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Interactions of Chloride and Formate at the Donor and the Acceptor Side of Photosystem II

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Chloride is required for the maximum activity of the oxygen evolving complex (OEC) while formate inhibits the function of OEC. On the basis of the measurements of oxygen evolution rates and the S_2 state multiline EPR signal, an interaction between the action of chloride and formate at the donor side of PS II has been suggested. Moreover, the $Fe^{2+}Q_A^-$ EPR signals were measured to investigate a common binding site of both these anions at the PS II acceptor side. Other monovalent anions like bromide, nitrate etc. could influence the effects of formate to a small extent at the donor side of PS II, but not significantly at the acceptor side of PS II. The results presented in this paper clearly suggest a competitive binding of formate and chloride at the PS II acceptor side.

KEY WORDS: Chloride; formate; electron paramagnetic resonance; photosystem II.

INTRODUCTION

Photosystem II (PS II), an integral membrane protein complex in chloroplast thylakoid membrane, carries out the initial steps in oxygenic photosynthesis. The oxygen evolving complex (OEC) of PS II requires calcium and chloride ions for its maximum activity. Chloride can be replaced by other monovalent anions like bromide (Ono *et al*., 1987).

Photosystem II mediates a four electron reaction as it cycles through five oxidation states called S states (S_n to S_{n+1}). The dark stable state is S_1 and after a single saturating flash of light, the S_2 state is generated. Chloride is bound near the OEC has been suggested by the fact that Cl^--depleted PS II membranes exhibit altered S_2 state EPR signals (Ono *et al*., 1986). It has been shown that the binding affinity for chloride depends on the oxidation state of the OEC, for which the affinity is lower in the S_2 state than in the S_1 state (Wincencjusz *et al*., 1998). S_2 state is characterized by a S_2 state multiline (ML) signal and a $g = 4.1$ signal. The spectral characteristics of the ML signal reflect the strength with which the Mn ions of the Mn complex are coupled with one another (Miller and Brudwig, 1991).

Quinone A (Q_A) is an electron acceptor in PS II. Photoreduced Q_A is a semiquinone anion radical and interacts with a nearby Fe^{2+} ion to produce an EPR signal at $g = 1.90$ and 1.64 or a narrower signal at $g = 1.82$ and 1.64. These two signals represent alternate forms of the $Fe^{2+}Q_A^-$ base pair, and are best observed at liquid helium temperature around 4–10 K (Miller and Brudwig, 1991).

The $Fe^{2+}Q_A^-$ EPR signal is however intrinsically small and it is difficult to quantify observed changes. No significant $Fe^{2+}Q_A^-$ EPR signal has been observed in Cl^--depleted and Cl^--sufficient PS II membranes, assuming that the PS II acceptor side is not affected by the presence or absence of chloride. On the other hand, formate anion has been shown to have effects both on the donor and the acceptor side of PS II (Feyziev *et al*., 2000).

Formate competes with the bicarbonate for the binding to the non-heme iron at the acceptor side of PS II and slows down the electron transfer from Q_A^- to Q_B (Petrouleas and Diner, 1990; Deligiannakis *et al*., 1994; Hienerwadel and Berthomieu, 1995). A role of chloride in reversing the inhibitory effects of formate and nitrite has been reported. Based on the measurement of Hill activity

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and chlorophyll *a* fluorescence, chloride was suggested to help bicarbonate to reverse the inhibitory effects of formate and nitrite on PS II (Jajoo and Bharti, 1993). Competition between formate and certain organic anions like glyoxylate, oxalate and glycolate has been shown by measuring the $Fe^{2+}Q_A^-$ EPR signal (Petrouleas *et al*., 1994). Competitive binding of acetate and chloride has been suggested on the PS II (Kuhne *et al*., 1999). They confirmed the existence of a chloride-insensitive acetate-binding site on the acceptor side and a chloride-sensitive acetate-binding site at the donor side of PS II. A possibility for the formate to replace chloride at the donor side of PS II was suggested (Feyziev *et al*., 2000).

This work has been carried out in order to confirm any interaction between formate and chloride at the donor and the acceptor sides of PS II. In this paper, we have tried to explore the effects of presence of chloride on the formate-induced S_2 state and $Fe^{2+}Q_A^-$ EPR signals. Changes in the rates of oxygen evolution with formate and chloride under different conditions have been measured in order to confirm the replacement of formate by chloride at the PS II donor side. Chloride has also been found to affect the S_2 state multiline signal as well as the $Fe^{2+}Q_A^-$ EPR signal which was induced by formate treatment. The results presented here suggest a competition between formate and chloride for the binding site as well as a site of action of chloride at the non-heme iron at the PS II acceptor site. This study will give a new insight on the role of chloride at the acceptor side of PS II, which has never been reported earlier.

