

Biosynthesis and Distribution of Phytoestrogens and Their Roles in Plant Defense, Signal Transduction, and Cell-to-Cell Signaling

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ABSTRACT

Phytoestrogens are natural products from plants that function as estrogen agonists. Here we briefly review the biochemistry, distribution, and proposed roles of phytoestrogens in plants. A primary role appears to be in host-microbe interactions. For example, results from our laboratory suggest that genistein plays multiple roles in soybean defense, including the initiation of hypersensitive (apoptotic) cell death and the activation or potentiation of the defense competency of soybean cells. Recent results from our laboratory suggest that mammalian estrogens and estrogen agonists (including other phytoestrogens and fungal estrogen agonists) and certain specific peroxisome proliferators can also potentiate defense competency in soybean. These findings suggest that some overlap in nuclear receptor or other lipid/steroid signaling pathways may exist between plants and animals.

BIOSYNTHESIS, DISTRIBUTION, AND ROLE OF PHYTOESTROGENS IN PLANTS

THE PREDOMINANT PHYTOESTROGENS in plants include the lignans and the isoflavones. The lignans are relatively simple metabolically and are the result of variations on a condensation of two phenylpropane units. The isoflavones also include a phenylpropane backbone, but with an additional ring structure that derives from acetate condensation. In contrast to the lignans, which are widely distributed, the isoflavones are evolutionarily late arrivals and are found mainly in one subfamily of the Leguminosae, the Papilionoideae. Within these plants, the specific isoflavones present vary widely and often accumulate only under specific stress conditions. One exception to this is the high constitutive level of the isoflavone

phytoestrogen, genistein, which is present in soybean seed and seedling organs as both glucosyl and malonylglucosyl conjugates (Graham, 1991). On the other hand, unlike the isoflavones, the closely related flavonols are widely distributed in plants at high constitutive levels. This raises the possibility that isoflavone formation could be engineered into food crops with the introduction of a few metabolic genes.

The endogenous functions of the isoflavones in plants are more thoroughly documented than those of the lignans. Predominant among these are roles in plant-microbe interactions, in which they can function as preformed toxins (phytoanticipins—see, for example, Rivera-Vargas et al., 1993); as precursors for induced antibiotics (phytoalexins—see for example, Ebel, 1986); as signals for chemoattraction of pathogens (Morris and Ward, 1992; Morris et

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al., 1994) or symbionts (Phillips et al., 1992; Hungria and Stacey, 1997) toward the host; or as signals for the induction of genes regulating infection by symbionts (see, for example, Hungria and Stacey, 1997). Recently we proposed that genistein also acts as an internal plant signal to trigger the initiation of hypersensitive cell death (a defense response in plants parallel in some ways to apoptosis in animals) and to establish the competency of soybean cells to accumulate antibiotic phytoalexins in response to inducing signals called elicitors from the cell wall of the pathogen (Graham and Graham, 1999). Thus genistein serves as a plant hormone-like signal that programs several aspects of cellular response. These various activities of genistein in soybeans may have intriguing parallels with signaling phenomena in animal cells.

A recent review provides a more complete description of the connections between hypersensitive cell death and the induction of cellular competency for phytoalexin accumulation in soybean (Graham and Graham, 1999). A brief summary of our working model is given here. In soybean, genistein is released from extracellular stores of its malonyl glucosyl conjugates after infection or wounding by an unusually specific isoflavone- β -glucosidase (Hsieh, 1997). The controlled and timely release of genistein leads to the activation of an extracellular peroxidase-like NADH oxidase (Nox II). Activation of Nox II is accompanied by the dismutation of superoxide by genistein to form hydrogen peroxide. It is proposed (Graham and Graham, 1999) that this generation of hy-

drogen peroxide contributes to the prolonged oxidative (determinative) phase of hypersensitive (HR) cell death, the equivalent in plants to apoptosis or programmed cell death. If the hydrogen peroxide is scavenged, however, through the action of lignin/suberin peroxidases and the subsequent formation of extracellular phenolic polymers, the cells are rescued from the HR cell death program and instead enter a competent state for phytoalexin accumulation in response to glucan elicitors from the pathogen. Thus genistein is proposed to be a primary signal in the initiation of at least two defense programs, HR cell death and phytoalexin elicitation competency.

Steroid and peroxisome proliferator response pathways in plants

The estrogenic activity of genistein prompted us to examine a series of nuclear receptor ligands, including mammalian estrogens and estrogen agonists and antagonists, for their effects on soybean defense responses. This led to the discovery that mammalian estrogens and estrogen agonists also strongly potentiate defense competency in soybean cells. As shown in Table 1, β -estradiol and estriol significantly increase the phytoalexin response of soybean cells in response to pathogen elicitor. The estrogen agonists, diethylstilbestrol, coumestrol, dienestrol, and zearalenone, are also effective, with coumestrol and zearalenone being most effective. Dienestrol is a simple synthetic estrogen agonist, whereas diethylstilbestrol and coumestrol are phytoestrogens and zearalenone is a fungal es-

TABLE 1. POTENTIATION OF SOYBEAN PHYTOALEXIN ELICITATION BY ESTROGENS AND ESTROGEN AGONISTS¹

<i>Pretreatment</i>	<i>% Increase</i>	<i>Pretreatment</i>	<i>% Increase</i>
None	0	Diethylstilbestrol	86
Genistein	231	Coumestrol	226
β -Estradiol	205	Zearalenone	242
Estriol	177	Ergosterol	260
Dienestrol	75	Ciprofibrate	199

