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Effect of polyamines on chlorophyll and protein contents, photochemical activity, and energy transfer in detached wheat leaves during dark incubation

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Abstract

Spermine as compared to putrescine or spermidine retarded the loss of chlorophyll and protein contents to a greater extent in wheat primary leaves during dark incubation. Activities of whole chain electron transport, photosystem (PS) 1 and PS 2, and absorbed excitation energy distribution in favour of PS 1 were protected by these amines in valency dependent manner during 72-h dark incubation.

Additional key words: chlorophyll fluorescence, *Triticum aestivum*.

Introduction

Polyamines (PAs) are ubiquitous in the plant kingdom and affect growth of several higher plants (Smith 1982, Kakkar *et al.* 2000). Natural PAs stabilize membranes and retard senescence. Evidence for stabilizing effect of PAs on cell membranes came from the studies with apple cells grown *in vitro*, storage tissue of Swedish turnip, and spinach leaf discs. Increased ion flux from these tissues induced by various aliphatic guanidines can be partially restored on adding spermine (Srivastava and Smith 1982). PAs also delay loss of chlorophyll (Chl) in oat seedling leaf segments (Shih *et al.* 1982).

Several studies were concentrated on the effects of PAs on the functional activity of thylakoid membranes, Chl degradation, and the rate of photosynthetic oxygen evolution (Iordanov *et al.* 1989, Bograh *et al.* 1997). PAs, between 0.05 and 1.00 mM, caused destruction of chloroplast envelope and declined activities of PS 1 and PS 2 (Cohen *et al.* 1979, Popovic *et al.* 1979). At low concentration, they positively affected photosynthetic activity and O_2 evolution, while at higher concentration, they reduced the rate of O_2 production (Beigbeder *et al.* 1995). Therefore, delay in loss of photochemical activity by PAs at low concentrations can be expected. We investigated the influence of PAs on electron transport activities and low temperature Chl *a* fluorescence emission spectra of thylakoids from wheat primary leaves during dark incubation.

Materials and methods

Primary leaf segments (4 - 5 cm long) were cut from 7-d-old wheat (*Triticum aestivum* L. cv. Kalyansona) seedlings grown under continuous "white" radiation of 30 - 35 μmol m^{-2} s^{-1}. Four sets consisting of 20 - 25 leaf segments were maintained in double distilled water, 20 μM putrescine, 20 μM spermidine, and 20 μM spermine solutions in separate test tubes. The tubes were kept at 25 ± 1 °C in dark for 96 h. Leaf segments were sampled from each set at 24-h intervals.

The Chl content was estimated according to Arnon (1949) and total protein content according to Lowry *et al.* (1951) using bovine serum albumin as standard.

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Abbreviations: Asc - ascorbate; Chl - chlorophyll; DCMU - 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; DCPIP - 2,6-dichlorophenol indophenol; ETC - electron transport chain; MV - methyl viologen; PAs - polyamines; *p*-BQ - *p*-benzoquinone; PS - photosystem.

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Thylakoid membranes were isolated in a medium containing 50 mM Hepes-NaOH, pH 7.8, 400 mM sucrose, 2 mM $MgCl_2$, and 5 mM KCl, by a procedure similar to that of Saha and Good (1970) as described in Swamy *et al.* (1995). Electron transport activities of thylakoid membranes were assayed according to Sabat *et al.* (1989) in 2 cm^3 reaction buffer (RB; 50 mM Hepes-NaOH, pH 7.5, 100 mM sucrose, 2 mM $MgCl_2$, and 5 mM KCl) using a *Hansatech* (Kings Lynn, England) electrode. PS 2 activity was measured by adding freshly prepared *p*-benzoquinone (*p*-BQ) to 2 cm^3 RB to a concentration of 0.5 mM. Whole chain electron transport was asssyed with 0.5 mM methyl viologen (MV). By using 0.1 mM 2,6-dichlorphenol indophenol (DCPIP), 0.5 mM MV, 5 mM ascorbate (Asc), and 10 mM 3-(3,4-dichlorphenyl)-1,1-dimethyl urea (DCMU), PS 1 activity was measured. In all the assays, thylakoid membranes equivalent to 40 μg Chl were used.

