EFFECT OF ABSCISIC ACID AND JASMONIC ACID (or JA-Me) ON THE PHOTOSYNTHETIC ELECTRON TRANSPORT AND OXYGEN EVOLVING REACTIONS IN PEA PLANTS

Liliana Maslenkova*, Sashka Toncheva, Yuzeir Zeinalov

Acad. M. Popov Institute of Plant Physiology, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria

Received October 24, 1995

Summary.The effect of prolonged ABA and JA-Me application to the growth medium of pea seedlings on PSII light reactions was assessed by monitoring of Hill reaction activity, fluorescence induction, kinetic behaviour of oxygen evolving PSII centres and polypeptide analysis of thylakoids and purified PSII oxygen evolving complexes (PSII OEC).
Results show the significant inhibitory effect of the two endogenous plant growth regulators on PSII activity and some specific alterations in the structural organization of chloroplast membranes.
Restoration of Hill reaction activity with DPC led to the conclusion that the used phytohormones have a direct effect on the PSII donor side and oxygen evolving enzyme complex itself. In addition, ABA and JA-Me treatment led to an increase in the number of $PSII_\beta$ centres situated in stroma lamellae regions, which are functioning by the cooperative mechanism for O_2 production. The polypeptide composition of grana and stroma lamellae vesicles confirmed this conclusion.

Key words: abscisic acid, chlorophyll fluorescence, electron transport, jasmonic acid, thylakoids, oxygen evolution, PSII OEC

Abbreviations: ABA – abscisic acid; DCPIP – 2,6 dichlorophenol-indophenol; DPC – diphenylcarbazide; JA – jasmonic acid; JA-Me – methyl jasmonate; PSII – photosystem II; PSII OEC – purified PSII oxygen evolving complexes; S-states – oxidation states of the water splitting system

*Corresponding author

Introduction

Although the effect of so-called classic phytohormone abscisic acid (ABA) on various steps of the photosynthetic process is well known there is no general agreement regarding the mechanism of ABA action. At least two possibilities are discussed in references, one of them concerning an indirect effect mediated by stomata closure (Dubbe et al., 1978) and the second one connected with a direct effect on the photosynthetic machinery (Raschke and Hedrich, 1985). In recent years jasmonic acid (JA) and its methyl ester, JA-Me have joined the group of endogenous plant growth regulators. To a certain extent, the action of JA and JA-Me on a number of photosynthetic parameters is similar to the effect of ABA. Plant treatment with 10^{-5} M ABA and JA reduced the rate of CO_2 fixation and the activity of ribulose biphosphate carboxylase, increased the rate of photorespiration and enhanced both CO_2 compensation point and stomata resistance (Popova and Vaklinova, 1988; Popova et al., 1988).

Our previous investigations (Maslenkova et al., 1989, 1990, 1992, 1993) show that 10^{-5} M ABA and JA application to the growth medium of barley seedlings has a marked inhibitory effect on PSII electron transport and O_2 evolving reactions in isolated thylakoid membranes and leads to specific alterations in thylakoid polypeptide patterns. These results support the idea of direct action of these two phytohormones on the photosynthetic apparatus. The assumption being that the established inhibition in Hill reaction activity and the changes in kinetic behaviour of PSII O_2 evolving centres when growing seedlings in the presence of 10 µM ABA and JA are determined by the observed structural reorganizations of chloroplast membrane leading to an increase in the number of $PSII_\beta$ centres in stroma partitions and an enhanced participation of a complementary cooperative mechanism for O_2 production (Zeinalov, 1982), which allow adaptation of photosynthetic apparatus to stress conditions.

These studies have now been extended by using purified OEC and isolated stroma and grana membranes as useful materials for assessing the changes in stacking behaviour of the thylakoid membranes and the possible reasons for the loss of PSII activity during *in vivo* treatment with ABA and JA. In addition, data about the conversion of $PSII_\alpha$ to $PSII_\beta$ centres are also presented. The results about the effect of ABA and JA on the thylakoid membranes and light-induced reactions connected with them could furnished useful information about the mechanism of action of these important substances.

Materials and Methods

Plant material

Seeds of pea (*Pisum sativum* L. cv. Ran) were germinated for 2 days in two layers of moist filter paper in vermiculite at 25°C in the dark. They were then transferred to pots containing 800 cm^3 distilled water for 2 days and of equal amounts of water so-

lution from 10 µM ABA or 50 µM JA-Me for the next 5 days. During the experimental period, seedlings grew in a growth chamber under white fluorescent lamps (35 $W.m^{-2}$), with 12 h light and dark periods. Day/night temperatures were 25/20°C; relative humidity was about 50%.

