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Effect of Sulfite on Chl Fluorescence in Barley(*Hordeum vulgare L.*) Seedlings in Light and Dark

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The effect of sulfite on barley seedlings was investigated through Chl content, the electron transport activity of the photosystem, and Chl fluorescence. Barley leaves were harvested every 12 hrs during greening periods, and were then treated with a sulfite solution in either light or dark conditions. In both cases, the Chl content decreased in comparison with the control at any greening period. After sulfite treatment in the light, the activity of PS I decreased slightly, yet that of PS II showed a decrease of about 15%. The values of Fv, qP and qE decreased, however, the value of qI increased compared with the control. In addition, the value of qE decreased in leaves greened more than 12 hrs compared with that of the control. This indicates that the photosynthetic complex involved in energy dependent fluorescence quenching is undeveloped in a 12 hrs greened leaf, accordingly, it was a hardly affected by sulfite. After sulfite treatment in the dark, the activities of PS II and PS I decreased slightly, there was a small change in the value of Fv, qP decreased, and qE and the ratio of qNP/q increased in comparison with the control. As a result, PS II and PS I were not inhibited, however, the redox of Q_A was inhibited, and the excited energy was lost through the nonphotochemical pathway. The effects of sulfite in light or dark conditions were not remarkably different according to the PS I and II activity measurement method yet were considerably different with the Chl fluorescence quenching analysis method. In both light and dark conditions, the value of qP significantly decreased with sulfite compared to that of the control. This implies that the redox of Q_A was inhibited by sulfite in both light and dark conditions.

Key words : sulfite, barley seedling, photosystem, Chl fluorescence, qP

1. Introduction

As plants are exposed to high concentrations of SO_2, chlorosis and tissue death occur, and even with low concentrations, although no visible disorder occurs, the physiological process, including photosynthesis, is affected (Silvius *et al.*, 1975). Chloroplast is sometimes affected by and yet sometimes tolerant of sulfite (Dittrich *et al.*, 1992). Accordingly, chloroplast can either oxidize sulfite to make sulfate and transport that sulfate quickly into vacuole or reduce sulfite into an amino acid containing sulfur using sulfite reductase and use that amino acid for protein synthesis. Chloroplast can also remove the reactive oxygen produced during sulfite metabolism by using the superoxide dismutase, ascorbate, and peroxidase within a cell (Tanaka and Sugahara, 1980; Ghisi *et al.*, 1990; Madamanchi and Alscher, 1991; Takahama *et al.*, 1992). Therefore, the

resultant will be different from the extracted chloroplast and intact leaves treatment with sulfite. The effect of sulfite differs in every plant development period because of changes in the development of chloroplast and the metabolic activity during these periods. Many studies on photosynthetic activity with SO_2 or sulfite treatment, however, have only focused on young plants after greening for a given time (Shimazaki and Sugahara, 1980; Chung, 1982; Veeranjaneyulu *et al.*, 1992), while few have investigated the effect of sulfite on chloroplast development and the difference this casues in inhibition. Most sulfite inhibition is a light-dependent reaction (Tanaka and Sugahara, 1980), yet photosynthetic activity is more inhibited by sulfite treatment in the dark than treatment in the light (Olszyk and Tingey, 1984; Veeranjaneyulu *et al.*, 1992). Based on this fact, it can be postulated that the effect of sulfite depends on the existence of light.

In this study, an etiolated barley seedling was greened and its leaves were the periodically harvested and sulfite treated in either the light or the dark. Thereafter, changes in Chl content, the electron transport activity of PS I and PS II, and Chl fluorescence were measured and compared.

2. Materials and Methods

2.1 Plant materials

Barley (*Hordeum vulgare* L, cv, Olbori) seeds distributed from an agricultural agent in Seung-ju Kun(Korea) were sowed after being washing in distilled water and cultured in the dark at a temperature of 22±2℃ and relative humidity of 70±5%. To measure the Chl (Chlorophyll) contents, electron transport activity, and Chl fluorescence, the tip of the first leaf was cut off at 1 cm, and the next 2 cm section was used.

2.2 Sulfite treatment

To investigate the effect of sulfite during greening periods, an etiolated barley seedling was greened in a plant growth cabinet (22±2℃, 60 $\mu mol \cdot m^{-2}s^{-1}$) and its leaves were harvested every 12 hrs. The leaf fragments were floated on a 50 mM Hepes buffer (pH 7.5) and 100 mM sulfite solution (50 mM Hepes buffer, pH 7.5, 100 mM Na_2SO_3) in a petri dish. Every control and experiment group was treated with the above solutions for 2 hrs in light (60 $\mu mol \cdot m^{-2}s^{-1}$) and dark conditions.

