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Sulfate Inhibition of Photosystem II Oxygen Evolving Complex

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ABSTRACT

Effect of sulfate on the oxygen evolution in barley Photosystem II membrane fraction was investigated. Purified Photosystem II membrane fraction was exposed to sulfate effect in dark or under dim light illumination. The presence of 10 mM $CaCl_2$ during the sulfate treatments prevented the loss of oxygen evolution. The protection was complete even at the concentration of 50 mM Na_2SO_4. However, light stimulated sulfate inhibition of oxygen evolution by the Photosystem II. After incubation with sulfate, we found there was depletion of 18 and 23 kDa polypeptides in Photosystem II complex. Here we provide new evidence that sulfate inhibition of oxygen evolution can be caused by depletion of chloride and calcium ions from the water splitting complex rather than by partial depletion of 18 and 23 kDa polypeptides from the complex.

Index Entries: Photosystem II; water splitting complex; sulfate inhibition; chloride; calcium; Photosystem II polypeptides; sulfur dioxyde effect.

INTRODUCTION

Extensive studies have been made on the effect of SO_2 on the physiological processes of plants (*1,2,3*). The light reactions of photosynthesis are known to be sensitive target of SO_2 inhibitory effect (*4,5,6,7,*). Ab-

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sorbed SO_2 in leaves is converted to bisulfite, sulfite, and sulfate anions in the plant cell (*5,8,9*). It is known that the stable sulfate anion accumulates in the plant cytosol. Some experiments showed that after SO_2 fumigation, sulfate is hardly eliminated from cells (*8,10*). Accumulation of sulfate in the cell should lead to presence of sulfate in the chloroplast (*11*). Although sulfate has been found to interfere with chloroplast activity, the site of its action in the chloroplast membrane is not precisely identified (*5,9*). There is some indication that Photosystem II (PSII) could be sensitive to sulfate accumulation in chloroplasts (*12*). In this report we attempt to characterize and localize the effect of sulfate on PSII. We used PSII membrane fraction to study the effects of sulfate on the structural and functional components of PSII complex. Taking into account previously reported data on SO_2 we provide new evidence that the effect of sulfate on PSII activity can be a part of SO_2 effect on plants.

METHODS

Barley thylakoids isolated as in (*13*) were resuspended in 0.4 M sucrose, 15 mM NaCl, 5 mM $MgCl_2$, 40 mM 2 [*N*-morpholino] ethanesulfonic acid (Mes) (pH 6.0), and Triton X-100 (20 mg/mg Chl) to a concentration of 1 mg chlorophyll/mL. The PSII membrane fraction was prepared by the procedure of Ikeuchi and Inoue 1986 (*14*), for which we modified time of Triton treatment and forces of centrifugation. After a 15 min incubation in the medium with Triton, coarse fragments and starch were removed from the mixture by centrifugation (4000 g for 4 min). The supernatant was centrifuged (35000 g for 35 min) to pellet the PSII membrane fraction. The particles were suspended in the same medium without Triton X-100 and kept stored on ice in the dark. All steps of this procedure were performed under dim light or in darkness at 0°C. These particles showed an oxygen evolution activity of 650–800 μmol O_2/mg Chl h with 2,5-dichlorobenzoquinone (DCBQ) as electron acceptor.

For sulfate treatments, samples of PSII membrane fraction containing 200 μg Chl was incubated in 1 mL of the assay medium consisting of: 0,4 M sucrose, 40 mM Mes (pH 6.0), and 0.1% digitonine and different concentrations of sodium sulfate. When indicated, calcium chloride was added to the assay medium by injecting 10 μL of freshly prepared 1 M $CaCl_2$ solution. All incubation treatments were done at 25°C and lasted 5 h. After the treatments, the particles were spun down and then resuspended in assay medium free of sulfate. Oxygen evolution was measured with a Clark-type electrode (*15*) at 23°C in an assay medium with Chl concentration of 10 μg/mL.

One minute before the measurements of oxygen evolution, 1 mM $CaCl_2$ was added to the PSII sample. DCBQ was used as an electron acceptor at a concentration of 350 μM.

