Planta 149, 355–360 (1980)

Planta

Analysis of the Regulation of Adenosine 5′-Phosphosulfate Sulfotransferase Activity in *Lemna minor* L. Using ^{15}N-Density Labeling*

Christoph von Arb and Christian Brunold
Pflanzenphysiologisches Institut der Universität, Altenbergrain 21, CH-3013 Bern, Switzerland

Abstract. The role of de novo synthesis in the regulation of adenosine 5′-phosphosulfate sulfotransferase activity by H_2S in *Lemna minor* L. was investigate using density labeling with ^{15}N applied as $^{15}NO_3^-$ in the culture medium. While adenosine 5′-phosphosulfate sulfotransferase activity was rapidly reduced by H_2S and rapidly recovered upon removal of H_2S, O-acetyl-L-serine sulfhydrylase (EC 4.2.99.8) did not show changes in extractable activity in response to H_2S and could therefore be used as an internal marker enzyme for density labeling. The incorporation of ^{15}N into adenosine 5′-phosphosulfate sulfotransferase was strongly reduced upon transfer of plants into a H_2S-containing atmosphere. Half-maximal labeling was reached only after 70–80 h compared to 40–50 h in the control. After removal of H_2S, adenosine 5′-phosphosulfate sulfotransferase activity increased to the initial level within 20 h, and the enzyme reached half-maximal labeling after only 15 h. The time course of the density increase of O-acetyl-L-serine sulfhydrylase was not affected very significantly by H_2S. These results provide evidence that de novo synthesis of enzyme protein is involved in the regulation of adenosine 5′-phosphosulfate sulfotransferase activity by H_2S.

Key words: Adenosine 5′-phosphosulfate sulfotransferase – *Lemna* – sulfate assimilation.

Introduction

One pathway of assimilatory sulfate reduction in algae and higher plants starts with adenosine 5′-phosphosulfate (APS), which is formed from SO_4^{2-} and ATP, catalyzed by ATP-sulfurylase (EC 2.7.7.4) (Schiff and Hodson 1973; Schmidt et al. 1974; Schmidt 1976). The sulfonyl group of APS is transferred via APSSTase to a carrier (Car-SH) to form Car-S-SO_3^- which can be reduced to Car-S-SH. The thiol group can then be incorporated into O-acetyl-L-serine (OAS) catalyzed by O-acetyl-L-serine sulfhydrylase (OASSase) (EC 4.2.99.8) to form cysteine.

Little has been published concerning the regulation of these enzymes in algae and higher plants. ATP-sulfurylase activity of cultured tobacco cells was repressed when sulfate, L-cysteine, or L-methionine were present in the growth medium. Derepression took place in the case of sulfur starvation or when cells were cultivated on djenkolate or reduced glutathione as a sole sulfur source (Reuveny and Filner 1977). Brunold and Schmidt (1976) examined the influence of H_2S on adenosine 5′-phosphosulfate sulfotransferase (APSSTase) activity in *Lemna minor*. They found a decrease in extractable enzyme activity at H_2S concentrations which did not have toxic effects on the organisms. After removal of H_2S from the air, APSSTase activity increased, reaching the initial level within about 20 h. Similar observations have been made in bean seedlings. Wyss and Brunold (1979) found that H_2S inhibited the increase of APSSTase activity in bean seedlings during chloroplast development. In green primary leaves, addition of 1 mM cysteine to the growth medium or of H_2S to the air caused a decrease in extractable APSSTase activity. However, there was no effect of H_2S on the extractable OASSase activity in these two plants.

These findings raise the question whether the control of APSSTase activity involves the de novo synthesis of this enzyme or is due to a post-translational modulation of its activity.

Lemna minor is an organism well suited for studying this question because it can be grown aseptically, having, under constant conditions, a constant doub-

* This is no. 9 in the series "Regulation of Sulfate Assimilation in Plants"

Abbreviations: APS = adenosine 5′-phosphosulfate; APSSTase = adenosine 5′-phosphosulfate sulfotransferase; BSA = Bovine serum albumine; DTE = dithioerythritol; OAS = O-acetyl-L-serine; OASSase = O-acetyl-L-serine sulfhydrylase; POPOP = 1,4-bis-(5-phenyl-2-oxazolyl)-benzene; PPO = 2,5-diphenyloxazole

0032-0935/80/0149/0355/$01.20

ling time which can be easily determined by counting the frond number of the culture, and because compounds can be fed directly to the green cells of the plants during the water or the gas phase (Brunold and Erismann 1974).

