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Inhibition of Photosystem II Precedes Thylakoid Membrane Lipid Peroxidation in Bisulfite-Treated Leaves of *Phaseolus vulgaris*[1]

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ABSTRACT

Exposure of leaves to SO_2 or bisulfite is known to induce peroxidation of thylakoid lipids and to inhibit photosynthetic electron transport. In the present study, we have examined the temporal relationship between bisulfite-induced thylakoid lipid peroxidation and inhibition of electron transport in an attempt to clarify the primary mechanism of SO_2 phytotoxicity. Primary leaves of bean (*Phaseolus vulgaris* L. cv Kinghorn) were floated on a solution of $NaHSO_3$, and the effects of this treatment on photosynthetic electron transport were determined *in vivo* by measurements of chlorophyll *a* fluorescence induction and *in vitro* by biochemical measurements of the light reactions using isolated thylakoids. Lipid peroxidation in treated leaves was followed by monitoring ethane emission from leaf segments and by measuring changes in fatty acid composition and lipid fluidity in isolated thylakoids. A 1 hour treatment with bisulfite inhibited photosystem II (PSII) activity by 70% without modifying Photosystem I, and this inhibitory effect was not light-dependent. By contrast, lipid peroxidation was not detectable until after the inhibition of PSII and was strongly light dependent. This temporal separation of events together with the differential effect of light suggests that bisulfite-induced inhibition of PSII is not a secondary effect of lipid peroxidation and that bisulfite acts directly on one or more components of PSII.

Sulfur dioxide is a wide-spread air pollutant that produces a variety of toxic effects in plants (26, 30). A primary site of action appears to be the photosynthetic apparatus as indicated by the inhibition of CO_2 fixation during the early stages of SO_2 toxicity (15, 30). This is supported by the observed inhibition of a number of partial reactions of photosynthesis after exposure to SO_2 or its related anionic forms, sulfite and bisulfite (1, 10, 12, 28, 29). Photosynthetic electron transport is particularly sensitive, apparently as a result of inhibition of PSII (12, 28, 29).

The mechanism of action of sulfur dioxide has not been clearly established, although it has been suggested that induction of thylakoid lipid peroxidation is involved (25). Indeed, there is considerable evidence that sulfur dioxide damages plant membranes by facilitating free radical-mediated lipid peroxidation (8, 24). The oxidation of sulfur dioxide in solution (as sulfite) is known to occur by a chain reaction that can be initiated by a variety of free radical-generating agents including illuminated chloroplasts (3, 26). During propagation of the reaction, the free radicals, OH· (hydroxyl), $SO_3^{\cdot-}$ (sulfite), and $O_2^{\cdot-}$ (superoxide), are thought to be generated (14, 26), although recent spin-trapping evidence suggests that $SO_3^{\cdot-}$ is more important than $O_2^{\cdot-}$ in the propagation of chloroplast-initiated oxidation of the sulfite ion (11). The free radicals generated are thought to be responsible for the observed peroxidation of thylakoid lipids exposed to sulfite (17).

Several studies, considered together, implicate thylakoid membrane lipid peroxidation in SO_2-induced injury to plants (3, 8, 16, 24, 25). In particular, there are several reports that exposure to SO_2 or bisulfite results in inhibition of photosynthetic electron transport, which could clearly be a consequence of lipid peroxidation in thylakoids (12, 28, 29). Alternatively, the possibility remains that inhibition of electron transport results from a more direct effect of SO_2 on the protein components of the thylakoid membrane. This could result from the known reaction of sulfite with disulfide bridges (4) or by reaction of membrane proteins with the free radicals generated by sulfite oxidation (13).

In the present study, we have examined the temporal relationship between bisulfite-induced thylakoid lipid peroxidation and inhibition of electron transport with a view to clarifying the mechanism of SO_2-mediated inhibition of photosynthesis. Electron transport capacity of bean leaves floated on a solution of $NaHSO_3$ was monitored *in vivo* by Chl *a* fluorescence measurements (for reviews see refs. 5 and 9) as well as *in vitro* by polarographic measurements of oxygen using thylakoids isolated from treated leaves. Lipid peroxidation was determined by measuring ethane emission from leaves and by fatty acid analysis of thylakoids isolated from bisulfite-treated leaves. The results indicate that sulfite-induced inhibition of PSII precedes measurable thylakoid membrane lipid peroxidation.