2.3 Measurement of Chl contents

Chl was extracted using the method of Hiscox and Israelstam (1979) ; 0.1 g leaf in 10 mL DMSO was put in a thermostat at 65℃ for 3 hrs. The Chl a and b contents were quantified by measuring the absorbance light at 663 nm and 645 nm according to Arnon (1949)'s method, and the carotenoid content was measured at 480 nm according to Liaaen-Jensen and Jensen (1971)'s method.

2.4 Preparation of chloroplast

The preparation of chroloplast was carried out using a modified version of Obokata (1987)'s method ; a 1 g sulfite-treated leaf fragment and 10

mL STN buffer solution (pH 8.0, 0.4 M sucrose, 0.05 M Tris, 0.01 M NaCl) were ground and crushed in a cooled mortar, and then filtrated through a double gauze. The solution was centrifuged at 300 *g* for 1 min and its supernatant was centrifuged at 600 *g* for 10 min. The resulting precipitate was then mixed with a 0.5 mL STN buffer solution (pH 7.8). The entire isolation process was performed at 0-4℃.

2.5 Measurement of electron transport activity

To determine the electron transport activity of PS I, the amount of oxygen consumed at 20℃ was measured with a Clark-typed oxygen electrode. The reaction solution consisted of a 50 mM Hepes buffer (pH 7.8, 100 mM sucrose, 2 mM $MgCl_2$, 10 mM NaCl). 2 mM methyl-viologen, 2 mM NaN_3, 1 μM DCMU[3,(3,4-dichlorophenyl)-1, 1-dimethylurea], 0.1 mM DCPIP (2,6-dichlorophenol indophenol), 5 mM ascorbate, 2 mM NH_4Cl, and 25 μg/mL Chl (Atal *et al.,* 1991).

To measure the electron transport activity of PSII, the DCPIP photoreduction rate was calculated using a spectrophotometer. The reaction solution containing the 50 mM Hepes buffer (pH 7.8) plus 30 μM DCPIP and 25 μg/mL Chl was put in a cuvette, and irradiated through a thermostat with 1,330 $\mu mol \cdot m^{-2}s^{-1}$ light. Thereafter, the change of light absorbance at 600 nm was measured and the photoreduction rate was calculated (Terashima *et al.*, 1989). The ultimate concentration of the electron donor, DPC (1,5-diphenylcarbazide) was established at 0.15 mM then PSII activity recovery was examined (Vernon and Show, 1969).

2.6 Measurement of Chl fluorescence

The fluorescence of Chl a was measured using a PAM Chl Fluorometer supplied by the Walz Corp. After the dark adaptation of an intact leaf for 20 min, a sample hold of 0.3×0.9 cm in size was irradiated using measured light (weak red light modulated into 1.6 kHz) and then the Fo (the fluorescence remaining after all the PSII reaction centers have been opened) was measured. Similarly, the sample hold was also irradiated by saturated light (3,000 $\mu mol \cdot m^{-2}s^{-1}$) and then the Fm (the fluorescence remaining after all the PSII reaction centers have been closed and the quinone electron receptors have been completely reduced) was measured. The amounts of Fo and Fm measured were used as indicators of PSII activity. The ratio of Fv to Fm was calculated using Fv, Fm minus Fo, then compared and analyzed (Driesenaar *et al.*, 1994). To determine the fluorescence quenching coefficient, the sample was irradiated continuously by actinic light (1,330 $\mu mol \cdot m^{-2}s^{-1}$) and simultaneously pulsed by saturated light for one second every 20 seconds. The fluorescence quenching coefficients were calculated as qP (photochemical quenching), qNP (nonphotochemical quenching), and qE (energy-dependent fluorescence quenching) using the method of Oxborough and Horton (1988), and qT (state-transitional dependent quenching) and qI (photoinhibitory quenching) using the method of Horton and Hague (1988).

3. Results and Discussions

3.1 Chl contents

As the etiolated barley seedlings greened, their

Table 1. Effects of sulfite on Chl and carotenoids contents and Chl a/b ratio of barley leaf fragments treated with sulfite solution (S) or buffer solution (B) under light (L) or dark (D) conditions. The treatments were conducted by floating the fragments on the solutions. The values are the means of three independent experiments.