Chl *a* fluorescence emission of control (0 h) and treated (72 h) leaf thylakoid membranes equivalent to 20 g(Chl) m^{-3} were investigated in presence or absence of 10 μM DCMU in a medium containing 50 mM Hepes-NaOH, pH 7.5, 100 mM sucrose, 2 mM $MgCl_2$, and 5 mM KCl on a *Jasco-FP 777* (Tokyo, Japan) spectrofluorometer at 25 ± 1 °C with an excitation wavelength of 440 nm. The excitation and emission slit width was 5 nm. Besides, 77 K fluorescence emission spectra were recorded on a *Hitachi MPF4* (Tokyo, Japan) spectrofluorimeter in samples prepared by mixing equal volume of 60 % glycerol and thylakoid membrane suspension. The samples were excited at 430 nm. The slit width of excitation and emission was 8 and 2 nm, respectively. Means, SD and Student *t*-test were used to draw inferences.

Results and discussion

Progressive decline in Chl and protein contents with time is a characteristic feature of leaf senescence. In the present study, decrease in both Chl and protein contents was observed when detached primary leaves were incubated in dark (Table 1). A decrease of 63 % Chl and 46 % protein was observed after 72 h indicating that Chl degraded at higher rate than proteins. Chlorophyllase and proteases might be responsible for these declines (Wittenbach 1978, Kuroki *et al.* 1987). Spermine delayed the loss of Chl and protein more than spermidine or putrescine (Table 1) implying the importance of valency of organic cations. No statistically significant difference was observed between dark control and PAs-treated leaves in case of Chl, but decreased percentage of Chl loss was observed in treated leaves in the following order putrescine, spermidine and spermine. Highly statistically significant difference ($P < 0.001$) was observed in protein content between dark control and PAs-treated leaves after 48, 72 and 96 h. The effect of PAs on Chl and protein contents could be due to stabilization of chloroplast thylakoid membranes and inhibition of enzyme mediated degradation as endogenous PAs bind strongly to proteases (Kaur-Sawhney *et al.* 1980, Besford *et al.* 1991, 1993).

Table 1. Effect of polyamines (putrescine, spermidine, and spermine) on chlorophyll [g(Chl) kg^{-1}(f.m.)] and total protein [g(protein) kg^{-1}(f.m.)] in primary leaves during dark incubation. The concentration of each polyamine was 20 μM. Values are means ± SD ($n = 4$), ** - $P < 0.01$, ***- $P < 0.001$.

	Treatment	0 h	24 h	48 h	72 h	96 h
Chlorophyll	H_2O	2.46 ± 0.12	1.91 ± 0.04	1.26 ± 0.05	0.90 ± 0.08	0.65 ± 0.05
	putrescine		1.89 ± 0.10	1.28 ± 0.04	0.95 ± 0.06	0.69 ± 0.08
	spermidine		2.08 ± 0.13	1.32 ± 0.05	0.95 ± 0.07	0.68 ± 0.09
	spermine		2.24 ± 0.10	1.43 ± 0.07	0.99 ± 0.02	0.74 ± 0.15
Protein	H_2O	26.66 ± 2.00	24.70 ± 1.50	17.14 ± 0.80	14.28 ± 0.40	9.50 ± 0.55
	putrescine		25.70 ± 1.80	22.86 ± 0.70***	18.09 ± 0.50**	17.14 ± 0.70***
	spermidine		26.60 ± 1.60	22.90 ± 0.70***	19.20 ± 1.00**	17.00 ± 0.58***
	spermine		26.70 ± 1.50	24.90 ± 1.00***	20.00 ± 0.50***	19.05 ± 0.60***

Photochemical activities of thylakoid membranes were drastically declined during dark incubation (Table 2) similarly as observed by Jenkins and Woolhouse (1981) in an *in vivo* study. PAs caused retention of the activity. Putrescine, spermidine, and spermine caused retention of the whole electron chain activity by 45, 47, and 56 %, respectively (after 72 h) (Table 2). Highly statistically significant difference ($P < 0.001$) was observed between dark control and spermine-treated leaves. Thus spermine was the most protective agent.

In order to ascertain the possible contribution of PS 1 and PS 2 separately to the observed loss in whole electron

transport chain, PS 1 and PS 2 activities of control and treated leaf thylakoids were measured. A decrease of 53 % of PS 2 and 17 % of PS I was found at 72 h in dark control and protection of these activities by PAs was also in a valency dependent manner (Table 2). PS 2 activity was more responsive to PAs treatments than PS 1 activity. At 72 h, spermine caused retention of PS 2 activity by 72 %, spermidine by 67 % and putrescine by 64 % ($P < 0.001$). In contrast to these results, Cohen *et al.* (1979) reported a loss of photochemical activities in barley leaf discs.

