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CYSTEINE DESULFHYDRASE ACTIVITY IN CUCURBIT PLANTS: STIMULATION BY PREINCUBATION WITH L- OR D-CYSTEINE

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Key Word Index—*Cucurbita pepo*; *Cucumis sativus*; Cucurbitaceae; cysteine catabolism; cysteine desulfhydrase; hydrogen sulfide; sulfur metabolism; light dependency.

Abstract—Cysteine desulfhydrase activity in leaf discs of cucurbit plants is enhanced 2–4-fold by preincubation with L- or D-cysteine. Preincubation with structural analogs of cysteine also stimulated the activity of the enzyme, but to a smaller extent. Maximal increase in cysteine desulfhydrase activity was observed by preincubation with 5 mM or higher concentrations of cysteine. Although not caused by activation, stimulation of the enzyme activity was half-maximal within less than 15 min. Whereas the increase in cysteine desulfhydrase activity by preincubation of leaf discs with cysteine was light independent, pretreatment of the entire plant with light or dark determined the leaf discs' potential for stimulation of the enzyme. Exposure to darkness for 4 hr reduced this potential by 60%. It is concluded that the potential for stimulation of cysteine desulfhydrase activity by preincubation with cysteine is regulated by a compound not synthesized, but metabolized, in the leaf tissue. This regulatory compound may be supplied to the leaves by long-distance transport.

INTRODUCTION

In a number of animal and plant species catabolism of L-cysteine has been shown to proceed via desulfhydration to sulfide, ammonium, and pyruvate [1–5]. This reaction is catalysed by the cysteine desulfhydrase (L-cysteine hydrogen sulfide-lyase, deaminating; EC 4.4.1.1), a pyridoxalphosphate dependent enzyme found in animal and microbial cells [1, 6, 7] as well as in cells of higher plants [3, 5, 8]. Kinetic and catalytic properties of cysteine desulfhydrase have been extensively studied with enzyme preparations from *Salmonella typhimurium* [9–11]. The enzyme is highly specific for L-cysteine and exhibits a positive co-operativity of the reaction with regard to this substrate [10, 11]. The reaction products ammonium or pyruvate alone do not inhibit cysteine desulfhydrase from *Salmonella typhimurium*, but sulfide alone as well as ammonium plus pyruvate are potent inhibitors at concentrations of 10^{-6} M [9–11]. At cysteine concentrations below 10^{-4} M, that do not inhibit growth of *Salmonella typhimurium* [12], cysteine desulfhydrase is relatively inactive (K_m 1.7–2.2 × 10^{-4} M [11]); the enzyme is active at higher cysteine concentrations that inhibit growth [12]. This growth inhibition is not caused by the sulfide produced by the enzymatic desulfhydration of L-cysteine, as inhibition of cysteine desulfhydrase by sulfide prevents an accumulation of damaging amounts of this compound [12]. These observations indicate that cysteine desulfhydrase functions in the removal of excess L-cysteine. The role of the D-cysteine specific cysteine desulfhydrase, recently reported in spinach and *Chlorella* [13], has still to be elucidated. In the presence of 0.5 mM L-cysteine the capacity of intact *Salmonella* cells to form sulfide from L-cysteine is increased [9, 12]. This increase could be attributed to *de novo* synthesis of cysteine desulfhydrase [7, 12]. In cells of higher plants cysteine desulfhydrase also appears to be an inducible enzyme. Preincubation of tobacco cells in suspension culture with L-cysteine enhanced the extractable activity of the enzyme 15–20-fold; during growth of the cells the specific activity of cysteine desulfhydrase roughly reflected the growth curve of the cultures with maximal capacity for L-cysteine degradation in late log phase [3]. In the present investigation stimulation of extractable cysteine desulfhydrase activity in higher plants by preincubation with L-cysteine is analysed in more detail in leaf tissue of cucurbit plants.

