Soybean Root Response to Symbiotic Infection Glyceollin I Accumulation in an Ineffective Type of Soybean Nodules with an Early Loss of the Peribacteroid Membrane

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Z. Naturforsch. **40c**, 179–181 (1985); received November 23, 1984

Glyceollin, *Glycine max*, Nitrogen Fixation, Nodules, Peribacteroid Membrane

A glyceollin I accumulation of about 6000 pmol · mg dry weight⁻, a tenfold increase above control root tissue. was found in one type of nodule from *Glycine max* which had been infected with a *fix*$^-$ strain (61-A-24) of *Rhizobium japonicum*. In nodules infected with one other ineffective (*fix*$^-$) strain of *Rhizobium japonicum* (RH 31-Marburg) or with two *fix*$^+$ strains of *Rhizobium japonicum* (61-A-101 and USDA 110) no increase in glyceollin I concentrations above control values was found at either 20 d or 34 d after infection. Nodules infected with *Rhizobium japonicum* 61-A-24 are distinguished by an early loss of the peribacteroid membrane in the infected host cell, whilst the bacteroids themselves remain stable.

Introduction

Nodule development in legumes is linked to derepression or induction of several distinct biochemical components. The functionally and quantitatively most obvious ones are nitrogenase (E.C. 1.18.2.1) in the *Rhizobium* bacteroids, leghaemoglobin, most likely located in the host cytoplasm and plant cell glutamine synthetase (E.C. 6.3.1.2) and uricase (E.C. 1.7.3.3) [1]. For several other nodule specific proteins, called "nodulines" functions have still to be found [2]. Nodule structure and functions are also significantly changed after infection with *nod*$^+$ *fix*$^-$ mutants of *Rhizobium*. No increase in plant cell glutamine synthetase activity was found in nodules of *Glycine max*, infected with the ineffective strain 61-A-24 of *Rhizobium japonicum*, compared to a 20 fold increase in *fix*$^+$ nodules [3]. The nodule type obtained with strain 61-A-24 is of special interest, since the peribacteroid membranes becomes unstable at very early stages of nodule development (20 d after infection). The bacteroids, conversely, remain stable and enzymatically active. Even 50 d after infection, specific activity of glutamine synthetase of bacteroids of *Rhizobium japonicum* 61-A-24 is about four times the activity in bacteroids from the *fix*$^+$ strain 61-A-101. The early loss of the peribacteroid membranes changes the symbiotic compartmentation in this type of nodule to a more parasitic type of interaction [4].

Treatment of soybeans with either phytopathogens or some of their components such as glucan elicitors prepared from purified mycelial walls of *Phytophthora megasperma* f. sp. *glycinea* induces a significant enhancement of enzymes of isoflavonoid biosynthesis and accumulation of glyceollin [5, 6]. This phytoalexin accumulates to a higher extent in the incompatible reaction (Hahn *et al.*, unpublished [7, 8, 15]). In this connection we were interested in analyzing glyceollin accumulation within soybean nodules after infection with several types of *fix*$^+$ anf *fix*$^-$ *Rhizobium japonicum* strains and to compare such nodules with uninfected roots.

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0341-0382/85/0300-0179 $ 01.30/0

Materials and Methods

Rhizobium japonicum strains 61-A-101 (*nod*$^+$ *fix*$^+$ and 61-A-24 (*nod*$^+$ *fix*$^-$) were originally received from Dr. Burton, Nitragin Company, Milwaukee. *Rhizobium japonicum* strain 110 USDA (*nod*$^+$ *fix*$^+$) was from Dr. Weber at the US Department of Agriculture, Beltsville. *Rhizobium japonicum* RH-31-Marburg is a *nod*$^+$ *fix*$^-$ mutant of 61-A-101 produced as described by [9]. Seedlings of *Glycine max* cv. Mandarin were grown and infected with a titer of 10^7 cells · ml^{-1} of *Rhizobium japonicum* as described previously [10, 11]. The method for determining nodule size distributions was that used by [12]. Nodules produced after infection of soybeans by *R. japonicum* RH 31-Marburg were analyzed in detail by [13].

Glyceollin I contents were determined in nodules harvested 20 and 34 d after infection. Nodules of ten plants were harvested, freeze-dried and homogenized by grinding to a powder with mortar and pestle. Samples (1 mg) of the nodule powder from each group of plants were each extracted with 1 ml of methanol for 1 h at room temperature. The insoluble material was subsequently removed by centrifugation and the supernatants evaporated to dryness under a stream of N_2. The dry residues were each redissolved in 1 ml of methanol. A 0.1 ml aliquot of each methanolic solution was diluted with 0.9 ml of distilled water. Serial 1 : 10 dilutions of the resulting solutions were made using 10% (v/v) methanol in distilled water. The amount of glyceollin I present in each of these solutions was determined using a radioimmunoassay specific for this phytoalexin [14].