MATERIALS AND METHODS

Sample Preparations

Oxygen evolving PS II membranes were prepared from market spinach using method as described in Kuwabara and Murata (1982). The membranes were stored at 77 K in a suspending buffer (0.2 M sucrose, 20 mM MOPS, 20 mM NaCl, and 50% (v/v) glycerol at pH 6.8) at chlorophyll concentrations of approximately 6 mg/mL, determined according to Porra (1990) until use. All steps of preparations were performed under dim green light.

Formate Treatment

The membranes were washed with solution A [50 mM MES-NaOH (pH 6.5) and 35 mM NaCl] in order to remove glycerol and centrifuged at 35000 *g* for 20 min. The pellets were suspended in solution B [50 mM MES-NaOH (pH 6.5), 5 mM NaCl, 5 mM $CaCl_2$, 0.3 M sucrose, 1 mM EDTA] including various concentrations of sodium formate and centrifuged. These samples were incubated at 0°C under the dark for 30 min to equilibrate PS II in a dark stable S_1 state, and then stored in liquid nitrogen until EPR measurements.

Oxygen Evolution

The oxygen-evolving activity of PS II was measured using a Clark-type electrode at 20°C under continuous illumination at the saturating intensity through a Toshiba R50 filter and 8 cm thick water filter. In the presence of 600 μM PPBQ as an electron acceptor, the rate of oxygen evolution in control samples at pH 6.5 was about 400 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}.

EPR Measurements

CW-EPR measurements were performed using a Bruker-300E X-band spectrometer, and an ST4102 standard cavity. An Oxford-900 continuous gas-flow cryostat and ITC-4 temperature controller were used to regulate the sample temperature at 6.0 K. Samples were illuminated with 500 W tungsten-halogen lamp at 200 K through an 8 cm thick water filter to observe the S_2 state EPR signals at 6 K.

RESULTS AND DISCUSSION

Chloride and formate both have been shown to have distinct effects on the donor side of PS II. The possibility that whether they interact with each other at the PS II donor side and influence the activity of the OEC in the presence of each other, was investigated. Table I shows the rates of oxygen evolution with different concentrations of formate and chloride under different conditions. Formate inhibits the activity of the OEC while chloride promotes the activity of the OEC, in a concentration dependent manner. When formate is added first to the medium and the chloride added later, predominant effects of chloride are observed, suggesting that chloride has been able to replace formate quite effectively. When chloride was added first to the medium and the formate added later, only little inhibition in the effects of chloride was observed, suggesting that formate does affect the binding of chloride in PS II but is unable to replace it significantly. The inhibitory effects of formate were largely masked by the presence of chloride. When equal concentrations (100 mM) of formate and chloride are added together, predominant effects of

Table I. Rates of Oxygen Evolution as Measured in Terms of μmole Oxygen Evolved in Formate and Chloride-Treated PS II Membranes

Treatment	μmol of O_2 evolved (mg Chl)$^{-1}$ h^{-1}
Control	400 ± 18 (100)
A.	
+50 mM $NaHCO_2$	216 ± 10 (54)
+100 mM $NaHCO_2$	160 ± 09 (40)
+250 mM $NaHCO_2$	116 ± 09 (20)
B.	
+50 mM NaCl	684 ± 18 (171)
+100 mM NaCl	860 ± 15 (215)
+250 mM NaCl	908 ± 18 (227)
C. Formate added and incubated first and chloride added later	
+100 mM $NaHCO_2$ + 50 mM NaCl	444 ± 11 (111)
+100 mM $NaHCO_2$ + 100 mM NaCl	572 ± 12 (143)
+100 mM $NaHCO_2$ + 250 mM NaCl	636 ± 12 (159)
D. Chloride added and incubated first and formate added later	
+100 mM NaCl + 50 mM $NaHCO_2$ added later	800 ± 15 (200)
+100 mM NaCl + 50 mM $NaHCO_2$ added later	764 ± 15 (191)
+100 mM NaCl + 50 mM $NaHCO_2$ added later	704 ± 17 (176)
E. 100 mM NaCl +100 mM $NaHCO_2$ added together	684 ± 22 (171)