Greening time (h)	Sulfite treatment (Light or Dark)	Pigment contents (μg/g fr wt) and Chl a/b ratio			
		Chl. a (%)	Chl. b (%)	Chl. a/b (%)	Carotenoid (%)
12	2hr L+B	138(100)	59(100)	2.34(100)	57(100)
12	2hr L+S	130(94)	57(97)	2.28(97)	56(98)
24	2hr L+B	350(100)	133(100)	2.63(100)	89(100)
24	2hr L+S	291(83)	133(100)	2.19(83)	79(89)
36	2hr L+B	448(100)	164(100)	2.73(100)	116(100)
36	2hr L+S	334(75)	151(92)	2.21(81)	97(84)
48	2hr L+B	513(100)	171(100)	3.00(100)	122(100)
48	2hr L+S	427(83)	161(94)	2.65(88)	105(86)
60	2hr L+B	555(100)	181(100)	3.07(100)	125(100)
60	2hr L+S	443(80)	161(89)	2.75(90)	106(85)
12	2hr D+B	131(100)	57(100)	2.30(100)	57(100)
12	2hr D+S	121(92)	54(95)	2.24(97)	50(88)
24	2hr D+B	352(100)	134(100)	2.63(100)	92(100)
24	2hr D+S	315(90)	123(92)	2.56(97)	79(86)
36	2hr D+B	468(100)	172(100)	2.72(100)	106(100)
36	2hr D+S	393(84)	148(86)	2.66(98)	95(90)
48	2hr D+B	511(100)	179(100)	2.86(100)	120(100)
48	2hr D+S	439(86)	162(91)	2.71(95)	101(84)
60	2hr D+B	557(100)	193(100)	2.89(100)	129(100)
60	2hr D+S	475(85)	168(87)	2.83(98)	115(89)

Table 2. Effects of sulfite on PS I and PS II activity of chloroplasts isolated from 60 hr greened barley leaves. The DCPIP and DPC concentrations were 30 μM and 0.5 mM, respectively. The values are the means of three independent experiments.

		Light		Dark	
		Control (%)	Sulfite (%)	Control (%)	Sulfite (%)
O_2 uptake (μmol O_2 · mg chl^{1} · h^{1})		270(100)	265(98)	270(100)	262(97)
DCPIP photoreduction (μmol · mg chl^{1} · h^{1})	-	75(100)	64(85)	74(100)	72(97)
	+DPC	81(100)	68(84)	80(100)	78(98)

leaves were harvested every 12 hrs and leaf fragments were made. After these fragments had floated for 2 hrs on a 50 mM Hepes buffer and 100 mM sulfite solution, their pigment contents were determined (Table 1). A longer greening time resulted in a smaller decrease in the Chl a content with sulfite, however, Chl a was more affected than Chl b. In case of a 60-hr-greened leaf, the contents of Chl a, Chl b, carotenoid, and Chl a/b ratio with sulfite decreased by about 20%, 11%, 15% and 10% respectively. When treated with sulfite in dark conditions, the pigment contents also decreased. In 60-hr-greened leaves treated with sulfite, the contents of Chl a, Chl b, and carotenoid decreased by about 15%, 13% and 11%, respectively. However, the Chl a/b ratio was similar to that in the control. The above results, therefore, indicate that sulfite treatment in light conditions has more affect on the Chl a and carotenoid content than on the Chl b content, whereas sulfite treatment in dark conditions has a similar affect on the contents of all three. When sulfite is absorbed through the leaf into the chloroplast, it seems to combine with the thylakoid membrane to produce several reactions. In particualr, the O_2^- produced in light-irradiated chloroplast forms chain reactions with sulfite and subsequently the amount of highly reactive O_3^- and $^1O_2$ increases. It has been previously shown that O_2^- destroys Chl a (Shimazaki et *al*.. 1980). accordingly, the dramatic decrease in the Chl a content after sulfite treatment in the light confirms that light and O_2^- accelerate the destruction of Chl a and that sulfite and $^1O_2$ react with the membrane protein and peroxided thylakoid membrane. (Shimazaki et *al*., 1980). The resulting damage to the membrane structure subsequently induced a decrease of pigment. This means that the photosynthetic pigment decreased in both the light and the dark.