¹Values represent the percent increase in glyceollin elicitation compared with a glucan elicitor control in the soybean cut cotyledon assay (cultivar Williams). Compounds were tested at 33 μ mol/L and applied immediately before the glucan elicitor and 10 μ mol/L orthovanadate, a competency coactivator. Each individual assay included ten cotyledons (subsamples) which were pooled for HPLC analysis. Values are the average of two separate experiments. The standard error was less than 18% of the average for all values. For comparative purposes, the values represent near saturation effects of these compounds. For instance, β -estradiol is active at concentrations as low as 100 nmol/L. More detailed dose-response data will be published separately.

trogen agonist. Coumestrol accumulates in elicitor-treated soybean plants and therefore could play a complementary role to genistein in defense activation. The activity of zearalenone prompted us to test ergosterol, a fungal steroid that has been reported to elicit defense responses in cucumber and tomato (Kauss and Jeblick, 1996; Granado et al., 1995). Ergosterol was also highly active. Thus, mammalian, plant, and fungal estrogens and estrogen agonists all potentiate isoflavone defense responses in soybeans.

To broaden our studies, we tested other nuclear receptor ligands, including several representative androgens, cortical steroids, thyroid hormones, and peroxisome proliferators (S. Landini, T.L. Graham, M.Y. Graham, unpublished data). Although these analyses are still underway, of all of these, the only class of molecules showing strong activity were several specific peroxisome proliferators. The activity of one of the classic peroxisome proliferators, ciprofibrate, is shown in Table 1.

Further examination of soybean response suggests that the potentiation of phytoalexin elicitation by these various compounds may at least in part involve "loading" of the pools of conjugates of the isoflavones daidzein and genistein in soybean tissues. This is supported by the fact that their application to soybean tissues in the absence of elicitor leads to significant increases in total isoflavones. For example, Table 2 presents the effects of the same compounds described previously on the accumulation of total daidzein conjugates. The loading of isoflavone pools can lead to defense potentiation in at least four ways. First, because daidzein is a precursor of the soybean phy-

toalexin, glycoellin, induced tissues may be directly metabolically primed for phytoalexin accumulation. Second, genistein is directly toxic to some pathogens (a phytoanticipin). Finally, increases in genistein conjugates could increase the capacity of tissues for both HR and phytoalexin elicitation competency. This raises the possibility that one of the activities of genistein may be an amplification of its own synthesis, an hypothesis that is very difficult to address in experiments involving genistein application *per se*.

How do these various nuclear receptor ligands work in the plant? Several possibilities exist. First, there could be homologs to nuclear receptors in plants. Using several approaches, we have performed an exhaustive search of cloned plant genes and genomic databases (including *Arabidopsis*) and found no clear homologs. We are currently designing probes against conserved regions of the estrogen and peroxisome proliferator-activated receptors (PPARs) to screen libraries of various soybean lines for homologs. It is possible that, like the isoflavones themselves, homologs of estrogen response pathways may be limited to the legumes.

A second possibility is that a redox-related pathway is involved. Because of the activation of the soybean NADH oxidase, Nox II, by genistein, we tested estradiol and estriol for their effects on this enzyme. At least these specific estrogens had no effect on Nox II (A.R. Rose, M.Y. Graham, and T.L. Graham, unpublished data). We have yet to test the other active compounds, but we believe that this mechanism is unlikely, because the activity of genistein in activation of this enzyme is a result of its unusual capacity

TABLE 2. LOADING OF DAIDZEIN CONJUGATE POOLS BY ESTROGENS AND ESTROGEN AGONISTS¹

Treatment	% Increase	Treatment	% Increase
None	0	Diethylstilbestrol	38
β -Estradiol	64	Coumestrol	54
Estriol	45	Zearalenone	58
Dienestrol	28	Ergosterol	60
		Ciprofibrate	52

¹Values represent the percent increase in total daidzein conjugate pools compared with a water control in the soybean cut cotyledon assay (cultivar Williams). Compounds were tested at 33 μ mol/L. Each individual assay included ten cotyledons (subsamples) which were pooled for HPLC analysis. Values are the average of two separate experiments. The standard error was less than 12% of the average for all values.

for superoxide dismutation. Another possibility that we are exploring is that the compounds are working through a homolog of old yellow enzyme (OYE), an NADH oxidase first isolated from yeast. OYE has an affinity for a number of phenolic ligands and was identified empirically as the estrogen-binding protein in *Candida albicans* (Madani et al., 1994; Buckman and Miller, 1998). A homolog of OYE in *Arabidopsis* (Schaller and Weiler, 1997) has been identified that functions as 12-oxophtyodienoate-10,11-reductase (OPR), an enzyme in the biosynthetic pathway to jasmonic acid, an established eicosanoid signal in plant defense and induced systemic resistance. OPR supplies precursors that, after several rounds of beta oxidation, finally lead to jasmonic acid.

Several natural ligands of the four different classes of PPARs have been identified. These include several specific fatty acids, leukotrienes, prostaglandins, and eicosinoids, including analogs of the animal "equivalent" of jasmonic acid, arachidonic acid (see, for example, Keller et al., 1993; Bocos et al., 1995; Krey et al., 1997; Forman et al., 1997; Gonzalez et al., 1998). To our knowledge activation of PPARs by jasmonate has not been examined. However, we previously established that jasmonic acid, like the compounds in Table 2, greatly potentiates the accumulations of the isoflavone conjugates (Graham and Graham, 1996), and fungal arachidonic and eicosapentaenoic acids also act as phytoalexin elicitors in solanaceous plants (Bostock et al., 1981).

Therefore, several possible signaling mechanisms exist for defense activation by the various compounds examined in this report. These include activation of homologs of the estrogen or PPAR nuclear receptors or action through OYE. It seems very likely that regulation of oxylipin metabolism plays a key signaling role.

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