MATERIALS AND METHODS

Plant Material

Bean seedlings (*Phaseolus vulgaris* L. cv Kinghorn) were grown in a controlled environment chamber in a mixture of

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sand, peat, and soil (1:1:2) under a 16-h photoperiod, fluorescent illumination of 26 W m^{-2}, and day/night temperatures of 25 and 20°C.

Bisulfite Treatment of Primary Leaves

Primary leaves were harvested 14 d after planting and floated adaxial side down on 20 mM $NaHSO_3$ (pH 3.5) or $NaHSO_4$ (pH 3.5) for up to 2 h in the dark or under fluorescent lighting (4.3 W m^{-2}). The leaves were rinsed in deionized water and in some cases floated on distilled water for a further 24 h under fluorescent lighting (4.3 W m^{-2}).

Isolation of Chloroplasts and Microsomal Membranes

Chloroplasts were isolated from 14-d-old primary leaves as described by McRae and Thompson (18). The leaves (10 g) were homogenized for 5 s in 60 mL of extraction buffer (0.3 M sorbitol, 10 mM $MgCl_2$, 0.1% BSA, and 50 mM Tricine [pH 8.0]) using a Sorvall Omnimixer. The homogenate was filtered through eight layers of cheesecloth and centrifuged for 2 min at 2000*g*. The resulting chloroplast pellet was resuspended in 20 mL of wash buffer (0.3 M sorbitol and 50 mM Tricine [pH 8.0]) and centrifuged again at 2000*g* for 2 min. This final pellet of chloroplasts was resuspended in 3 mL of wash buffer. Thylakoid membranes were obtained by resuspending the final chloroplast pellet in 20 mL of 50 mM Tricine (pH 8.0), and centrifuging for 2 min at 2000*g*. This procedure was repeated three times. The final preparation of isolated thylakoids was resuspended in 3 mL of wash buffer.

Microsomal membranes were isolated from the primary leaves as described by McKersie and Thompson (20).

Electron Transport Measurements

Measurements of electron transport were carried out essentially as described by McRae *et al.* (19). Rates of noncyclic, PSI, and PSII electron transport was measured using a Yellow Springs Instrument Oxygen electrode (Clark type) and a slide projector providing a light intensity of 100 W m^{-2} at the cuvette surface. Rates of oxygen consumption or evolution in the dark were subtracted from corresponding rates in the light.

The basic reaction mixture used for all electron transport assays contained 0.1 M sorbitol, 50 mM Tricine, 10 mM $MgCl_2$, 1 mM ADP, 1 mM K_2HPO_4 (pH 8.0), and thylakoid membranes (67 μg Chl·mL^{-1}). For PSI measurements, the reaction mixture contained in addition 80 μM DCIP[4], 1 mM ascorbate, 0.5 mM MV, and 0.1 mM DCMU. For measurements of PSII, the reaction mixture also contained 5 mM $K_3Fe(CN)_6$, 50 μM Trifluralin, and 0.5 mM PD_{ox}. For total noncyclic electron transport, 0.5 mM MV was added to the basic reaction mixture.

Fluorescence Induction

Fluorescence induction curves for control and treated leaves were recorded at room temperature after a 30 to 45 min period of dark adaptation using an SLM 8000 spectrofluorimeter fitted with an electronic shutter (Ealing Scientific Ltd., U.K.). Rectangular leaf samples were sandwiched between cover slips fitted diagonally in a water-filled 1.0 cm^2 fluorescence cuvette. Samples were illuminated with 400 nm (16 nm bandwidth) light. Fluorescence from the sample was passed through a 630 nm long-pass filter (Corning 2-59) and a grating monochromator set at 685 nm (16 nm bandwidth) and was detected by a photomultiplier tube. Fluorescence transients were recorded by microcomputer using an analog-to-digital converter. This allowed accurate measurement of the initial fluorescence, F_o, in the first few ms after the shutter opened, as well as recording of the slower phases of fluorescence induction.

