Plant Physiol. (1992) 100, 1114–1119
0032-0889/92/100/1114/06/$01.00/0

Received for publication April 13, 1992
Accepted June 4, 1992

# Induction of UDP-Glucose:Salicylic Acid Glucosyltransferase in Oat Roots[1]

**Nasser Yalpani*[2], Nelson E. Balke, and Margot Schulz[3]**

*Department of Agronomy, University of Wisconsin, Madison, Wisconsin 53706*

## ABSTRACT

**A UDP-glucose:salicylic acid 3-*O*-glucosyltransferase (EC 2.4.1.35) (GTase) from oat (*Avena sativa* L. cv Dal) root extracts was assayed in vitro using [$^{14}$C]salicylic acid (SA) and an ion exchange column to separate SA from β-glucosylsalicylic acid. The GTase, present at a very low constitutive level, was inducible to 23 times the constitutive level. When excised roots were exposed to SA at pH 6.5, the specific activity of the enzyme increased within 1.5 h, peaked after 8 to 10 h, and then declined. The increase in specific activity depended on the concentration of SA in the induction medium. Among 16 phenolics and phenolic derivatives tested, GTase induction showed high specificity toward SA and acetylsalicylic acid. Specific activity of the enzyme was induced to higher levels in roots from 7-d-old seedlings than roots from younger plants. GTase activity was less inducible in basal compared with median or apical root sections. Induction of GTase activity was a result of de novo RNA and protein synthesis. Candidate peptides for the GTase were identified by comparison of two-dimensional electrophoresis gels of proteins labeled with [$^{35}$S]methionine during incubation of roots in the presence or the absence of SA and a gel of a partially purified GTase preparation.**

Recently, SA[4] was identified as a regulator in the flowering of thermogenic *Arum* lilies (24) and also as a signal molecule in the activation of resistance responses of plants to pathogens (20, 21). These findings stress the need to understand the processes that regulate SA accumulation. Reports of the formation of SA-glucosides in a number of plants (3, 7, 17) suggest that SA conjugation could play an important role in determining the size of the free SA pool.

Ion uptake by cereal roots is inhibited by exogenous applications of SA (9, 10, 12). Oat roots can recover from this inhibition. It has been suggested that this recovery is due to the induction of the activity of a GTase (2). The GTase catalyzes the formation of GSA, a compound less inhibitory to $Cl^-$ absorption than SA (2). The GTase has been partially purified from oat roots, and some of its properties have been described (2, 29). The protein is soluble, has a native $M_r$ of approximately 50,000, has an isoelectric point of approximately 5, and shows high specificity toward UDP-glucose and SA (29). In this paper, we report our initial studies concerning the induction of GTase activity in oat (*Avena sativa* L.) roots.

## MATERIALS AND METHODS

### Plant Material and Chemicals

Oat (*Avena sativa* L. cv Dal) seeds were germinated on cheesecloth stretched over a 4-L beaker containing 3 L of continuously aerated 10 mM $CaSO_4$ in the dark at room temperature. [7-$^{14}$C]SA (1.96 GBq mmol$^{-1}$) and [$^{35}$S]methionine (>40.7 TBq mmol$^{-1}$) were purchased from New England Nuclear and Amersham, respectively.

### Induction of GTase

Roots from 5-d-old seedlings were excised, rinsed with distilled $H_2O$, and incubated in 30 mL g$^{-1}$ of roots of induction buffer (0.5 mM KCl, 0.25 mM $CaSO_4$, 0.5 mM SA, 25 mM Mes/Tris [pH 6.5]) at 25°C for the specific times indicated in "Results." SA was added in DMSO to a final DMSO concentration of 0.05% (v/v). SA uptake from the induction solution was measured by a spectrophotometric procedure adopted from Brodie et al. (5). Induction solution (1 mL) was mixed with 1.2 mL of 25 mM $Fe_2(SO_4)_3$ in 0.1 M glycine/HCl (pH 2.0). The resulting colored SA-iron complex was measured spectrophotometrically at 530 nm. Induction was stopped by decanting the induction solution, washing the roots in cold water for 2 min, blotting them dry, and freezing them with liquid $N_2$. Roots were stored at −80°C for not more than 6 d before GTase extraction. Extractable GTase activity was not affected by storage of roots for several months at this temperature.

