Phytochemistry, Vol. 21, No. 9, pp. 2173–2178, 1982.
Printed in Great Britain.

0031-9422/82/092173-06$03.00/0

HYDROGEN SULFIDE EMISSION BY CUCUMBER LEAVES IN RESPONSE TO SULFATE IN LIGHT AND DARK

JIRO SEKIYA*, AHLERT SCHMIDT†, HEINZ RENNENBERG, LLOYD G. WILSON and PHILIP FILNER‡

MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, U.S.A.

(*Received* 5 *June* 1981)

Key Word Index—*Cucumis sativus*; Cucurbitaceae; cucumber; sulfur compounds; hydrogen sulfide; biosynthesis; photosynthesis; sulfate reduction.

Abstract—Young leaf discs of cucumber (*Cucumis sativus*) emit H_2S at 50–100 pmol/min/cm^2 in response to 25 mM K_2SO_4 and light. The light-dependent H_2S emission was inhibited by cyanazine, atrazine and DCMU, each of which also inhibits CO_2 fixation. However, H_2S emission was markedly more sensitive to DCMU, and exhibited small differences in sensitivity to the triazines, compared to CO_2 fixation. Incorporation of ^{35}S from SO_4^{2-} into organic sulfur (S) compounds such as cysteine, methionine, glutathione and proteins occurred in darkness, but at a rate 40% less than in light. In the light 0.1 mM cyanazine inhibited incorporation of ^{35}S from SO_4^{2-} into organic S compounds also by 40%. This was much less than the inhibition of H_2S emission and CO_2 fixation rates caused by the same treatments. These results indicate that the path of SO_4^{2-} assimilation leading to emitted H_2S is heavily dependent on photosynthetic electron transport but in a manner which differs significantly from the dependence of CO_2 fixation. Furthermore, the path of assimilation of SO_4^{2-}into organic S compounds functions quite well in darkness; i.e. without simultaneous production of reductants by light reactions. Dithioerythritol (10 mM), which does not cause H_2S emission by itself, greatly stimulated H_2S emission in dark or light in response to 25 mM K_2SO_4. However, assimilation of SO_4^{2-} into organic S compounds was inhibited by DTE. These results indicate that DTE provides access to an alternative reducing pathway leading to H_2S which can function in light or darkness. DTE may cause H_2S production from SO_4^{2-} by diverting sulfur from the normal pathway of carrier-bound intermediates, forming a free sulfite pool instead, which could give rise to H_2S by the action of sulfite reductase.

INTRODUCTION

Sulfate (SO_4^{2-}) is the most common oxidized form of sulfur in nature. It meets the sulfur requirements of higher plants, algae and other micro-organisms which are capable of reducing SO_4^{2-} and incorporating it into reduced organic sulfur (S) compounds such as L-cysteine, L-methionine and glutathione. This pathway, known as the assimilatory SO_4^{2-} reduction pathway [1,2] is stimulated by light and is localized in chloroplasts [3–5]. Furthermore, ferredoxin can serve as reductant in the reaction catalysed by thiosulfonate reductase the key reductive step in the assimilatory sulfate pathway which employs carrier-bound sulfur intermediates [1]. Therefore, it is highly probable that the reductants for light-dependent assimilatory SO_4^{2-} reduction in leaves are generated photosynthetically.

When leaves or roots were exposed to relatively high concentrations of SO_4^{2-}, hydrogen sulfide (H_2S) emission from the leaves was observed in response to light at rates comparable to rates of sulfate assimilation into leaf protein [6, 7]. The path of synthesis of the emitted H_2S has not yet been determined. The most likely candidates for the immediate precursor of H_2S formed by light-dependent reduction of sulfate are carrier-bound or free sulfide formed from carrier-bound or free sulfite, respectively. If free sulfide is the natural precursor of L-cysteine in leaves as it appears to be in some micro-organisms, free sulfide, hence H_2S, would be a normal intermediate of sulfate assimilation. However, there is evidence that carrier-bound sulfide is the immediate precursor in green algae and higher plants [1] and H_2S might arise as a result of decomposition of the carrier-sulfide conjugate. Still a third possible origin of H_2S is desulfhydration of L-cysteine, a reaction which Harrington and Smith [8] encountered in cultured plant cells. We have recently shown it to occur in leaves [9]. L-Cysteine is also desulfhydrated during synthesis of β-cyanoalanine from L-cysteine and cyanide, in a reaction catalysed by β-cyanoalanine synthase, an enzyme which has been encountered widely in plants [10].