Ethane Measurements

Discs (5.1 cm diameter) were cut from 14-d-old primary leaves and floated, adaxial side down, on 50 mL of 20 mM $NaHSO_3$ (pH 3.5), 20 mM $NaHSO_4$ (pH 3.5) or distilled water in sealed 100 mL glass jars for 2 h under fluorescent lighting (4.3 W m^{-2}) at the surface of the jar. At the end of the incubation period, the discs were washed with distilled water and floated on 50 mL of distilled water in sealed 100 mL glass jars for an additional 24 h under fluorescent lighting (4.3 W m^{-2}). For ethane measurements, 1-mL gas samples were withdrawn from each jar at the beginning and end of each treatment. The gas samples were analyzed for ethane under isothermal conditions (65°C) using a Perkin-Elmer Series 900 GC fitted with an $A1_20_3$ column and a flame ionization detector. Ethane identification and quantitation was by comparison to an authentic standard (Canlab).

Chl and Fatty Acid Determinations

Total Chl (*a* plus *b*) was determined by diluting an aliquot of chloroplast suspension with 2.9 mL of 80% acetone and reading the absorbance at 652 nm. The concentration of Chl was calculated as described by Arnon (2). Lipids were extracted from isolated thylakoid and microsomal membranes as described by Bligh and Dyer (7), and total fatty acids were measured according to McRae *et al.* (19).

Fluorescence Depolarization

Fluorescence depolarization measurements of thylakoid membranes isolated from bisulfite-treated and control leaves were carried out as described by McRae *et al.* (19) using the fluorophore, diphenylhexatriene, and an SLM 8000 spectrofluorimeter.

RESULTS AND DISCUSSION

The effects of bisulfite treatment on photosynthetic electron transport were monitored *in vitro* by recording Chl fluorescence induction curves of leaf segments after a period of dark adaptation. Typical induction curves for untreated leaves and for leaves floated on 20 mM $NaHSO_4$ or $NaHSO_3$ for 2 h in the light are shown in Figure 1. Untreated leaves gave rise to a two-peaked curve typical of those reported previously for

[4] Abbreviations: DCIP, 2,6-dichloroindophenol; F_o, initial fluorescence; F_p, maximal fluorescence; F_v, variable fluorescence; MV, methyl viologen; PD_{ox}, oxidized phenylenediamine.

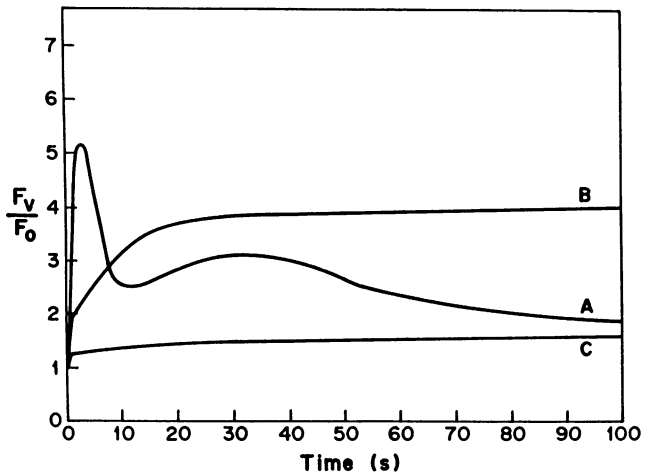

Figure 1. Typical fluorescence induction curves for untreated and sulfur anion-treated leaves. Curve A is for an untreated leaf. Leaves were floated on 20 mM $NaHSO_4$ (pH 3.5) (curve B) or 20 mM $NaHSO_3$ (pH 3.5) (curve C) for 2 h under fluorescence illumination. The induction curves are normalized to the initial fluorescence level, F_o.