Material and Methods

Lemna minor L., strain number 6580 of Landolt's collection of Lemnacae (Landolt 1957), was cultivated aseptically in a device described by Erismann and Brunold (1973) on E-NO_3 medium (Erismann and Finger 1968), as previously described (Brunold and Schmidt 1976). For density labeling, $Ca(^{14}NO_3)_2$ was replaced by $Ca(^{15}NO_3)_2$ (99.5 atom% ^{15}N).

Permeation tubes (Metronics, Palo Alto, Cal., USA) served as H_2S-sources. H_2S concentrations were calculated from the loss of weight of the permeation tubes and the flow rate of the air. Direct measurement of H_2S with a Jonoflux (Hartmann and Braun, Frankfurt, West Germany) gave identical results.

The doubling time of *Lemna minor* was estimated by counting the fronds on two different occasions in a separate culture vessel. The doubling time, t_d (h), was calculated according to:

$$t_d = \frac{\ln 2 \cdot t}{\ln a_x - \ln a_0}$$

$t(h) = t_x - t_0$
a_x = number of fronds at time t_x
a_0 = number of fronds at time t_0

Preparation of the Extract. 1 g of plants was homogenized in a chilled glass homogenizer with 8 ml 0.1 M tris-HCl buffer (pH 8.0) containing 0.1 M KCl, 0.02 M MgCl, and 0.01 M DTE. The homogenate was filtered through Miracloth and centrifuged at 32,000 *g* for 10 min at 4° C. Part of the supernatant was used for the determination of APSSTase, OASSase, and protein. To the rest, solid $(NH_4)_2SO_4$ was added to 70% saturation. The precipitated protein was sedimented at 10,000 *g* for 10 min at 4° C and resuspended in 5 ml $(NH_4)_2SO_4$ solution (70% saturation) containing 0.05 M tris-HCl (pH 9.0) and 0.01 M DTE. The protein suspension was stored at −20° C. There was no appreciable loss of OASSase activity during either storage or density gradient centrifugation. APSSTase was stable as an $(NH_4)_2SO_4$ precipitate at −20° C, but during density gradient centrifugation there was a loss of 95% of the activity. This loss was comparable to APSSTase extracted from *Lemna minor* cultivated either with air or with 6.4 µl H_2S l^{-1} air.

Density Gradient Centrifugation. The stored protein suspension was centrifuged at 10,000 *g* for 10 min at 4° C and the pellet dissolved in 2 ml 0.05 M tris-HCl (pH 9.0) containing 0.01 M DTE. An aliquot containing 1 mg protein was adjusted to 4.5 ml with 0.05 M tris-HCl buffer (pH 9.0) containing 0.01 M DTE. To this mixture 1.87 g CsCl and 50 µg of catalase were added. The tubes were centrifuged in a vertical rotor (Sorvall TV 865) at 40,000 min^{-1} for 50 h at 3° C. Gradients were fractionated through a 50 µl micropipet (Corning) which had been lowered to the bottom of the tube and which was connected to a peristaltic pump through a silicon tube (1,3 mm internal diameter). Single-drop fractions were collected.

Every tenth fraction was used for measuring the refractive index with an Abbé refractometer (Zeiss) at 20° C. The remaining fractions were used for measuring either APSSTase, OASSase, or catalase activity. The refractive index values were converted to density units using a calibration curve which had been established on a DMA digital densitometer (Anton Paar, Graz, Oesterreich) with CsCl solutions containing tris-HCl, DTE, and protein, as in the density gradients.

Enzyme Assays. APSSTase was measured by the production of sulfite-[^{35}S], assayed as acid-volatile radioactivity from $AP^{35}S$ in the presence of an active thiol (Schiff and Levinthal 1968; Hodson and Schiff 1971). DTE was used as thiol. Routinely, the incubation time was 30 min, for the assay of the gradient fractions it was 60 min. The counting fluid was toluene-Triton X-100 (2:1, v/v) with 4 g l^{-1} PPO and 120 mg l^{-1} POPOP.

