Quantitative Histochemistry of Nematode-Induced Transfer Cells

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ABSTRACT

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Determinations of metabolites in *Meloidogyne*-induced giant cells and *Heterodera*-induced syncytia were made by Lowry's ultra-micro analytical techniques. Samples of 5-50 ng were dissected from lyophilized frozen sections and metabolite concentrations were determined by quantitative conversion to pyridine nucleotides. These in turn were subjected to "enzymatic cycling" and measured fluorometrically. Both giant cells and syncytia in soybean had concentrations of ATP, G-6-P, and protein comparable to those of actively growing root tip tissue but contained about four times more glucose. Free amino acids were more concentrated in giant cells than in syncytia. Compared to controls, giant cells of garden balsam had higher concentrations of ATP, G-6-P, and glucose, and slightly higher concentrations of free amino acids. From these data we conclude that both pathogen and host influence the altered metabolite concentrations in giant cells and syncytia.

Additional key words: metabolites of nematode-induced transfer cells.

The large multinucleate cells induced by sedentary endoparasitic nematodes reflect altered morphogenesis of plant cells. The nematodes influence cell response both by injecting gland contents and by removing portions of the host cytoplasm. Although details of the morphology have been adequately recorded, it has not been possible to show how the metabolism of these cells differs from that of nontransformed cells.

Only a few descriptions of biochemical changes in plant tissues infected with root-knot nematodes have been published. Reduced concentrations of amino and organic acids and increased sugar concentrations were found in root exudates and xylem sap of infected tomato plants. Exudates from galled roots had much larger amounts of a glucose polysaccharide than those from healthy plants (22). Owens and Specht (19) compared the composition of galled and healthy tomato roots from the same plants. Galled tissues had decreased carbohydrates, pectins, cellulose, and lignins, but increased hemicellulose, organic acids, free amino acids, protein, nucleotides, nucleic acids, lipids, and minerals. Owens and Rubinstein (18) reported higher rates of glycolysis in galls and higher rates of intermediary metabolism, particularly that leading to protein and nucleic acid synthesis. DeMott (2) estimated that the hexose monophosphate pathway was 1.4 - 1.8 times more active in galled tomato roots than in adjacent noninfected tissue. Lewis and McClure (15) found differences in several free amino acids after infection of cotton with root-knot nematodes; proline increased nearly 20-fold in a susceptible cultivar but only 1.5 times in a resistant cultivar. Owens and Specht (19) also reported high increases in proline and showed that giant cells were especially rich in ninhydrin-positive compounds. Hanounik and Osborne (7) found that the increased amino acids of infected tobacco roots were not the same as those in tomato or cotton.

With the exception of Owens and Specht's histochemical observations, all of the above determinations were made on homogenates of whole galls. This method does not permit conclusions on the particular changes within giant cells.

In a study of *Meloidogyne*-induced changes, Endo and Veech (5) demonstrated a number of enzyme activities by histochemical methods. Increased activities of malate, isocitrate, succinate, glucose-6-phosphate, and 6-phospho-gluconate dehydrogenases and NAD and NADP diaphorases occurred in cells adjacent to both anterior and posterior regions of the nematode. Giant cells were very active, but cells along larval migration pathways also displayed higher than normal enzyme activity. Veech and Endo (21) showed that giant cells also have increased activities of alkaline and acid phosphatases, nonspecific esterases, peroxidase, adenosine triphosphatase, and cytochrome oxidase. In short, cells stimulated by the nematode to become giant cells display a generalized increase of enzyme activity compared with adjacent nonstimulated cells.

There is one report of increased amino acid concentrations in susceptible beet roots infected with

Heterodera schachtii (3), but no others on biochemical changes in infections with *Heterodera*.

We set out to compare metabolite concentrations in giant cells induced by *Meloidogyne* with those in syncytia induced by *Heterodera* in soybeans and to determine whether each nematode's regulation results in distinctive host cell metabolism. Further, we compared *Meloidogyne*-induced giant cells in two hosts: garden balsam and soybean, to determine differences in host species responses to the same nematode.

MATERIALS AND METHODS

The methodology used was developed by Lowry and coworkers (16). The following steps are followed: (i) tissue is fixed in liquid nitrogen to preserve original concentrations of metabolites and enzymes as fully as possible; (ii) fixed tissue is sectioned in a cryostat at −20 to −30 C; (iii) sections are lyophilized at −35 C, after which the sections under vacuum can be warmed to room temperature for dissection; (iv) samples are accurately weighed on a fishpole balance; (v) metabolites are quantitatively converted to the equivalent pyridine nucleotides in small volumes (0.1 to 5 μliters) under oil in Teflon oil wells; and (vi) the pyridine nucleotide from (v) is amplified by "enzymatic cycling" and measured fluorometrically. We followed published procedures for determination of the following metabolites in our samples of 5 to 50 ng dry weight: glucose, glucose-6-phosphate (G-6-P), fructose-1, 6-diphosphate (FdP), 6-phosphogluconate (6-P-G) and adenosine triphosphate (ATP) (14, 16).