In this report, we present some findings on the light dependence of H_2S emission in young cucumber leaf discs in response to sulfate, compared to the light

*Present address: Department of Agricultural Chemistry, Yamaguchi University, Yamaguchi 753, Japan.

†Present address: Botanisches Institut der Universität Diaeresis München, Menzinger Strasse 67, D-8000 München, West Germany.

‡To whom correspondence should be addressed.

dependence of synthesis of organic S compounds, and to the light dependence of CO_2 fixation. The results point to the existence of two routes from sulfate to H_2S.

RESULTS

Light-dependency of H_2S *emission in response to* SO_4^{2-}

Young cucumber leaf discs exposed to 25 mM K_2SO_4 emitted H_2S and other sulfur volatiles (Fig. 1) in the same light-dependent manner as leaves on intact plants which had been exposed to sulfate through the roots or detached leaves which had been exposed to sulfate through the cut petiole [11]. The maximal rates of emission of H_2S were 50–100 pmol/min/cm^2.

The air which passed by the discs thereby acquired the odor of H_2S and this was confirmed by GC analysis using an S detector and by the fact that the volatile sulfur could be trapped by a zinc acetate solution, which is characteristic of H_2S. The trapped material formed methylene blue when reacted with dimethyl-*p*-phenylenediamine (DMD), a reaction specific for sulfide [12]. The amount of H_2S trapped by zinc acetate and determined chemically with DMD accounted on average for 88% of the amount of volatile sulfur detected by the GC sulfur detector. When known amounts of H_2S were analysed by both methods, the methylene blue method gave values equal to 95–98% of the sulfur analyser values. Therefore, it became evident that the volatile sulfur emitted in response to SO_4^{2-} and light was almost entirely H_2S.

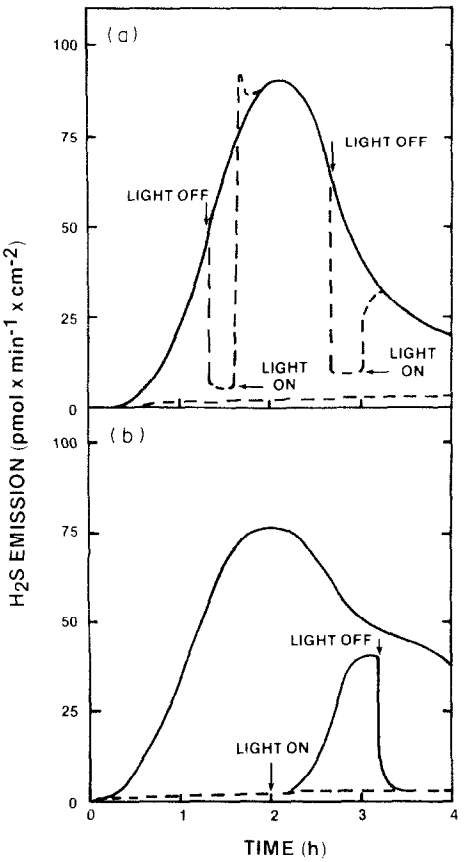

Fig. 1. Light dependence of H_2S emission by discs from young cucumber leaves in response to 25 mM K_2SO_4. Three groups of eight discs (18 cm^2 leaf area) were obtained from a single leaf and each group of discs was floated on 10 ml K_2SO_4 (pH 6) in a 125 ml Erlenmeyer flask coupled to a sulfur analyser through an automatic channel selector. One group of discs was kept in darkness; one group was kept in light (8 mW/cm^2) continuously; the third group was illuminated, but the illumination was interrupted for brief periods of darkness.