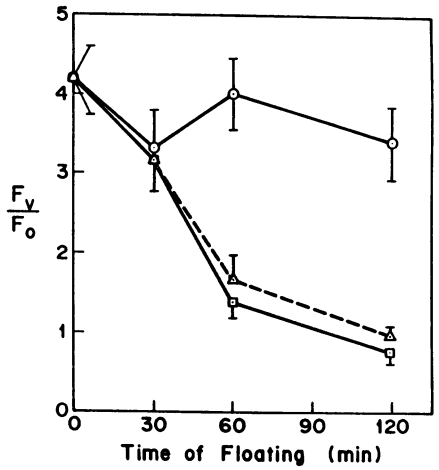

Figure 2. Values of F_v/F_o for bisulfate-and bisulfite-treated bean leaves. Samples were floated on 20 mM bisulfate in light (○); bisulfite in light (□); and bisulfite in darkness (△). Standard errors are indicated; $n = 12$.

higher plants (5, 9). An important indicator of PSII activity is the F_v, which is the difference between the F_p and the F_o, measured a few milliseconds after the shutter is opened. By expressing F_v relative to F_o (*i.e.* F_v/F_o), differences in Chl content between leaf segments are normalized. Although the interpretation of changes in F_v can be difficult, the rise in fluorescence from F_o to F_p is thought to reflect the photoreduction of plastoquinone by PSII (9). Consequently, a decrease in F_v can be interpreted as indicating a decline in the ability of PSII to reduce plastoquinone (5, 9).

Treatment of leaves with $NaHSO_4$ (pH 3.5) results in a fluorescence induction curve that rises monotonically from F_o (Fig. 1B). This change in shape of the induction curve relative to that obtained for untreated leaves may be due to an effect of sulfate on photophosphorylation (27) or possibly on the activity of Calvin cycle enzymes (23). Alternatively, sulfate may act directly on PSII, as has been recently reported (6), or there may be an effect on CHl fluorescence induction due simply to the low pH of the solution.

Although the shape of the fluorescence induction curve of $NaHSO_4$-treated leaves is altered, there is only a small drop in F_v/F_o relative to corresponding values obtained for untreated leaves (Figs. 1A and 2, time = 0). By contrast, for leaves treated with bisulfite, pH 3.5 for 2 h, the parameter F_v/F_o was drastically reduced (Fig. 1C). The time course for this reduction is shown in Figure 2. For bisulfate-treated leaves, F_v/F_o remained above 80% of corresponding 0-time control values, whereas F_v/F_o for bisulfite-treated leaves decreased to one-third and one-fifth of corresponding control values after 1 and 2 h, respectively (Fig. 2). A similar decline in F_v/F_o has been reported for SO_2-fumigated leaves of various species (21), and it evidently reflects inhibition of PSII activity (12, 28). The time course for the decline in F_v/F_o is essentially identical whether bisulfite treatment is carried out in the light or in darkness (Fig. 2).

Time-dependent Chl fluorescence measurements provide a rapid monitor of the effects of sulfur anions on photosynthetic

Table I. *Rates of Photosynthetic Electron Transport in Isolated Chloroplasts*

Chloroplasts were isolated from untreated primary bean leaves and from leaves floated for 1 h on 20 mM $NaHSO_4$ (pH 3.5) or 20 mM $NaHSO_3$ (pH 3.5) in the light. Standard errors of the means are indicated in parentheses; $n = 3$ to 4. Rates of electron transport for untreated leaves are 54, 76, and 72 μmol ½ O_2·mg Chl^{-1}·h^{-1} for H_2O to MV, for 2,6-DCIP to MV and for H_2O to PD_{ox}, respectively. Rates for chloroplasts from treated leaves are expressed relative to those from untreated control leaves, which have arbitrarily been assigned a value of 100.

Assay	Treatment	Relative Rate of Electron Transport
H_2O to MV	Control	100
	Bisulfate	122 (9)
	Bisulfite	70 (12)
DCIP to MV	Control	100
	Bisulfate	99 (<1)
	Bisulfite	98 (2)
H_2O to PDox	Control	100
	Bisulfate	121 (3)
	Bisulfite	30 (11)

electron transport in intact leaf tissue. The results of these measurements were confirmed by more direct *in vitro* assays of electron transport using isolated thylakoids. The effects of treating leaves with bisulfite on noncyclic electron transport through PSII and PSI, PSI-driven electron transport, and PSII-driven electron transport are illustrated in Table I. Treatment of leaves for 1 h in the light with 20 mM $NaHSO_3$ resulted in a 70% reduction in PSII activity and a 30% reduction in noncyclic electron transport activity of isolated thylakoids. It is notable that the rate of PSII-dependent electron transport from H_2O to PD_{ox} dropped below that of whole chain electron transport. This has been noted previously for chloroplasts from bisulfite-treated spinach leaves (28) and triazine-resistant pigweed (22). As proposed previously (22), these observations may reflect a decrease in the binding constant for PD_{ox} without a proportionate change in the binding constant for