Sodium docecyl sulfate (SDS) polyacrylamide gel electrophoresis was carried out in a slab gel apparatus using a discontinuous buffer system as

described in (*16*) with our modifications: 6 M urea was added to the gel and polyacrylamide concentration of 15% was used in the resolving gel. A Clifford Densicomp model-445 scanner was used to detect bands of protein colored with Coomassie brilliant blue R-250. Changes in protein amount were evaluated by comparing the peak area obtained from the protein bands, after they have been previously normalized to the peak area of 28 kDa protein band. Chlorophyll concentration was evaluated by the method of Arnon (*17*).

RESULTS

The oxygen evolution of PSII particles was measured after 5 h of incubation in the dark or under illumination with different concentrations of sulfate. Figure 1 shows the effect of sulfate on the oxygen evolution measured after dark incubation. The presence of 10 mM $CaCl_2$ during the sulfate treatments prevented the loss of oxygen evolution. The protection was complete even at the concentration of 50 mM Na_2SO_4. In the absence of $CaCl_2$ the sulfate inhibition of oxygen evolution was evident. At 50 mM sulfate, only 40% of oxygen evolution remained, compared to the control.

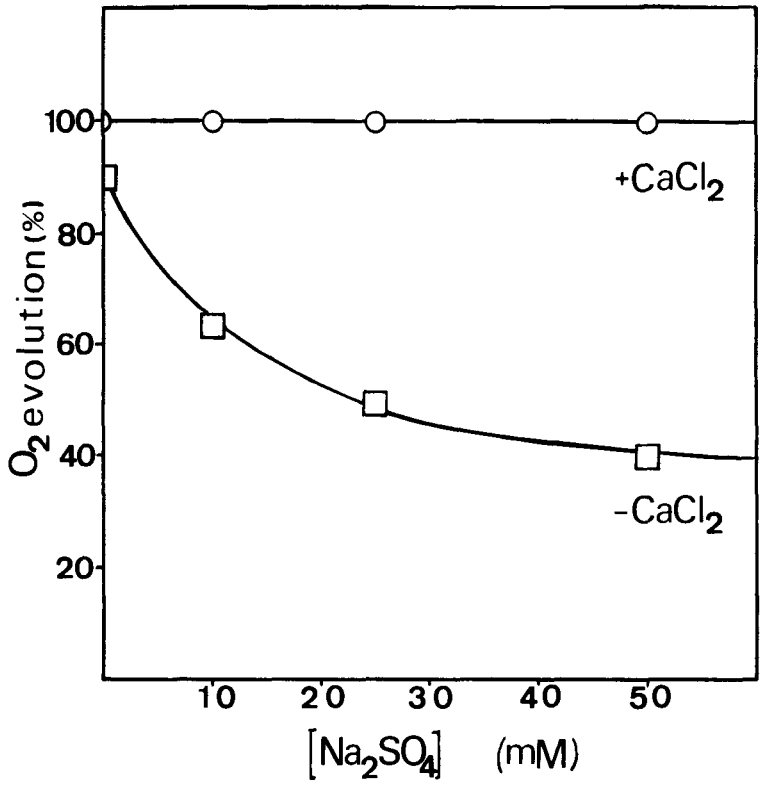

Fig. 1. Sulfate inhibition of oxygen evolution by PSII particles. The oxygen evolution of PSII particles incubated with 10 mM $CaCl_2$ (circles) or without (squares) during the sulfate treatment. *See* details in Methods.