OASSase was measured according to Pieniazek et al. 1973, but doubling the buffer concentration and using OAS hydrochloride instead of OAS. Routinely, the incubation time was 10 min and 20 min for the gradient fractions. Catalase was measured according to Aebi (1970) using 3 ml buffer solution per assay. Proteins were determined turbidimetrically (Fankhauser 1978) using BSA as a standard. $AP^{35}S$ was prepared according to Tsang et al. 1976. Catalase from beef liver and O-acetyl-L-serine hydrochloride were obtained from Sigma, Triton X-100 from Fluka, Buchs, Switzerland. ^{35}S-labeled SO_4^{2-} was purchased from the Radiochemical Centre, Amersham, England. $Ca(^{15}NO_3)_2$ (99.5 atom%) was obtained from B.O.C., London, UK.

The distributions of the activities of APSSTase, OASSase, and catalase in the density gradients were calculated using the computer programm BMDP 3R (Dixon 1975). This program determines the parameters a, b, and c of the exponential function

$$y(x) = a e^{-b(x-c)^2}$$

so that the sum

$$S = \sum_{i=1}^{n} (y_i - f(x_i))^2$$

becomes minimal.

x = fraction number;
a = maximal activity;
c = fraction number containing the maximal activity;
b = $\frac{1}{2d^2}$, where d represents the numbers of fractions between the fraction containing the maximal activity and the turning point of the exponential function;
y_i = measured enzyme activity in fraction i;
$f(x_i)$ = Enzyme activity in fraction i calculated with the exponential function.

A theoretical time course of increase in enzyme density was calculated in each density labeling experiment. This calculation is based on the fact that in an exponentially growing culture new enzyme molecules must be synthesized to maintain a constant enzyme level. If it is assumed (i) that the protein precursor pool is immediately saturated with the added ^{15}N after the transition from $^{14}NO_3^-$ to $^{15}NO_3^-$, (ii) that there is no turnover of the ^{14}N-enzymes, and (iii) that there are no significant precursor pools of inactive enzyme, the increase in density, d_t, of the enzymes can be calculated according to:

$$d_t = d_{max}\left(1 - e^{-\frac{\ln 2}{\tau} \cdot t}\right)$$

$000\Delta d_t$ = density increase at time t;
t = time after addition of $^{15}NO_3^-$
Δd_{max} = maximal density increase
τ = doubling time.

Maximal density increase was determined after CsCl density gradient centrifugation of extracts from *Lemna minor* L. cultivated for 9 generations on medium containing $^{14}NO_3^-$ or $^{15}NO_3^-$ (99.5 atom%) as the N source.

Table 1. Densities (ρ) of peak fraction of adenosine 5′-phosphosulfate sulfotransferase (APSSTase) and O-acetyl-L-serine sulfhydrylase (OASSase) after CsCl density gradient centrifugation of extracts from plants cultivated for 9 generations on medium containing $^{14}NO_3^-$ or $^{15}NO_3^-$ as sole N source. Catalase from beef liver was added as a density marker. Mean values of 5 independent experiments ± standard error are presented

	$^{14}NO_3^-$ ρ(g cm^{-3})	$^{15}NO_3^-$ ρ(g cm^{-3})	increase in $^{15}NO_3^-$ ρ(g cm^{-3})
APSSTase	1.302 ± 0.0012	1.314 ± 0.0015	0.012 (0.9%)
OASSase	1.289 ± 0.0011	1.301 ± 0.0013	0.012 (0.9%)
Catalase	1.308 ± 0.0010	1.307 ± 0.0014	−0.001 (0.08%)

Results

The densities of APSSTase and OASSase extracted from *Lemna minor* and cultivated on $^{14}NO_3^-$ or $^{15}NO_3^-$ for 9 generations are presented in Table 1. The difference in density between the unlabeled (^{14}N-) and the ^{15}N-labeled enzyme was about 12 mg cm^{-3}. Thus, there is a 0.9% increase for APSSTase under conditions which allow maximal labeling of these enzymes.

It is evident from Table 1 that catalase is an ideal marker enzyme since its density is very close to the density of both APSSTase and OASSase. This is also illustrated in Fig. 1 which shows density changes of APSSTase and OASSase extracted at different times after replacement of $^{14}NO_3^-$ by $^{15}NO_3^-$ in the culture medium. In Figure 1, the APSSTase peak moves from the right side of the catalase peak to the left side and the OASSase peak approaches the marker peak. Figure 1 also shows that compared to catalase there is an initial band-broadening for APSSTase and OASSase during the labeling with ^{15}N which disappears at later stages. However, for both enzymes the changes of width at half-peak height were too small to be used in the further analysis.