RESULTS AND DISCUSSION

Cysteine desulfhydrase activity in leaf discs of cucurbit plants is doubled by preincubation with 10 mM L-cysteine; preincubation with D-cysteine enhanced the activity of the enzyme to the same extent (Table 1). Preincubation with glutathione (GSH) or coenzyme A slightly inhibited cysteine desulfhydrase activity, whereas 2-thiouracil did not have a significant effect on the activity of this enzyme (Table 1). Structural analogs of cysteine, i.e. L-homocysteine, L-methionine, L-cysteic acid and S-methyl-L-cysteine, stimulated cysteine desulfhydrase activity when used in preincubation experiments, but to a smaller extent than L- or D-cysteine. Preincubation with L-cystine did not affect cysteine desulfhydrase activity (Table 1). These observations show that the enhanced cysteine desulfhydrase activity obtained by preincubation of leaf discs from cucurbit plants with cysteine cannot be attributed to a general thiol effect. Although non-stereospecific, stimulation of cysteine desulfhydrase activity seems to be highly dependent on the structure of the cysteine molecule.

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Table 1. Effect of preincubation with sulfur compounds on cysteine desulfhydrase activity

Preincubation in	Concn (mM)	Cysteine desulfhydrase activity (% of control ± s.e.)
Water	—	112 ± 4
L-Cysteine	10	199 ± 16
D-Cysteine	10	203 ± 9
GSH	10	73 ± 18
Coenzyme A	10	64 ± 23
2-Thiouracil	satd (20°)	119 ± 8
L-Cystine	satd (20°)	100 ± 11
L-Homocysteine	10	157 ± 19
S-Methyl-L-cysteine	10	128 ± 10
L-Cysteic acid	10	133 ± 12
L-Methionine	10	140 ± 16

Two groups of two leaf discs were punched from the first leaf of a 16-day-old pumpkin plant, each from one half of the leaf. One group of leaf discs was immediately assayed for cysteine desulfhydrase activity (control, 100%), the other group of leaf discs was floated for 3 hr on 10 ml of one of the preincubation solutions indicated. Cysteine desulfhydrase activity of controls ranged between 4.1 and 6.4 nmol H_2S/min·g fr. wt.

Stimulation of cysteine desulfhydrase activity by preincubation of leaf discs from cucurbit plants with L-cysteine is dependent on the L-cysteine concentration and the length of exposure to this compound. Whereas preincubation with L-cysteine concentrations up to 3 mM did not enhance the enzyme activity significantly, optimal stimulation was observed with 5 mM L-cysteine. Preincubation with higher L-cysteine concentrations did not enhance cysteine desulfhydrase activity any further (Fig. 1). The time course of stimulation of cysteine desulfhydrase activity by preincubation with L-cysteine was studied in leaf discs from the first leaf of 12-day-old pumpkin plants. Discs from these leaves exhibited a high potential for stimulation of cysteine desulfhydrase activity [8]. In these discs a preincubation period of less than 15 min was sufficient to obtain half-maximal stimulation; full stimulation was obtained by preincubation with L-cysteine for 60–90 min (Fig. 2). The fast stimulation of cysteine desulfhydrase activity suggests that preincubation with cysteine causes an activation of the enzyme. If the observed stimulation of cysteine desulfhydrase activity is mediated directly by an activating compound, generated during preincubation with cysteine, this compound, present in homogenates from leaf discs exposed to cysteine, should activate cysteine desulfhydrase in homogenates not exposed to cysteine. However, Fig. 3 shows that such an activating compound is not present in homogenates of leaf discs preincubated with cysteine. Mixing of homogenates from leaf discs floated on 10 mM L-cysteine with homogenates from leaf discs floated on water exhibited simple additivity of the enzyme activities. This result also rules out the possibility of the presence of a compound inhibiting cysteine desulfhydrase in homogenates not exposed to L-cysteine.

No difference in the stimulation of cysteine desulfhydrase activity was observed, when leaf discs of cucurbit plants were exposed to 10 mM L-cysteine in the light or in the dark (Table 2). However, pretreatment of the entire plant with light or dark had a considerable effect on the potential of discs from the leaves of these plants for stimulation of cysteine desulfhydrase activity by preincubation with L-cysteine. When leaf discs were punched from plants, exposed to light for 5 hr, subsequent preincubation of leaf discs with 10 mM L-cysteine doubled the cysteine desulfhydrase activity (Table 2). When plants were chosen for such an experiment that were kept in the dark after a light period of 5 hr, the potential for stimulation of cysteine desulfhydrase activity decreased with increasing length of the dark period. Only 1 hr dark after 5 hr light was sufficient to slightly reduce the potential for stimulation of cysteine desulfhydrase activity; 4 hr dark after 5 hr light reduced this potential by 60% (Table 2). If a 4 hr dark period was followed in such an experiment by light, the dark effect was reversed and the potential for stimulation of cysteine desulfhydrase activity recovered (Table 2). Exposure of plants to 0.5 hr