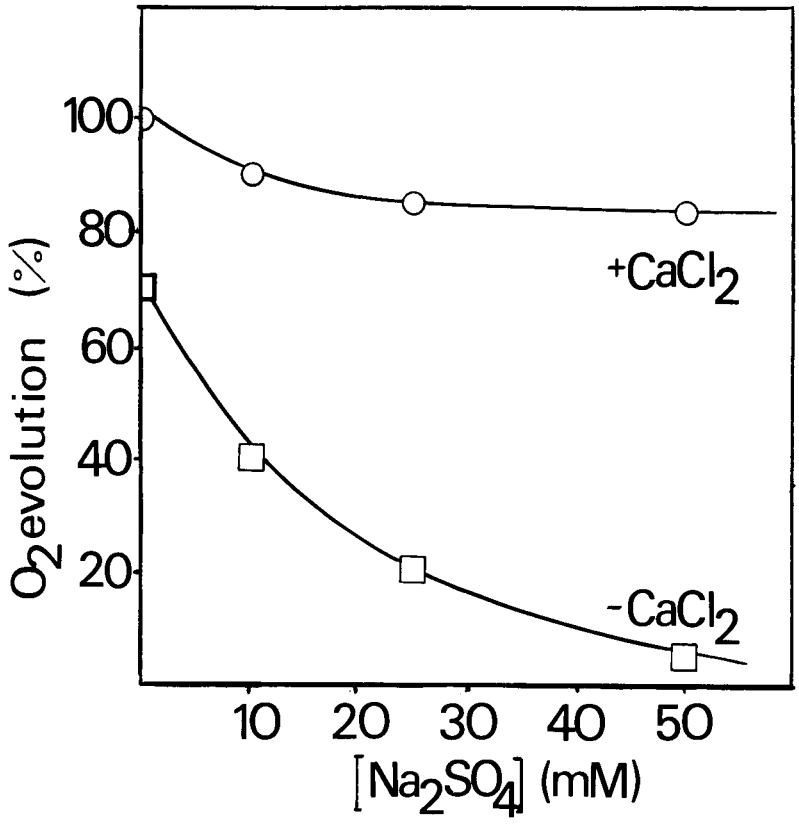

Fig. 2. Effect of light on sulfate inhibition of PSII oxygen evolution. Sample of PSII were treated with sulfate under dim illumination. Oxygen evolution of PSII incubated with 10 mM $CaCl_2$ (circles) or without (squares) during the treatment. *See* details in Methods.

The effect of light on the sulfate inhibition was studied by exposing the sample to dim light (1 mW/cm^2) during the sulfate treatments. A low light intensity was used to avoid photoinhibition effect on the PSII activity. Figure 2 shows the effect of sulfate treatments with illumination on the oxygen evolution. The results demonstrate the sulfate inhibitory effect, either in the absence or presence of 10 mM $CaCl_2$. The inhibition of oxygen evolution by sulfate treatments in the presence of $CaCl_2$ was 15% compared to the control sample. However, in the absence of $CaCl_2$, the rate of oxygen evolution was inhibited by 95% compared to the control.

To localize the effect of sulfate on PSII structure, we studied the polypeptide composition after sulfate treatments. Figure 3 shows densitograms of colored protein bands separated by SDS-polyacrylamide gel electrophoresis. The control sample profile is shown in Fig. A, whereas the sample treated with 50 mM sulfate is presented in Fig. B. The comparison of the gel densitograms demonstrates a relative decrease of 18 and 23 kDa polypeptides after sulfate treatments. It appears that sulfate directly effects the oxygen evolution system since these two polypeptides are parts

of the water splitting complex of PSII. Table 1 shows the approximative depletion of these proteins as evaluated from the gel densitograms for each concentration of sulfate used. As seen, no depletion of polypeptides was observed under 25 mM sulfate concentration. The extent of removal of both proteins remained unchanged whether $CaCl_2$ was present or not during incubation. Nor was any effect on light on protein depletion observed (data not shown). These results show that the depletion of the 18 and 23 kDa polypeptides from PSII complex does not strongly inhibit ox-