Using catalase in each gradient as a reference for normalization, the density increases of APSSTase and OASSase were compared to the theoretical density increase calculated on the basis of a doubling time of 36 h and a maximal density increase of 12 mg cm^{-3} (Table 1, Fig. 2). The observed time courses show an initial lag during which an increase of only about 1 mg cm^{-3} was observed in both enzymes. During the ensuing 7 h there was an increase of 4.0 and 2.2 mg cm^{-3} for APSSTase and OASSase, respectively. These values by far exceed the value of 1.5 mg cm^{-3} expected from the theoretical curve. Half-maximal labeling of both enzymes was obtained after 40 to 50 h.

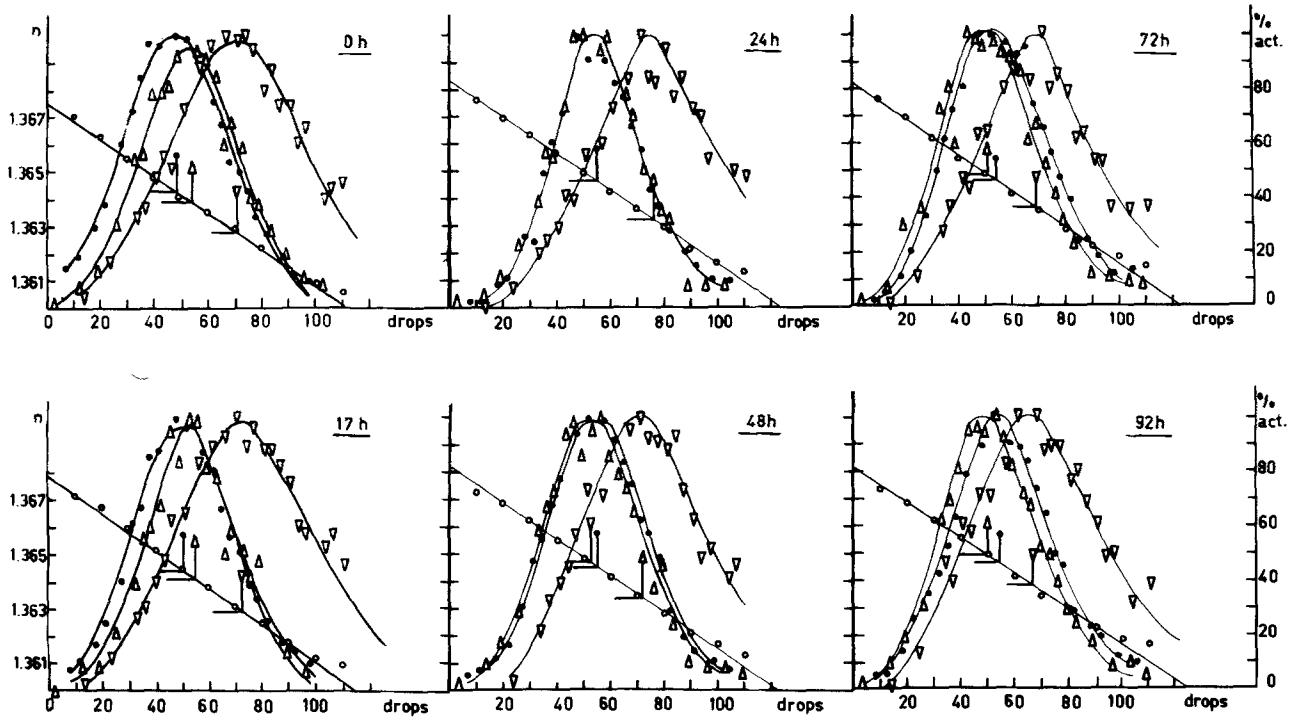

Fig. 1. Distribution of the activity of adenosine 5′-phosphosulfate sulfotransferase (APSSTase, –△–△–, and O-acetyl-L-serine sulfhydrylase (OASSase, –▽–▽– at various times after transfer from $^{14}NO_3^-$ to $^{15}NO_3^-$. Catalase (–●–●–) served as a density marker; refractive index n (–○–○–)

