Responses of Sulfur-Containing Compounds in *Lemna paucicostata* Hegelm. 6746 to Changes in Availability of Sulfur Sources

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**ABSTRACT**

The steady state concentrations of S-containing compounds formed in *Lemna paucicostata* Hegelm. 6746 in response to variations in source and concentrations of sulfur were measured. Neither growth rates nor protein accumulation were markedly affected by the various growth conditions. Ignoring complications due to possible compartmentation, the results are consistent with internal pools of both SO₄²⁻ and cyst(e)ine (or products of their metabolism), but not methionine, being effectors of regulation of high affinity SO₄²⁻ uptake. As SO₄²⁻ in the growth medium was increased to 10 mM, down-regulation of high affinity SO₄²⁻ uptake was more than compensated for by unregulated uptake via the "non-saturating" uptake system. Tissue inorganic SO₄²⁻ accumulated but formation of reduced sulfur remained constant. Some conversion of L-cystine to SO₄²⁻ occurred. Presence of L-cystine in the medium (a) down-regulated high affinity SO₄²⁻ uptake and (b) decreased the rate of SO₄²⁻ organification. The net results were decreased (7 µM L-cystine) or normal (14 µM L-cystine) total tissue SO₄²⁻ and dose-dependent accumulation of soluble cyst(e)ine and glutathione, but not of soluble methionine. L-Methionine was not metabolized to cyst(e)ine or its products. Presence of L-methionine in the medium led to increased total tissue sulfur, accounted for almost wholly by manifold increases in soluble methionine, L-Met, and S-methylmethionine sulfonium. Soluble cyst(e)ine increased slightly.

In this paper, we report upon the responses of S-containing compounds in *Lemna paucicostata* to variations in the availability of several external sulfur sources. The study was initiated to gain insight into effectors which might regulate the high affinity SO₄²⁻ uptake system of *Lemna*. This system is specifically regulated by the level of SO₄²⁻ in the growth medium. Growth in L-cystine, but not L-methionine, also regulates the high affinity system (4). It is not known whether these regulatory effects are due to changes in the number of uptake sites or to changes in the rate of operation of a constant number of such sites (see 11, 17 for discussion). In the course of this work, we have gained further understanding of the responses of this plant system to variations in the chemical forms and concentrations at which sulfur is made available. A preliminary report upon this work has been made (3).

**MATERIALS AND METHODS**

**Chemicals.** Chemicals were obtained as previously specified (2). Tritiated compounds used as markers were prepared as described in (8). Protosol, L-[³⁵S]cystine, L-[³⁵S]methionine, and L-[³²S]SO₄ were purchased from New England Nuclear. L-[³⁵S]Cystine was separated from impurities present in the preparation by adsorption onto a Dowex 50-H⁺ column. The column was washed with 0.4 N HCl and L-[³⁵S]cystine was eluted in 3 N NH₄OH. The preparation was taken to dryness, redissolved in H₂O, and filter-sterilized for addition to growth medium. Chromatography of an aliquot of the preparation in solvent A showed that the single peak of radioactivity (containing at least 85% of the counts) migrated with authentic L-cystine. L-[³⁵S]Methionine was subjected to preparative chromatography in solvent B before use. The peak of radioactivity corresponding to L-methionine was eluted in N₂-bubbled H₂O. The eluate was lyophilized and the residue was incubated with 2-mercaptoethanol to reduce any L-methionine sulfoxide present to methionine (10). The preparation was stored frozen in 2-mercaptoethanol. Preparations of L-methionine were subjected to reduction by 2-mercaptoethanol in a parallel fashion. Just before use, both preparations were taken to dryness and dissolved in N₂-bubbled H₂O. Aliquots were removed for chromatography and the remainder of the preparations were filtered-sterilized. The [³⁵S]methionine was 92% radiopure as judged by chromatography in solvent B; the only other peak of radioactivity was coincident with L-methionine sulfoxide, detected by ninhydrin.

Concentrated stock solutions of [³⁵S]SO₄²⁻, molybdate, and amino acids (except L-cystine) were filter-sterilized and suitable aliquots added aseptically to autoclaved medium. L-Cystine and SO₄²⁻ were dissolved directly in growth medium at the desired final concentrations and the resulting solutions were filter-sterilized and aseptically transferred to sterile growth flasks.