Protein determinations were based on the measurement of amino acids released by acid hydrolysis. These were allowed to react with orthophthalaldehyde and βmercaptoethanol in a small volume (10 μliters) followed by dilution in 1 ml 0.5 N NaOH for fluorometric measurement of the products. Concentrations of free amino acids in the samples were estimated in a similar way by omitting the hydrolysis step and heating in distilled water only. Bovine plasma albumin and alanine served as standards (1).

TABLE 1. Concentrations of metabolites (mM kg^{-1} dry weight) and of protein and free amino acids (% of dry weight) at different times after infection in *Heterodera glycines* syncytia and *Meloidogyne incognita* giant cells in soybean or garden balsam (*Impatiens balsamina*) and in root tip tissue[a]

	Metabolite concentrations							
	Heterodera-stimulated syncytia in soybean		*Meloidogyne*-stimulated giant cells in soybean		*Meloidogyne*-stimulated giant cells in garden balsam		Controls[g]	
Metabolite	Day		Day		Day		Root tip of soybean	Root tip of garden balsam
ATP	15	17.1 ± 1.3 [10]	18	11.0 ± 1.1 [8]	14	19.1 ± 1.4 [17]	11.4 ± 0.5 [5]	13.3 ± 1.5 [5]
	18	10.6 ± 0.8 [10]	29	8.8 ± 0.9 [9]	19	18.1 ± 1.1 [14]		
	22	5.7 ± 0.8 [4]	39	8.8 ± 0.6 [8]	30	20.3 ± 0.9 [12]		
	25	2.9 ± 0.8 [9]			36	19.5 ± 1.6 [12]		
					42	18.1 ± 0.9 [17]		
FdP[b]	15	<0.2 [4]	29	<0.2 [4]			<0.2 [4]	
G-6-P[c]	15	9.1 ± 0.9 [15]	18	6.9 ± 0.4 [15]	19	31.6 ± 1.8 [12]	7.9 ± 0.7 [9]	4.7 ± 0.3 [4]
	18	7.8 ± 0.7 [13]	29	6.8 ± 0.8 [14]	30	14.3 ± 0.7 [12]		
	22	3.1 ± 0.6 [10]	39	3.5 ± 0.4 [15]	36	10.6 ± 0.8 [9]		
	25	3.4 ± 0.4 [15]			42	8.9 ± 0.8 [10]		
Glucose[d]	15	202.9 ± 17.3 [9]	15	158.4 ± 9.7 [9]	19	104.7 ± 15.6 [4]	54.8 ± 4 [10]	31.7 ± 1.8 [9]
	18	202.0 ± 10.3 [10]	29	197.2 ± 8.9 [10]	30	99.2 ± 5.7 [10]		
	22	105.1 ± 12.2 [5]	39	73.6 ± 10.2 [4]	36	151.4 ± 10.2 [9]		
	25	55.2 ± 21.4 [9]			42	151.8 ± 8.8 [10]		
6-P-G	15	<0.3 [4]	29	<0.3 [4]			<0.3 [4]	
Protein[e]	13	38.5 ± 4.8 [3]	18	51.6 ± 3.0 [5]	19	52.2 ± 0.8 [6]	51.4 ± 1.6 [10]	43.6 ± 0.9 [5]
	15	43.2 ± 2.9 [9]	29	53.6 ± 2.9 [8]	30	53.3 ± 1.7 [8]		
	18	40.3 ± 1.3 [9]	39	56.9 ± 2.3 [9]	36	51.6 ± 2.6 [7]	16.4 ± 2.3 [5][f]	5.6 ± 0.5 [7][e]
	22	45.5 ± 3.1 [9]			42	52.9 ± 2.1 [6]		
	25	5.3 ± 1.2 [5]						
Free amino acids	15	1.8 ± 0.2 [7]	29	6.3 ± 0.4 [6]	36	6.3 ± 0.4 [5]	1.1 ± 0.1 [7]	4.4 ± 0.2 [5]

[a]Numbers in brackets = number of determinations.
[b]Not corrected for dihydroxyacetone phosphate.
[c]Not corrected for glucose-1-phosphate.
[d]Not corrected for glucose-6-phosphate and UDP-glucose.
[e]Not corrected for free amino acids.
[f]Cortical tissue.
[g]Soybean root tips were harvested on day 18 and those of garden balsam on day 19.