### Distribution of GTase in Root and Shoot Sections

Roots (12 cm long) from 5-d-old seedlings were divided into three equal lengths. These sections and the 3-cm-long shoot from the seedlings were exposed to 0.5 mM SA for 12 h before GTase extraction.

[1] Support to N.E.B. by U.S. Department of Agriculture Competitive Research Grants Office grant No. 85-CRCR-1-1572, University of Wisconsin-Madison Graduate School project No. 900317, and the College of Agricultural and Life Sciences, University of Wisconsin-Madison.

[2] Present address: Center for Agricultural Molecular Biology, Rutgers, The State University of New Jersey, Cook College, P.O. Box 231, New Brunswick, NJ 08903-0231.

[3] Present address: Institut fur Landwirtschaftliche Botanik, Meckenheimer Allee 176, D-5300 Bonn, Federal Republic of Germany.

[4] Abbreviations: SA, salicylic acid; 2-D, two-dimensional; GSA, β-glucosylsalicylic acid; GTase, UDP-glucose:salicylic acid 3-*O*-glucosyltransferase (EC 2.4.1.35); mU, milliunit.

### Inhibition of RNA and Protein Synthesis

Roots were preincubated for 1 h in induction buffer containing 50 $\mu$g mL$^{-1}$ of actinomycin D, chloramphenicol, cycloheximide, or puromycin. After 1 h, 0.5 mM SA was added, and induction was continued for an additional 12 h before extraction of GTase. The control lacked inhibitor but contained SA.

### Extraction of GTase

All procedures were at 0 to 4°C. Induced roots (1 g) were submerged under liquid $N_2$ and homogenized with a mortar and pestle. The resulting powder was thawed in 1.5 mL of buffer (25 mM Tris-Mes, 10% [v/v] glycerol, 20 mM 2-mercaptoethanol, 1 $\mu$M leupeptin [pH 6.5]) containing 10% (w/w of root) Dowex-1 and 1% (w/w of root) polyvinylpolypyrrolidone. The extract was centrifuged at 15,000$g$ for 20 min, and the resulting supernatant was assayed for GTase activity.

### Partial Purification and Assay of GTase

GTase was partially purified from oat roots by pH fractionation and anion exchange, gel filtration, and chromatofocusing chromatography as detailed elsewhere (29). Assays of GTase activity and separation of [$^{14}$C]SA from the product [$^{14}$C]GSA by Polyamide 6 chromatography were performed as described previously (29). One unit of GTase activity is defined as the amount of enzyme required to catalyze the synthesis of 1 $\mu$mol of GSA min$^{-1}$ under assay conditions.

### In Vivo Protein Labeling

Roots (1.5 g) from 5-d-old seedlings were incubated with 50 $\mu$g mL$^{-1}$ of chloramphenicol in 45 mL of induction buffer in the absence or the presence of 0.5 mM SA at 25°C. After 2 h, 44.4 MBq of [$^{35}$S]methionine was added to the induction buffer and the incubation continued for either 1 or 2.5 h. Alternatively, roots were incubated in the absence or presence of 0.5 mM SA for 1 h and then labeled with the addition of 18.5 MBq [$^{35}$S]methionine for 12 h. After labeling, roots were rinsed with ice-cold 1 mM unlabeled methionine in $H_2O$ for 2 min, blotted dry, frozen in liquid $N_2$, and stored at −80°C until extracted for 2-D PAGE.

### 2-D PAGE

Roots were extracted as described above. Proteins in the 15,000$g$ supernatant were extracted for electrophoresis and separated by 2-D PAGE as described previously (29). About 5 $\mu$g of total unlabeled protein or 5.6 kBq of $^{35}$S-labeled protein were loaded on prefocused, first-dimension, isoelectric focusing tube gels. $^{35}$S-labeled proteins were visualized on silver-stained gels (11) by fluorography with Amplify (Amersham) according to the manufacturer's directions. The gels were dried on Whatman 3 MM paper and exposed to Kodak X-OMAT AR x-ray film at −80°C for 3 d.

### Protein Determination

Protein was determined using Bio-Rad protein dye reagent and BSA as the standard.

