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MANGANESE INDUCED PEROXIDATION OF THYLAKOID LIPIDS AND CHANGES IN CHLOROPHYLL-*a* FLUORESCENCE DURING AGING OF CELL FREE CHLOROPLASTS IN LIGHT

SUNAKAR PANDA, A. K. MISHRA and U. C. BISWAL
Laboratory of Biochemistry and Biophysics, Sambalpur University, Jyoti Vihar 768019, Orissa, India

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Key Word Index—*Triticum aestivum*; Graminae; wheat; isolated chloroplasts; aging, lipid peroxidation; manganese; chlorophyll-*a* fluorescence.

Abstract—Photoinduced peroxidation of thylakoid lipids in wheat chloroplasts is correlated with thylakoid membrane disorganisation as probed by Chl. *a* fluorescence during aging of cell free organelles. Manganese, at its different redox states, modulates lipid peroxidation differently with its consequent effect on Chl. *a* fluorescence.

INTRODUCTION

Lipid peroxidation is one of the major factors leading to the disorganisation of membrane structure [1] and loss in photochemical activities during aging of chloroplasts *in vitro* [2–5]. Cations, namely Ca^{2+} and Mg^{2+}, are known to enhance membrane lipid peroxidation by screening surface negative charge of the thylakoid membrane [6–8] which makes the membrane permeable to $\cdot O_2^-$ [9], the primary reactive species for peroxidation. Manganese ions could also screen the surface negative charge of the thylakoid membrane. But because of its variable multiple oxidation states, its action is likely to be different from that of other divalent cations like Ca^{2+} and Mg^{2+}. Effect of exogeneous Mn^{2+} on lipid peroxidation and the consequent effect of the process on thylakoid membrane disorganisation has not yet been systematicalally studied during aging although a preliminary report on its role in thylakoid lipid peroxidation is recently examined by us [10].

In this paper an attempt is made to examine whether there exists any correlation between the effect of exogenous Mn^{2+} at its different redox states on photoperoxidation of membrane lipids and structural disorganisation of thylakoid membrane in wheat chloroplasts as probed by Chl. *a* fluorescence.

RESULTS AND DISCUSSION

Aging of cell free wheat chloroplasts is characterized by rapid loss of DCPIP photoreduction by the isolated organelles [10]. Aging causes an increase in the level of thylakoid membrane lipid peroxidation (Table 1). Manganese, which exists as Mn^{2+} at neutral pH and in mixed oxidation state of Mn^{2+}, Mn^{3+} and Mn^{4+} at pH 9.0 [10, 11], stimulated peroxidation at both the pH 7.0 and 9.0 (Table 1). It is already proposed [10] that depending on the stability of $\cdot O_2^-$ at pH 7.0 and 9.0 [12], it can generate other potent species such as $^1O_2$, $\cdot OH$ and H_2O_2 in the reaction system leading to peroxidation. At pH 7.0, Mn^{2+} may be oxidised to Mn^{3+} by the oxidised species accumulated at the donor side of PS II with simultaneous generation of $\cdot O_2^-$. Mn^{3+}, being unstable may return to Mn^{2+} with oxidation of $\cdot O_2^-$ to $^1O_2$. At pH 9.0 photoinduced oxidation of Mn^{2+} to Mn^{4+} by the oxidised centers at the donor side of PS II [10] may also lead to the formation of $\cdot O_2^-$ which may give rise to H_2O_2 and $\cdot OH$ [10]. The immediate enhancement of lipid peroxidation at 3 hr in pH 9.0 may be attributed to the reaction of O_2^{2-} (peroxide) generated by the pH induced oxidation of Mn^{2+} to Mn^{4+} as shown in Eqn 1.