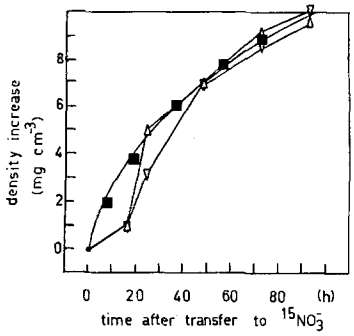

Fig. 2. Time courses of density shifts shown in Fig. 1. (APSSTase, –△–△–; OASSase, –▽–▽–). The time course of the theoretical density increase calculated on the basis of a doubling time of 36 h and a maximal density increase of 12 mg cm^{-3} is included for comparison (–■–■–). The extractable activity of both enzymes remained constant (APSSTase: 250 to 350 nmol mg^{-1} protein h^{-1}; OASSase: 150 to 250 nmol mg^{-1} protein min^{-1})

When *Lemna minor* was exposed to 6.4 µl H_2S l^{-1}, the extractable APSSTase activity decreased rapidly while the OASSase activity remained constant (Fig. 3A). After 24 h the APSSTase activity was about 5% of its initial value and finally a low constant level of activity was reached. At the beginning of the H_2S-treatment, $^{14}NO_3^-$ was replaced by $^{15}NO_3^-$ in the culture medium, thus, the increase of density of APSSTase and OASSase could be determined. For APSSTase only a small density shift was observed during the first 50 h (about 15% of the maximal value). Afterwards, a more rapid increase in density occurred. The time course of OASSase density, however, showed only a slight inhibitory effect of H_2S; half-maximal density increase was reached after about 55 h. The theoretical curve of the density increase illustrated in Fig. 3B was calculated on the basis of a doubling time of 45 h, which is somewhat longer than that in Fig. 2 due to growth inhibition by H_2S.

When *Lemna minor* was permanently cultured with 6.4 µl H_2S l^{-1}, the activity of both enzymes remained constant at about 10 nmol mg^{-1} protein h^{-1} (APSSTase) and about 200 nmol mg^{-1} protein

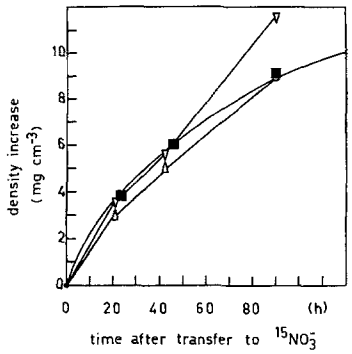

Fig. 4. Time course of the density shift of adenosine 5′-phosphosulfate sulfotransferase (APSSTase, –△–△–) and O-acetyl-L-serine sulfhydrylase (OASSase, –▽–▽–) in plants grown in the presence of 6.4 µl H_2S l^{-1} air after transfer to $^{15}NO_3^-$. Prior to transfer the plants had been grown in an atmosphere containing 6.4 µl H_2S l^{-1} air on $^{14}NO_3^-$ medium for 9 generations. The extractable activity of both enzymes remained constant (APSSTase: 10 to 20 nmol mg^{-1} protein h^{-1}; OASSase 150 to 250 nmol mg^{-1} protein min^{-1}). The time course of the theoretical density increase calculated on the basis of a doubling time of 45 h and a maximal density increase of 12 mg cm^{-3} is included for comparison (–■–■–)

min^{-1} (OASSase). The doubling time was 45 h. Under these conditions, the time course of density changes follow the theoretical curve between 22 and 44 h after transfer to $^{15}NO_3^-$ (Fig. 4). A half-maximal density shift was reached after about 50 h, which is comparable to the control (Fig. 2).

After omission of H_2S, the extractable APSSTase activity increased to its original level of about 250 nmol mg^{-1} protein h^{-1} within 20 h and subsequently remained constant (Fig. 5A). During the same period there was no change in extractable OASSase activity. With the omission of H_2S, $^{14}NO_3^-$ had been replaced by $^{15}NO_3^-$. The resulting density shift of APSSTase was markedly faster than that of OASSase, reaching a half-maximal value after 15 h as compared to 40 h for OASSase. The doubling time for the calculation of the theoretical time course of density increase was 38 h (Fig. 5B).