### Uniformity of Data

Induction experiments were carried out with triplicate 1-g root samples for each treatment. Triplicate GTase assays were performed on each root sample. Experiments were repeated at least twice with similar results. Results of representative experiments are shown along with the SE for each mean.

## RESULTS

### Induction

GTase activity in the 15,000$g$ supernatant prepared from oat root homogenates was constitutively present at about 0.1 mU g$^{-1}$ fresh weight of tissue. When roots were exposed to a solution containing 0.5 mM SA at pH 6.5, an increase in GTase specific activity was detected after a lag of about 1.5 h (Fig. 1). The increase of GTase activity reached a maximum rate (0.6 mU mg$^{-1}$ of protein h$^{-1}$) between 1.5 and 4 h of exposure to SA. Enzyme activity reached a maximum 23-fold induction compared to constitutive activity about 8 to 10 h after application of SA and then declined slightly. Concurrently with GTase induction, the SA concentration in the incubation medium decreased. GTase activity decayed with a half-life of about 20 h after transfer of roots that had been incubated in 0.5 mM SA for 18 h to buffer lacking SA and could subsequently be reinduced in such roots upon reexposure to SA (data not shown).

Induction of GTase activity was not due to tissue injury during root excision. Roots (10 cm long) cut into 0.5-cm sections just before a 12-h incubation in the absence or presence of 0.5 mM SA had 0.15 and 3.10 mU of GTase activity mg$^{-1}$ of protein, respectively, whereas uncut roots induced with SA had 4.32 mU mg$^{-1}$ of protein.

As the SA concentration in the induction buffer was in-

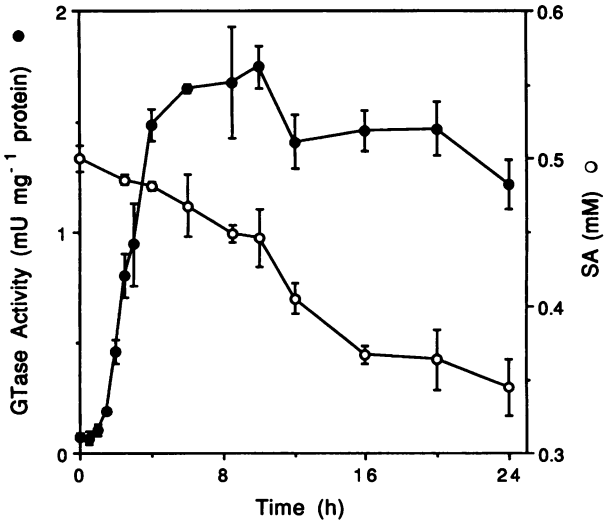



**Figure 1.** Time course of induction of GTase activity and disappearance of SA from induction medium. Excised roots were exposed to 0.5 mM SA at pH 6.5 for 30 min to 24 h. SA concentration in the induction buffer (○) and extracted protein assayed for GTase activity (●) were determined. Results are means ± SE.

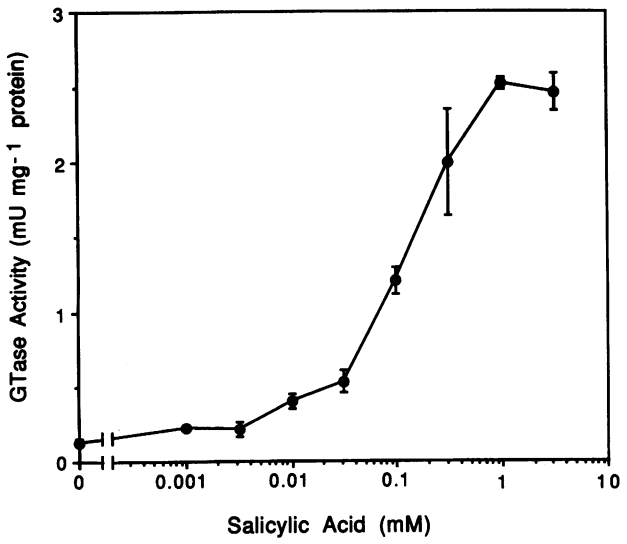



**Figure 2.** Dependence of GTase induction on SA concentration. Roots were exposed to SA concentrations of 1 μM to 3.14 mM in induction buffer at pH 6.5 for 12 h. Results are means ± SE.

creased, increasing levels of GTase activity were induced (Fig. 2). At least 10 μM SA was required to elicit GTase induction. The response saturated at SA concentrations >1 mM. Saturation of the response may reflect the presence of a saturable SA-perception mechanism and/or result from SA toxicity (12).

