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STIMULATION OF LIPASE AND *N*-ACETYL-β-D-GLUCOSAMINIDASE ACTIVITIES IN *EUPHORBIA CHARACIAS* LATEX BY CONTACT OF FREE FATTY ACIDS WITH ROOTS

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Abstract—A transitory increase in the basal levels of lipase and *N*-acetyl-β-D-glucosaminidase activity was observed in the latex of *Euphorbia characias* plants with their roots in contact with the free fatty acids, butyric, 3-butenoic and caprylic. Incorporation of [^{14}C-1] -butyric acid into latex was also demonstrated.

INTRODUCTION

Ultrastructural studies have shown that non-articulated laticifers consist of a single giant cell, the central vacuole of which contains a fluid of lysosomal nature [1] called latex, in which many secretion products, such as terpenes are accumulated [2–4]. Latex in these laticifers undoubtedly plays a physiological role, the exact nature of which has not yet been elucidated. For instance, we do not know whether substances, such as terpenes, included in the laticifers are used again in plant metabolism or whether they must be considered as waste matter. Many enzymes, especially hydrolases, are present in latex [1, 5–8], so that many biosynthetic or degradative biochemical pathways may exist in laticifers. Recently, lipase activity was described in the latex of *Euphorbia characias* [9, 10]. Because rubber synthesis is linked to lipid metabolism, the question arises as to whether fatty acids may be used as nutrients by plants and whether these substances may influence laticifer composition; several organic substances are present in the humic–clay complex [11]. However, only a few of these, such as benzoic or 3-butenoic acids, have been found to be directly absorbed by the roots [12].

The aim of the present work was to examine the effects of short-chain free fatty acids introduced into the culture medium on the lipase and *N*-acetyl-β-D-glucosaminidase (GlcNAcase) activities levels of *E. characias* latex.

RESULTS AND DISCUSSION

Stimulation of enzymatic activities

Considerable variability was observed in lipase (1200–3800 IU ml^{-1}) and GlcNAcase (0.6–4.5 U ml^{-1}) activities from 24 wild plants before they were incubated in sterile water. In all plants during the first 6 days in water, the latex lipase and GlcNAcase activities decreased very rapidly to zero. After preincubation for 10 days in sterile water of adventitious roots of six *E. characias* plants, as described in Experimental, these roots were exposed to butyric acid, 3-butenoic acid, caprylic acid and HCl (control to verify effect of pH-shock). We observed that after each addition of free acid, lipase and GlcNAcase activities which occurred in the latex increased consistently (Table 1), suggesting, in turn, that plant metabolism was stimulated by this exposure. Table 1 shows the average variations in lipase and GlcNAcase activities with addition of the free acid. An increase of lipase activity (stimulation factors 8 ± 1 after first addition [no. 1], 6 ± 1 after second addition [no. 2] and 2.5 ± 0.5 after third addition [no. 3]) from the basal level (time zero) was observed after the first, second and third additions of butyric acid. On the other hand, a smaller stimulation factor for lipase activity was observed after the first addition of 3-butenoic acid (1.2 ± 0.2), as compared with that obtained after the first addition of butyric acid (8.0 ± 1.0). After the second addition of 3-butenoic or butyric acids, the stimulation factors were similar (5.6 ± 0.5 and 6.0 ± 1, respectively). Otherwise, the addition of caprylic acid involved an increase of lipase activity (stimulation factor 2.4 ± 0.2).

Except after the first addition of butyric acid (2.73 U ml^{-1}; stimulation factor 1.1 ± 0.2), we observed a stimulation of GlcNAcase activity after addition of free fatty acids (stimulation factor 1.3 ± 0.2 to 11 ± 1, according to the nature of free fatty acid used). Addition of the water-insoluble fatty acid, caprylic acid, produced a high GlcNAcase stimulation factor (11.0 ± 1.0). Control experiments with HCl give a similar stimulation for lipase and GlcNAcase activities (stimulation factors