plastoquinone, the endogenous electron acceptor for PSII in the whole chain reaction. By contrast, PSII and noncyclic electron transport were both stimulated by about 20% in thylakoid membranes from $NaHSO_4$-treated leaves. PSI activity was unaffected by either treatment, which is in agreement with previous studies (12, 28). Thus, *in vivo* and *in vitro* measurements both indicate a bisulfite-specific inhibition of PSII within 1 h of the treatment being initiated. This inhibition is not reversed by the routine washing of thylakoid membranes during preparation, and it appears to be independent of light intensity within the range of 0 to 4.3 W m^{-2} (Fig. 2).

Previous studies have indicated that sulfur dioxide or bisulfite treatment of leaves induces lipid peroxidation in thylakoid membranes attributable to free radical production during sulfite oxidation (15, 25). This raises the possibility that sulfur dioxide-induced inhibition of PSII may be a secondary effect of lipid peroxidation (25). The possible relationship between bisulfite-induced inhibition of PSII and bisulfite-induced lipid peroxidation was examined further in the present study by monitoring changes in fatty acid composition and lipid fluidity of thylakoid membranes over the 2 h treatment with bisulfite that inhibits photosynthetic electron transport. The fatty acid composition of thylakoid membranes isolated from control leaves and leaves treated for 2 h in the light with bisulfate or bisulfite is illustrated in Table II. No significant changes were apparent among membranes from control, bisulfate-treated and bisulfite-treated leaves during this initial 2 h period. However, when the leaves were treated in the same fashion and floated in the light on distilled water for an additional 24 h prior to thylakoid isolation and lipid extraction, major changes in fatty acid composition were observed (Table II). Specifically, the most abundant fatty acid of thylakoid membranes, linolenic acid, declined in relative abundance from approximately 80% (w/w) in thylakoids from control and bisulfate-treated leaves to 66% (w/w) in thylakoids from bisulfite-treated leaves. This selective decline in linolenic acid, a highly unsaturated fatty acid, is indicative of lipid peroxidation. Bisulfite-induced peroxidation of lipids does not appear to be limited to thylakoid membranes since similar changes in fatty acid composition were also observed in microsomal membrane preparations (Table III).

This decrease in fatty acid unsaturation and accompanying lipid peroxidation should result in decreased fluidity of the membrane lipid bilayer. This was confirmed for thylakoids by measurements of fluorescence polarization after labeling the isolated membranes with the fluorophore diphenylhexatriene. It has been shown previously that there is no significant transfer of energy from diphenylhexatriene to chloroplast pigments, which could confound measurements of polarization (19). Following treatment with bisulfite, the polarization values for thylakoid membranes rose from 0.185 to 0.320, whereas treatment with bisulfate resulted in only a small increase to 0.204 (Table IV). [Polarization is defined as $\frac{(I_\parallel/I_\perp) - 1}{(I_\parallel/I_\perp) + 1}$ where $I_\parallel$ and $I_\perp$ are the fluorescence emission intensities parallel and perpendicular to the plane of polarization, respectively.]

Measurements of ethane production provide a nondestructive, *in vivo* monitor of lipid peroxidation (8, 24, 25). With a view to confirming the results of the fatty acid analyses, ethane production from leaf discs was measured immediately after a 2 h treatment in light or darkness with bisulfite or bisulfate as well as during a subsequent 24 h incubation in light or darkness on distilled water. Total ethane production from control and bisulfate-treated leaf discs did not exceed 3 pmol cm^{-2} of leaf surface (Table V). However, when leaves were incubated on distilled water for 24 h after treatment, accumulation of ethane from bisulfite-treated leaves showed a roughly 17-fold enhancement over control and bisulfate-treated leaves (Table V). This effect was strongly dependent on light. If the 2 h treatment and 24 h incubation were carried out in darkness, the bisulfite-induced ethane production was about 3 pmol cm^{-2}, about 15-fold less than that for the corresponding treatment and incubation in light (Table V).