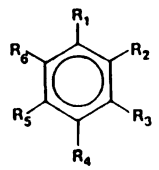
A number of phenolics and other substances (0.5 mM, 12 h exposure) were tested as potential inducers of the GTase. GTase induction showed high specificity toward SA (Table I). *m*- or *p*-Hydroxylated benzoic acid resulted in significantly lower induction than SA (*o*-hydroxybenzoic acid). Acetylsalicylic acid had similar activity as SA. No obvious structural requirements for inducing ability could be determined. Extractable GTase activity did not correlate with the octanol/water partition coefficients of the compounds.

The level of inducible GTase activity depended on the age of the seedling when roots from plants that ranged from 3 to 7 d old were compared. Both the level and the specific activity of inducible GTase increased even though the protein concentration decreased with age (Fig. 3).

The distribution of inducible GTase varied along roots. The specific activity of the enzyme was lowest in basal compared with median or apical root sections (Fig. 4). In excised shoots, GTase was induced to a lower specific activity than in root tip or median root sections (Fig. 4).

The increase in GTase activity upon exposure to SA was

**Table I.** *Effect of Various Benzoic Acid Derivatives and Other Phenolic Compounds on GTase Induction*
Roots from 5-d-old seedlings were exposed to induction buffer containing 0.5 mM test compound for 12 h.




| Inducer | Substituents | | | | | | SA-Induced GTase Activity[a] |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | |
| | | | | | | | % |
| Acetylsalicylic acid | COOH | $OCOCH_3$ | | | | | 101 |
| *o*-Hydroxybenzoic acid (SA) | COOH | OH | | | | | 100 |
| Benzoic acid | COOH | | | | | | 45 |
| Methylsalicylate | $COOCH_3$ | OH | | | | | 29 |
| *p*-Coumaric acid | CH=CHCOOH | | | OH | | | 23 |
| *trans*-Cinnamic acid | CH=CHCOOH | | | | | | 22 |
| Saligenin | $CH_2OH$ | OH | | | | | 21 |
| Vanillin | CHO | | $OCH_3$ | OH | | | 19 |
| 2,6-Dihydroxybenzoic acid | COOH | OH | | | | OH | 18 |
| *o*-Coumaric acid | CH=CHCOOH | OH | | | | | 12 |
| Ferulic acid | CH=CHCOOH | | $OCH_3$ | OH | OH | | 11 |
| *p*-Hydroxybenzoic acid | COOH | | | OH | | | 10 |
| Thiosalicylic acid | COOH | SH | | | | | 10 |
| Gentisic acid | COOH | OH | | | OH | | 7 |
| *m*-Hydroxybenzoic acid | COOH | | OH | | | | 6 |
| *p*-Nitrophenol | OH | | | $NO_2$ | | | 0 |
| Control (15 μL of DMSO/30 mL) | | | | | | | 6 |

[a] SA-induced GTase activity = 4.22 mU $mg^{-1}$ protein.

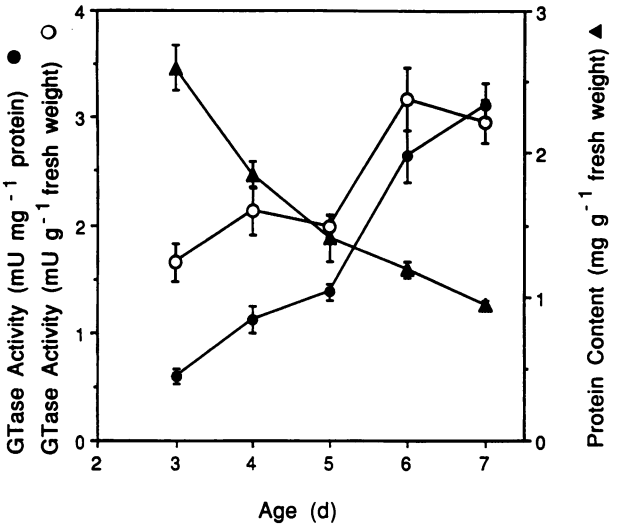



**Figure 3.** Effect of seedling age on protein level and GTase induction. Roots were exposed to 0.5 mM SA for 12 h. Results are means ± SE.