Timed *Heterodera glycines* Ichinohe infections were produced by Endo's agar cone technique (4). Root-knot infections were made by inoculating seedlings in vitro with *Meloidogyne incognita* (Kofoid and White) Chitw. for 48 hr and transplanting to vermiculite. The host plants were soybean cultivar Williams and *Impatiens balsamina* 'Tom Thumb'. Control tissues were taken from actively growing roots just behind the root tip of noninfected seedlings.

RESULTS

Heterodera.—The data are presented in Table 1 and Fig. 1. Between 15 and 18 days after infection there is a sharp decrease in the concentration of ATP, followed immediately (18-22 days postinfection) by decreases in G-6-P and glucose. The decrease in protein only starts 22 days after infection. This coincides with maturation of the female which was white at day 18 and yellowish at day 22. Protein concentrations in the active syncytia are slightly lower than in the control (root tip tissue) and the concentrations of free amino acids increase (1.7 times) in 15-day-old syncytia. These cells have 1.75 times more ATP, 1.3 times more G-6-P, and 4.4 times more glucose than control tissue. Concentrations of all metabolites decrease sharply as the nematode approaches the end of its life cycle.

Meloidogyne.—Concentrations of ATP, glucose, and G-6-P in *Meloidogyne*-induced giant cells show the same trend in time as those in *Heterodera*-induced syncytia. However, the life cycle of *Meloidogyne* extends beyond 39 days in soybean. The most striking difference is the large concentration of free amino acids in *Meloidogyne*-induced giant cells. The ratio of concentration of free amino acids relative to the control is 5.7 in soybean and 1.4 in balsam. In soybean giant cells, ATP, G-6-P, and glucose are fairly constant from day 18 to day 29. At day 39, all three values decline. In giant cells of garden balsam both ATP and G-6-P concentrations are higher than controls.

DISCUSSION

The set of determinations on syncytium and giant cell tissue shown here is a first step towards a quantitative

