ascorbate peroxidase (34 kDa), guaiacol peroxidase (33 kDa), and extensin peroxidases (34–37 kDa) (10). On the other hand, pI values (8.7 and 8.9) of peroxidase 1 and 2 indicated that they are cationic peroxidases.

Among endogenous flavones tested, peroxidases 1 and 2 showed the highest H$_2$O$_2$-metabolizing activity with BA, and peroxidase 1 displayed somewhat higher activity than peroxidase 2. In contrast, both peroxidases could not oxidize other endogenous flavones including BAG. Scutellaria cells contain much more BAG than BA, indicating that H$_2$O$_2$ metabolism depends significantly on the hydrolysis of BAG. These findings provided a reasonable elucidation for the result that SAL treatment extensively reduced the ability of Scutellaria cells to metabolize H$_2$O$_2$. It is notable that H$_2$O$_2$ is effectively metabolized using luteolin as a proton donor. Neither luteolin nor its glycosides have been identified in S. baicalensis, but they do occur in numerous plants (30), thus suggesting that a similar detoxification of H$_2$O$_2$ may occur commonly in the plant kingdom.

Anhalt and Weissenböck (23) reported a metabolic pathway of luteolin glucuronide in rye leaves, where luteolin 7-O-di-glucuronyl-4'-O-glucuronide is hydrolyzed by endogenous β-glucuronidase, and the hydrolysate luteolin 7-O-diglucuronide is finally polymerized by a peroxidase reaction.

Flavonoid polymerization by peroxidase is also reported for daidzein, the isoflavone of P. lobata. Park et al. (26) demonstrated that in vitro peroxidase reaction with daidzein gives dimeric daidzein and unidentified polymers. We attempted similar reaction with BA and peroxidases, but such polymerization was not recognized, and only DBA was quantitatively produced. Therefore, we concluded that neither BA nor DBA is polymerized by peroxidases, contrary to the metabolism of luteolin 7-O-diglucuronide and daidzein, although the precise mechanism of DBA metabolism has remained still unclear.

Concerning the roles of cell wall peroxidases in other plants, the insolubilization of extensin (7, 8) and the biosynthesis of the cell wall including lignification (31) have been hitherto reported. Our study unequivocally demonstrated a novel function in which cell wall peroxidases rapidly metabolize a huge amount of H$_2$O$_2$ produced by the oxidative burst. These peroxidases and β-glucuronidase pre-exist in Scutellaria cells, and neither activity was increased by the oxidative burst. The constitutive presence of these enzymes, in addition to a large amount of BAG, possibly enables cells to immediately induce the H$_2$O$_2$ degradation system.

Ascorbate peroxidase and catalase are well known as H$_2$O$_2$-metabolizing enzymes. SAL is not an inhibitor for both enzymes, although the SAL treatment induces a serious damage in the elicited Scutellaria cells. Therefore, H$_2$O$_2$ detoxification by ascorbate peroxidase and catalase seems less effective as compared with the cell wall peroxidases. BA belongs to a quite different class of natural products from ascorbic acid, which is required for H$_2$O$_2$ metabolism by ascorbate peroxidases, but it is interesting that both compounds act as a proton donor. The cell wall peroxidases of S. baicalensis and ascorbate peroxidase may metabolize H$_2$O$_2$ by a similar mechanism because both peroxidase reactions afforded a product (DBA and dehydroascorbic acid) with a diketone moiety.

In conclusion, BAG and BA were shown to be involved in the protection of Scutellaria cells against oxidative stress, whereas other interesting roles were also suggested for BAG metabolism. It seems particularly important that BA is rapidly formed in response to the elicitor because BA has antibacterial (12) and antiviral (13) effects. We also confirmed that BA shows antibacterial activity for Clavibacter michiganensis subsp. nebraskense and C. michiganensis subsp. michiganensis, suggesting that BA may contribute to a chemical defense against pathogens. Because more DBA was produced than BA during the oxidative burst, we are now examining the antimicrobial activity of DBA as well as BA using various pathogens.

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