Sodium Bisulfite in Chloroplast Electron Transport

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Introduction

As shown by Trebst and Schmidt (14) and Schmidt and Trebst (9), sulfite is an intermediate in the photoreduction of sulfate to sulfide by class I chloroplasts. They found this process to be ferredoxin-dependent. Schmidt and Schwenn (8) describe several enzymatic steps involved in this process. Hennies (5) purified the ferredoxin-linked sulfite reductase 125 times. Schwenn et al. (11) found evidence for the participation of both stromal and membrane-bound enzymes in sulfate reduction in chlorophasts. Schwenn and Depka (10) investigated the regulatory influence of adenosine mono- and diphosphates on the first 3 enzymatic steps of sulfate reduction and found that the regulatory step was located at the ATP-sulfurylase reaction site.

Libera *et al.* (6) studied the effect of sodium bisulfite on chloroplast electron transport. They found stimulation of ferricyanide and NADP reduction by bisulfite. Using Tris-washed chloroplasts, they could show electron donation to photosystem II by bisulfite, which was DCMU-sensitive. Asada and Kiso (1) implicate the superoxide anion as the initiator of the DCMU-sensitive sulfite oxidation in illuminated chloroplasts. Univalent oxidation of oxygen is necessary before sulfite can be oxidized.

Several studies dealing with the inhibition of electron transport reactions by SO_2 or sulfide (7,12,13) come to the conclusion that the effect is somewhere between the donor site in Tris-treated chloroplasts and the plastoquinone pool, an effect on the primary donor and the reaction center in PS II not exluded.

In view of the variety of possible bisulfite effects on chloroplasts, this study was undertaken to find out which partial reactions were most severely affected by sodium bisulfite in the sucrose-NaCl chloroplasts. The ultimate aim was to pinpoint the site(s) of action of bisulfite. The inhibition of all PS II reactions by bisulfite has not been reported before.

Materials and Methods

Spinach chloroplasts were isolated from market spinach as previously described (2). Oxygen evolution or uptake were measured with a Clark-type electrode. Reaction rates were recorded with a Sargent-Welch recorder. Reaction components are given in Figure legends.

Tris treatment of chloroplasts to destroy water oxidation was according to Yamashita and Butler (15).

Results and Discussion

The bisulfite stimulation of electron transport reported here is given by lower concentrations (0.5mM, Figure 1) than previously used to demonstrate the ferredoxin-dependent sulfate reduction in chloroplasts (5,6,8,9,10,11,14). Libera *et al.* (6) used 1mM sodium bisulfite to stimulate DCMU-sensitive ferricyanide reduction and 3mM bisulfite to donate electrons to Tris-washed chloroplasts. According to Asada and Kiso (1), the optimum pH for aerobic sulfite oxidation in chloroplasts is 6.8 but it is higher for bisulfite in this study, as shown in Figure 2, where the optimum lies between pH 7 and 7.5

