

THERMOLUMINESCENCE AND OXYGEN EVOLUTION IN JA-TREATED BARLEY (*Hordeum vulgare* L.)

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Received 27 July 1999

Summary. The effect of jasmonic acid on PSII reactions was assessed by changes in kinetic characteristics of O₂ evolution and thermoluminescence glow curves in barley leaves and isolated thylakoid membranes. In comparison to the control preparation, JA-treated samples showed reduced efficiency in the utilization of oxidizing equivalents generated at PSII reaction centres and retardation of S-state transitions. S₂ and S₃ states in JA-treated samples were found to be significantly unstabilized.

Key words: jasmonic acid, oxygen evolution, PSII, PSII OEC, S-state transitions, thermoluminescence, thylakoids

Abbreviations: JA – jasmonic acid, PSII – photosystem II, PSII OEC – purified PSII oxygen evolving complexes, S-states – oxidation states of the water splitting system, TL – thermoluminescence

Introduction

Endogenous plant growth regulator jasmonic acid (JA) and its methyl ester, JA-Me, are widespread in plants (Meyer et al., 1984; Sembdner et al., 1985), but little is known about their function and physiological action.

Some investigations suggest that one of the physiological functions of JA may be to directly or indirectly inhibit certain photosynthetic reactions (Popova et al., 1989). It was shown that plants treated with JA reveal changes in a number of photosynthetic parameters, such as decrease in the rate of photosynthetic CO₂ fixation and in the

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activity of Rubisco. There are considerable increases in the rates of dark respiration, in the CO₂ compensation point value and in stomata resistance (Popova et al., 1988).

Ensuing from the phytohormonal nature of JA and JA-Me, it is possible to speculate that these substances, much like a number of other phytohormones, have an indirect effect on photosynthesis, mediated by stomatal closure. However, not all changes observed can be ascribed to this effect alone. Popova et al. (1988) adduced experimental data about the direct effect of JA on the biosynthesis of the total soluble protein, in particular on Rubisco synthesis in barley leaves. JA influenced light induced reactions of photosynthesis and the function of PSII reaction centres (Maslenkova et al., 1990). Photochemical activity of PSII was markedly reduced in chloroplasts and purified PSII particles isolated from JA treated barley seedlings (Maslenkova et al., 1995). JA application to the growth medium of the plants leads to specific alterations in thylakoid polypeptide patterns (Maslenkova et al., 1992, 1995). The assumption being that the established inhibition in Hill reaction activity and the changes in kinetic behaviour of PSII O₂ evolving centres are determined by the observed structural reorganization of chloroplast membranes, leading to an increase in number of PSII_β centres in stroma partitions and an enhanced participation of a complementary cooperative mechanism for O₂ production (Zeinalov, 1982), which allows adaptation of the photosynthetic apparatus to stress conditions.

In the present study we have further investigated PS II in leaves and chloroplasts isolated from JA-treated barley seedlings in terms of the O₂ evolution under flash irradiation and thermoluminescence (TL) glow curves. Our observations show that JA treatment results in a reduced efficiency in the utilization of oxidizing equivalents generated at the reaction centres and in a retardation of S-state transitions. Furthermore, S₂ and S₃ states of JA treated samples were found to be significantly more unstable. These data are in accordance with the changes in polypeptide composition of chloroplasts, especially depletion in 24 and 17 kDa bands (Maslenkova et al., 1995). These proteins belong to the PSII O₂-evolving enzyme system and are assumed to be required for an effective turnover of higher S-states (Boussac et al., 1985).

Materials and Methods

Seeds of barley (*Hordeum vulgare* L. var. Alfa) were germinated and treated with 10 μM JA as previously described (Maslenkova et al., 1990). Fresh chloroplasts (thylakoids) were isolated from control and treated barley seedlings and were suspended in a medium containing 0.4 M sucrose, 10 mM NaCl, 5 mM MgCl₂ and 50 mM Tricine–NaOH pH 7.8. The O₂ flash yields were recorded at room temperature with a rate electrode of the Joliot type (Joliot et al., 1968). The turnover kinetics of the O₂ evolving centres were estimated using a slightly modified method (Kok et al., 1970; Bouges-Bocquet, 1973). Desactivation of the S₂ and S₃ states was investigated after

reaching steady state distribution according to the method of Forbush et al. (1971). Thermoluminescence measurements were made using a set up described by Ichikawa et al. (1975). After 1 h dark adaptation the samples – leaf peaces with equal size or chloroplasts suspension with 0.3 mg Chl per ml were exposed to short saturating flasher (8 μ s, 4J) at 5 °C. The heating rate was 1 °C/s. TL Q-band was obtained by infiltration of the leaves with 2.10⁻⁵M DCMU and flashing at -10 °C.