Chloroplast membrane preparation

Pea thylakoids and purified OEC were prepared according to Chapman et al. (1988). The subchloroplasts fractions (grana and stroma lamellae) were separated according to Leto et al. (1985).

Measurements of oxygen evolution

The activity of the electron transport from water to DCPIP was measured at 25°C in a medium containing 0.4 mM sucrose, 10 mM NaCl, 5 mM $MgCl_2$ and 40 mM HEPES-NaOH (pH 7.5). The concentration of the artificial electron acceptor DCPIP was 30 µM and chloroplasts equivalent to 15 µg Chl/ml were used. The reduction of the dye was measured at 580 nm. The initial oxygen burst (induction curves) and the oxygen flash yields were recorded at room temperature with an oxygen rate electrode and an universal polarograph OH-105 (Radelkis, Hungary). Samples (75 µl) with 22.5 µg Chl were excited either with continuous white light (135 $W.m^{-2}$) or with saturating flashes (4J, $t_{1/2}$ = 8 µs) spaced by 0.5 s. The initial S_0 and S_1 distribution and the values of misses (α) and double hits (β) were calculated according to the model of Kok et al. (1970). The turnover kinetics of the O_2 evolving centres were estimated using a slightly modified method of Bouges–Bocquet (1973) and Kok et al. (1970).

Chlorophyll fluorescence measurement

Chlorophyll fluorescence induction kinetics were measured with chloroplast suspension (10 µg Chl/ml) using equipment containing an excitation light source (250 W halogen lamp), a 436 nm interference filter (τ_{max}=42%, $\Delta\lambda_{1/2}$ = 10 nm), a monochromator, a photomultiplier (FEU-39, USSR), an oscilloscope C-13 (USSR), an analog-digital converter and a personal computer. Chlorophyll fluorescence was measured at 685 nm. The kinetic analysis of the area over the fluorescence induction curve in the presence of 20 µM DCMU for the calculation of $PSII_\alpha$ and $PSII_\beta$ centres was performed according to Melis and Homann (1978).

Gel electrophoresis

One-dimensional gel electrophoresis was carried out according to the procedure of Laemmli (1970). Samples of membrane fractions were solubilized in a sample buffer containing 62.5 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 5% (w/v) β-mercaptoethanol and 10% (w/v) glycerol for 30 min at room temperature, SDS:Chl = 20:1.

Results and Discussion

Prolonged 10 µM ABA and 50 µM JA-Me application to the root medium of pea seedlings had marked inhibitory effect on the photosynthetic PSII reactions. Table 1 shows that Hill reaction activity in isolated thylakoids decreased up to 26% with 10 µM ABA and 20% with 50 µM JA-Me. In the case of purified PSII OEC the effect of the two phytohormones was even more pronounced. The addition of DPC as an artificial PSII electron donor resulted in a near complete recovery of electron transport activity that may indicate a relatively greater damage of the donor side of PSII. The results from chlorophyll *a* fluorescence measurements showed lower values of F_v/F_{max} ratio in the photosynthetic membranes from ABA and JA-Me treated plants. This points to some structural disruptions (Krause and Weis, 1984) and as a consequence functional alterations in PSII complexes (Berry and Björkman, 1980).

Table 1. Photosynthetic parameters of pea chloroplasts and PSII OEC isolated from untreated, 10 µM ABA and 50 µM JA-Me treated plants[a]

Treatments	Hill reaction activity[b] (%)		F_v/F_{max}[c]	Chl *a*/*b*	$PSII_\alpha$ (%)
	−DPC	+DPC			
Chloroplasts:					
Control	100	100	0.70±0.03	2.55±0.10	100
10 µM ABA	73.7±2.1	97.3±1.7	0.54±0.07	2.63±0.13	56.3
50 µM JA-Me	79.6±5.4	91.1±2.3	0.56±0.01	2.57±0.08	59.0
PSII OEC:					
Control	100	100	0.83±0.11	1.57±0.05	
10 µM ABA	49.9±4.8	91.1±4.1	0.72±0.03	1.61±0.02	
50 µM JA-Me	51.7±6.1	89.2±3.4	0.77±0.03	1.65±0.14	

[a] Values are averages of four separate experiments

[b] Oxygen evolution with DCPIP in thylakoids from untreated plants was 94.5 µmol O_2/mg Chl.h; in PSII OEC it was 249.0 µmol O_2/mg Chl.h

[c] F_v – variable fluorescence; F_{max} – the peak of maximum level fluorescence

To obtain more information about the mechanisms of ABA and JA action on PSII structure and reactions we have investigated kinetic behaviour of PSII oxygen evolving centres in thylakoids and purified OEC using Kok's model and polypeptide structure of the membranes.