Note. The normalized values have been written in the brackets. The assay medium consisted of 50 mM MES-NaOH (pH 6.5), 5 mM NaCl, 5 mM $CaCl_2$, 0.3 M sucrose, 1 mM EDTA with 600 μM PPBQ as an electron acceptor.

chloride are observed. Formate-treated PS II membranes were treated with other monovalent anions like bromide, iodide and nitrate, in order to test the specificity of the above-noted chloride effects. Rates of oxygen evolution were measured in the presence of 100 mM formate and 100 mM anion (Table II). It is clear from the results that when formate and chloride are added together, the effects of chloride predominate. Bromide also acts in almost similar manner. Iodide and nitrate inhibit the rates of oxygen evolution and the inhibition is found to be more pronounced in the presence of formate. Thus at the donor side of PS II, interaction of different anions is evident. There seems to be a common binding site which has different binding affinities for various anions, being maximum for chloride.

Table II. Rates of Oxygen Evolution as Measured in Terms of μmole Oxygen Evolved in Formate and Anion-Treated PS II Membranes

Treatment	μmol of O_2 evolved (mg Chl)$^{-1}$ h^{-1}
Control	400 ± 18 (100)
100 mM $NaHCO_2$ alone	160 ± 9 (40)
100 mM NaCl alone	860 ± 15 (215)
100 mM NaCl +100 mM $NaHCO_2$	684 ± 17 (171)
100 mM NaBr alone	805 ± 12 (200)
100 mM NaBr +100 mM $NaHCO_2$	320 ± 5 (80)
100 mM NaI alone	183 ± 5 (46)
100 mM NaI +100 mM $NaHCO_2$	152 ± 4 (38)
100 mM $NaNO_3$	290 ± 5 (72)
100 mM $NaNO_3$ +100 mM $NaHCO_2$	200 ± 5 (50)

Note. The formate and the anions were added together. The normalized values have been written in the brackets. Assay medium same as in Table I.

These observations do suggest an interaction between formate and chloride (and other anions too) and a common anion binding site at the donor side of PS II. It is also inferred from the above data that formate and chloride compete for the same binding site, which preferentially binds to chloride. These observations led us to confirm the effects at the donor side by EPR spectroscopy and to investigate any type of interaction between formate and chloride at the acceptor side of PS II too.

The charge separation at 200 K produces the S_2 state multiline and a $g = 4.1$ EPR signal from the Mn_4 cluster as well as the Q_A^- Fe^{2+} EPR signal.

In chloride sufficient sample, a large ML signal between 2500 and 4000 G and a $g = 4.1$ signal has been observed between 1200 and 2200 G. In chloride-depleted PS II membranes, almost no ML signal was photo induced and only a broad signal at $g = 4.1$ was observed (data not shown). The $Fe^{2+}Q_A^-$ EPR signal is however intrinsically small and it is difficult to quantify observed changes. The effects on chloride depletion and re-addition of chloride and other anions on the $Fe^{2+}Q_A^-$ signals are unnoticeable.