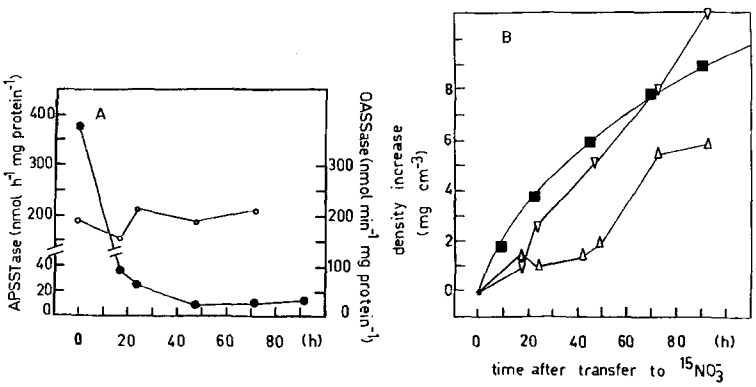

Fig. 3A and B. Time course of adenosine 5′-phosphosulfate sulfotransferase (APSSTase) and O-acetyl-L-serine sulfhydrylase (OASSase) activity and density increase after transfer to 6.4 µl H_2S l^{-1} air and to $^{15}NO_3^-$ instead of $^{14}NO_3^-$. **A** activity of APSSTase (–●–●–) and OASSase (–○–○–). **B** density increase of APSSTase (–△–△–) and OASSase (–▽–▽–). The time course of the theoretical density increase calculated on the basis of a doubling time of 45 h and maximal density increase of 12 mg cm^{-3} is included for comparison (–■–■–)

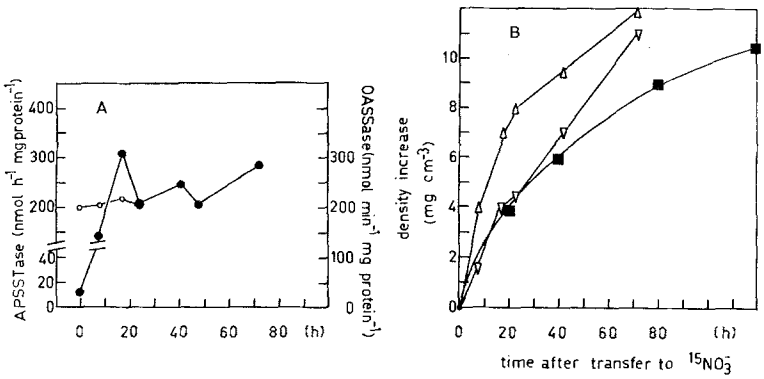

Fig. 5A and B. Time course of adenosine 5′-phosphosulfate sulfotransferase (APSSTase) and O-acetyl-L-serine sulfhydrylase (OASSase) activity and density increase after removal of H_2S and transfer to $^{15}NO_3^-$ instead of $^{14}NO_3^-$.
A activity of APSSTase (–●–●–) and OASSase (–○–○–).
B density increase of APSSTase (–△–△–) and OASSase (–▽–▽–).
The time course of the theoretical density increase calculated on the basis of a doubling time of 38 h and a maximal density increase of 12 mg cm^{-3} is included for comparison (–■–■–)

Discussion

The technique of density labeling has been applied to the investigation of the mechanism involved in the increase of an enzyme activity in response to a stimulus such as a hormone (Filner and Varner 1967), a substrate (Zielke and Filner 1971), or light (Schopfer and Hock 1971). The interpretation of density labeling experiments has been discussed by Acton et al. (1974) Lamb and Rubery (1976) and by Johnson (1977).

In the present paper, the density labeling technique was used to study the mechanism involved in the regulation of APSSTase by H_2S in *Lemna minor*. OASSase was chosen as an internal marker for mainly two reasons: (i) the enzyme has a similar time course of increase in density to that of APSSTase when *Lemna minor* is cultivated in air; (ii) though it is an enzyme of assimilatory sulfate reduction (Hodson and Schiff 1971; Schmidt 1979), its extractable activity is not affected by H_2S, a possible end product of this pathway (Wilson et al. 1978).

The increase in density of about 12 mg cm^{-3}, which was determined for APSSTase and OASSase after extensive incorporation of ^{15}N, is comparable to the values found for nitrate reductase from tobacco cells (Zielke and Filner 1971) and to the value of 13 mg cm^{-3} given by Johnson (1977) as a maximal increase which can be introduced into a protein of typical amino acid composition.