3.2 Electron transport activity

To observe the effects of sulfite on the electron transport activity of photosynthesis, the chloroplast of a sulfite-treated leaf fragment was isolated and its PS I and PS II activities were calculated. The quantity of oxygen consumption was measured to determine the PS I activity and the DCPIP photoreduction rate was measured to determine the PS II. Table 2 shows the PS I and PS II activities in 60 hr greened fragments after sulfite treatment, when the development of chloroplast was almost complete (Park and Chung,

Table 3. Effects of sulfite on the Chl fluorescence yield of barley leaf fragments treated with a sulfite solution (S) or buffer solution (B) under light (L) or dark (D) conditions. The values are the means of three independent experiments.

Greening time (h)	Sulfite treatment	Chlorophyll fluoroscence		
		Fo (%)	Fv (%)	Fv/Fm (%)
12	2hr L+B	1.88(100)	4.57(100)	0.71(100)
12	2hr L+S	1.78(95)	3.82(84)	0.68(96)
24	2hr L+B	1.85(100)	4.87(100)	0.73(100)
24	2hr L+S	1.86(101)	4.16(85)	0.69(95)
36	2hr L+B	1.70(100)	5.25(100)	0.76(100)
36	2hr L+S	1.75(103)	4.25(81)	0.71(93)
48	2hr L+B	1.68(100)	5.62(100)	0.77(100)
48	2hr L+S	1.70(101)	4.25(76)	0.71(92)
60	2hr L+B	1.58(100)	5.72(100)	0.78(100)
60	2hr L+S	1.61(102)	4.32(76)	0.73(94)
12	2hr D+B	1.89(100)	4.60(100)	0.71(100)
12	2hr D+S	1.88(100)	4.47(97)	0.70(99)
24	2hr D+B	1.85(100)	5.74(100)	0.76(100)
24	2hr D+S	1.86((101)	5.15(90)	0.74(97)
36	2hr D+B	1.75(100)	6.10(100)	0.78(100)
36	2hr D+S	1.76(101)	5.83(96)	0.77(99)
48	2hr D+B	1.68(100)	6.20(100)	0.79(100)
48	2hr D+S	1.68(100)	6.15(99)	0.79(100)
60	2hr D+B	1.60(100)	6.30(100)	0.80(100)
60	2hr D+S	1.61(101)	6.05(96)	0.79(99)

1996).

The PS I activity was only slightly inhibited, and showed a small difference between the light and the dark conditions (2%, 3%). The PS II activity decreased by 15% in the light and 3% in the dark conditions. This indicates that sulfite treatment in light conditions substantially inhibites the electron transport activity of PS II. Shimazaki and Sugahara (1979) also reported that PS II electron transport was inhibted in spinach treated with SO_2. Plus this corresponds with the findings of Veeranjanegula *et al.* (1992) that PS II electron transport was not damaged in bean leaves treated with sulfite in dark conditions. In contrast, Covello *et al.* (1989) reported that PS I was not affected by bisulfite whereas PS II was seriously damaged, which has nothing to do with light. Although DPC, the electron donor of PS II, was added to the reaction solution containing the chloroplast isolated from the leaf treated with sulfite in light conditions, the DCPIP photoreduction rate did not recover to the same extent as that of the control. Accordingly, the inhibited site is thought to be the reaction center of PS II or the reduction site of PS II, not the oxidation site of PS II. Therefore, sulfite treatment in dark conditions had a slight affect on the activities of both PS I and PS II, while treatment in the light had a small affect PS I activity yet a significant effec on PS II. In particular, this shows that electron transport is inhibited at either the reaction center of PS II or the reduction area of PS II. Therefore, Chl fluorescence was measured for more details on the effects of sulfite.