Illumination appears to be more important during the 24

Table II. *Fatty Acid Composition of Thylakoid Membranes*

Data are shown for thylakoids isolated from control (untreated) leaves, for leaves floated for 2 h in the light on 20 mM $NaHSO_4$ (pH 3.5), for leaves floated in the light on 20 mM $NaHSO_4$ (pH 3.5) and for an additional 24 h on distilled water in the light, for leaves floated for 2 h in the light on 20 mM $NaHSO_3$ (pH 3.5), and for leaves floated in the light on 20 mM $NaHSO_3$ (pH 3.5) and for an additional 24 h on distilled water in the light. Standard errors of the means are indicated in parentheses; $n = 3$.

Fatty Acid[a]	Untreated Leaves	Bisulfate-Treated Leaves		Bisulfite-Treated Leaves	
		2 h	2 h + 24 h on H_2O	2 h	2 h + 24 h on H_2O
			% w/w		
14:0	1.3 (0.1)	1.1 (0.1)	ND[b]	1.1 (0.1)	0.6 (0.6)
16:0	10.5 (0.4)	10.2 (0.4)	9.9 (2.2)	10.9 (0.4)	13.3 (0.4)
16:1	3.7 (0.1)	4.0 (0.2)	3.7 (0.7)	4.2 (<0.1)	5.6 (1.1)
18:0	2.0 (0.5)	1.4 (<0.1)	0.9 (0.2)	1.8 (0.1)	3.8 (0.9)
18:1	1.8 (1.0)	0.9 (0.2)	0.4 (0.3)	1.0 (0.1)	2.4 (0.8)
18:2	3.3 (0.9)	2.7 (0.3)	2.8 (0.2)	3.7 (0.1)	8.7 (0.9)
18:3	77.3 (3.0)	79.8 (0.9)	82.4 (3.2)	77.4 (0.5)	65.7 (1.6)

[a] 14:0, myristic acid; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18.2, linoleic acid; 18:3 linolenic acid. [b] Not detectable.

Table III. *Fatty Acid Composition of Microsomal Membranes*

Data are shown for microsomal membranes isolated from control (untreated) leaves, from leaves floated in the light on 20 mM $NaHSO_4$ (pH 3.5) and for an additional 24 h on distilled water in the light, and from leaves floated in the light on 20 mM $NaHSO_3$ (pH 3.5) and for an additional 24 h on distilled water in the light. Standard errors of the means are indicated in parentheses: $n = 3$.

Fatty Acid[a]	Untreated Leaves	Bisulfate-Treated Leaves	Bisulfite-Treated Leaves
		% w/w	
14:0	0.1 (<0.1)	0.03 (<0.1)	1.3 (1.5)
16:0	25.3 (0.7)	25.7 (0.5)	29.9 (1.6)
16:1	3.0 (1.1)	2.9 (1.5)	5.9 (1.1)
18:0	8.3 (1.6)	7.9 (1.4)	8.7 (2.0)
18:1	3.3 (1.5)	2.6 (1.5)	4.5 (0.3)
18:2	17.5 (1.3)	18.7 (0.5)	17.1 (0.6)
18:3	42.9 (3.0)	42.2 (2.8)	32.5 (4.8)

[a] 14:0, myristic acid; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid.

Table IV. *Fluorescence Polarization Values for Thylakoids from Bisulfate- and Bisulfite-Treated Leaves*

Thylakoids were isolated from control (untreated leaves), from leaves floated in the light on 20 mM $NaHSO_4$ (pH 3.5) and for an additional 24 h on distilled water in the light, and from leaves floated in the light on 20 mM $NaHSO_3$ (pH 3.5) and for an additional 24 h on distilled water in the light. Standard errors of the means are indicated in parentheses; $n = 3$.