$$Mn^{2+} + O_2 + 4OH^- \rightarrow Mn(OH)_4 + O_2^{2-}. \quad (1)$$

The room temperature fluorescence spectra of freshly isolated chloroplasts either at pH 7.0 and 9.0 shows a distinct peak at 685 nm (F_{685}) and a shoulder at 735 nm (F_{735}) (Fig. 1). On aging of isolated chloroplasts, there is a significant decline of F_{685} and F_{735}. And at 6 hr of aging, F_{735} almost disappears which could be due to the faster disorganisation of PS I than PS II of thylakoid membranes. In addition, the decline of F_{685} is faster at pH 9.0 than at pH 7.0 (Table 2). This can be explained in the term of interaction between PS II reaction center and reactive oxygen species that participate in thylakoid lipid peroxidation. It is proposed that F_{685} emission may arise either from the fluorescence of an exciton reinjected back in PS II core antenna or from a direct decay of the excited reaction center of PS II (P_{680}^*) [13, 14]. At pH 7.0, the oxidised reaction centers generated by the damage of oxygen evolving system as reported by us earlier [10] may be utilised in oxidising $\cdot O_2^-$ to $^1O_2$. But at pH 9.0, the entry of the O_2^- to the side of PS II reaction centre at the inner side of thylakoid membrane is relatively less because of a negatively charged potential barrier at the outer surface of thylakoid. It seems that as aging proceeds, relatively more oxidised centers (D^+) accumulates at side of PS II at pH 9.0 leading to lowering of fluorescence emission at F_{685}.

In contrast, exogenous Mn^{2+} stimulates aging induced loss in F_{685} emission at pH 7.0 whereas at pH 9.0 it stimulates F_{685} only at the intial phase but retards F_{685} at 6 hr of aging. The Mn^{2+} induced decline of F_{685} emission could be either due to the accumulation of oxidised

Table 1. Effect of Mn^{2+} on malondialdehyde (MDA) formation during aging of cell free chloroplasts in light

Aging (hr)	pH 7.0 MDA (nM)		pH 9.0 MDA (nM)	
	Control	Mn^{2+}	Control	Mn^{2+}
0	1.94	1.94	1.94	1.94
3	4.64	6.97	5.66	10.31
6	7.42	14.51	6.91	12.50

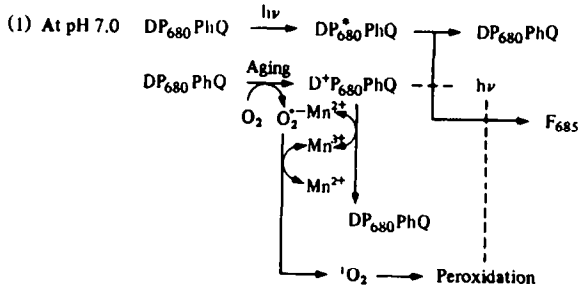
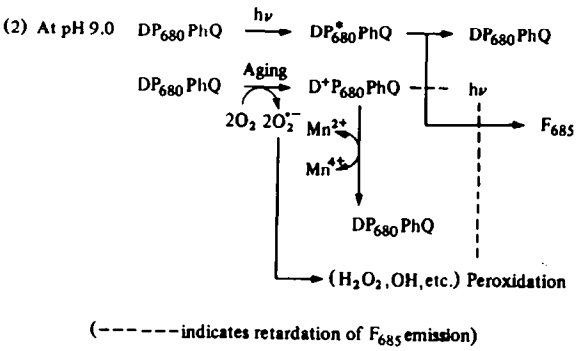


Table 2. Effect of Mn^{2+} on F_{685} emission during aging of cell free chloroplasts

Aging (hr)	pH 7.0 F_{685} (arbitrary units)		pH 9.0 F_{685} (arbitary units)	
	Control	Mn^{2+}	Control	Mn^{2+}
0	112	110	100	102
3	65.6	45.2	45.2	36.3
6	48.2	16	25.3	26.9