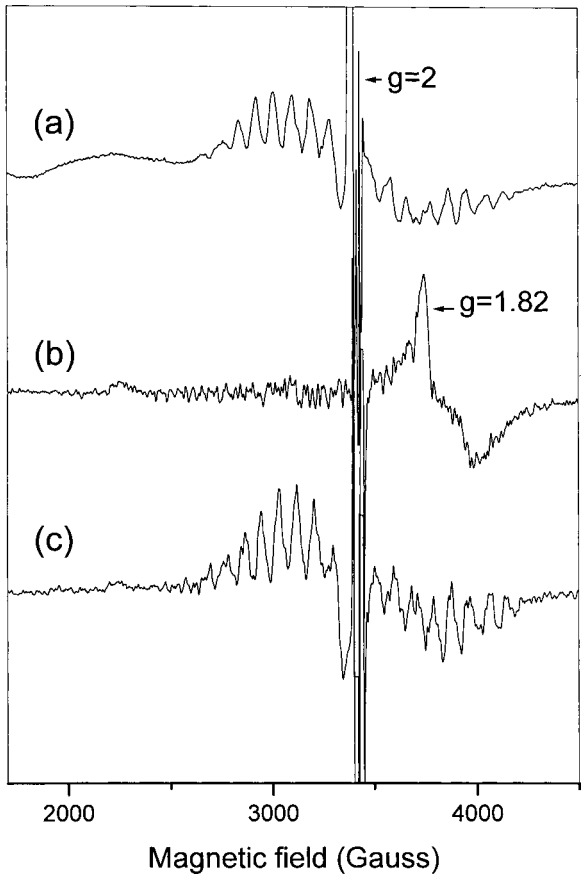

Fig. 1. S_2 state EPR spectra of untreated (a), 100 mM formate-treated (b), 100 mM chloride-treated (c), PS II membranes. Chlorophyll concentrations used are 8–10 mg/mL. All spectra are the (200 K illuminated-minus-dark) difference spectrum. Experimental conditions: microwave power 3.2 mW, modulation amplitude 10 G, temperature 6 K. Each of the spectra is a result of four scans.

Significant $Fe^{2+}Q_A^-$ EPR signal has been observed only in case of formate-treated PS II membranes.

Figure 1 shows the S_2 state EPR signal in control (untreated), formate-treated and chloride-treated PS II membranes. Increase in the amplitude of the ML signal and no $Fe^{2+}Q_A^-$ EPR signal has been observed in case of chloride-treated PS II membranes, while a distinct $Fe^{2+}Q_A^-$ EPR signal with the reduced ML signal amplitude has been observed in case of the formate-treated PS II membranes. Binding of formate stimulates the EPR signal at $g = 1.82$ due to photoinduced $Fe^{2+}Q_A^-$ state of the PS II acceptor complex (Vermaas and Rutherford, 1984).

In order to link these results with specific binding sites on the donor and acceptor sides of PS II, the S_2 state multiline signal and the $Q_A^-Fe^{2+}$ EPR spectra were measured as a function of formate concentration in the presence and absence of chloride. Formate inhibited the formation of the S_2 state multiline signal concomitant with stimulation of the $Q_A^-Fe^{2+}$ signal observed at $g = 1.82$. A concentration response of formate on the S_2 state EPR spectra and the $Q_A^-Fe^{2+}$ signal obtained in the absence of any additional chloride (Fig. 2A) and in the presence of additional 100 mM chloride has been measured. All spectra were (200 K illuminated-minus-dark) difference spectra and their intensities have been normalized by the $Y_D^\bullet$ radical signal. A pronounced formate effect as evident from a strong $Q_A^-Fe^{2+}$ signal and a reduced ML has been observed in the absence of any additional chloride. On the other hand, in the presence of additional 100 mM chloride in the medium, formate effects on the ML and the $Q_A^-Fe^{2+}$ signal seems to be subdued. The yield of the S_2 state ML signal decreases with increase in formate concentration, and the effects are modulated by chloride, as evident from an increased ML signal after addition of chloride. In the same way, the yield of the $Q_A^-Fe^{2+}$ EPR signal increases in the presence of formate, but is affected by the presence of chloride. This suggests interference of chloride with the formate for binding to the donor as well as the acceptor side of PS II.

In order to investigate the binding affinity of chloride or formate on the PS II donor and acceptor sides, we added one anion, incubated it in dark for 30 min, and then added the other anion. The S_2 state ML and the $Fe^{2+}Q_A^-$ EPR signals were then measured in such samples (Figs. 3A and 3B). Figure 3A shows the S_2 state ML and the $Fe^{2+}Q_A^-$ EPR signals in the PS II membranes which were first treated with formate (100 mM) and different concentrations of chloride anion were then added to it. The results show that chloride is largely able to reverse the effects of formate, as shown by a gradual increase in the ML intensity and a decrease in the $Fe^{2+}Q_A^-$ EPR signal, with the increase in the concentration of chloride. This shows that chloride is able to remove formate from the binding site to a large extent. On the other hand, when chloride (100 mM) was added first to the PS II membranes and the formate (varying concentrations) added later, formate was unable to induce its characterstic EPR signals (Fig. 3B). A very small $Fe^{2+}Q_A^-$ EPR signal has been induced at 250 MM formate. The results indicate a relatively tight binding of chloride to the binding site. A competition between formate and chloride for binding at the acceptor side of PS II has been reflected in the $Q_A^-Fe^{2+}$ EPR signal. The effect of other monovalent anions like bromide, nitrate etc. on the EPR signals of PS II membranes was also investigated (Fig. 4). Formate (100 mM) was added first to the PS II membranes and the other monovalent anions (100 mM) added later. In order to make a clear comparison, results of 100 mM chloride (from Fig. 3A) have also been included. It is evident from the results that only chloride is capable