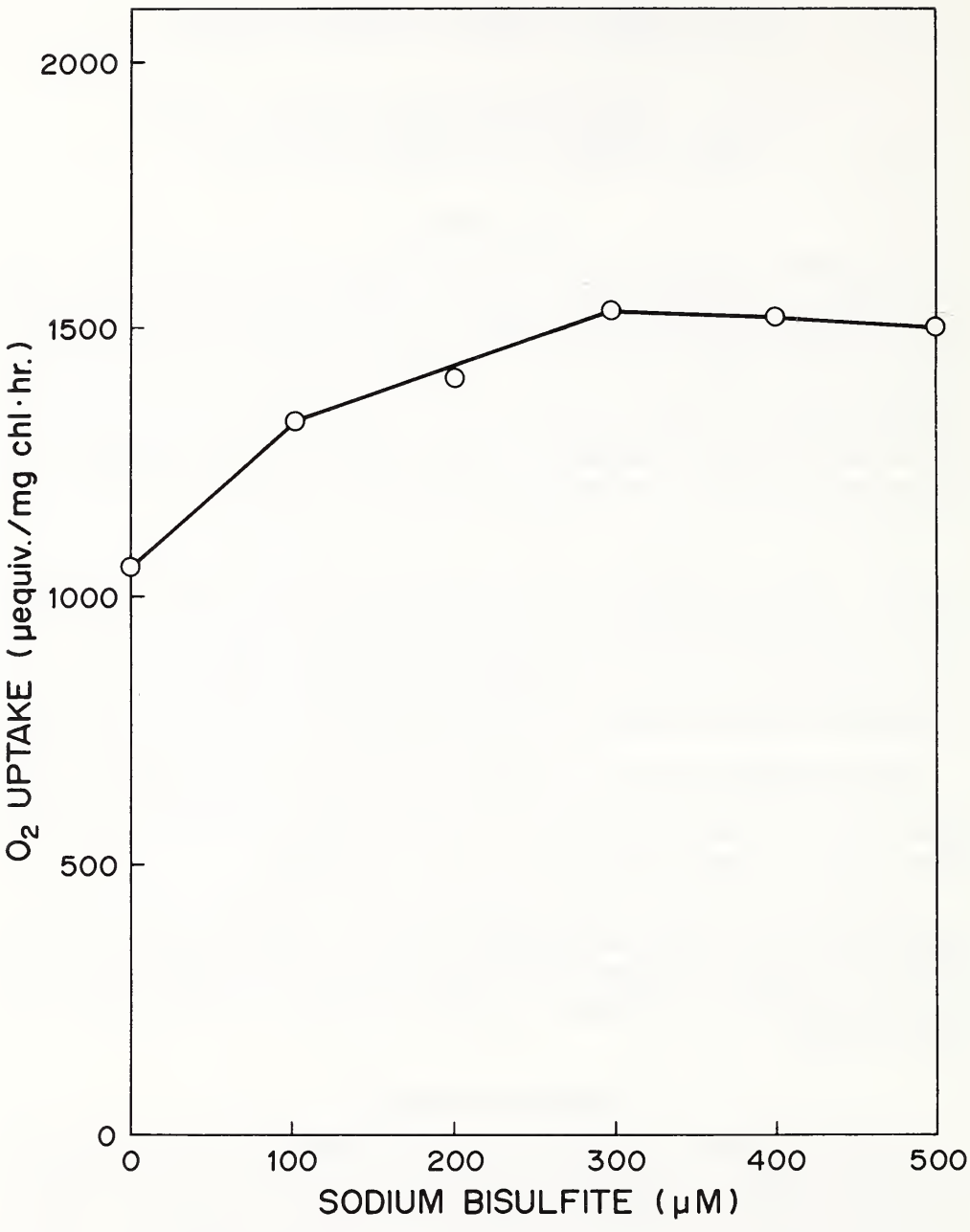

FIGURE 1. *Stimulation of Electron Transport in Spinach Chloroplasts by Various Concentrations of Sodium Bisulfite. The reaction mixture contained chloroplasts (0.05 mg chlorophyll), buffer (25 mM Tris-Mes, pH 7), 0.5 mM Na azide, 0.5 mM methylviologen, and Na bisulfite in concentrations indicated.*

Sodium bisulfite can stimulate the overall electron transport from water to methylviologen up to 50% (Table I) and to about 30% in PS I donor reactions (Table II). Using specific inhibitors, such as DCMU, dibromothymoquinone or polylysine (Table III), which inhibit between the 2 photosystems, the stimulation site can be localized in the region of the plastoquinone pool, i.e. between the dibromothymoquinone and the polylysine inhibition sites. The stimulation sites cannot be located betwen the DCMU and dibromothymoquinone inhibition sites, because the stimulation of electron transport is seen in PS I reactions, where DCMU blocks electron flow from the water oxidation or oxidizing side of PS II.