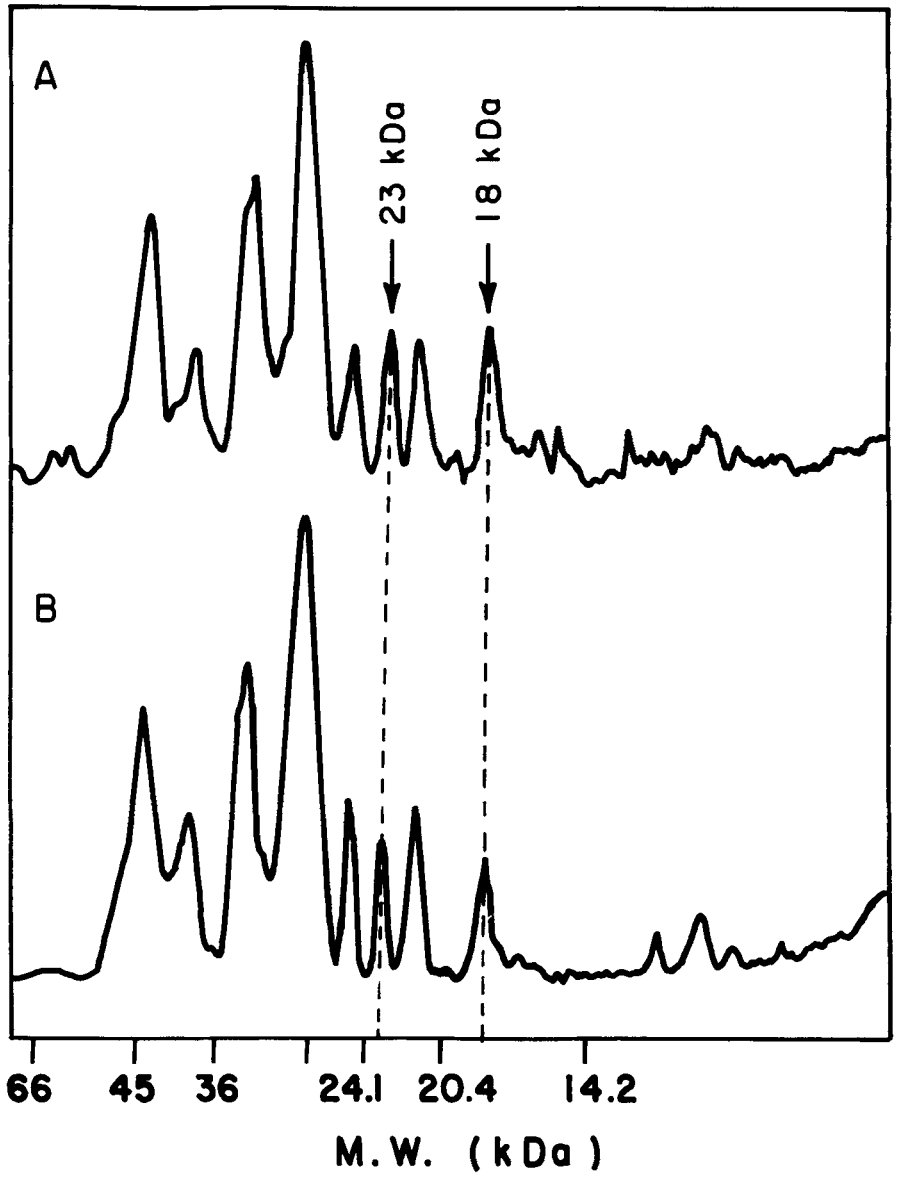

Fig. 3. Polypeptides depletion in the PSII complex by sulfate incubation of PSII particles. A) gel densitogram of the control sample and B) gel densitogram of PSII particles treated with 50 mM sodium sulfate. *See* details in Methods.

Table 1
Effect on Sulfate Incubation on the 23 kD and 18 kD Proteins[a]

Na_2SO_4, mM	18 kD, %	23 kD, %
0	100	100
10	100	100
25	80	90
50	40	65

[a]Values indicate the percentage of proteins remaining after the treatment compared to the control sample. These values are evaluated from the densitograms of the gels, as indicated in Methods.

ygen evolution if 10 mM $CaCl_2$ is present during the incubation. Figure 4 represents the comparison of data on PSII oxygen evolution with depletion rates for 18 and 23 kDa proteins affected by sulfate treatments. It is evident that there is not complete interdependence between the inhibition of the oxygen evolution and the PSII polypeptide depletion rate