Results and Discussion

It was previously demonstrated that chloroplast membranes, isolated from JA-stressed barley plants have lower electron-transport capacity of PSII (Maslenkova et al., 1990, 1993). Like the control samples, oxygen flash yields in JA-treated samples exhibit a period of four oscillations, with a maximum on the third flash. 10 μ M JA appreciably lowered the amplitude of oxygen yields and accelerated damping of oscillation. Exogenous JA application brought about essential changes in the total number of active O₂ evolving centres and in their kinetic parameters, leading to the observed changes in the flash pattern. This behaviour may be explained by the data in Table 1, showing a rise in the values of “misses” (α) and of “double hits” (β). The total number of centres (S_0+S_1) is presented in relative units and shows a considerable decrease of nearly 60%.

Table 1. Effect of JA on TL characteristics and oxygen evolution in thylakoids, isolated from control and treated barley seedlings

Sample	S_0+S_1 (%)	Misses (α)	Double hits (β)	TL“B” band		TL“Q” band	
				ampl.	peak t°	ampl.	peak t°
Control	100	0.149	0.039	47.6	34.3	55.6	13.1
10 μ M JA	39.7	0.288	0.022	38.2	31.6	40.3	12.6

In order to obtain more precise information about the possible site(s) of inhibition of the PSII electron-transport chain we performed measurements of flash induced TL in paralel to O₂ evolving reactions.

Analyses of TL glow curves allow detection in the charge pairs, created as a result of electron transport from manganese to Q_A and Q_B independent of O₂ production. The glow curves of barley leaves when excited by one short flash exhibit a characteristic band, so-called B-band with a peak position at around 23 °C, which arises from the S₂Q_B⁻ recombination (Fig. 1A). It is generally accepted that peak position is a measure of energetic stabilisation of separated charges, while the peak amplitude of the B-band is proportional to the number of centres in S₂Q_B⁻. When DCMU is added to block electron transport between Q_A and Q_B another band, the so-called Q-band is

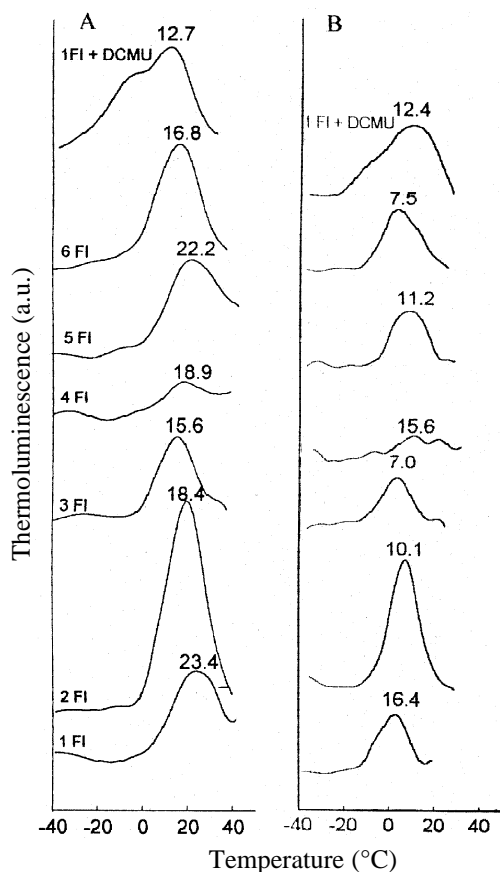


Fig. 1. Effect of Jasmonic acid on TL characteristics of B-band and Q-band in barley leaves. A – control plants; B – plants treated with 10 μ M JA