**Chromatography.** The following chromatography solvents were used: solvent A, 2-propanol:88% formic acid:H₂O (7:1:2, v/v/v); solvent B, 1-butanol:propionic acid:H₂O (200:124:175, v/v/v) containing 10 mM 2-mercaptoethanol; solvent C, 0.1 M sodium phosphate buffer, pH 6.8, containing 60 g (NH₄)₂SO₄, and 2 ml 2-propanol/100 ml (20).

**Plant Growth.** Cultures of *Lemna paucicostata* Hegelm. 6746 were grown mixotrophically under standard conditions in medium 4 with 20 µM SO₄²⁻ (5).

**Labeling to Isotopic Steady State.** Plants were pregrown in supplemented medium for at least 4 doublings to ensure that steady state growth had been attained before cultures were initiated in control or supplemented medium containing [³⁵S]-labeled compound(s). Cultures were harvested after they had undergone 3.7 to 4.6 doublings in the presence of the isotopically labeled

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compound(s). Inoculum size and volume of medium were adjusted so that sufficient plant material for analysis was obtained yet depletion of amino acid supplement in the medium did not exceed 20% at time of harvest. For example, for growth in 0.7 μM L-methionine, six fronds were inoculated into 2.5 L of medium; 11% of the L-methionine was taken up in the subsequent 163 h during which the frond number increased to 130 (4.4 doublings).

For cultures grown with SO₄²⁻ as the sole sulfur source, SO₄²⁻ was labeled and analysis of these plants permitted measurement of SO₄²⁻ uptake and assessment of their concentrations of S-containing compounds. For cultures grown with an additional sulfur source (L-cystine or L-methionine), four labeled cultures were obtained for each growth condition. In one, only SO₄²⁻ was labeled and analysis of these plants permitted measurement of SO₄²⁻ uptake. In a second, only L-cystine or L-methionine was labeled and analysis of these plants permitted measurement of uptake and subsequent metabolism of the organic molecule. In the final duplicate incubations, both SO₄²⁻ and sulfur amino acid were labeled with 35S of the same specific radioactivity (on a sulfur basis). Analysis of these plants permitted assessment of their concentrations of S-containing compounds since all sulfur sources available to the plants were of the same specific radioactivity.

Analysis of S-Containing Compounds. Colonies were homogenized in 10% TCA and the extracts were separated into TCA-soluble and -insoluble fractions. Total TCA-insoluble 35S was measured after the pellet was dissolved in Protosol (4). 35S in protein cyst(e)ine and methionine was determined as described previously (8). Analysis of 35S-containing compounds in the TCA-soluble extracts were made essentially as previously described in (6, 8).

The method for analysis of AdoMet² (6) was modified to permit simultaneous assay of S-methylmethionine. Aqueous extract, to which was added carrier and tritiated AdoMet and S-methylmethionine, was electrophoresed at pH 2.9 as described and the single peak containing both compounds was eluted and subjected to the boiling procedure which converts AdoMet to MTA (6). The eluate was taken to dryness and redissolved in 0.2 ml H₂O. MTA contained in the eluate was oxidized by incubation with H₂O₂ (30%, 0.04 ml) at room temperature for 20 min. Oxidation was stopped by the addition of HBr (48%, 0.02 ml). Upon re-electrophoresis at pH 2.9, S-methylmethionine again migrated about 25 cm toward the cathode, while MTA was migrated about 11 cm toward the cathode. Each compound was eluted using the 1H markers as guides and chromatographed in solvent A. The peak corresponding to S-methylmethionine (Rf 0.34) was eluted for determination of 35S/3H. The peak corresponding to MTA, (Rf 0.44) was eluted and chromatographed in solvent C. The peak containing MTA was eluted for determination of 35S/3H.