3.3 Chl fluorescence

Table 3 shows the change of Fo, Fv and Fm with sulfite treatment in light or dark conditions. After sulfite treatment in light conditions, the value of Fo increased slightly yet this was high in comparision with the control except for a 12 hr greened leaf, and Fv deceased significantly. Fo proved the transport of excited energy to the reaction center from the antenna pigment, and Fv confirmed the redox state of the PS II electron acceptor. This result shows that the excited energy transport from the antenna pigment changed slightly and the accumulated reduction of Q_A increased the closing of the reaction center As Fv/Fm decreased, which reflected the photochemical efficiency of the PS II reaction center, electron transport from P_{680} to Q_A was also inhibited. After sulfite treatment in dark conditions, Fo was similar to the control, and Fv and Fv/Fm decreased slightly in comparison with the control. This shows that sulfite treatment in dark conditions only had a slight affect on excited energy transport from the antenna pigment, the redox state of the PS II electron acceptor, and the photochemical efficiency of the PS II reaction center. Therefore, since sulfite had more affect on PS II electron transport activity in light conditions than in dark (Table 2), it also had the same affect on Fv and Fv/Fm. After sulfite treatment in light conditions, when the greening lasted longer, the value of Fv was similar to that of the control. This means that PS II electron transport was damaged by sulfite while it used light to perform photosynthesis in the chloroplast.

Table 4 shows the results of the fluorescence induction curve and an analysis of the fluorescence quenching element after sulfite treatment in light or dark conditions during greening periods. After sulfite treatment in light conditions, the value of

Table 4. Chl fluorescence quenching characteristics of barley leaf fragments treated with sulfite solution (S) or buffer solution (B) under light (L) or dark (D) conditions. q is total quenching. qT is state transition dependent quenching due to LHC II phosphorylation. qI is caused by the photoinhibition of PS II. qP, qNP. and qE are described in the section on Materials and Methods. The values are the means of three independent experiments.

Greening Time (h)	Sulfite trement	Quenching coefficients							
		qP (%)	qNP (%)	qE (%)	qT (%)	qI (%)	q (%)	qP/q (%)	qNP/q (%)
12	2 hr L+B	0.60(100)	0.78(100)	0.51(100)	0.24(100)	0.40(100)	0.91(100)	0.66(100)	0.86(100)
12	2 hr L+S	0.12(20)	0.75(96)	0.56(110)	0.00(0)	0.45(113)	0.77(85)	0.16(24)	0.97(113)
24	2 hr L+B	0.70(100)	0.80(100)	0.56(100)	0.29(100)	0.38(100)	0.91(100)	0.75(100)	0.88(100)
24	2 hr L+S	0.07(10)	0.65(81)	0.21(38)	0.00(0)	0.52(137)	0.67(74)	0.10(13)	0.97(110)
36	2 hr L+B	0.68(100)	0.82(100)	0.52(100)	0.30(100)	0.35(100)	0.92(100)	0.74(100)	0.89(100)
36	2 hr L+S	0.08(12)	0.63(77)	0.20(39)	0.03(10)	0.50(143)	0.64(70)	0.13(18)	0.98(110)
48	2 hr L+B	0.68(100)	0.81(100)	0.52(100)	0.30(100)	0.32(100)	0.91(100)	0.75(100)	0.89(100)
48	2 hr L+S	0.08(12)	0.60(74)	0.18(35)	0.05(17)	0.48(150)	0.62(68)	0.13(17)	0.97(109)
60	2 hr L+B	0.65(100)	0.77(100)	0.53(100)	0.28(100)	0.31(100)	0.90(100)	0.72(100)	0.86(100)
60	2 hr L+S	0.10(15)	0.59(77)	0.20(38)	0.16(57)	0.43(139)	0.62(69)	0.16(22)	0.95(111)
12	2 hr D+B	0.67(100)	0.70(100)	0.49(100)	0.23(100)	0.33(100)	0.92(100)	0.73(100)	0.76(100)
12	2 hr D+S	0.14(21)	0.74(106)	0.67(137)	0.00(0)	0.12(36)	0.77(84)	0.18(25)	0.96(126)
24	2 hr D+B	0.71(100)	0.69(100)	0.45(100)	0.22(100)	0.39(100)	0.93(100)	0.76(100)	0.74(100)
24	2 hr D+S	0.13(18)	0.69(100)	0.63(140)	0.02(9)	0.15(39)	0.72(77)	0.18(24)	0.96(130)
36	2 hr D+B	0.64(100)	0.67(100)	0.44(100)	0.19(100)	0.35(100)	0.92(100)	0.70(100)	0.73(100)
36	2 hr D+S	0.10(16)	0.62(93)	0.51(116)	0.04(21)	0.16(46)	0.66(72)	0.15(21)	0.94(129)
48	2 hr D+B	0.63(100)	0.66(100)	0.38(100)	0.20(100)	0.31(100)	0.87(100)	0.72(100)	0.76(100)
48	2 hr D+S	0.14(22)	0.59(89)	0.49(129)	0.05(25)	0.16(52)	0.65(75)	0.22(31)	0.91(120)
60	2 hr D+B	0.58(100)	0.65(100)	0.40(100)	0.17(100)	0.28(100)	0.86(100)	0.67(100)	0.76(100)
60	2 hr D+S	0.11(19)	0.54(83)	0.45(113)	0.05(29)	0.17(61)	0.59(69)	0.19(28)	0.92(121)