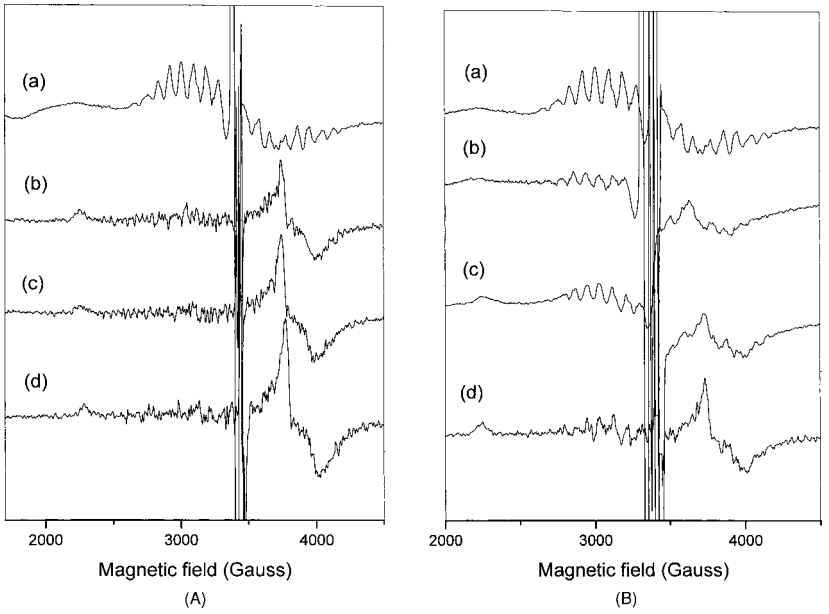

Fig. 2. S_2 state EPR spectra after treating with various concentrations of formate in the absence (A) and presence (B) of chloride (100 mM). Untreated (a), 50 mM formate treated (b), 100 mM formate treated (c), 250 mM formate treated (d). All other experimental conditions are same as described in the legend of Fig. 1.

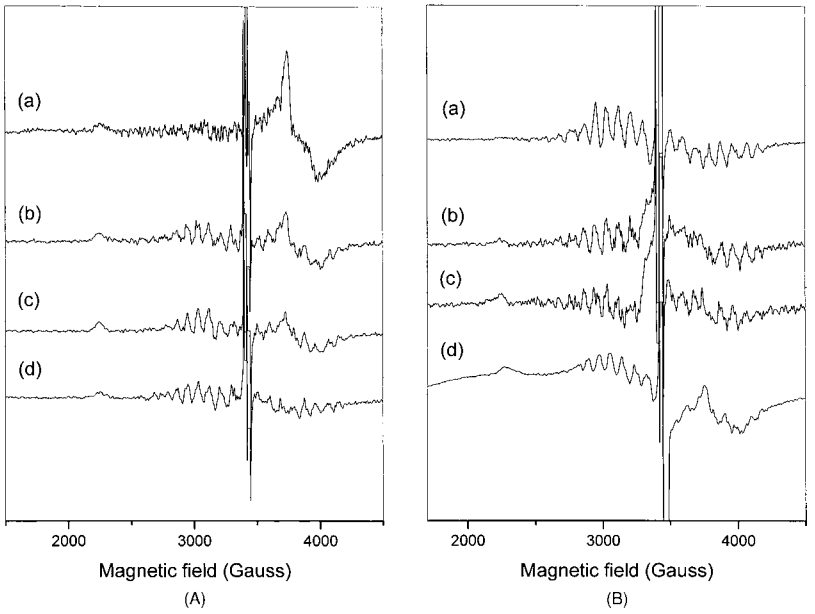

Fig. 3. S_2 state EPR spectra after treating PS II membranes with (A) Formate (100 mM) added first, chloride added later in varying concentration; 100 mM formate alone (a), 100 mM formate + 50 mM chloride (b), 100 mM formate + 100 mM chloride (c), 100 mM formate + 250 mM chloride (d). (B) Chloride (100 mM) added first and formate added later in varying concentrations; 100 mM chloride alone (a), 100 mM chloride + 50 mM formate (b), 100 mM chloride + 100 mM formate (c), 100 mM chloride + 250 mM formate (d). All other experimental conditions are same as described in the legend of Fig. 1.