RESULTS

Concentrations of S-containing compounds were measured in *Lemma* grown in steady states under conditions chosen for their effects on the high affinity SO₄²⁻ uptake system, and were compared to the concentrations present in *Lemma* grown under our standard conditions (Table I). Also shown in Table I is the high affinity SO₄²⁻ uptake of plants grown under the various conditions, expressed as a percentage of that of plants grown under standard conditions. For standard plants ("None" in the table) grown with 20 μM SO₄²⁻ as the sole sulfur source, protein sulfur and inorganic SO₄²⁻ account for most (>90%) of the total tissue sulfur, while the soluble sulfur amino acids make up about 4%. Of the amino acids quantitated here, glutathione is present in greatest amount, while cyst(e)ine, methionine, S-methylmethionine, and AdoMet are present at lower and about equal concentrations. These results are in accord with our earlier studies (2, 6).

Growth in Increased SO₄²⁻, L-Cystine, or L-Methionine. The concentrations of these sulfur sources provided in the present experiment did not lead to marked changes in growth rates (although 2 μM L-methionine consistently decreased the multiplication rate by 10 to 20% [2, 10]). Nor were major changes observed in protein sulfur/frond. Growth in increased SO₄²⁻ raised total tissue inorganic SO₄²⁻, but produced only small, inconsistent rises in soluble cyst(e)ine which, further, were not dose-dependent. Unexpectedly, the concentration of glutathione decreased. Growth in L-cystine did produce rises in soluble cyst(e)ine and glutathione, but little or no change in soluble methionine. Growth in L-methionine led chiefly to increases in soluble methionine and its products, AdoMet and S-methylmethionine. Smaller increases occurred in soluble cyst(e)ine and glutathione. These and other changes are addressed further in "Discussion."

Growth in PAG and L-Lysine plus L-Threonine. Either PAG or the combination of L-lysine plus L-threonine can limit endogenous methionine formation in *Lemma* by decreasing flux through cystathionine γ-synthase. The former compound is an irreversible inactivator of cystathionine γ-synthase (25), whereas L-lysine plus L-threonine indirectly inhibit formation of O-phosphohomoserine, the co-substrate (with cysteine) of cystathionine γ-synthase (2). In the present studies, after suitable adaptation (26), *Lemma* were grown in steady states in sublethal concentrations of these inhibitors. It was hoped that such plants would contain decreased concentrations of soluble methionine and that study of their high affinity SO₄²⁻ uptake would provide further insight into amino acid effectors of this uptake. In plants grown in PAG, a slight decrease in soluble methionine (to 40% of control) occurred, accompanied by only a small decrease in specific high affinity SO₄²⁻ uptake (Table I). In plants grown in L-lysine plus L-threonine, a similar change occurred in soluble methionine. In this situation, the decrease in high affinity uptake was not demonstrated to be specific. In each instance, slight increases may have occurred in soluble cyst(e)ine, but these were so small as not clearly to be outside of experimental error.

Metabolism of L-Cystine and L-Methionine. The amount of radioactivity appearing in sulfur compounds isolated from *Lemma* grown in SO₄²⁻ and either L-[³⁵S]cystine or L-[³⁵S]methionine is shown in Table II. Each amino acid was provided at a 15 nM concentration (i.e. a "tracer" amount) to plants grown in standard medium and at one of the concentrations used for the experiment shown in Table I to plants pregrown in that concentration of the amino acid for at least four doublings. 35S from both 5 nM and 14 μM L-[³⁵S]cystine was readily converted to inorganic SO₄²⁻ by *Lemma*, and radioactivity was present in all fractions examined. In contrast, when L-[³⁵S]methionine was present in the growth medium, no 35S was unequivocally detected in inorganic SO₄²⁻, glutathione, soluble cyst(e)ine, or in protein cyst(e)ine in those plants grown in 4 nM L-[³⁵S]methionine. Of the total 35S taken up by plants grown in 2 μM L-[³⁵S]methionine, 0.2% was detected in protein cyst(e)ine.

Growth in Molybdate. When molybdate is increased from 6 μM in the standard medium to 52 μM, *Lemma* grow in a steady state but display a high frond to colony ratio, typical of plants undergoing SO₄²⁻ limitation (2, 5). High affinity SO₄²⁻ uptake is not significantly up-regulated (4). S-containing compounds were measured in plants grown in medium containing 6 and 52 μM molybdate. It was later found that the Mes buffer used in this medium contained contaminating SO₄²⁻, so that the SO₄²⁻ con-

² Abbreviations: AdoMet, S-adenosylmethionine; S-methylmethionine, S-methylmethionine sulfonium; MTA, S'-methylthioadenosine; PAG, dl-propargylglycine (i.e. dl-2-amino-4-pentyenoic acid).