There is definite similarity in the effects of ABA and JA-Me. The two inhibitors bring about essential changes in the kinetics of dampening the oscillations connected with the rise in the values of misses (α) and double hits (β) parameters (Ta-

ble 2). The increase in the values of "α" and "β" after 10 µM ABA and 50 µM JA-Me treatment explains the observed decrease in the rate constants of the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ state transients according to Kok's scheme. Furthermore, our previous data (Maslenkova et al., 1990) showed that under the influence of JA (10 µM) there appeared an acceleration of the deactivation of highly oxidized S_2 and S_3 states – an effect similar to the well know effect of so-called ADRY reagents (Renger, 1972) which react directly with the trapped holes (the oxidizing equivalents) of the water-splitting enzyme system. We showed however that JA and ABA had no direct effect on isolated photosynthesizing membranes and consequently did not represent ADRY reagents (Maslenkova et al., 1989, 1990).

Table 2. S_i-state parameters in chloroplasts and PSII OEC estimated according to the Kok's model[a]

Treatments	$S_0 + S_1$[b] (%)	Misses (α)	Double hits (β)	Turnover k (s^{-1}) $S_1 \rightarrow S_2$	$S_2 \rightarrow S_3$
Chloroplasts:					
Control	100	0.158±0.008	0.034±0.001		
10 µM ABA	55.8±2.2	0.257±0.003	0.046±0.001		
50 µM JA-Me	60.4±2.1	0.234±0.011	0.044±0.003		
PSII OEC:					
Control	100	0.251±0.007	0.036±0.003	317.9	987.0
10 µM ABA	45.3±2.1	0.320±0.013	0.049±0.007	152.8	471.8
50 µM JA-Me	48.0±2.7	0.341±0.009	0.047±0.007	142.5	490.1

[a] Values are averages of four separate experiments
[b] S_0+S_1 – the sum of effective oxygen evolving centres working through the non-cooperative (Kok's) mechanism

Many different causes could explain the observed changes in the activity and the kinetic characteristics of the PSII O_2-evolving centres. In earlier studies (Maslenkova et al., 1989, 1990; Popova et al., 1989) we obtained evidences and suggested that the prolonged application of these two growth substances to the root system of higher plants resulted in disruption of the fine structure of chloroplasts, respectively to the blocking of grana formation. $PSII_\alpha$ centres in grana lamellae operate by non-cooperative Kok's mechanism for O_2 production and are especially sensitive to stress factors. On the contrary, there is an increase in the number of stroma situated $PSII_\beta$ centres that are functioning by a cooperative O_2 evolving mechanism. This statement was confirmed by the results presented on Tables 1 and 2 showing the rate of Hill reaction (reflecting the full oxygen evolving capacity of the photosynthesizing system i.e. cooperative and non-cooperative mechanisms) is less affected than the O_2 flash

yield amplitudes (or the number of effective O_2 evolving centres S_0+S_1, working in the grana partitions – $PSII_\alpha$ centres). The data from fluorescence induction analysis also show that upon ABA and JA-Me application to the growth medium there is a significant decrease in the number of $PSII_\alpha$ centres as compared to the untreated plants (Table 1).

Changes in the organization of the photosynthetic membranes under the influence of ABA and JA were detected not only by the electron transport and fluorescence measurements but also by SDS–PAGE and subfractionation analyses (Fig. 1). Compared to the control, the thylakoid membranes and the stroma and grana lamellae vesicles from treated plants show characteristic changes in PSII polypeptides, including the subunits of water-oxidizing complex – 33-, 24- and 17 kDa polypeptides and some other unidentified bands with low molecular weights. The polypeptide pattern of subchloroplast fractions revealed that there is an enrichment of stroma lamellae particles from treated plants with PSII specific polypeptide bands.

Fig 1. SDS-polyacrilamide gel electrophoresis of thylakoids (A), stroma lamellae vesicles (B), grana lamellae vesicles (C) and PSII OEC (D) isolated from control pea plants (1) and plants treated with 10 μM ABA (2) and 50 μM JA-Me (3). The samples were loaded on an equal (10 μg) chlorophyll basis

According to our hypothesis the observed ABA- and JA-dependent modifications in PSII polypeptide composition of the thylakoids, and grana and stroma lamellae are part of a physiological mechanism connected with the conversion of $PSII_\alpha$ and $PSII_\beta$ centres and the higher degree of participation of a complementary cooperative mechanism for oxygen production which is more resistant to stress. The as-

sumption is that exogenously applied ABA and jasmonates, as mediators for plant stress response, provoked some specific modifications in photosynthetic membrane structure and function that allow adaptation of the photosynthetic apparatus to different environmental conditions.