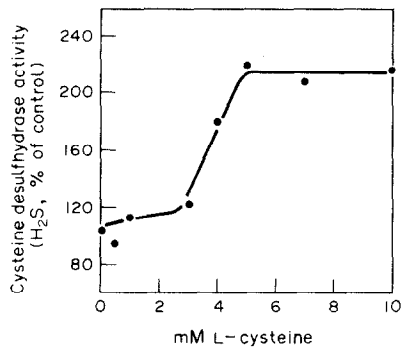

Fig. 1. Concentration-dependency of stimulation of cysteine desulfhydrase activity by preincubation with L-cysteine. Two groups of two leaf discs were punched from the cotyledons of a 14-day-old pumpkin plant, each from one cotyledon. One group of leaf discs was immediately assayed for cysteine desulfhydrase activity (control, 100%), the other group of leaf discs was floated for 3 hr on 10 ml of a preincubation solution containing one of the cysteine concentrations indicated. Cysteine desulfhydrase activity of controls ranged between 1.4 and 1.8 nmol H_2S/min·g fr. wt.

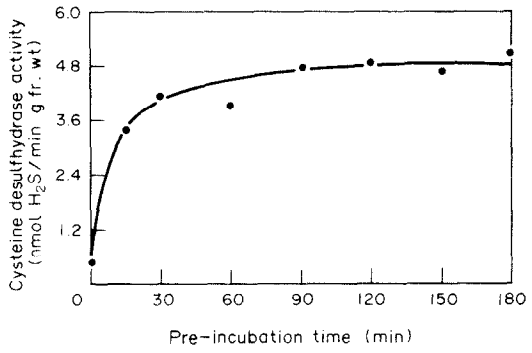

Fig. 2. Time-dependency of stimulation of cysteine desulfhydrase activity by preincubation with L-cysteine. Groups of two leaf discs were punched from the first leaf of a 12-day-old pumpkin plant, and floated on 10 ml of a 10 mM L-cysteine solution for the times indicated. After this preincubation period, cysteine desulfhydrase activity was analysed.

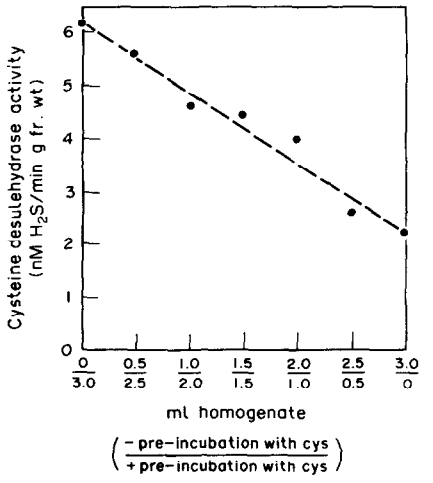

Fig. 3. Influence of homogenates from pumpkin tissue, preincubated with L-cysteine, on cysteine desulfhydrase activity of homogenates from untreated pumpkin tissue. Two groups of 12 leaf discs were punched from the first leaf of a 16-day-old pumpkin plant, each from one half of the leaf. One group of leaf discs was floated for 3 hr on water, the other group of leaf discs on 10 mM L-cysteine for the same time. After this preincubation period each group of leaf discs was homogenized in 15 ml buffer (0.1 mM potassium phosphate, pH 7.5, 1% ascorbic acid). The homogenates were mixed as indicated and the cysteine desulfhydrase activity of the mixtures determined. (- - -) Theoretical line for additivity of the enzyme activities.

light after 4 hr dark was insufficient for such a recovery, 2 hr light after 4 hr dark caused full recovery to a potential for stimulation of cysteine desulfhydrase activity observed without dark-treatment of the plants. In all cases it did not make any difference whether the leaf discs were preincubated with L-cysteine in the light or in the dark. Therefore, the potential for stimulation of cysteine desulfhydrase activity by preincubation with cysteine is determined by the treatment of the entire plant with light or dark before the tissue is excised and exposed to L-cysteine. Thus, it can be concluded that the process of stimulation of cysteine desulfhydrase activity by itself is not dependent on light. However, a prerequisite produced in the light is necessary to maintain the tissue's potential for stimulation of cysteine desulfhydrase activity. This factor appears not to be produced in the leaf tissue, but seems to be metabolized rapidly in the leaf cells: once the tissue is taken from the plant, light does not affect stimulation of cysteine desulfhydrase activity any more. It, therefore, can be assumed that a component not synthesized in the leaves, but supplied to the leaf tissue by long-distance transport participates in the regulation of cysteine desulfhydrase activity in higher plants. The nature of this compound is under investigation.