Darkness after illumination caused a quick decrease in H_2S emission within 1 min (Fig. 1). Reillumination after a brief period of darkness caused a quick increase in H_2S emission usually with a transient overshoot for *ca* 10 min after which the emission rate returned to the rate which would have been achieved in continuous light. This was rigorously established by monitoring the emission rates from duplicate samples of leaf discs from the same leaf one of which was kept in continuous light and the other placed in darkness for a short interval and observing the superposition of the emission rate curves (Fig. 1). A transient undershoot occurs when the irradiance is reduced to an intermediate level (data not shown). Both overshoots and undershoots also occur when attached or detached cucumber leaves are used. The occurrence of these transient variations in rate in cut discs and the rapidity of their occurrence indicate that they probably do not reflect changes in SO_4^{2-} translocation in the vascular system.

It is noteworthy that the rate of emission of H_2S is greater by discs exposed to SO_4^{2-} for the same times but which had been illuminated then subjected to darkness (Fig. 1). This result indicates that illumination makes possible a change in the leak (perhaps accumulation of a precursor or reductant) which enhances H_2S emission in darkness. Sulfate has to be present for the change to occur since the leaves were illuminated prior to the start of the sulfate treatments.

The effects of inhibitors of photosynthetic electron transport on light-dependent H_2S emission were compared with their effects on CO_2 fixation (Fig. 2). 3(3',4'-Dichlorophenol) - 1,1 - dimethylurea (DCMU), atrazine and cyanazine, which inhibit between photosystem II and plastoquinone, each caused inhibition of light-dependent H_2S emission, but the dose–response curves differed from those for CP_2 fixation in each case. Hydrogen sulfide emission was far more sensitive to DCMU than was CO_2 fixation (Fig. 2a). Only 5 μM DCMU was required for 50% inhibition of H_2S emission, while 45 μM DCMU was required for 50% inhibition of CO_2 fixation.

The dose–response curves with cyanazine as the inhibitor are qualitatively similar for H_2S emission and CO_2 fixation, but H_2S emission was consistently more strongly inhibited, in terms of per cent of total activity which was inhibited (Fig. 2b). However, neither CO_2 fixation nor H_2S emission was totally inhibited even at high concentrations of cyanazine. If one subtracts the components of CO_2 fixation and H_2S emission which were not inhibited by cyanazine, the differences in sensitivity disappear.

In the case of inhibition by atrazine, CO_2 fixation was slightly more sensitive than H_2S emission at low doses, but at higher doses, the two processes had essentially identical sensitivities (Fig. 2c).