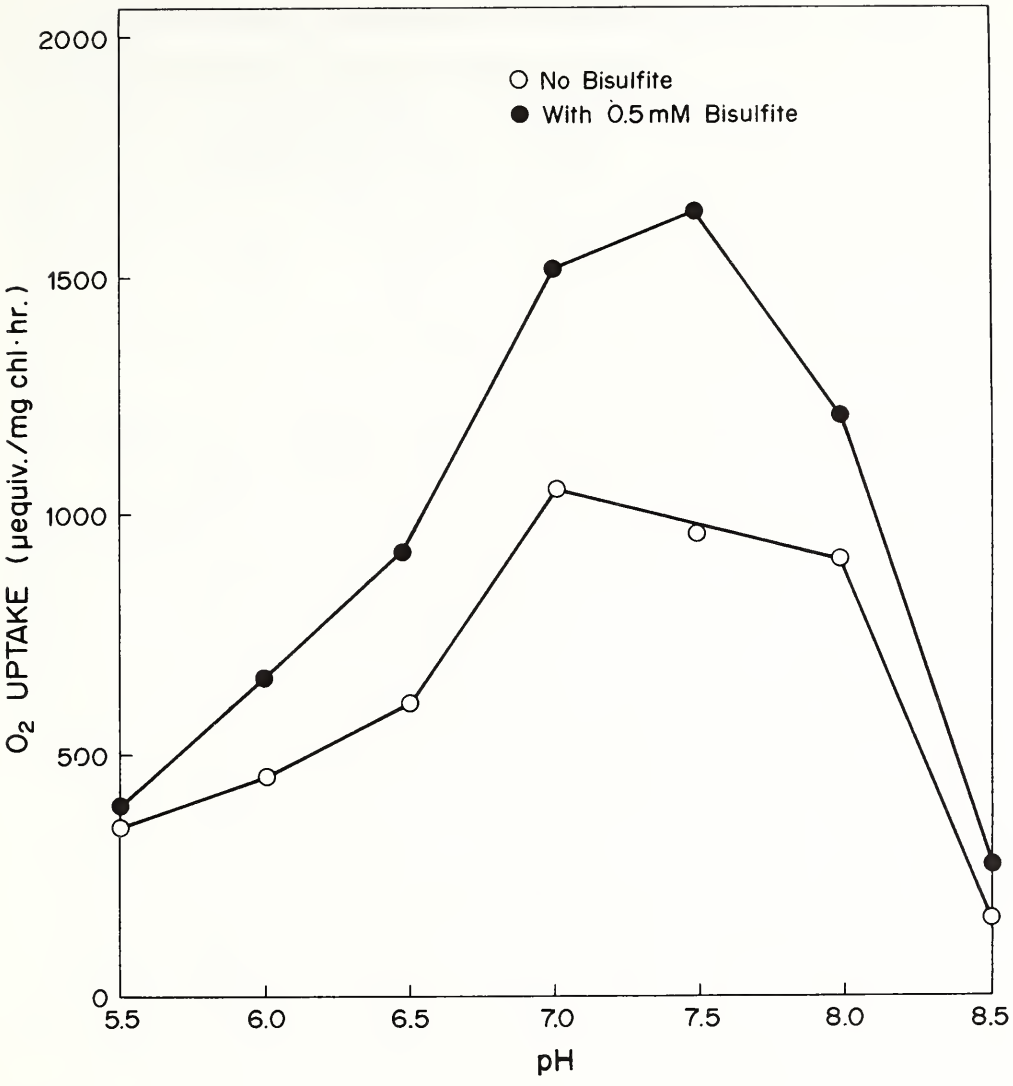

FIGURE 2. *The Effect of pH on Sodium Bisulfite Stimulation of Electron Transport. The reaction mixtures were as in Fig. 1, except the pH of the buffer varied as indicated.*

TABLE I. *Stimulation of Electron Transport by Sodium Bisulfite.*

Reaction and conditions	Bisulfite conc. (mM)	Electron transport rate − bisulfite (μ equiv./mg chl•hr)	Electron transport rate + bisulfite (μ equiv./mg chl•hr)	Stimulation (%)
H_2O → MV, pH 7	—	671	—	—
H_2O → MV, pH 7	0.1	—	872	30
H_2O → MV, pH 7	0.5	—	1009	50
In Tris-treated chloroplasts	—	124	—	—
In Tris-treated chloroplasts	0.5	—	237	91
Without chloroplasts	0.5	0	0	0
Without light	0.5	0	0	0