the result of de novo synthesis of the enzyme. GTase induction was reduced by 81% by the RNA synthesis inhibitor actinomycin D. The protein synthesis inhibitors cycloheximide and puromycin inhibited GTase induction by 96 and 35%, respectively. Chloramphenicol, an inhibitor of protein synthesis on 70S ribosomes, resulted in only 20% reduction of GTase activity. These data indicate that induction of GTase is the result of RNA and protein synthesis and not due to GTase activation or induction of contaminating microbial enzymes.

### In Vivo Labeling of GTase

Because the increase in oat root GTase activity is the result of de novo synthesis, we used [$^{35}$S]methionine in the induction medium and 2-D PAGE to help us identify the peptide representing the enzyme. Roots were $^{35}$S-labeled for 2.5 h in the absence (control) and presence of SA during the period when root GTase activity increases at the maximum rate. Total $^{35}$S incorporated into protein was essentially the same with or without SA, 22.2 and 29.6 MBq mg$^{-1}$ of protein, respectively. Because the GTase is negatively charged at neutral pH, as determined by its retention on DEAE-Sephacel at pH 7 (28), the enzyme should be present on 2-D gels containing proteins that are separated using a pH 7.1 to 4.3 gradient in the first dimension. A number of peptides were labeled more intensely in SA-induced tissue than in the untreated control (Fig. 5, A and B). The proteins marked as different in Figure 5, A and B, also showed the same relative pattern in extracts of tissue [$^{35}$S]methionine labeled with or without SA for 1 or 12 h (data not shown). Three peptides with $M_r$ 54,500, 54,000, and 48,000 (Nos. 1, 2, and 3, respectively, in Fig. 5) were consistently present in SA-treated roots but were undetectable on fluorograms of control roots. A fourth, $M_r$ 25,500 peptide (No. 4 in Fig. 5), was less intensely labeled in control than in SA-induced tissue. These four peptides were also present on silver-stained 2-D gels of a GTase preparation that had been purified 178-fold by pH fractionation, ion exchange, gel filtration, and chromatofocusing chromatography (28) (Fig. 5C). The $M_r$ of the native GTase on gel filtration columns is like that of many other plant glucosyltransferases, approximately 50,000 (29). Whereas some glucosyltransferases are monomeric (1, 25), others consist of a dimer of peptide subunits (13, 15, 16). Thus, peptides Nos. 1 to 4 are all likely GTase candidates.

## DISCUSSION

Metabolic detoxication of xenobiotics by inducible P-450 monoxygenases or glutathione-*S*-transferases has been the subject of a number of reviews (18, 23). In this paper, we report an inducible glucosyltransferase that helps protect plants from SA toxicity. The action of the GTase may not only be important in the detoxication of exogenously applied SA but may regulate endogenous SA levels at which transient increases in SA concentration are reported to control physiological processes (20, 21, 24). It is noteworthy that oat roots that have not been treated with SA contain 500 ng of SA per g fresh weight (N. Yalpani, unpublished data) and also low constitutive levels of GTase activity (Fig. 1).

The GTase is rapidly induced by its substrate SA (Fig. 1). This effect of SA on protein synthesis is more rapid than the induction of a similar GTase in suspension-cultured *Mallotus japonicus* cells (26) or SA stimulation of the synthesis of certain pathogenesis-related proteins that may be associated with disease resistance in tobacco and tomato (27, 28).

The level of SA-inducible GTase varies with the region and the age of root tissue (Figs. 3 and 4). Our data may reflect differences in the total rate of metabolic activity and protein synthesis among actively growing, younger cells and fully differentiated tissue. It is also possible that tissue sensitivity to exogenously applied SA varies.

The exact mechanism of the induction process is unknown.