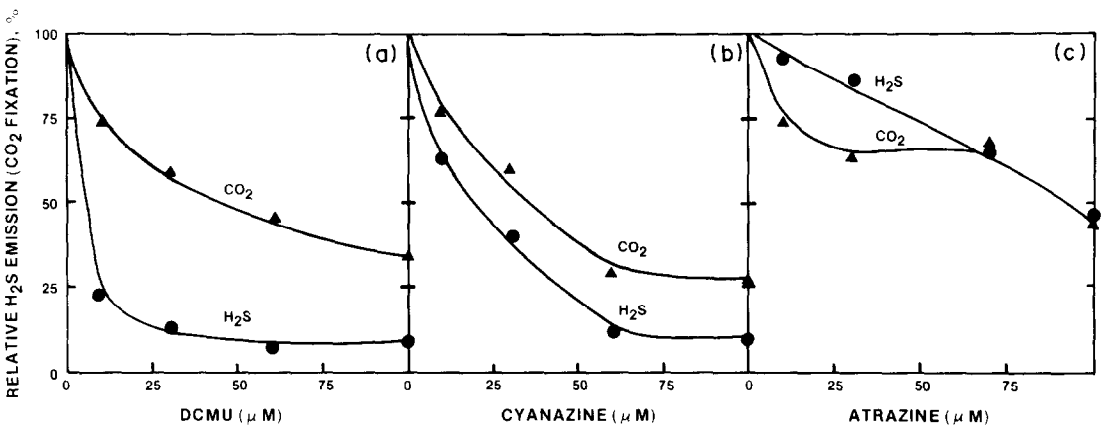

Fig. 2. Effects of inhibitors of photosynthetic electron transport on H_2S emission and CO_2 fixation by discs from young cucumber leaves exposed to 25 mM K_2SO_4. For each inhibitor concentration tested, two groups of eight discs (18 cm^2 of leaf area) from one half of an expanding leaf were placed in a pair of matched leaf chambers and the CO_2 fixation and H_2S emission monitored intermittently for 4 hr under continuous illumination (4 mW/cm^2). One group of leaf discs was floated on 10 ml 25 mM K_2SO_4 containing one of the inhibitor concentrations indicated. The other group of leaf discs was floated on 10 ml 25 mM K_2SO_4 solution without inhibitors. CO_2 fixation or H_2S emission without inhibitor was taken as 100%. Within the 30 experiments performed, 100% CO_2 fixation was in the range 0.7–1 μmol CO_2/hr/cm^2 of leaf, and 100% H_2S emission was in the range 1.2–3.5 nmol H_2S/hr/cm^2 of leaf.

In the light, leaf discs took up *ca.* 8–10 mol of SO_4^{2-} per g fr. wt from the treatment solution during the first 2 hr (Table 1). The rate of SO_4^{2-} uptake was 50% lower in darkness, but cyanazine in the light did not inhibit sulfate uptake (Table 1), so addition of cyanazine is not entirely equivalent to turning off the light. Assimilation of SO_4^{2-} into reduced organic S compounds such as cysteine, methionine, glutathione and proteins was determined by measurement of incorporation of ^{35}S from $^{35}SO_4^{2-}$ into those compounds. In the light, total ^{35}S in reduced organic S compounds was 6–10% of ^{35}S taken up from external 25 mM $^{35}SO_4^{2-}$ (Table 1). The major fates of assimilated ^{35}S were incorporation into glutathione and cystine (4–6% and 1.5–3% respectively of ^{35}S taken up). The rate of incorporation into organic S compounds in the dark, as per cent of ^{35}S taken up, was about half of that in the light (Table 1). In light, cyanazine inhibited by 40% the incorporation of ^{35}S from $^{35}SO_4^{2-}$ into organic S compounds. In all cases, 80–90% of ^{35}S taken up could be recovered from the discs as SO_4^{2-}.

Stimulation by dithioerythritol (DTE) of H_2S emission in response to SO_4^{2-} in light and dark

The enzyme APS sulfotransferase can catalyse the transfer of the sulfo moiety from APS to a suitable carrier thiol such as GSH; or it can catalyse the release of the sulfo moiety as sulfite in the presence of another thiol compound such as DTE in what is probably an abortive sulfo transfer reaction [1]. Therefore DTE might abort the formation of H_2S *in vivo* if the synthesis from sulfate proceeded via a bound sulfur pathway. However, leaves also contain

Table 1. Sulfate uptake and assimilation into cysteine and its metabolites by young cucumber leaf discs

Treatment	Light or dark	SO_4^{2-} uptake (μ mol/g fr. wt)	S assimilated, as % total ^{35}S taken up				
			Cysteine	Methionine	GSH	Protein	Total
Experiment 1							
SO_4^{2-} alone	L	8.39	3.2	0.7	6.2	0.5	10.6
SO_4^{2-} + DTE	L	6.29	2.3	0.4	4.4	0.2	7.3
SO_4^{2-} alone	D	4.93	2.2	0.3	2.4	0.6	5.5
SO_4^{2-} + DTE	D	4.36	1.5	0.7	1.2	0.2	3.5
Experiment 2							
SO_4^{2-} alone	L	8.98	1.5	0.2	4.1	0.1	5.9
SO_4^{2-} + cyanazine	D	9.63	1.4	0.3	2.2	0.1	3.9