The lag in the increase in density of APSSTase and OASSase after the replacement of $^{14}NO_3^-$ by $^{15}NO_3^-$ (Fig. 2) indicates that the amino acids in the protein precursor pool are equilibrated with ^{15}N only after some time (Zielke and Filner 1971). Furthermore, labeling of active APSSTase molecules could be delayed due to the presence of an inactive precursor of APSSTase (Lamb and Rubery 1976). In the subsequent phase of rapid labeling at constant enzyme activity, the observed density increase of both APSSTase and OASSase appreciably exceeded the calculated value, indicating that one of the assumptions made in calculating the theoretical time course of density increase, the lack of turnover, was incorrect. This means that both enzymes are continuously inactivated and/or degraded even under optimal growth conditions. Evidence for the occurrence of these processes is also provided by the density increase of OASSase which exceeded the theoretical value after prolonged labeling periods (Fig. 3, 4 and 5). Degradation and/or inactivation (half-life approx. 5 h) was furthermore demonstrated during the period of rapidly decreasing extractable APSSTase activity caused by H_2S. Without this process, and even with a complete inhibition of APSSTase synthesis, the specific activity of the enzyme would only decrease by dilution through growth (half-life 45 h). It is not clear from the present results whether the rate of enzyme disappearance is influenced by H_2S. It is clear, however, from the time course of the density shift in Fig. 3B, that the rate of APSSTase synthesis is greatly reduced in the presence of H_2S. One has to bear in mind that an increase in enzyme inactivation by H_2S on the background of an unchanged synthesis would lead to an enhancement of ^{15}N labeling of the enzyme rather than a delay. The time course of OASSase labeling in the presence of H_2S is rather close to that in air (Fig. 2), indicating that protein precursor pools are not greatly altered by H_2S. The same conclusions can be drawn from experiments done in the steady-state established in the presence of H_2S, in which APSSTase activity was only 5% of the control, while OASSase activity and rate of density labeling were essentially unchanged. If the rates of APSSTase synthesis, degradation, and/or inactivation were the same as in air, the turnover rate of APSSTase would be increased about twenty fold. This would result in faster density labeling of APSSTase as compared to OASSase. It is clear, however, from Fig. 4 that the time courses of density labeling of both enzymes are comparable, at least up to 44 h, indicating again that the rate of APSSTase syn-

thesis in the presence of H_2S is lower than in air. The very rapid density labeling of APSSTase, exceeding the theoretical curve which was observed after removal of H_2S, indicates that the increase in extractable enzyme activity is not due to the activation of previously inactivated APSSTase molecules, but rather to a very pronounced increase in the rate of APSSTase synthesis. The rate of OASSase labeling during the first 8 h after removal of H_2S was about 60% less than of APSSTase, but it was still faster than in steady-state cultures in air. This effect may be partly due to the fact that after removal of H_2S the doubling time decreased from 45 to 38 h.

Taken together the presented results lead to the conclusion that H_2S regulates the rate of synthesis of APSSTase. It is not clear from our experiments, however, whether this regulation is at transcription or at postranscriptional steps.

We thank Dr. P. Schopfer, Freiburg i.B., West Germany, for his help in amending this paper and Dr. P. Filner, East Lansing, Michigan, USA, for useful comments. Dr. M. Cunningham checked the English of the manuscript. This work was supported by grant 3.610-0.75 and 3.371-0.78 from the Swiss National Science Foundation.

References

Acton, G.J., Drumm, H., Mohr, H. (1974) Control of synthesis de novo of ascorbate oxidase in the mustard seedling (*Sinapis alba* L.) by Phytochrome. Planta **121**, 39–50

Aebi, H. (1970) Katalase. In: Methoden der enzymatischen Analyse, vol. 1, pp. 636–640, Bergmayer, H.-U., ed. Chemie, Weinheim

Brunold, Chr., Erismann, K.H. (1974) H_2S als Schwefelquelle bei *Lemna minor* L.: Einfluß auf das Wachstum, den Schwefelgehalt und die Sulfataufnahme. Experientia **30**, 465–467

Brunold, Chr., Schmidt, A. (1976) Regulation of adenosine 5′-phosphosulfate sulfotransferase activity by H_2S in *Lemna minor* L. Planta **133**, 85–88

Brunold, Chr., Schiff, J.A. (1976) Studies of sulfate utilization by algae. 15. Enzymes of assimilatory sulfate reduction in *Euglena* and their cellular localization. Plant Physiol. **57**, 430–436

Dixon, W.J. (1975) BMDP Biomedical Computer Programs; University of California Press, Berkeley Los Angeles London

Erismann, H.H., Finger, A. (1968) Lemnaceen in kontinuierlicher Kultur. Ber. Schweiz. Bot. Ges. **78**, 5–15

Erismann, H.H., Brunold, Chr. (1973) Die Verwendung einer neuen Lemna-Kulturanlage in Wachstums- und Stoffwechseluntersuchungen mit gelösten und gasförmigen Schwefelverbindungen. Ber. Schweiz. Bot. Ges. **83**, 213–222