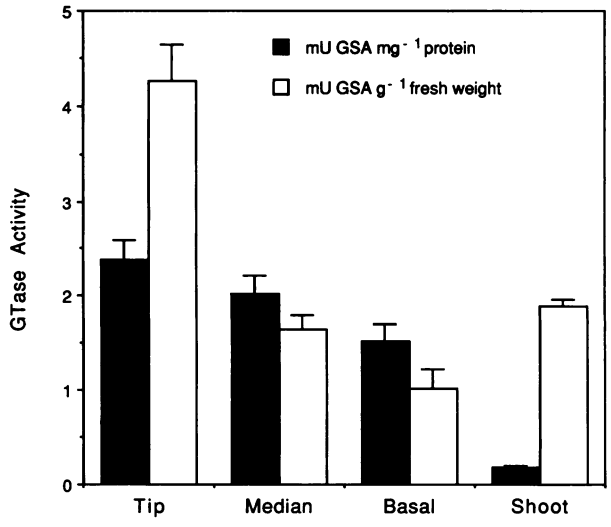



**Figure 4.** Distribution of inducible GTase in root and shoot sections after exposure to 0.5 mM SA for 12 h. Results are means ± SE.

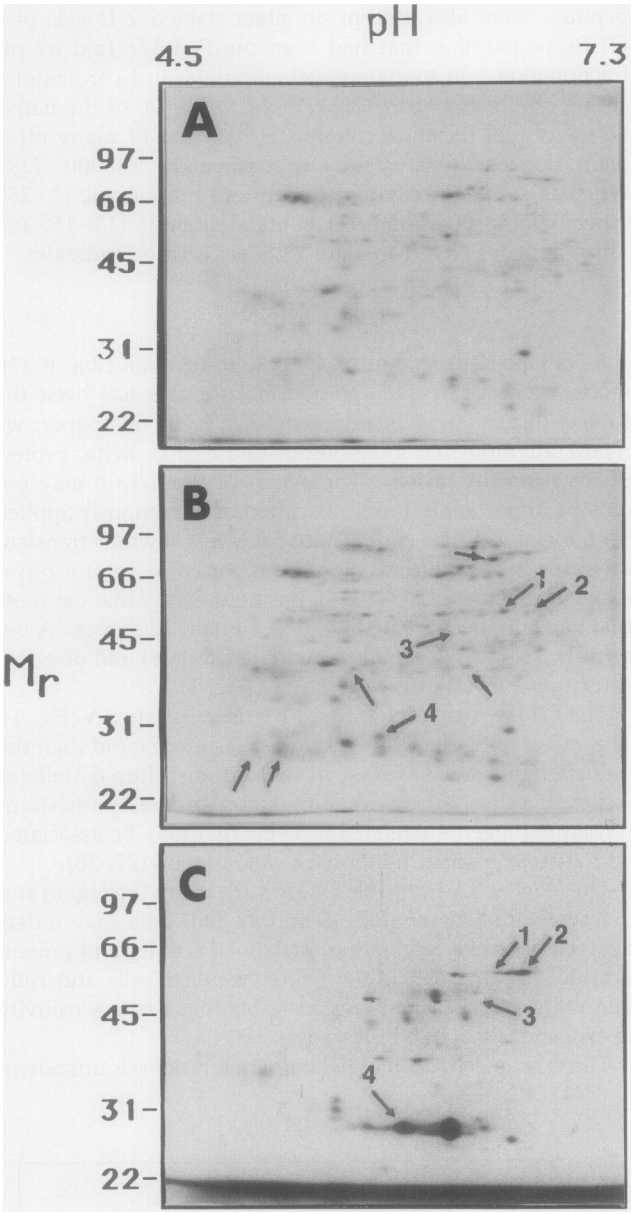



**Figure 5.** 2-D PAGE analysis of root proteins labeled with [$^{35}$S]-methionine in the absence or the presence of SA; comparison with partially purified GTase. Fluorogram of root proteins labeled with [$^{35}$S]methionine for 2.5 h after a 2-h preincubation in the absence (A) or presence (B) of SA. Arrows denote peptides that were consistently labeled more intensely in samples of SA-induced roots than control roots. The numbered arrows denote candidate peptides for the GTase that are also present in a silver-stained, partially purified GTase preparation (C). Ordinate, $M_r$ in thousands.