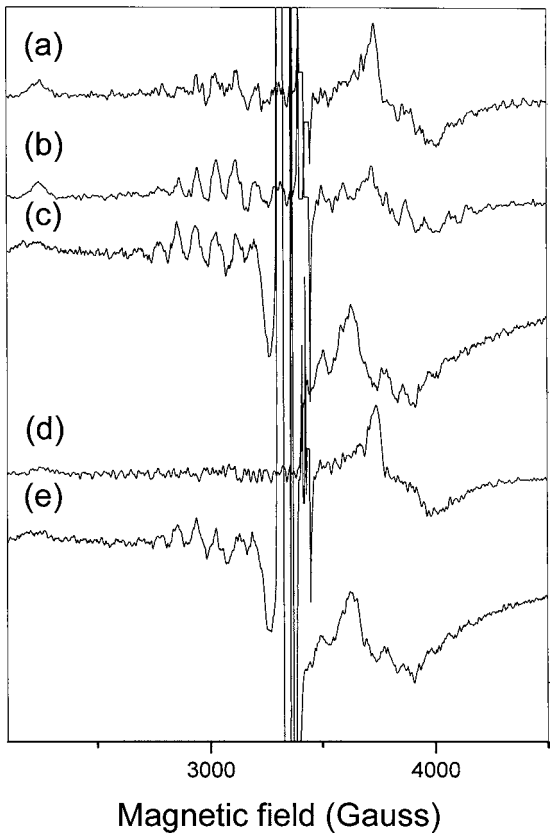

Fig. 4. S_2 state EPR spectra after treating PS II membranes with 100 mM formate first (a) and 100 mM other monovalent anions like chloride (b), bromide (c), iodide (d), nitrate (e) added later. Other experimental conditions are same as described in the legend of Fig. 1.

of significantly reversing the formate-induced changes in the S_2 state ML and the $Q_A^-Fe^{2+}$ EPR signal, while other anions fail to do so. Small increase in the amplitude of S_2 state ML has been observed with other monovalent anions, but the $Q_A^-Fe^{2+}$ EPR signal remained largely unaffected. These results provide evidence to support the idea that at the acceptor side of PS II, effects of chloride are quite specific, unlike its effects on the donor side of PS II.

Chloride-induced changes in the S_2 state ML and the $Fe^{2+}Q_A^-$ EPR signals in the formate-treated PS II membranes provides evidence for a competitive binding of chloride and formate for certain sites on PS II. Based on the EPR measurements, this study clearly indicates an interaction between the chloride and formate at the PS II donor and the acceptor side, which has not been reported earlier.

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REFERENCES

Deligiannakis, Y., Petrouleas, V., and Diner, B. A. (1994). *Biochim. Biophys. Acta* **1188**, 260–270.
Feyziev, Y. M., Yoneda, D., Yoshii, T., Katsuta, N., Kawamori, A., and Watanabe, Y. (2000). *Biochemistry* **39**, 3848–3855.
Hienerwadel, R., and Berthomieu, C. (1995). *Biochemistry* **34**, 16288–16297.
Jajoo, A., and Bharti, S. (1993). *Physiol. Plant.* **88**, 78–84.
Kuhne, H., Szalai, V. A., and Brudwig, G. W. (1999). *Biochemistry* **38**, 6604–6613.
Kuwabara, T., and Murata, N. (1982). *Plant Cell Physiol.* **23**, 533–539.
Miller, A. F., and Brudwig, G. W. (1991). *Biochim. Biophys. Acta* **1056**, 1–18.
Ono, T., Nakayama, H., Gleiter, H., Inoue, Y., and Kawamori, A. (1987). *Arch. Biochim. Biophys.* **256**, 618–624.
Ono, T., Zimmermann, J. L., Inoue, Y., and Rutherford, A. W. (1986). *Biochim. Biophys. Acta* **851**, 193–201.
Petrouleas, V., and Diner, B. A. (1990). *Biochim. Biophys. Acta* **1015**, 131–140.
Petrouleas, V., Deligiannakis, Y., and Diner, B. A. (1994). *Biochim. Biophys. Acta* **1188**, 271–277.
Porra, R. J. (1990). *Biochim. Biophys. Acta* **1019**, 137–141.
Vermaas, W. F. J., and Rutherford, A. W. (1984). *FEBS Lett.* **175**, 243–248.
Wincencjusz, H., Yocum, C. F., and Van Gorkom, H. J. (1998). *Biochemistry* **37**, 8595–8604.