EXPERIMENTAL

Plant material. Expts were performed with pumpkin (*Cucurbita pepo* L. cv Small Sugar Pumpkin) or cucumber plants (*Cucumis sativus* cv Chipper). Seeds were planted directly into a steam-sterilized mixture of sand, soil, peat and Perlite and

Table 2. Light-dependency of stimulation of cysteine desulfhydrase activity by preincubation with L-cysteine (cys.)

Preincubation of plants	Preincubation of leaf discs	Cysteine desulfhydrase activity (% of control)
—	Water, light	114 ± 6
—	Water, dark	114 ± 9
—	10 mM cys., light	202 ± 8
—	10 mM cys., dark	195 ± 12
1 hr dark	10 mM cys., light	165 ± 15
1 hr dark	10 mM cys., dark	170 ± 16
2 hr dark	10 mM cys., light	143 ± 16
2 hr dark	10 mM cys., dark	153 ± 18
4 hr dark	10 mM cys., light	138 ± 12
4 hr dark	10 mM cys., dark	139 ± 14
4 hr dark, 0.5 hr light	10 mM cys., light	137 ± 16
4 hr dark, 0.5 hr light	10 mM cys., dark	131 ± 13
4 hr dark, 1 hr light	10 mM cys., light	151 ± 18
4 hr dark, 1 hr light	10 mM cys., dark	162 ± 15
4 hr dark, 2 hr light	10 mM cys., light	190 ± 21
4 hr dark, 2 hr light	10 mM cys., dark	189 ± 15

Cucumber plants were grown for 24 days in an environmental growth chamber as previously described [4] with 16 hr light–8 hr dark; plants were removed from the growth chamber for the preincubations indicated 5 hr after the beginning of the light period. Subsequently to one of the preincubations, two groups of leaf discs were punched from the cotyledons of a plant, each group from one cotyledon. One group of leaf discs was immediately assayed for its cysteine desulfhydrase activity (control, 100%), the other group of discs was preincubated for 3 hr under one of the conditions indicated, before the cysteine desulfhydrase activity was measured. Cysteine desulfhydrase activity of controls ranged between 1.2 and 1.7 nmol H_2S/min·g fr. wt.

watered with a modified Hoagland nutrient soln [14]. Plants were grown in an environmental growth chamber operating 16 hr at 33° with full light (fluorescent and incandescent lamps, 7.5 mW/cm^2) and 8 hr at 15° with 2 hr light (incandescent lamps, 2.4–4.7 mW/cm^2) at the beginning and the end of the cool period [14].

Determination of cysteine desulfhydrase activity. Two groups of leaf discs were punched from a leaf, each group from one half of the leaf. As a control, one group of leaf discs was assayed immediately; the other group of leaf discs was floated under the preincubation conditions indicated separately for each expt. In a standard expt four leaf discs (2.65 cm^2 each) were homogenized for 2 min in an ice-cold mortar with 3 ml 0.1 mM KPi buffer, pH 7.5, containing 1% (w/v) ascorbic acid. Cysteine desulfhydrase activity was measured directly in the crude homogenates. Homogenates were placed at 30° in a 25 ml filter flask which was connected to a flame photometric sulfur analyser (Monitor Labs, San Diego; Model 8450) in such a way that room air was drawn through the head space of the flask and into the sulfur analyser. The reaction was started by addition of 0.6 ml 100 mM L-cysteine. Cysteine desulfhydrase activity was calculated as an average emission rate for the first 5 min. Enzymes using L-cystine, formed by oxidation of L-cysteine in the reaction mixture, as a substrate [10, 15], did not interfere with this assay, as H_2S emission from leaf tissue of cucurbit plants has not been observed in response to L-cystine [5]. Emission of H_2S by resuspended $(NH_4)_2SO_4$ ppts proceeded at rates similar to those observed with crude homogenates. Therefore, spontaneous or enzymatic liberation of H_2S from mixed disulfides (X-SSH) by addition of cysteine can be excluded.

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