Whereas our experiments with protein and RNA synthesis inhibitors suggest nuclear-encoded, cytoplasmic de novo synthesis of the GTase as a result of SA exposure, it is not possible to distinguish between a direct induction of the GTase gene by SA or activation of the gene by processes or events set in motion by SA.

The mechanism of GTase induction by SA seems to differ from that of inhibition of ion uptake. Based on the relation between the structure of a series of benzoic acid derivatives and their activity, inhibition of ion uptake in barley roots is thought to result from integration of SA into membranes and is observable within 1 min (10, 12). Comparison between chemical structure of candidate inducers and extractable GTase activity reveals a high specificity of the induction mechanism toward SA and acetylsalicylic acid (Table I). The inducing ability of test compounds does not correlate with their octanol/water partition coefficients, suggesting that induction of GTase synthesis is not simply a result of SA disturbing the integrity of cellular membranes. However, this analysis is complicated by the effects of uptake and metabolism. For example, SA may be formed from deacetylation of acetylsalicylic acid, $\beta$-oxidation of *o*-coumaric acid, or *o*-hydroxylation of benzoic acid (6, 22). It is also possible that the different phenolics induce distinct glucosyltransferases with broad substrate specificities that happen to include SA.

Our in vivo labeling experiments (Fig. 5) indicate that SA affects the synthesis or turnover of several proteins. This is consistent with the reported SA stimulation of the de novo synthesis of a number of proteins associated with defense responses of plants to pathogen attack, including $\beta$-1,3-glucanases and chitinases (see ref. 4 for review). SA has also been shown to induce synthesis of alternative oxidase of the cyanide-insensitive respiration pathway (8), affect the activity of nitrate reductase in maize seedlings (14), and inhibit ethylene-forming enzyme (19).

Using $^{35}$S labeling and 2-D PAGE, we have tentatively identified four candidate peptides that could represent the GTase (Fig. 5). To confirm the identity of this protein we will attempt to use more highly purified GTase preparations. Identification of the GTase will be one step in the study of the signal-response path leading to the induction of this enzyme.

## ACKNOWLEDGMENTS

The authors thank T. Ciolkosz and C.C. Lee for technical assistance.

## LITERATURE CITED

1. **Bajaj KL, De Luca V, Khouri H, Ibrahim RK** (1983) Purification and properties of flavonol-ring B glucosyltransferase from *Chrysosplenium americanum*. Plant Physiol **72:** 891–896
2. **Balke NE, Davis MP, Lee CC** (1987) Conjugation of allelochemicals by plants. Enzymatic glucosylation of salicylic acid by *Avena sativa*. *In* CR Waller, ed, Allelochemicals: Role in Agriculture and Forestry. American Chemical Society Symposium series, Vol 330. American Chemical Society, Washington, DC, pp 214–227
3. **Ben-Tal Y, Cleland CF** (1982) Uptake and metabolism of [$^{14}$C]-salicylic acid in *Lemna gibba* G3. Plant Physiol **70:** 291–296
4. **Bol JF, van Kan JAL** (1988) The synthesis and possible functions of virus-induced proteins in plants. Microbiol Sci **5:** 47–52
5. **Brodie BB, Undenfriend S, Coburn AF** (1944) The determination of salicylic acid in plasma. J Pharmacol Exp Ther **80:** 114–117
6. **Chadha KC, Brown SA** (1974) Biosynthesis of phenolic acids in tomato plants infected with *Agrobacterium tumefasciens*. Can J Bot **52:** 2041–2047
7. **Cooper-Driver G, Corner-Zamodits JJ, Swain T** (1972) The metabolic fate of hydroxybenzoic acids in plants. Z Naturforsch **27b:** 943–946