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4.0 ± 0.5 and 4.7 ± 0.5, respectively). Maximum enhancement of activities was observed between 1 hr and 2.5 hr (data not shown). After the first addition of 250 μmol butyric acid (initial pH of incubation medium, 4.8), lipase activity was 4000 IU ml^{-1}, as compared with 500 IU ml^{-1} at time zero. After 24 hr, the pH was 6, which indicates that butyric acid was absorbed. After the second addition of butyric acid (500 μmol) at 24 hr, the pH had increased from 4.2 to 5.5. After the third addition of butyric acid (750 μmol), the pH increased from 4 to 4.7. Although the butyric acid concentration increased in the culture medium after these additions, the pH variations observed versus time became smaller, which suggests that perhaps absorption by the adventitious roots had reached saturation. In view of the low solubility of caprylic acid in water, the pH measurement was not relevant here.

Table 1. Average stimulation factors recorded with various free acids and controls (HCl) on lipase and GlcNAcase activities in latex from six *E. characias* plants kept in sterile water

Addition		Stimulation factor	
Free acid	no.	Lipase	GlcNAcase
Butyric	1	8.0 ± 1.0	1.1 ± 0.2
	2	6.0 ± 1.0	3.5 ± 0.5
	3	2.5 ± 0.5	1.5 ± 0.2
3-Butenoic	1	1.2 ± 0.2	1.3 ± 0.2
	2	5.6 ± 0.5	1.4 ± 0.2
Caprylic	1	2.4 ± 0.2	11.0 ± 1.0
HCl	1	4.0 ± 0.5	4.7 ± 0.5

*The stimulation factor is the ratio of the maximal parameter value (activity) measured after each addition of acid to the value of the same parameter measured before the addition (mean value ± standard error).

Since the control experiments involving addition of HCl yielded similar pH variations and stimulation factors, pH-shock might be responsible for the stimulation of lipase and GlcNAcase. Addition of weak acids, such as free fatty acids, or strong acids such as HCl, caused a fall in pH which might be responsible for the stimulation of lipase and GlcNAcase activities.

Absorption of fatty acids by roots

After incubating adventitious roots of four plants with [^{14}C-1] -labelled and unlabelled butyric acid (500 μmol) for 5 days in incubation medium, the latex was tapped from whole stems (0.3 ml). After performing ^{14}C-counts on latex samples, we observed that *ca* 62% of the initial radioactivity was present in the crude latex. This value was derived from the ratio between the radioactivity measured in the latex (estimated volume *ca* 0.4 ml) and the radioactivity incorporated in the plant. The latter was calculated from the difference between the radioactivity in the incubation medium at the start and end of the experiment. The detection of ^{14}C in the latex indicates that labelled butyric acid was directly incorporated into the plant, then metabolized.

Recently, a lipidic cofactor necessary for lipase activity was partially purified from *E. characias* latex [13]. After lipid extraction from the latex [10], this lipidic cofactor was also labelled (*ca* 10% of total latex radioactivity). From the extracted leaf lipids, it was established that 10.6% of the initial radioactivity was also present in the leaves. Separation of leaf lipids on silica gel TLC indicated that ^{14}C was incorporated into polar compounds (phospholipids, phosphatidyl-*N*,*N*-dimethylethanolamine, phosphatidic acid and free acids) (Table 2). These data were obtained from four plants devoid of damaged roots.

Table 2. TLC fractionation of [^{14}C]-labelled leaf lipids on silica gel

Lipid component	R_f Authentic	R_f Extract	% ^{14}C
Unknown	—	0	28.4 ± 1.2
Phosphatidylcholine	0.33	0.37	44.0 ± 2.0
Sulfoquinosyl-diacylglycerol	0.40	0.43	5.8 ± 0.6
Unlnown	—	0.47	trace
Phosphatidyl-*N*, *N*-dimethyl-ethanolamine	0.52	0.54	10.3 ± 1.0
Unknown	—	0.66	trace
Phosphatidic acid	0.74	0.74	5.2 ± 0.3
Unknown	—	0.84	trace
Fatty acids	0.95	0.94	4.4 ± 0.1
Pigments	0.98	0.98	trace

*Solvent: chloroform–methanol–water (65:25:4). The average % of [^{14}C]-labelled-lipids was calculated after counting the positive anilinonaphthalene sulphonate spots on the chromatogram. Values presented show the mean ± standard error of results from triplicate experiments. Roots washed in sterile water for 10 days were placed in a mixture of 500 μmol unlabelled butyric acid and 0.34 μmol of [^{14}C-1] butyric acid under stirring for 5 days.