Results and Discussion

Glyceollin I concentration in fix^+ and fix^- types of nodules of *Glycine max* and from roots are summarized in Table I. A significant accumulation is obvious only in nodules infected with *Rhizobium japonicum* 61-A-24. 20 d after infection more than 6000 pmol glyceollin I per mg dry weight are present. This is equivalent to about 1 µmol per g fresh weight of nodule tissue. The accumulation of glyceollin in this type of nodules is thereby in the same order of magnitude as in roots of soybeans, 24 h after infection with zoospores of *Phytophthora megasperma* f. sp. *glycinea* [15]. Two weeks later, the glyceollin I concentration has dropped to about 1600 pmol per mg dry weight, still four times the amount present in control root tissue of the same age. The significant stimulation of glyceollin I synthesis in the nodules infected with strain 61-A-24 is not a consequence of the lack of nitrogenase activity (ineffective nodules). Nodules infected with the fix^- strain RH 31-Marburg contain the lowest glyceollin I concentration of all nodule types studied, even lower than in the fix^+ types 61-A-101 and USDA 110 (Table I).

Some other characteristics of the four nodule types studied are summarized in Table II. The nodule type infected with *Rhizobium japonicum* RH 31-Marburg is an intermediate between the nodules infected with strain 61-A-101 and those infected with strain 61-A-24: the RH 31-Marburg nodules have an intermediate leghaemoglobin content, an intermediate increase of nodule number per plant during development within 50 d after infection. Also the nodule size distribution appears intermediate between the other types. These parameters are apparently not correlated with glyceollin I accumulation, since the RH 31-Marburg infected nodules show the same negative response as the two fix^+ nodules. In the time period between 20 and 30 d after infection, only the peribacteroid membranes are disappearing in the 61-A-24 nodules, leaving the bacteroids in direct contact with the host cell cyto-

Table I. Glyceollin accumulation in nodule and root tissue of *Glycine max* var. Mandarin, infected with $nod^+ fix^+$ and $nod^+ fix^-$ strains of *Rhizobium japonicum*

Tissue	Infection with *Rhizobium japonicum* strain	Days after infection (nodules) and after germination (roots)	Glyceollin I [pmol · mg dry $weight^{-1}$]
Nodule	61-A-101 (fix^+)	20	140
Nodule	61-A-101 (fix^+)	34	< 50
Nodule	110 USDA (fix^+)	20	630
Nodule	110 USDA (fix^+)	34	< 50
Nodule	RH 31-Marburg (fix^-)	20	< 50
Nodule	RH 31-Marburg (fix^-)	34	< 50
Nodule	61-A-24 (fix^-)	20	6250
Nodule	61-A-24 (fix^-)	34	1600
Root	–	20	650
Root	–	34	410

Table II. Biochemical and structural characters of four types of soybean nodules (+ + high, + intermediate, – absent or very low, n. d.: not determined)

Infection by	*R. japonicum* 61-A-101 (*fix*$^+$)	*R. japonicum* 110 USDA (*fix*$^+$)	*R. japonicum* Rh 31-Marburg (*fix*$^-$)	*R. japonicum* 61-A-24 (*fix*$^-$)
Nitrogenase activity	+ +	+ +	–	–
Leghaemoglobin content	+ +	+ +	+	–
nodule number plant^{-1} during development	constant	small increase	significant increase	continuous increase
percent of nodules in the size class				
1.6 mm	18	n. d.	30	85
2.0 – 2.2 mm	19	n. d.	28	–
2.6 – 2.8 mm	20	n. d.	–	–
Lysis of bacteroids during nodule growth	–	–	partial	–
Stability of peribacteroid membranes	+ +	+ +	+ +	–
Accumulation of glyceollin	–	–	–	+ +

plasm and cell organelles [3]. Thus, the instability of the peribacteroid membrane seems to be a decisive structural characteristic of this nodule in order for the plant to synthesize and accumulate this phytoalexin. This correlation, if generalizable, implies an important role for the peribacteroid membrane in preventing the induction of the plants disease defense mechanisms by the invading bacteria. Our results also indicate that the accumulation of phytoalexins in nodules of legumes could be used as a quantitative biochemical indicator for the loss of the peribacteroid membrane system in the bacteroid zone of the nodules. This can be also concluded from the result, that only very small concentrations of pisatin ($0.1 - 10\ \mu g \cdot ml^{-1}$ tissue concentration) have been found in effective pea nodules [16].

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