Fankhauser, H. (1978) Untersuchungen zur Lokalisierung der assimilatorischen Sulfatreduktion bei Spinat (*Spinacia oleracea* L.). Dissertation, Berne, Switzerland

Fankhauser, H., Brunold, Chr. (1979) Localization of O-acetyl-L-serine sulfhydrylase in *Spinacia oleracea* L. Plant Sci. Lett. **14**, 185–192

Filner, P., Varner, J.E. (1967) A test for de novo synthesis of enzymes: density labelling with $H_2{}^{18}O$ of barley α-amylase induced by gibberellic acid. Proc. Natl. Acad. Sci. USA **58**, 1520–1526

Hodson, R., Schiff, J.A. (1971) Studies of sulfate utilization by algae. 9. Fractionation of a cell-free system from *Chlorella* into two activities necessary for the reduction of adenosine 3′-phosphate 5′-phosphosulfate to acid-volatile radioactivity. Plant Physiol. **47**, 300–305

Johnson, C.B. (1977) Use of density labelling techniques. In: Regulation of enzyme synthesis and activity in higher plants, pp. 225–243, Smith H., ed. Academic Press, London New York San Francisco

Lamb, C.J., Rubery, P.H. (1976) Interpretation of the rate of density labelling of enzymes with ^2H_2O. Biochim. Biophys. Acta **421**, 308–318

Landolt, E. (1957) Physiologische und ökologische Untersuchungen an Lemnaceen. Ber. Schweiz. Bot. Ges. **67**, 271–410

Pieniazek, N.J., Stephien, P.P., Pazewski, A. (1973) An *Aspergillus nidulans* mutant lacking cystathionine β-synthase: Identity of L-serine sulfhydrolase with cystathionine β-synthase and its distinctness from O-acetyl-L-serine sulfhydrolase. Biochim. Biophys. Acta **297**, 37–47

Reuveny, Z., Filner, P. (1977) Regulation of adenosine triphosphate sulfurylase in cultured tobacco cells. J. Biol. Chem. **252**, 1858–1864

Schiff, J.A., Levinthal, M. (1968) Studies of sulfate utilization by algae. 4. Properties of a cell-free sulfate-reducing system from *Chlorella*. Plant Physiol. **43**, 547–554

Schiff, J.A., Hodson, R.C. (1973) The metabolism of sulfate. Annu. Rev. Plant Physiol. **24**, 381–414

Schmidt, A., Abrams, W.R., Schiff, J.A. (1974) Reduction of adenosine 5′-phosphosulfate to cysteine in extracts from *Chlorella* and mutants blocked for sulfate reduction. Eur. J. Biochem. **47**, 423–434

Schmidt, A. (1976) The adenosine 5′-phosphosulfate sulfotransferase from spinach (*Spinacia oleracea* L.) Planta **130**, 257–263

Schmidt, A. (1979) Photosynthetic assimilation of sulfur compounds. In: Encyclopedia of plant physiology, vol. 6, pp. 481–495, Gibbs, M., Latzko, E., eds. Springer, Berlin Heidelberg New York

Schopfer, P., Hock, B. (1971) Nachweis der Phytochrom-induzierten de novo-Synthese von Phenylalanin Ammoniumlyase (PAL, EC 4.3.1.5) in Keimlingen von *Sinapis alba* L. durch Dichtemarkierung mit Deuterium. Planta **96**, 248–253

Tsang, M.L.-S., Lemieux, J., Schiff, J.A., Bojarski, T.B. (1976) Preparation of adenosine 5′-phosphosulfate (APS) from adenosine 3′-phosphate 5′-phosphosulfate (PAPS) prepared by an improved procedure. Anal. Biochem. **74**, 623–626

Wilson, G.L., Bressan, R.A., Filner, P. (1978) Light-dependent emission of hydrogen sulfide from plants. Plant Physiol. **61**, 184–189

Wyss, H.-R., Brunold, Chr. (1979) Regulation of adenosine 5′-phosphosulfate sulfotransferase activity by H_2S and cysteine in primary leaves of *Phaseolus vulgaris* L. Planta **147**, 37–42

Zielke, H.R., Filner, P. (1971) Synthesis and turnover of nitrate reductase induced by nitrate in cultured tobacco cells. J. Biol. Chem. **246**, 1772–1779

Received 22 January; accepted 4 March 1980