It is well known that the function of roots is to absorb inorganic nutrients [14]. Ours results suggest that *E. characias* plants growing under natural conditions are also able to absorb water-soluble fatty acids, such as fulvic acid [15], which may stimulate metabolism in the roots during the slow mineralization of the humic-complex. This may amplify plant metabolic processes, which in turn may also have an impact on latex content.

EXPERIMENTAL

Plant material and culture conditions. Twenty four *E. characias* plants obtained from the field were surface-sterilized for 15 min with 5% (W/V) NaOCl soln and washed for 10 days in sterile H_2O) under experimental temp. (25°) and light (12 000 lux) conditions; sterile H_2O was renewed every 12 hr for 10 days. Roots were then washed with sterile H_2O, plants maintained at constant temp. (25°) in sterile H_2O and constantly stirred with a magnetic bar. Free fatty acids, butyric, 3-butenoic or caprylic, were added to the H_2O (0.5 l) bathing the roots, by making 3 successive additions (250, 500 and 750 μmol or 0.5, 1.0 and 1.5 mM) at 24 hr intervals. Six plants were used in expts with each free fatty acid. After incubating roots in 0.5 l sterile H_2O, 250 μmol butyric acid and 0.34 μmol of [^{14}C-1] butyric acid (15 mCi mmol^{-1}) were added with stirring for 5 days. Upon tapping the stems, 0.3 ml of latex was recovered. A ^{14}C count was carried out on a 2 μl aliquot. Selective extraction of latex lipids was performed using a previously described method [10]. Total leaf lipid extraction was carried out as described in ref. [16]. [^{14}C]-labelled leaf and latex lipids were fractionated by TLC using $CHCl_3$–MeOH–H_2O (65: 25: 4). After, staining with anilinonaphthalene sulphonate, lipids were scraped off from the silica gel and radiolabelling measured.

Enzyme assays. Lipase (EC 3.1.1.3) and *N*-acetyl-β-D-glucosaminidase (EC 3.2.1.36), were measured with latex collected for 3.5 hr from the stems of 6 plants with their roots in contact with water-soluble (butyric and 3-butenoic) or water-insoluble (caprylic) acids under the same exptal conditions as those previously described [9, 17]. Lipase activity associated with suspension latex particles [9] were selected on account of its strong galactolipase activity on polar lipids, such as MGDG (Moulin and Piéroni, unpublished results). GlcNAcase activity of the supernatant was tested on account of its relation with catabolic processes which play a major role in the first steps towards cell wall synthesis [18]. Control expts were carried out with HCl (10^{-4} M) added to sterile H_2O. Synthetic *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide was used as substrate. The standard incubation medium (0.6 ml) used to measure GlcNAcase activity contained 0.1 M succinate (pH 5.5) and 2.5 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide as substrate. After 120 min incubation at 30°, the reaction was stopped by adding 0.25 ml 1 M Na_2CO_3 and *A* of the *p*-nitrophenol formed was read at 400 nm against a control tube containing no enzyme. One enzyme unit (U) is defined as the amount of the enzyme which liberated 1 μmol of *p*-nitrophenol min^{-1}. Lipase activity was determined with mechanically-stirred emulsions of tributyroylglycerol (600 mM) as substrate in 10 ml of 0.15 M NaCl [9]. Lipase activity was monitored titrimetrically at pH 8 with a pH-stat equipped with a thermostatted (37°) vial. In all assays, hydrolysis rates were derived from initial slopes of kinetic curves and expressed as international units (IU). One IU corresponds to 1 μmol of fatty acid liberated min^{-1}.

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