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Table 1. The Steady State Concentrations of Sulfur-Containing Compounds in Lemma

Concentrations of components in plants grown with no additions to the medium were measured in four samples. Values shown here are the highest and the lowest found for each component. For all growth conditions, concentrations are for individual samples. Analyses of S-methylmethionine and AdoMet were made on a single sample. Values were judged to be significant to two places and have been rounded off accordingly. Mean multiplication rates (5) for the four (i.e., "None") or duplicate (in all other cultures) were: none, 206 (SD ± 10, SE ± 5); 5 mM SO\(^4\)\(^{-2}\), 220 (SD ± 4, SE ± 3); 10 mM SO\(^4\)\(^{-2}\), 207 (SD ± 11, SE ± 8); 7 µM L-cystine, 214 (SD ± 13, SE ± 4); 14 µM L-cystine, 223 (SD ± 4, SE ± 3); 0.7 µM L-methionine, 200 (SD ± 5, SE ± 4); 2 µM L-methionine, 177 (SD ± 5, SE ± 4); 10 nM PAG, 174 (SD ± 1, SE ± 1); 40 nM PAG, 160 (SD ± 1, SE ± 1); and 36 µM L-lysine plus 3 µM L-threonine, 114 (SD ± 33, SE ± 23). The mean wet weight of control Lemma fronds ("None") is 509 pg/ frond (n = 89, SD ± 52, range 334–665). A few determinations were made of wet weights of plants grown under the experimental conditions listed and no striking changes were noted, i.e., all determinations were within the control range. The maximum deviation seemed to occur in the case of growth in 14 µM cystine where wet weight may have decreased 20 to 30% below the mean control value. Note that values for sulfur are given as pg atoms sulfur/frond, a unit the same as pmol/frond for all compounds except cystine and protein. ND, not determined.

<table>
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<th>Component</th>
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<th>10</th>
<th>7</th>
<th>14</th>
<th>0.7</th>
<th>2</th>
<th>10</th>
<th>40</th>
<th>36 plus 3</th>
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<td>830</td>
<td>1300</td>
<td>780</td>
<td>1200</td>
<td>110</td>
<td>150</td>
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<td>220</td>
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<td>7.1</td>
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<td>10.5</td>
<td>10.5</td>
<td>10.5</td>
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<td>2300</td>
<td>2300</td>
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<td>3500</td>
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<td>3500</td>
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</tr>
</tbody>
</table>

% of control\(c\)

\(a\) Homocyst(e)ine contained in this fraction was measured in selected samples. The amounts found are minimum values since correction for losses occurring in steps prior to the final two chromatographic procedures was not made. The amounts found (in pg atoms sulfur/frond) for duplicate samples from plants grown with the indicated additions to the medium were: none, 0.4, 0.7; 0.7 µM L-methionine, 6.9, 6.6.; and 2 µM L-methionine, 22, 5, 13, 9.

<table>
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<th>Protein</th>
<th>2000</th>
<th>2300</th>
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<tr>
<td>Total</td>
<td>3200</td>
<td>3500</td>
<td>3500</td>
<td>3500</td>
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</tr>
</tbody>
</table>

\(b\) S-Methylcysteine, if present would be contained in this fraction. In the selected samples examined, no S-methylcysteine was detected and values in parentheses indicate the maximum amount (in pg atoms sulfur/frond) which could have been present and gone undetected: control (<1); 7 µM L-cystine (<3); 14 µM L-cystine (<10).

\(c\) Analyzed by the modified procedure described in the present paper.

\(d\) Analyzed by the procedure described in (6).

\(e\) Results from (4), and this paper (data not shown). Uptake expressed on a frond-d basis.

\(f\) This decrease judged not to be specific down-regulation since uptake, based on surface area of the fronds or on their ability to take up other organic compounds, was not decreased (4).