TABLE II. *Stimulation of Photosystem I Reactions by Sodium Bisulfite.*

Reaction	Bisulfite conc. (mM)	Electron transport rate − bisulfite (μ equiv./mg chl•hr)	Electron transport rate + bisulfite (μ equiv./mg chl•hr)	Stimulation (%)
Ascorbate + DAD → MV, pH 8	0.5	1674	1945	16
Ascorbate + TMPD → MV, pH 8	0.5	767	953	14
Ascorbate + ferrocyanide → MV, pH 8	0.5	2012	2057	2

TABLE III. *The Effect of Inhibitors on Sodium Bisulfite Stimulation of Photosystem I Reactions in Spinach Chloroplasts.*

Reaction	Inhibitor	Conc. (μM)	Electron transport rate − inhibitor (μ equiv./mg chl•hr)	Electron transport rate + inhibitor (μ equiv./mg chl•hr)	Stimulation or inhibition (%)
Ascorbate + DAD → MV	None	—	2345	—	—
Ascorbate + DAD → MV	DBMIB	2.5	—	395	-78[a]
Ascorbate + DAD → MV	None	—	1945	—	—
Ascorbate + DAD → MV	Polylysine[b]	10 μg/ml	—	209	-87
Ascorbate + TMPD → MV	None	—	953	—	—
Ascorbate + TMPD → MV	DBMIB	2.5	—	1009	+32
Ascorbate + TMPD → MV	None	—	861	—	—
Ascorbate + TMPD → MV	Polylysine[b]	10 μg/ml	—	367	-57
Ascorbate + ferrocyanide → MV	None	—	1567	—	—
Ascorbate + ferrocyanide → MV	DBMIB	2.5	—	1274	-19
Ascorbate + ferrocyanide → MV	None	—	2057	—	—
Ascorbate + ferrocyanide → MV	Polylysine[b]	10 μg/ml	—	2125	+6

[a] + indicates stimulation, - inhibition of rate

[b] M.W. 30,000

TABLE IV. *Inhibition of Photosystem II Reactions by Sodium Bisulfite.*

Reaction	Bisulfite conc. (mM)	Electron transport rate − bisulfite (μ equiv./mg chl•hr)	Electron transport rate + bisulfite (μ equiv./mg chl•hr)	Inhibition (%)
H_2O → SM (+DCMU), pH 6	0.10	522	508	3
H_2O → SM (+DCMU), pH 6	0.50	522	428	18
H_2O → SM (+DCMU), pH 8	0.10	256	130	49
H_2O → SM (+DCMU), pH 8	0.50	256	0	100
H_2O → FeCN, pH 6	0.10	444	383	14
H_2O → FeCN, pH 6	0.50	444	428	4
H_2O → FeCN, (+DBMIB), pH 8	0.10	137	62	55
H_2O → FeCN, (+DBMIB), pH 8	0.50	137	0	100
H_2O → DMBQ (+DBMIB), pH 7	0.10	742	727	2
H_2O → DMBQ (+DBMIB), pH 7	0.50	742	259	65

The phenomenon observed in Table IV, which shows the inhibition of PS II partial reactions by sodium bisulfite, especially at higher pH values (pH 7-8), is harder to explain, unless bisulfite acts as a competitive inhibitor of electrons generated in the water oxidation process or at the well-known bicarbonate site on the B protein between the 2 photosystems (4). Another possibility, which cannot be disregarded, is that bisulfite may form addition products with quinones (3). Its inhibition of PS II partial reactions may be consistent with bisulfite modification of plastoquinone A, rendering it incapable of redox reactions. Further studies are necessary to distinquish between these alternatives.

In summary, this study shows that sodium bisulfite may have 2 sites of action in chloroplast electron transport: 1) acting as an inhibitor of several PS II partial reactions and 2) stimulating electron transport at a site between the 2 photosystems. The mechanism of stimulation by sodium bisulfite can be explained on the basis of electron donation to chloroplasts.

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