Leaf discs prepared from a single young cucumber leaf were floated on 2 ml 25 mM K_2SO_4 containing 10–15 μCi of $Na_2{}^{35}SO_4$ with or without 10 mM DTE or 0.1 mM cyanazine in Petri dishes in the light (8 mW/cm^2) or in the dark for 2 hr. ^{35}S compounds in leaf discs were extracted and fractionated as described in the Experimental, to determine ^{35}S in each fraction and ^{35}S in reduced organic S compounds. SO_4^{2-} uptake was calculated from total ^{35}S taken up and specific radioactivity of the treatment solution.

the enzyme sulfite reductase which catalyses the reduction of sulfite and sulfide so it is conceivable that, even if DTE released sulfite from APS, the final product could still be H_2S. A third possibility is that DTE might be able to serve as reductant for sulfate in the dark. Still, a fourth possibility is that the thiols of DTE might themselves serve as precursors of H_2S. These various possibilities prompted us to see if DTE affected H_2S emission by leaf discs.

DTE stimulated H_2S emission by illuminated leaf discs which were also exposed to SO_4^{2-}, while a negligible amount of H_2S emission was observed in response to 10 mM DTE alone (Table 2 and Fig. 3). The extent of stimulation increased with the concentration of DTE (Table 2). Remarkably, SO_4^{2-} uptake by illuminated leaf discs was inhibited *ca.* 20% by 10 mM DTE (Table 1). Also, the rate of incorporation of ^{35}S from $^{35}SO_4^{2-}$ into organic S compounds in light was inhibited 30% by DTE (Table 1). In this case, *ca.* 85% of ^{35}S from $^{35}SO_4^{2-}$ still remained in the form of SO_4^{2-}.

Although exposure to 25 mM SO_4^{2-} alone did not elicit significant H_2S emission in darkness, leaf discs exposed to 25 mM SO_4^{2-} in the presence of 10 mM DTE in darkness emitted H_2S at a low rate, but still well above the almost negligible rate in darkness without DTE. During the first 10 hr, discs exposed to SO_4^{2-} and DTE emitted in darkness only *ca.* 10% as much H_2S as illuminated discs (Fig. 3). Also, the pattern of H_2S emission in response to SO_4^{2-} and DTE in darkness was different from the pattern in light. The emission rate in darkness increased gradually throughout the first 10 hr, while the emission rate in light reached its maximum after 2 hr and then decreased. In the presence of DTE the rates of SO_4^{2-} uptake and of incorporation of ^{35}S from $^{35}SO_4^{2-}$ into organic S compounds were lower in darkness than in light. Furthermore, compared to darkness alone, DTE in darkness inhibited SO_4^{2-} uptake and incorporation of ^{35}S into organic S compounds. Thus, while DTE stimulated H_2S emission in response to SO_4^{2-} in both light and dark, SO_4^{2-} uptake and incorporation of the sulfur in SO_4^{2-} into organic S compounds were being inhibited.

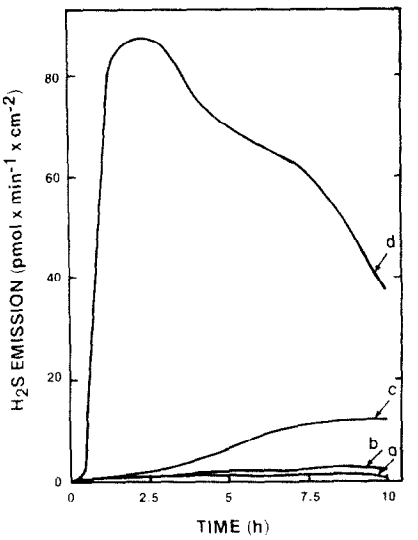

Fig. 3. Effect of DTE on H_2S emission by young cucumber leaf discs in response to 25 mM K_2SO_4. Groups of eight leaf discs prepared as in Fig. 2 were floated on 10 ml 25 mM K_2SO_4 solution with or without 10 mM DTE in 125 ml Erlenmeyer flasks either in light (8 mW/cm^2) or in darkness. The flasks were connected to a sulfur analyser, and the H_2S emission was monitored intermittently by means of an automatic sample selector. (a), 25 mM K_2SO_4, dark; (b), 10 mM DTE, light or dark; (c), 25 mM K_2SO_4 + 10 mM DTE, dark; (d), 25 mM K_2SO_4 + 10 mM DTE, light.