\(g\) This decrease judged to be specific down-regulation based on the observation that plants preadapted to growth in 40 nM PAG took up [\(^{14}\)C]PAG as well as did unadapted plants (26).

\(h\) It is not known if this decrease is specific down-regulation as these plants were not tested for uptake of other compounds and their area may be smaller than that of standard plants.
RESPONSES OF S-CONTAINING COMPOUNDS TO SULFUR AVAILABILITY

Table II. Distribution of Radioactivity in S-Containing Compounds after Growth of Lemma in L-[35S]Cystine and L-[35S]Methionine

<table>
<thead>
<tr>
<th>Component</th>
<th>Addition to Growth Medium</th>
<th>% of total tissue [35S]</th>
<th>L-[35S]Cystine</th>
<th>L-[35S]Methionine</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.005 µM</td>
<td>14 µM</td>
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<tr>
<td>Soluble</td>
<td></td>
<td></td>
<td>14.2</td>
<td>31.5</td>
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<tr>
<td>Nonamino acid</td>
<td></td>
<td></td>
<td>5.1</td>
<td>16.9</td>
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<tr>
<td>SO42−</td>
<td></td>
<td></td>
<td>2.4</td>
<td>12.4</td>
</tr>
<tr>
<td>Amino acid</td>
<td></td>
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<tr>
<td>Acidic oxidation</td>
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<td>ND</td>
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<tr>
<td>products</td>
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<td>Glutathione</td>
<td>ND</td>
</tr>
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<td>ND</td>
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</table>

centration was approximately 40, not 20 µM. The values found for duplicate cultures of these plants (6 and 52 µM molybdate, respectively) were: multiplication rate 200, 229, and 167, 175; frond to colony ratio 3.9, 3.7, and 7.1, 7.1; and, on a frond basis as a % of the mean value for plants grown in 6 µM molybdate: high affinity SO42− uptake 86, 114 and 50, 60; inorganic SO42− 99, 101 and 44, 46; glutathione 92, 107 and 109, 119; soluble cyst(e)ine 84, 114 and 78, 92; soluble methionine 96, 104 and 87, 102; protein 85, 115 and 75, 86; total 91, 110 and 66, 76.

DISCUSSION

Effects of SO42− Uptake. In previous studies with cultured tobacco cells, the sulfur amino acids were implicated in regulation of SO42− uptake (16). Further studies subsequently indicated that SO42−, rather than the sulfur amino acids, was the effector and that cysteine affected uptake by virtue of its conversion to SO42− (22, 23). In Lemma minor, 12-h exposure to 5 mm cysteine decreased SO42− uptake by about 70% and increased soluble tissue cyst(e)ine 10 times (1), but the SO42− pool size was not measured. As pointed out by Smith (23), unequivocal conclusions as to the identity of effectors of regulation of SO42− uptake based on observations of total tissue content of S-containing compounds are difficult to make in the absence of knowledge of the extent of compartmentation of the critical metabolites. Studies of kinetics of metabolite labeling have shown that soluble cyst(e)ine exists in two compartments in Lemma, and that cystathionine, homocyst(e)ine and methionine are much less compartmented than is cyst(e)ine (18). A pulse-chase experiment indicated that tissue SO42− in Lemma is composed of rapidly and slowly turning over components (4). It was concluded from compartmental analysis of Lemma (grown in a considerably higher SO42− concentration than used in the above mentioned studies) that the major portion of SO42− was localized in the vacuoles (24). Further experiments which correlate compartmental distribution of the S-containing components with regulatory effects will be necessary to conclusively establish the identity of the effectors in question.

Bearing these limitations in mind, the results of measurements of total tissue content of components presented here are, however, consistent with the possibility that in Lemma high affinity SO42− uptake is regulated, directly or indirectly, by the internal pools of both cyst(e)ine and SO42−. Plants grown in 14 µM L-cystine, where the high affinity SO42− uptake system was down-regulated to 17% of control, contained increased soluble cyst(e)ine but no more inorganic SO42− than did standard plants. Plants grown in 10 mM SO42−, where the high affinity SO42− uptake system was down-regulated to 6% of control, contained increased SO42−, but no more soluble cyst(e)ine than did standard plants. The failure of plants grown in 52 µM molybdate and containing lowered total tissue SO42− to up-regulate high affinity SO42− uptake is amenable to several possible explanations, and, in our opinion, pending further investigation this finding does not constitute strong evidence against the suggested regulation by SO42−.