qP was much lower than that of the control during greening periods. The qNP was similar to the control in a 12 hr greened leaf yet otherwise exhibited a distinct difference. This significant decrease of qP compared with the control shows that sulfite seriously inhibited the redox of Q_A. The qE decreased subtantially more than that of the control except in a 12 hr greened leaf. Electron transport induced the active transport of protons over the thylakoid membrane, consequently, the value of ΔpH between the thylakoid membranes was very large and qE showed a high value. The extremely low quantity of qE found in a leaf treated with sulfite in light conditions and greened longer than 24 hrs revealed that the ΔpH between the thylakoid membranes had a very small value. This result suggests an inhibition of either the PSII water splitting system or the PQ pool electron transport. The value of qE decreased in a 12 hr greened leaf compared to that of the control in light conditions. Accordingly, the mechanism controlling fluorescence quenching is thought to be less affected by sulfite due to the immature development after 12 hrs of greening. Since q, the total quenching coefficient, indicates the level of fluorescence quenching, the fact that q was smaller than the control after treatment with sulfite shows that the excited energy which reached the reaction center was not exhausted but continued to leak via florescence through photosynthesis or other processes. Plus in the case of the experiment with sulfite treatment, qNP/q was higher than qP/q and qNP/q higher than the control by calculating qP to q and qNP to q. This means that most fluorescence was quenched during the nonphotochemical process through photosynthesis. qT is the fluorescence quenching induced by protein phosphorylation, where the molecular mechanism is referred to as light harvesting Chl II (LHC II) phosphorylation. qI is the fluorescence quenching involved in photoinhibition and caused by damage to PSII (Horton and Hague, 1988). After sulfite treatment in light conditions, qT initially increased in the greened leaf older than 36 hrs and thereafter continuously increased during greening periods. This means that the sulfite treatment preceeding photosynthesis via the development of chloroplast caused LHC II phosphorylation. After sulfite treatment in light conditions, the value of qI was higher than that of the control. This inidcates damage to PSII, which corresponds with the inhibition of PSII after sulfite treatment in light conditions (Table 2). Therefore, PSII was photoinhibited by sulfite treatment in light conditions. As a result, sulfite treatment in light conditions decreased the value of Fv, qP, and qE yet increased qI more than that of the control. This shows that the damaged PSII inhibited both the electron transport of photosynthesis and the redox of Q_A

After sulfite treatment in dark conditions, the value of qP decreased significantly in comparision with the control, and the value of qE increased more than that of the control. It has been previously assumed that sulfite in dark conditions seriously damages electron transport after Q_A and increases ΔpH between thylakoid membranes. After sulfite treatment in dark conditions, as in light conditions, q, the total quenching coefficient, was lower and qNP/q higher, yet qT and qI were lower than that of the control. Therefore, qE covered most of qNP in dark conditions. In particular, the value of qE in leaves that were 12 and 24 hrs old was substantially more than that in leaves which

were 36, 48, and 60 hrs old. Since the metabolism of sulfite is light-dependent and requires several enzymes, in dark conditions sulfite is accumulated, consequently, when undeveloped chloroplast is treated with sulfite the pH in the stroma increases (Plans et *al.*, 1987), ⊿pH is enlarged, and qE increases. Therefore, after sulfite treatment in dark conditions, Fv showed little change. Due to the decreased qP and increased qE in dark conditions, the process after the PQ pool or thylakoid membrane was more affected than the PSⅡ reaction center. Perhaps this resulted from a combination of the direct reaction between the accumulated sulfite and photosynthetic complex, and the indirect effect from the change of pH. Once again, the electron transport within the PSⅡ reaction center was not inhibited yet the redox of Q_A was, and the excited energy which reached the reaction center was lost through a nonphotochemical method and fluorescence. In particular, the value of qP decreased seriously after treatment with sulfite compared with that of the control with or without light. This means that sulfite inhibits the redox of Q_A with or without light, thus the redox of Q_A is inhibited by sulfite treatment in light or dark conditions.

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