Table 2. Effect of DTE on H_2S emission by illuminated young cucumber leaf discs exposed to 25 mM K_2SO_4 in the light

Treatment	H_2S emitted, (%)
25 mM K_2SO_4	100
+ 1 mM DTE	141
+ 5 mM DTE	192
+ 10 mM DTE	394
10 mM DTE alone	2

Each treatment was tested in a separate experiment. In each experiment, two groups of ten leaf discs were punched from each half of a single young cucumber leaf. The discs were floated on 10 ml 25 mM K_2SO_4 with or without DTE (pH 6) in a pair of matched leaf disc chambers coupled to a sulfur analyser through an automatic channel selector. H_2S emissions by leaf discs with K_2SO_4 (control) and leaf discs with K_2SO_4 and DTE in the light (8 mW/cm^2) were monitored by a sulfur analyser. For each experiment, H_2S emitted for 4 hr is presented as a percentage of emission by the control in that experiment.

DISCUSSION

Leaf discs emit H_2S as well as detached whole leaves or intact plants with injured roots [7] but they have the very great advantage that replicate samples of discs from a single leaf behave virtually identically. Individual leaves vary substantially from plant to plant, even when the plants have been raised identically. The reproducibility of the leaf discs' responses makes it possible for the first time to detect subtle responses of the H_2S emission system of leaves to various treatments.

Photosynthetic electron transport appears to be involved in the light-driven step leading to H_2S emission in response to SO_4^{2-}. This conclusion is based on the fact that H_2S emission in response to SO_4^{2-} and light was inhibited by cyanazine, atrazine and DCMU when these inhibitors of photosynthetic electron transport were inhibiting CO_2 fixation. However, none of the inhibitors had quantitatively the same effect on CO_2 fixation and H_2S emission. Quantitatively identical effects would be expected only if certain criteria were met: (a) photosynthetic electron transport was the sole source of reductant for each pathway; (b) the reductant for both pathways was obtained from the same point in photosynthetic elec-

tron transport after the point of inhibition, e.g. reduced ferredoxin or NADPH from the end of photosystem I; (c) the reductant-limited enzymes of CO_2 fixation and H_2S emission had equal access to the limiting reductant; (d) the reductant-limited enzymes in the respective pathways had the same K_ms for the reductant.

The H_2S emitted in response to SO_4^{2-} is synthesized largely by reduction of SO_4^{2-} to the sulfide level since *ca.* 60% of sulfur in the H_2S emitted is derived from sulfur in the SO_4^{2-} applied [11]. There are at least two possible pathways of reduction of SO_4^{2-} to the sulfide level: (a) reduction of carrier–SO_3^- to carrier–S^- in what is believed to be the normal SO_4^{2-} assimilatory pathway, or (b) reduction of free SO_3^{2-} to free sulfide [1,2]. Reduction of SO_4^{2-} and incorporation of the reduced sulfur into cysteine in leaf tissues or isolated chloroplasts are highly stimulated by illumination [14]. Reduction *in vitro* of carrier–SO_3^- and of free SO_3^{2-} to the sulfide level have been shown to occur in the presence of reduced ferredoxin [4,5,15–19] or in some cases reduced pyridine nucleotide [16,19,20].

If the reduction of SO_4^{2-} which leads to H_2S proceeded via free SO_3^{2-}, and an appreciable pool of free HSO_3^-/SO_3^{2-} developed, injury to the leaf tissue would have been expected because of the toxicity of HSO_3^-/SO_3^{2-}. In another study being reported elsewhere [9], we have observed that leaves are injured by L-cysteine which was desulfhydrated in leaf tissue, and H_2S was emitted as a result. As the desulfhydration proceeded, a pool of HSO_3^-/SO_3^{2-} developed in the tissue. The rate of H_2S emission accompanying desulfhydration of L-cysteine was comparable to the rate of H_2S emission accompanying sulfate reduction. Therefore, the lack of injury associated with sulfate-dependent H_2S emission is evidence against the participation of free SO_3^{2-} in the pathway.