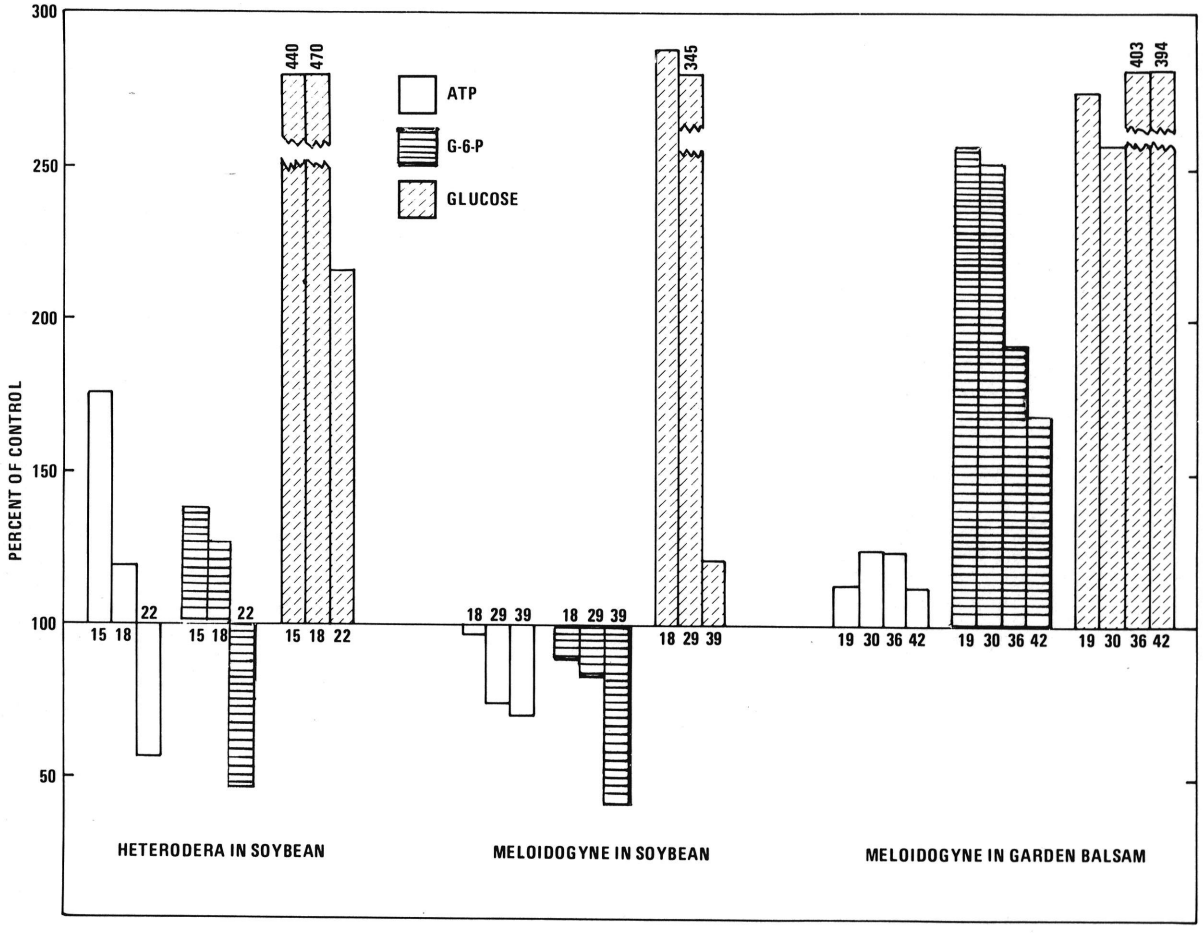

Fig. 1. Relative concentrations of adenosine triphosphate, glucose-6-phosphate, and glucose in *Heterodera glycines* syncytia and in *Meloidogyne incognita* giant cells in soybean or *Impatiens balsamina*. Values are calculated on the basis of protein concentrations employing root-tip tissue as a control. Protein concentration of root tip tissues is expressed as 100%. The numbers near the 100% line indicate days after infection. Actual determinations (also protein) were done on samples of 5 to 50 ng.

description of the metabolism of these nematode-induced transfer cells. Reports in the literature on quantitative and qualitative differences between infected and control tissues were based on homogenates of whole galls or noninfected tissue from infected plants (3, 5, 7, 15, 18, 19, 21). Homogenates represent considerable dilution of enzyme and metabolite concentrations in giant cells and syncytia by the contents of adjacent cells. Histochemical comparisons with adjacent tissue also are subject to error, because the vacuole in parenchyma cells is about 90% of their volume and only 30% in a giant cell (13). There may be differences in metabolic activities per unit of cytoplasm between nematode-induced transfer cells and adjacent tissues. In addition, gall tissues and tissues adjacent to *Heterodera*-induced pathologies probably differ from comparable tissues of noninfected plants. We took actively elongating root tip tissue of noninfected plants as a reference for the analyses of portions of the cytoplasm of giant cells and syncytia.

Protein concentrations in syncytia and giant cells are high and very similar to each other and to the controls. The slightly lower percentage found in the syncytia probably reflects substantial amounts of cell wall in the samples. The giant-cell samples were dissected to eliminate cell walls and thus to have the highest protein concentrations. Owens and Specht (19), using histochemical procedures, found a 3- to 20-fold increase in protein in giant cells of tomato over cortical tissue. Our data show increases of 2.5-fold for *Heterodera*-induced syncytia, 3.3-fold for *Meloidogyne*-induced giant cells in soybean, and a 10-fold increase in *Meloidogyne*-induced giant cells of garden balsam, all with reference to cortical cells.

Free amino acid concentrations of *Heterodera*-induced syncytia are about 1.7 times that of the controls. This compares to approximately 1.5-fold increase in giant cells in garden balsam and a 6-fold increase in soybean giant cells. In general, this confirms the reports from the literature both for *Meloidogyne* and *Heterodera* (3, 6, 15, 19). Techniques such as those described in Neuhoff (17) will permit more precise descriptions of the qualitative and quantitative composition of free amino acids. The big difference in amino acid concentrations in giant cells and syncytia in the same plant indicates a difference in metabolic activities between *Heterodera*-induced syncytia and *Meloidogyne*-induced giant cells. This suggests active intervention by the nematode in the metabolic activities of their feeding sites.

Glucose concentrations were much higher in each of the nematode-induced transfer cells than in control tissue. It would be of great interest to determine whether such concentrations occur generally in plant transfer cells or are confined to nematode-induced transfer cells.

The nematode-induced transfer cell is a useful model for investigation of the morphology, physiology, and biochemistry of transfer cells in general (6, 9, 10, 11, 12, 13, 20). They are large, infections can be timed, and the details of morphology differ among the examples induced by the various sedentary nematode parasites. Each type represents the interaction of the genetics of both host and parasite, which in a few cases are beginning to be known (8). Application of micromethods (16, 17) will be useful to obtain general descriptions of transfer cell metabolism and to delineate the degree of control exerted by the nematode inducers.

In conclusion, our experience with micromethods strongly suggests that such methods can be used to measure changes in plant cells both immediately adjacent to pathogens and at some distance from the site of infection.

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