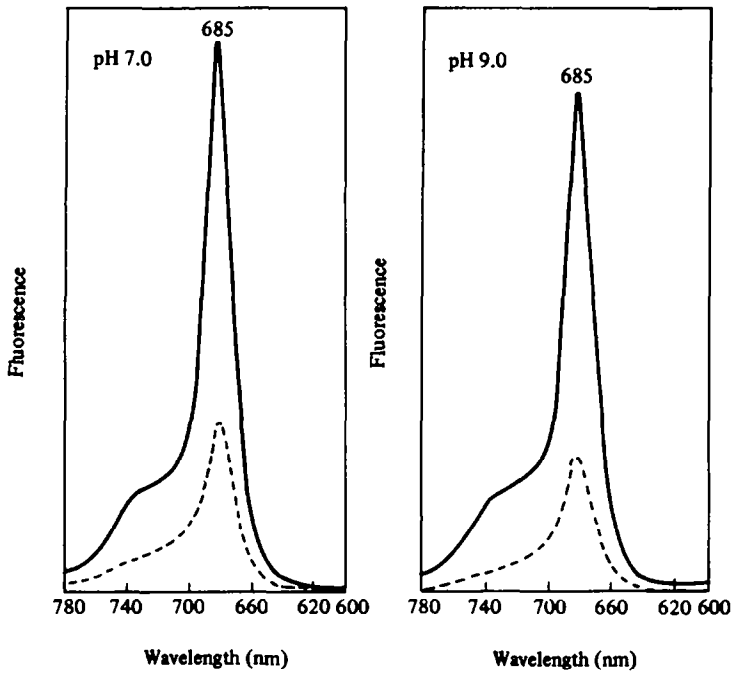


Fig. 1. Qualitative changes in Chl. *a* fluorescence spectra of isolated chloroplasts excited at 450 nm at pH 7.0 and 9.0. The chloroplasts equivalent to 5-7 μg/ml of chlorophyll in 50 mM Tris–HCl buffer containing 175 mM NaCl are taken for scanning. 0 hr aging (—), 6 hr of aging (---).

species at the donor side of PS II and/or peroxidation induced structural modification of thylakoid membranes. At pH 7.0, the reduction of oxidised centers at PS II (D^{+}) by the oxidation of Mn^{2+} to Mn^{3+} may not be irreversible because of the relative instability of Mn^{3+} [10] and such a process may logically lead to an accumulation of D^{+} resulting thereby a stimulation in the loss of F_{685} emission. At pH 9.0, photoinduced reduction of oxidised

centers at the donor side of PS II by the oxidation of Mn^{2+} to Mn^{4+} [9] is irrversible and such a process may reduce the effective concentration of D^+ resulting in a retardation in the loss of F_{685} emission.

At pH 7.0, Mn^{2+} induced generation of $^1O_2$ rapidly disorganises the thylakoid membrane system by peroxidation [10] which could lead to the lowering of F_{685} emission. But at pH 9.0, although Mn^{2+} induced peroxidation is significant at 6 hr of aging, the emission is retarded. A significant loss induced by Mn^{2+} in F_{685} emission at 3 hr could be correlated to high rate of peroxidation induced by $O^2{}_2{}^-$ (Eqn 1) formed at pH 9.0 [10]. At pH 9.0, it is proposed that both Mn^{2+} induced photoperoxidation and Mn^{2+} induced reduction of D^+ occur simultaneously and the peroxidation induced disorganisation predominates the other process at 3 hr. The manganese induced enhancement of lipid peroxidation and the consequent effect on the lowering of F_{685} emission at pH 7.0 and 9.0 may be proposed as in Scheme I.

EXPERIMENTAL

Chloroplasts were isolated in 50 mM Tris–HCl buffer containing 175 mM NaCl from the leaves of 7-day-old seedlings of wheat (*Triticum aestivum* L. emend Thell CV. Sonalika), grown under continuous illumination at $25 \pm 2°$ by the method described in ref. [15]. The chloroplasts were incubated in petri dishes (d = 5 cm) with or without $MnCl_2$ (5 mM) in continuous white light at $25 \pm 2°$. Aliquots were taken for different measurements.

Lipid peroxidation was measured by estimating MDA (malondialdehyde), by the method described is ref. [16]. Chlorophyll was estimated according to ref. [17].

The room temperature fluorescence spectra were measured by spectrofluorimeter (Hitachi model 650-40, Japan). The chloroplasts equivalent to 5–7 μg/ml of chlorophyll in Tris–HCl buffer were excited at 450 nm and the fluorescence emission was measured at 685 nm (F_{685}).

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