In the dark, while H_2S emission from leaf discs in response to 25 mM SO_4^{2-} was at a negligible level, SO_4^{2-} was nevertheless assimilated into organic S compounds, at a rate equal to about one-quarter of the absolute assimilation rate in the light. This corresponds to about one-half the assimilation rate in the light if correction is made for the 50% lower rate of sulfate uptake in the dark. Cyanazine (0.1 mM) inhibited incorporation of ^{35}S from SO_4^{2-} into organic S compounds by illuminated leaf discs, but the degree of inhibition was less than the degrees of inhibition of H_2S emission and CO_2 fixation. The substantial rates of SO_4^{2-} assimilation in darkness or when photosynthetic electron transport is inhibited could have two different explanations. Firstly, there may be two assimilation pathways, one light-dependent and the other light-independent. The former reduction system, coupled to photosynthetic electron transport, would be responsible for H_2S emission when unusually high levels of SO_4^{2-} are taken up. In the light-independent system, reduction would be driven by an alternative reducing source, such as must occur in roots [21,22] and it would not be responsible for H_2S emission in response to SO_4^{2-} and light.

The second possibility is that there is one reduction system and over-production by it of sulfide (free or bound form) results in H_2S emission. This reduction system would mostly have to utilize a light-dependent reducing source, but it also must be capable of using an alternative reducing source which is available in darkness. With such a system, SO_4^{2-} would be reduced and incorporated into cysteine in the dark, and there would be little excess reduced sulfur to emit as H_2S. Sulfide would be over-produced at high rates only in the light when the supply of sulfate and reductant exceeded the capacity for cysteine synthesis.

Emission of H_2S in response to SO_4^{2-} was greatly stimulated by DTE in light or darkness compared to the emission in response to SO_4^{2-} alone. However, assimilation of SO_4^{2-} into organic S compounds was inhibited by DTE. DTE has been reported to stimulate the formation of acid-labile SO_3^{2-} (probably DTE–SO_3^-) [23,24], the reduction of carrier–SO_3^- [15] and free sulfite formation [25,26]. Thus it is conceivable that DTE might act in leaf tissue by stimulating the formation of either carrier–sulfide, or of free sulfide through free SO_3^{2-}. However, DTE inhibited the incorporation of SO_4^{2-} into organic S compounds, which is inconsistent with stimulation of reduction of carrier–SO_3^-. Therefore, it seems more likely that in the presence of DTE, sulfide and hence H_2S is formed via free SO_3^{2-}, by sulfite reductase, rather than via the carrier-bound sulfur pathway. The reduction of free sulfite to sulfide would have to be capable of being driven by reductant generated in light or dark. This hypothesis is supported by the observation that H_2S emission in response to SO_2 during dark periods occurs at rates which are a higher percentage of the light-dependent H_2S emission rates, than are H_2S emission rates in response to SO_4^{2-} in darkness compared to those in light. The fits with the idea that sulfate is reduced via carrier-bound intermediates, but $SO_2/HSO_3^-/SO_3^{2-}$ is reduced via free SO_3^{2-}; and that DTE acts by diverting SO_3^{2-} from the bound to the free sulfur pathway. If the resultant of reduction, free sulfide, is less suitable as a precursor of cysteine than is carrier-bound sulfide, this would account for the fact that it is released as H_2S rather than incorporated into cysteine.

EXPERIMENTAL

Plant materials. Cucumber plants (*Cucumis sativus* L. cv Chipper) were grown for 30–40 days in a growth chamber [9]. Young cucumber leaves which were the second or third leaves from the top and actively growing were used for the experiments.