***Acknowledgements*:** This study was supported by Grant K-511/1995 from the National Science Fund, Bulgaria.

References

Berry, J.A., O. Björkman, 1980. Photosynthetic response and adaptation to temperature in higher plants. Annu. Rev. Plant Physiol., 31, 491–543.

Bouges-Bocquet, B., 1973. Limiting step in photosystem II and water decomposition in *Chlorella* and spinach chloroplasts. Biochim. Biophys. Acta, 292, 772–785.

Chapman, D.J., K. Gounaris, J. Barber, 1988. Electron transport properties of the isolated D1-D2-cytochrome b-559 photosystem II reaction centre. Biochim. Biophys. Acta, 933, 423–431.

Dubbe, D.R., C.D. Farquhar, K. Raschke, 1978. Effect of abscisic acid on the grain of feedback loop involving carbon dioxide and stomata. Plant Physiol., 62, 413–417.

Kok, B., B. Forbush, M. McGloin, 1970. Cooperation of charges in photosynthetic O_2 evolution. I. A linear four step mechanism. Photochem. Photobiol., 11, 457–475.

Krause, G.H., E. Weis, 1984. Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. Photosynth. Res., 5, 139–157.

Laemmli, U.K., 1970. Cleavage of structure of proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680–685.

Leto, K.J., E. Bell, L. McIntosh, 1985. Nuclear mutation leads to an accelerated turnover of chloroplast-encoded 48 kd and 34.5 kd polypeptides in thylakoids lacking photosystem II. The EMBO Journal, 4, 1645–1653.

Maslenkova, L.T., Yu. Zanev, L.P. Popova, 1989. Effect of abscisic acid on the photosynthetic oxygen evolution in barley chloroplasts. Photosynth. Res., 21, 45–50.

Maslenkova, L.T., Yu. Zanev, L.P. Popova, 1990. Oxygen-evolving activity of thylakoids from barley plants cultivated on different concentrations of jasmonic acid. Plant Physiol., 93, 1316–1320.

Maslenkova, L.T., T.S. Miteva, L.P. Popova, 1992. Changes in the polypeptide patterns of barley seedlings exposed to jasmonic acid and salinity. Plant Physiol., 98, 700–707.

Maslenkova, L.T., Yu. Zanev, L.P. Popova, 1993. Adaptation to salinity as monitored by PSII oxygen evolving reactions in barley thylakoids. J. Plant Physiol., 142, 629–634.

Melis, A., P.H. Homann, 1978. A selective effect of Mg^{2+} on the photochemistry at one type of reaction centre in photosystem II of chloroplasts. Arch. Biochem. Biophys., 190, 523–530.

Popova, L.P., S.G. Vaklinova, 1988. Effect of jasmonic acid on the synthesis of ribulose-1,5-bisphosphate carboxylase-oxygenase. J. Plant Physiol ., 133, 210–215.

Popova, L.P., T.D. Tsonev, S.G. Vaklinova, 1988. Changes in some photosynthetic and photorespiratory properties in barley leaves after treatment with jasmonic acid. J. Plant Physiol., 132, 257–261.

Popova, L.P., L.T. Maslenkova, Yu. Zanev, A.G. Ivanov, A. Christov, 1989. Stomatal and non-stomatal response of photosynthesis in barley leaves after treatment with abscisic acid and jasmonic acid. Colloquia Pflanzenphysiologie der Humboldt Universitat zu Berlin 13, Stomata'89, Ed.Goring, 13–15.

Raschke, K., R. Hedrich, 1985. Simultaneous and independent effects of abscisic acid on stomata and photosynthetic apparatus in whole leaves. Planta, 163, 105–118.

Renger, G., 1972. The acceleration of the deactivation kinetics in the water splitting enzyme system of photosynthesis by chemicals. Vol. I. Proceedings of the 2nd International Congress of Photosynthesis Researches, Stresa, Italy, Ed. Dr. J. Wunk, 53–59.

Zeinalov, Yu., 1982. Existence of two different ways for oxygen evolution in photosynthesis and photosynthetic unit concept. Photosynthetica, 16, 27–35.