Treatment	Polarization
Control	0.185 (0.012)
Bisulfite	0.320 (0.025)
Bisulfate	0.204 (0.010)

h incubation than during bisulfite treatment. If bisulfite-treated leaves are incubated 24 h in the light (after a 2 h dark treatment), ethane accumulation is about threefold higher than for dark incubation. It is likely that bisulfite is not completely washed out by the rinse in distilled water following treatment and remains in the leaf during the 24 h light incubation. In contrast, illumination during bisulfite treatment followed by dark incubation has a negligible (or possibly negative) effect on ethane production compared to dark treatment followed by dark incubation (Table V). This, bisulfite-induced ethane production reflecting lipid peroxidation appears to be strongly light-dependent, particularly in the period following the inhibition of PSII. It is possible that this reflects light-dependent oxidation of bisulfite remaining in the leaf (25). Alternatively, it may reflect the light dependence of other processes involved in ethane production.

These data suggest that bisulfite inhibits PSII without modifying PSI activity and that this inhibitory effect is not light dependent (Fig. 2; Table 1). By contrast, the onset of lipid peroxidation, as detected by ethane production, is strongly light dependent and is not detectable until well after there has been extensive inhibition of PSII activity. This temporal separation of events together with the differential effects of light suggests that bisulfite-induced inhibition of PSII is not a secondary effect of lipid peroxidation and that bisulfite acts directly on one or more PSII elements either through reactions involving free radicals (13) or by reacting with disulfide bridges (4). It is to be noted, however, that the measurements of ethane production and changes in fatty acid composition pertain to peroxidation of bulk lipid. It remains possible, therefore, that preferential oxidation of a small fraction of thylakoid membrane lipid, which is not evident from fatty acid analysis, may contribute to inhibition of PSII function. Further work is required to examine the effects of chronic exposure to SO_2 on the proteins and partial reactions that make up PSII.

It is also possible that bisulfite-induced inhibition of PSII actually contributes to the induction of thylakoid membrane lipid peroxidation. The generation of free radicals during aerobic oxidation of sulfite to sulfate is known to initiate peroxidation in thylakoid membrane lipids (25). Sulfite oxidation itself is initiated by numerous free radical-generating agents including illuminated chloroplast membranes (3 and

Table V. *Ethane Production by Leaf Discs Treated with Bisulfite or Bisulfate*

Leaf discs were floated in the light or in darkness on distilled water (the control), 20 mM $NaHSO_4$ (pH 3.5), or 20 mM $NaHSO_3$ (pH 3.5) for 2 h and then in the light or in darkness for an additional 24 h on distilled water. Cumulative ethane production during the initial treatment (0–2 h) and during the subsequent incubation on distilled water (2–26 h) was measured. Values for two separate experiments are shown.

Illumination Conditions	Experiment	Ethane (pmol/cm² of leaf surface)								
		Control			Bisulfite-treated Disks			Bisulfate-treated Disks		
		0–2 h	2–26 h	Total[a]	0–2 h	2–26 h	Total	0–2 h	2–26 h	Total
0–2 h, light; 2–26 h, light	A	2.7	ND[b]	2.7	2.7	37.9	40.6	1.3	1.3	2.6
	B	ND	ND	ND	1.3	50.4	51.7	2.2	ND	2.2
0–2 h, light; 2–26 h, darkness	A	1.3	ND	1.3	0.13	1.3	1.4	ND	1.8	1.8
	B	0.89	ND	0.89	0.89	1.8	2.7	ND	2.2	2.2
0–2 h, darkness; 2–26 h, light	A	ND	ND	ND	ND	8.9	8.9	ND	1.3	1.3
	B	ND	1.3	1.3	ND	11.2	11.2	ND	2.7	2.7
0–2 h, darkness; 2–26 h, darkness	A	0.36	0.89	1.25	ND	3.6	3.6	ND	1.3	1.3
	B	ND	ND	ND	ND	2.7	2.7	ND	1.3	1.3

[a] Cumulative ethane production over the 26 h period. [b] Not detectable.

references therein). It seems likely that this free radical generation would be enhanced in functionally disrupted PSII complexes where excitation energy is not directed towards reduction in plastoquinone. This would accelerate the oxidation of bisulfite and ensuing peroxidation of thylakoid membrane lipids and would also explain the strong light dependence observed for ethane production in bisulfite-treated leaves.

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