L-Methionine is not an effector of the high affinity system. Plants with greatly increased soluble methionine, brought about by growth in L-methionine, were not specifically down-regulated in their high affinity SO42− uptake. Plants with decreased soluble methionine, brought about by growth in PAG, were not up-regulated in their high affinity uptake.

Adjustment to External SO42−. From measurements of the tissue SO42− concentrations and rates of uptake mediated by the high affinity and linear, nonsaturating SO42− uptake systems (4; present paper), it is possible to describe the adjustments made by Lemma in response to growth in concentrations of external SO42− ranging from 20 µM to 10 mM. At 20 µM SO42−, about 99% of the total SO42− uptake is via the high affinity system and the amount taken up provides sufficient sulfur so that neither growth rate nor protein synthesis is limited. As the external SO42− concentration is increased, the high affinity system is down-regulated but the linear, nonsaturating system is not. At 10 mM SO42− uptake by the nonsaturating system accounts for about 95% of the total uptake, and this total is about twice that of standard plants. The excess accumulates as SO42−, and there is essentially no change in the amount of reduced sulfur. Such a constancy in sulfate reduction has been noted before (6) and may be the result of close regulation of ATP sulfurylase, the enzyme which catalyzes the first step in SO42− reduction (19).

L-Cystine Metabolism. In addition to its major function in higher plants as precursor of methionine, glutathione and protein cysteine (9), L-cyst(e)ine can also be degraded to sulfide, SO42−, and other compounds (15, 21–23). In cultured tobacco cells, the first step in such degradation is due to an inducible cysteine desulhydrase (or, perhaps predominantly, a cystine lyase [13]). The present studies show that Lemma plants also metabolize L-cystine to SO42− (Table II). Since this metabolism occurs not only in plants adapted to growth in L-cysteine but also in standard plants given a trace amount of L-[35S]cysteine, the reactions may well be constitutive in Lemma. A minimal value for the rate of cyst(e)ine desulfihydration in plants grown in standard medium was estimated as follows. Of the ^3S taken up as L-[35S]cysteine, 2.4% ends as tissue inorganic SO42− (Table II). Of the ^3S taken up as SO42−, 30% ends as tissue ^3S2O4− ("None" in Table I). Therefore, approximately 2.4 + 0.3 = 8% of cysteine sulfur is converted to SO42−. Cyst(e)ine desulfihydration might well be higher if account could be taken of sulfide formed from cyst(e)ine, but reconverted to organic sulfur without passage through SO42−.

As L-cystine is increased in the growth medium, it becomes the dominant source of sulfur. At 7 µM L-cystine, about 1900 pg atoms sulfur/frond (i.e. two-thirds of the total sulfur in the plants) is provided by L-cystine. Down-regulation of the high affinity
SO\textsubscript{4}\textsuperscript{2-} uptake system occurs under these conditions. The net results are slight increases in soluble cyst(e)ine and glutathione, a decrease in tissue inorganic SO\textsubscript{4}\textsuperscript{2-}, and only a marginal change in total tissue sulfur. At 14 \mu M L-cystine, about 3100 pg atoms sulfur/frond (i.e. more than 80% of the total sulfur of the plant) is provided by L-cystine. In spite of further down-regulation of high affinity SO\textsubscript{4}\textsuperscript{2-} uptake, total tissue sulfur now rises above normal. The increment between plants grown in 7 and 14 \mu M cystine is comprised of relatively small rises in soluble cyst(e)ine, glutathione, and neutral oxidation products, but a larger increment in inorganic SO\textsubscript{4}\textsuperscript{2-}. By calculations analogous to those above, it was estimated that the conversion of L-cystine sulfur to SO\textsubscript{4}\textsuperscript{2-} is 15 and 23% at 7 and 14 \mu M L-cystine. Organification of SO\textsubscript{4}\textsuperscript{2-} sulfur continues but very likely at rates below that occurring in control plants. For example, control plants organify about 2500 pmol/frond of SO\textsubscript{4}\textsuperscript{2-} sulfur, whereas plants grown in 14 \mu M L-cystine organify only about 360 pmol/frond, consistent with the reported down-regulations of ATP sulfurylase (19), and APS sulfotransferase (1) by cysteine.