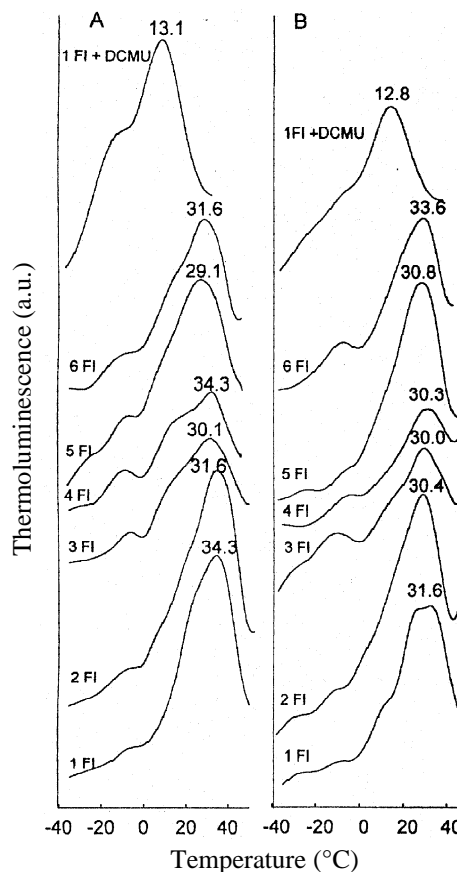


Fig. 2. Effect of Jasmonic acid on TL characteristics of B-band and Q-band in isolated chloroplasts from control plants (A) and from plants treated with 10 μ M JA (B)

observed around 13 °C (Fig. 1A), which originates as a result of S_2Q_A charge pairs recombination. Since any change in the redox state of electron donors and acceptors is reflected in the position of the main bands in TL glow curves, we can study the possible modifications in the components of PSII electron-transport chain during any stress conditions. A comparative study of TL characteristics of excised leaf pieces of barley plants showed that JA-treated plants have considerably lower B-band temperature and amplitude (Fig. 1B). Q-band temperature and amplitude were changed to a relatively lower extent. Analogous inhibitory effect of JA on TL characteristics was also observed in chloroplasts, prepared from treated plants (Fig. 2A, B). These data proved our previous suggestion that JA action is on the membrane level and thus a mechanism other than that on stomata function can be involved in the inhibition of photosynthesis (Maslenkova et al., 1990).

Since TL emission temperature is a function of free energy of stabilization of the PSII charge pairs, the relative downshifts in peak temperature of the $S_2Q_A^-$ and $S_2Q_B^-$ recombinations, when 1 flash was given, show a decrease in their stability. TL peak after 2 flashes, corresponding to the $S_3Q_B^-$ - recombination is also shifted to lower temperature, suggesting also a destabilization of the S_3 state.

In both control and JA treated plants the TL B-band oscillates with a period of four and maximum on the second flash. The TL intensity of JA-treated samples (Fig. 1 and 2) is lower than that of the control. Most likely this reduction is a result of increasing the number of inactive PSII oxygen evolving centres, as well as values of “misses”, calculated from the oscillations of O_2 yields. Obviously, increased damping under JA treatment means that PSII centres can not reach their higher oxidation states, S_3 and S_4 . In order to clarify the effect of JA treatment on the turnover time of the S-states, the O_2 yields from the first four flashes, generated in a group, involving generation of the dark interval between two neighbouring flashes from 300 to 0.1 ms were investigated. The individual turnover times calculated on the basis of Kok’s model using the “trial-error” method are presented in Table 2. The results show that JA treatment leads to a significant increase of the average turnover time of all the investigated S states. The estimated deactivation times of S_2 and S_3 presented on the same table show an increase in the rate of deactivation. Data in Table 2 show the effect of JA on the turnover time of S-states, calculated on the basis of Kok’s model. The results show that in treated preparations the turnover half-time of S-states increased and the stability of the S_2 and S_3 -state decreased.

Consistent with the decreased TL emission temperature after JA-treatment the half-time of B-band ($S_2Q_B^-$) also decreases ($t_{1/2}$ 44.5 s vs $t_{1/2}$ 34.5 s).

The observed effect of JA on the kinetic behaviour of S-states of PSII oxygen-evolving centres could be considered as indicative for an effect of this plant growth regulator on PSII donor side, which may be related to the specific changes in the polypeptide pattern (Maslenkova et al., 1995). On the other hand, since the S_2

Table 2. Effect of JA on the average turnover parameters of PSII oxygen-evolving centres and on the deactivation of S_i -states

Parameters	Control	JA-treated
turnover ($t_{1/2}$ (ms))		
$S_0 \rightarrow S_1$	0.9	1.4
$S_1 \rightarrow S_2$	1.4	2.3
$S_2 \rightarrow S_3$	1.3	2.2
Deactivation ($t_{1/2}$ (s))		
S_2	97.6	80.6
S_3	36.5	20.9

state of Mn-containing water-oxydizing complex is common for both $S_2Q_A^-$ and $S_2Q_B^-$ charge pairs, respectively for the appearance of both, B and Q TL bands, the more pronounced destabilisation of $S_2Q_B^-$ could be considered as a more pronounced effect of JA on the acceptor side of PSII.

Acknowledgements: This work was supported by Grant K-511/1997 from National Science Fund, Bulgarian.

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