Replicate leaf tissue samples. A major difficulty in earlier work on H_2S emission in our laboratory was the large variation in emission rates among supposedly comparable leaves from physically equivalent positions on plants grown side by side from seed [7]. This variation made it virtually impossible to obtain whole leaf replicates which could serve as control leaf and treated leaf, in experiments designed to detect subtle differences in behavior. We have found, however, that replicate samples of leaf discs punched from a single leaf, usually one set of discs from each half-leaf, give virtually identical responses to agents which cause H_2S emission or modulate that emission. Therefore, we now use leaf discs instead of whole leaves. We found in a study of the difference between young and mature leaves, in their resistance to injury by SO_2, that young leaves were much more active emitters of H_2S than were mature leaves [27]. This also holds true for H_2S emission in response to SO_4^{2-} (H. Rennenberg, unpublished observations).

Continuous measurements of volatile sulfur emission. Leaf discs (2.65 cm^2 diameter) were punched from young

leaves with a cork borer and floated on 10 ml of 25 mM K_2SO_4 (pH 6) with or without additions in Petri dishes in matched Plexigas leaf disc chambers. The temp. of the chamber was controlled with water at constant temp. usually circulating at 25 ml/min. The leaf disc chambers were coupled to a sulfur analyser (Monitor Labs model 8450) through an automatic channel selector and volatile sulfur emission was monitored [9]. A phosphor-coated metal halide lamp (400 W) provided illumination (8 mW/cm^2, unless noted). To measure CO_2 concn a CO_2 analyser (Beckman, model 865), which is a non-destructive device, was connected between the automatic channel selector and the sulfur analyser, which is destructive. It should be noted that the flow cell of the CO_2 analyser, which is gold plated and is supposed to be inert, initially absorbed appreciable amounts of H_2S when it was present in the flowing air in the 10000–500000 ppm range of concn. It was therefore necessary to saturate the CO_2 analyser's adsorption capacity before it would be used in-line preceding the sulfur analyser without affecting the measure concn of volatile sulfur. The CO_2 analyser was operated as an absolute concn analyser, with N_2 in the reference cell. The analyser was calibrated with dilutions in N_2 of a 349 ppm primary CO_2 standard (Matheson Co.). The dilutions were made at a constant pressure drop across a proportioner which was achieved by means of a compensatory variable resistance downstream from the proportioner. This made it possible to obtain true additivity of the volume flows measured by the proportioner.

GC analysis. Samples (2 ml) containing volatile sulfur were injected into a gas chromatograph (Varian 3700) equipped with a 6 ft × 1/8 in. Teflon column packed with Chromosil 330 (Supelco) and flame photometric detector for sulfur [27]. The Supelco column temp. was 40°, and the carrier was N_2 at 15 ml/min. Retention times in min for standard components were 0.75 (H_2S), 1.55 (MeSH), 2.45 (SO_2) and 3.0 (MeSMe) under the standard conditions [25].

SO_4^{2-}-uptake and assimilation of SO_4^{2-} into organic S compounds. Young leaf discs (0.6 cm^2/disc) were treated for 2 hr in light or dark with 25 mM K_2SO_4 containing 15 μCi $Na_2{}^{35}SO_4$ (sp. act. *ca.* 400 μCi/mmol) and additions. Leaf discs were washed twice with 100 ml H_2O after the treatment and subjected to extraction and fractionation into EtOH-soluble, TCA-soluble and residual fractions [9]. The ^{35}S in each fraction was determined by liquid scintillation counting. The EtOH-soluble and TCA-soluble fractions were subjected to TLC [9] and ^{35}S in cysteine, methionine and glutathione was determined. SO_4^{2-} uptake was calculated from the ^{35}S in the three extract fractions and the sp. act. of $^{35}SO_4^{2-}$ initially placed in the treatment soln.

Acknowledgements—This work was supported by the U.S. Department of Energy under contract DE-AC02-ERO-1338. H. R. was the recipient of a Deutsche Forschungsgemeinschaft Fellowship. Cyanazine was a gift from Shell Development Co.

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