8. **Elthon TE, Nickels RL, McIntosh L** (1989) Mitochondrial events during development of thermogenesis in *Sauromatum guttatum* (Schott). Planta **180:** 82–89
9. **Glass ADM** (1973) Influence of phenolic acids on ion uptake. 1. Inhibition of phosphate uptake. Plant Physiol **51:** 1037–1041
10. **Glass ADM** (1975) Inhibition of phosphate uptake in barley roots by hydroxy-benzoic acids. Phytochemistry **14:** 2127–2130
11. **Görg A, Postel W, Weser J, Gunther S, Strahler JR, Hanash SM, Somerlot L** (1987) Elimination of point streaking on silver stained two-dimensional gels by addition of iodoacetamide to the equilibration buffer. Electrophoresis **8:** 122–124
12. **Harper JR, Balke NE** (1981) Characterization of the inhibition of $K^+$ absorption in oat roots by salicylic acid. Plant Physiol **68:** 1349–1353
13. **Hrazdina G** (1988) Purification and properties of a UDPglucose:flavonoid 3-*O*-glucosyltransferase from *Hippeastrum* petals. Biochim Biophys Acta **81:** 802–806
14. **Jain A, Srivastava HS** (1981) Effect of salicylic acid on nitrate reductase activity in maize seedlings. Plant Physiol **59:** 339–342
15. **Jonsson LMV, Aarsman MEG, van Diepen J, de Vlaming P, Smit N, Schram AW** (1984) Properties and genetic control of anthocyanin 5 *O*-glucosyltransferase in flowers of *Petunia hybrida*. Planta **160:** 341–347
16. **Keil U, Schreier P** (1989) Purification and partial characterization of a UDP-glucose:phenyl-β-D-glucosyltransferase from papaya fruit. Phytochemistry **28:** 2281–2284
17. **Klämbt HD** (1962) Conversion in plants of benzoic acid to salicylic acid and its β-*d*-glucoside. Nature **196:** 491
18. **Lamoureaux GL, Russness DG** (1986) Xenobiotic conjugation in higher plants. *In* GD Paulson, J Caldwell, DH Hutson, eds, Xenobiotic Conjugation Chemistry. American Chemical Society Symposium series 299. American Chemical Society, Washington, DC, pp 62–105
19. **Leslie CA, Romani RJ** (1986) Inhibition of ethylene biosynthesis by salicylic acid. Plant Physiol **88:** 833–837
20. **Malamy J, Carr JP, Klessig DF, Raskin I** (1990) Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. Science **250:** 1002–1004
21. **Metraux JP, Singer H, Ryals J, Ward J, Wyss-Ban M, Gaudin J, Raschdorf K, Schmid E, Blum W, Inverardi B** (1990) Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. Science **250:** 1004–1006
22. **Morgan AN, Truitt EB Jr** (1965) Evaluation of acetylsalicylic acid esterase in aspirin metabolism. J Pharmacol Sci **54:** 1640–1646
23. **O'Keefe DP, Romesser JA, Lets KJ** (1987) Plant and bacterial cytochromes P-450: involvement in herbicide metabolism. *In* JA Saunders, L Kosak-Channing, EE Conn, eds, Phytochemical Effects of Environmental Compounds. Plenum, New York, pp 151–173
24. **Raskin I, Ehmann A, Melander WR, Meeuse** JD (1987) Salicylic acid: a natural inducer of heat production in *Arum* lilies. Science **237:** 1601–1602
25. **Sandermann H, Schmitt R, Eckey H, Bauknecht T** (1991) Plant biochemistry of xenobiotics: isolation and properties of soybean *O*- and *N*-glucosyl and *O*- and *N*-malonyltransferases for chlorinated phenol and anilines. Arch Biochem Biophys **287:** 341–350
26. **Tanaka S, Hayakawa K, Umetani Y, Tabata M** (1990) Glucosylation of isomeric hydroxybenzoic acids by cell suspension cultures of *Mallotus japonicus*. Phytochemistry **29:** 1555–1558
27. **Van Loon LC** (1983) The induction of pathogenesis-related proteins by pathogens and specific chemicals. Neth J Plant Pathol **89:** 265–273
28. **Vera P, Conejero V** (1989) The induction and accumulation of the pathogenesis-related P69 proteinase in tomato during citrus exortis viroid infection and in response to chemical treatments. Physiol Mol Plant Pathol **34:** 323–334
29. **Yalpani N, Schulz M, Davis MP, Balke NE** (1992) Partial purification and properties of an inducible uridine 5′-diphosphate-glucose:salicylic acid glucosyltransferase from oat roots. Plant Physiol **100:** 457–463