L-Methionine Metabolism. The present tracer studies show that in control plants no more than 0.1% of the L-methionine sulfur taken up is converted to the sulfur of cyst(e)ine, glutathione, or SO\textsubscript{4}\textsuperscript{2-} (Table II). Nonetheless a rapid rate of such conversion, induced by growth in the presence of 2 \mu M L-methionine, sufficient to raise the tissue soluble methionine concentration some 300-fold. Under the latter conditions, no more than 0.4% of \textsuperscript{35}S taken up as L-[\textsuperscript{35}S]methionine ends in cyst(e)ine and its products. This is less than 1% of the amount needed in order that methionine serve as a sole sulfur source for Lemna. These experiments directly demonstrate that methionine is not rapidly metabolized in the direction of homocyst(e)ine to cyst(e)ine in vivo in Lemna, and are in agreement with our conclusion based on inhibition (2) and enzyme (7) studies that in many plant tissues such transsulfuration is not likely to be a quantitatively important metabolic pathway (9). This conclusion may not apply to all plant tissues. Known exceptions occur in two legumes. In Astragalus, some \gamma-cleavage of cystathionine has been noted (12); and, in cultured soybean cotyledons, L-methionine can serve as the sole sulfur source (14).

When 0.7 \mu M L-methionine is present in the growth medium, high affinity uptake of SO\textsubscript{4}\textsuperscript{2-} is moderately decreased (in this case nonspecifically [4]) to about 60% of control (Table I and [4]). L-Methionine provides sufficient sulfur so that total tissue sulfur is raised approximately 1100 pg atoms/frond. Within experimental uncertainty, this increment is accounted for by increases in soluble methionine (600 pmol/frond), S-methylmethionine (240 pmol/frond), AdoMet (40 pmol/frond), and acidic oxidation products (130 pg atoms/frond). At 2 \mu M L-methionine, sulfate uptake is not further down-regulated and L-methionine uptake now contributes sufficient sulfur so that total tissue sulfur is raised by about 2600 pg atoms/frond. Again the increment is approximately accounted for by rises in soluble methionine (2350 pmol/frond), S-methylmethionine (380 pmol/frond), AdoMet (80 pmol/frond), and acidic oxidation products (280 pg atoms/frond).

Changes in soluble methionine concentration correlate with reciprocal changes in the specific activity of cystathionine \gamma-synthase. In 36 \mu M \textsuperscript{L}-l-lysine plus 3 \mu M \textsuperscript{L}-threonine, soluble methionine falls to about half its control concentration (Table I), and cystathionine \gamma-synthase rises to 180% of control (26). In 0.7 \mu M \textsuperscript{L}-methionine, soluble methionine is increased some 80-fold (Table I), and cystathionine \gamma-synthase decreases to 15% of control (27). Further increases in soluble methionine (Table I) were not accompanied by further down-regulation of cystathionine \gamma-synthase (27). When measured, changes in AdoMet and S-methylmethionine qualitatively reflected changes in soluble methionine. Thus, the present results do not distinguish whether the effect for regulation of cystathionine \gamma-synthase is methionine itself, or one of its metabolites.

The decreases in cystathionine \gamma-synthase in plants grown in increased methionine may well account for the observed rises in its substrate, soluble cyst(e)ine and (secondarily) glutathione (Table I). Direct conversion of methionine to cyst(e)ine is ruled out by isotopic experiments (Table II). Curiously, the accumulation of soluble cyst(e)ine under these circumstances does not produce specific down-regulation of high affinity SO\textsubscript{4}\textsuperscript{2-} uptake comparable to that resulting from a similar accumulation of soluble cyst(e)ine produced by l-cysteine administration. Further work will be needed to explain this surprising result.

LITERATURE CITED

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RESPONSES OF S-CONTAINING COMPOUNDS TO SULFUR AVAILABILITY