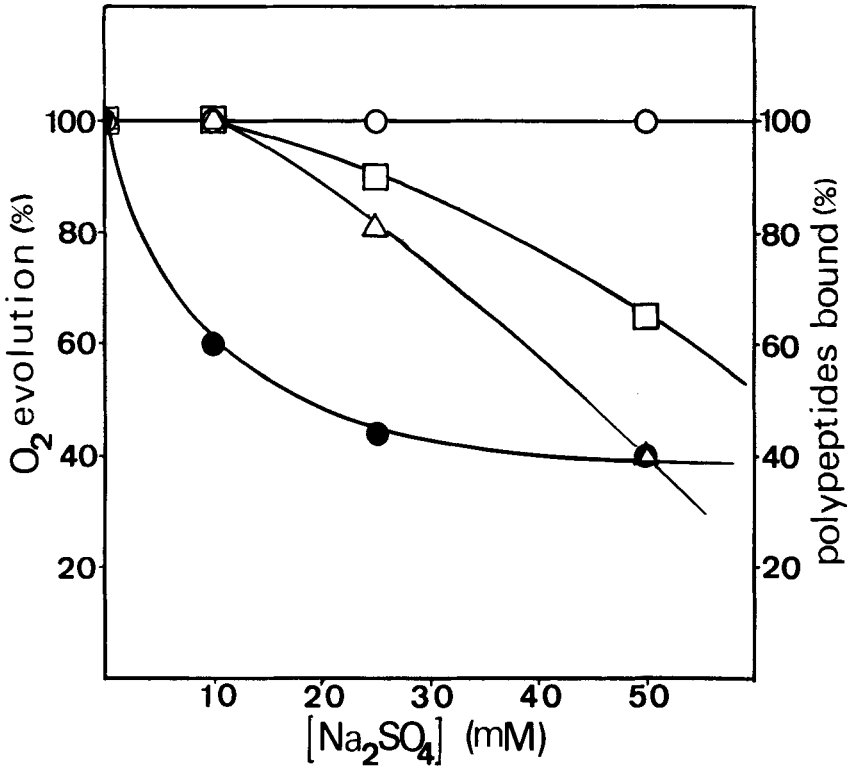

Fig. 4. The comparison of polypeptides depletion with the inhibition of oxygen evolution in PSII particles. The oxygen evolution after sulfate treatment is indicated by dark circles. Open circles represent the oxygen evolution in PSII particles treated with sulfate in the presence of 10 mM $CaCl_2$. Squares represent percentage of 23 kD protein bound to the PSII and triangles indicate percentage of 10 kD remained bound after sulfate treatment. *See* details in Methods.

caused by the sulfate incubation. This indicates the complexity of the sulfate effects on the functions and structure of PSII components.

DISCUSSION

In our study, we found evidence of sulfate inhibition of the oxygen evolution in PSII membrane fraction. This inhibition was increased by light, but suppressed in the presence of $CaCl_2$. It was previously reported that light can stimulate the sulfate competition with chloride ions for a chloride binding site in the PSII complex (*12,21*). It appears that the addition of 10 mM $CaCl_2$ provided sufficient chloride to compete with sulfate and maintain the activity of the water splitting complex. In a recent report, the protective status of chloride was interpreted to be owing to its influence on the structure of the water splitting complex (*22*).

Furthermore, we observed the depletion of 18 and 23 kDa in the PSII complex after 5 h incubation with a sulfate concentration higher than 10 mM. These polypeptides are both part of the water splitting complex and are attached to the inner surface of the photosynthetic membrane. They can be easily removed from the membrane by treatments with high concentration of salts (*23,24*). It thus appears that sulfate incubation released the proteins by increasing the ionic strength of the medium. The loss of 18 and 23 kDa polypeptides from the PSII complex results in a decrease of oxygen evolution (*25,26*). It was assumed that the role of the two polypeptides is to provide the necessary concentration of chloride and calcium for PSII functions (*26,27*). The depletion of these calcium and chloride "concentrator" polypeptides will consequently decrease the concentration of these ions near PSII; it can thus participate in the inhibition of the oxygen evolution (*28,29*). Our study indicated that the sulfate inhibition of oxygen evolution was induced by the loss of chloride and calcium closely associated with PSII rather than by the partial depletion of 18 and 23 kDa polypeptides.

SUMMARY

We conclude that sulfate treatments of PSII membrane fraction can induce two effects related to the water splitting complex. The first is the inhibition of the oxygen evolution activity dependent on chloride and calcium ions. This inhibition was stimulated by light and suppressed by the presence of 10 mM $CaCl_2$. The second sulfate effect on PSII membrane fraction, shown for the first time, was the partial depletion of 18 and 23 kDa polypeptides from the water splitting complex. These conclusions consequently contribute to explain some earlier observations of sulfur effect on plants in in vivo conditions. It was found for plants exposed to SO_2 that sulfate accumulated in cytosol cannot be easily eliminated (*8,10*).

The chloroplasts isolated from SO_2 treated plants show low activity of the water spliting system (*4*). It has also been reported that the addition of chloride to chloroplasts isolated from SO_2 treated plants can recover the oxygen evolution activity (*1*). Therefore, our data on sulfate inhibition of the oxygen evolution and protective role of $CaCl_2$ are in agreement with these reports.

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