Table 2. Effect of 20 μM putrescine, spermidine, or spermine on whole chain electron transport [mmol(O_2 consumed) kg^{-1}(Chl) s^{-1}], and PS 2 [mmol(O_2 evolved) kg^{-1}(Chl) s^{-1}] and PS 1 [mmol(O_2 consumed) kg^{-1}(Chl) s^{-1}] activities in primary leaves during dark incubation. Means ± SD ($n = 4$), * - $P < 0.05$, ** - $P < 0.01$, ***- $P < 0.001$.

	Treatment	0 h	24 h	48 h	72 h	96 h
ETC	H_2O	28.35 ± 1.89	21.87 ± 2.02	17.01 ± 1.08	10.82 ± 0.54	-
	putrescine		24.30 ± 1.62	18.63 ± 1.35	12.69 ± 0.54*	-
	spermidine		25.92 ± 1.08*	20.52 ± 0.95*	13.39 ± 0.27**	-
	spermine		26.46 ± 1.35*	21.47 ± 0.81**	15.80 ± 0.54***	-
PS 2	H_2O	48.60 ± 2.70	44.28 ± 3.78	37.80 ± 2.16	22.95 ± 1.89	17.28 ± 1.08
	putrescine		45.90 ± 2.43	40.50 ± 2.43	31.05 ± 1.35***	18.90 ± 0.81
	spermidine		47.52 ± 1.62	40.23 ± 1.89	32.40 ± 1.62***	20.52 ± 1.08*
	spermine		48.06 ± 1.08	44.55 ± 1.89**	35.10 ± 1.89***	22.14 ± 1.35**
PS 1	H_2O	133.00 ± 7.80	124.20 ± 6.75	117.00 ± 6.75	110.00 ± 4.86	104.00 ± 4.05
	putrescine		126.90 ± 4.32	120.40 ± 5.67	113.00 ± 4.32	106.00 ± 4.59
	spermidine		125.50 ± 4.86	122.00 ± 5.13	115.60 ± 4.86	105.30 ± 4.73
	spermine		130.40 ± 5.13	129.30 ± 5.40	126.90 ± 4.05**	118.50 ± 3.51**

Table 3. Effect of 20 μM putrescine, spermidine, or spermine (72-h treatment) on the ratio of fluorescence emission of thylakoid membranes in the presence and absence of 10 μM DCMU. Means ± SD ($n = 3$), ***- $P < 0.001$.

Treatment	F_{685} +DCMU/-DCMU
H_2O (0 h)	1.65 ± 0.02
H_2O (72 h)	1.14 ± 0.02
Putrescine	1.43 ± 0.01***
Spermidine	1.52 ± 0.02***
Spermine	1.53 ± 0.01***

Chl *a* fluorescence emission is a sensitive index of thylakoid organization and can be used to test its efficiency under stress (Biswal *et al.* 1979, Govindjee 1995, Murthy *et al.* 1995). DCMU (10 μM), an inhibitor of electron transport at plastoquinone level, caused a 65 % increase in fluorescence intensity in control leaf thylakoids (0 h) while only a 14 % at 72-h dark incubation (Table 3), suggesting that dark incubation induced alterations in thylakoid membranes (Prakash *et al.* 1998). However, in spermine-treated leaves the enhancement was 53 % which is near to the value of 0 h control, indicating stabilization of thylakoid organization.

In order to show the influence of PAs on relative distribution of energy between the two photosystems, we

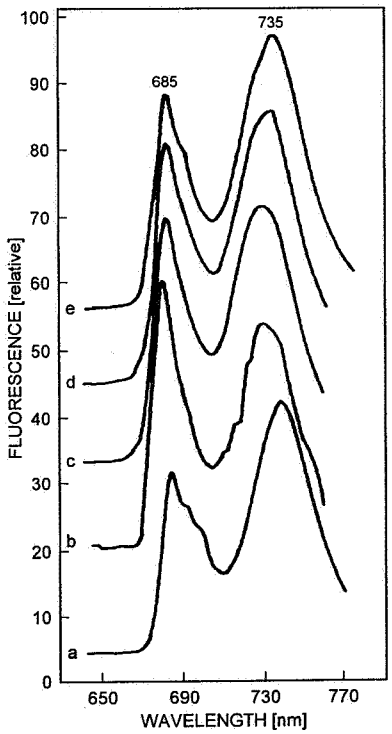

Fig. 1. Chlorophyll *a* fluorescence emission spectra (77 K) of thylakoids from control (0 h; *a*), dark incubated control (72 h; *b*), putrescine (*c*), spermidine (*d*), and spermine (*e*) treated leaf segments for 72 h. Excitation was at 430 nm.

recorded low temperature emission spectra and assessed the relative ratio of F_{735}/F_{685}. This ratio (Fig. 1) was decreased from 1.43 at 0 h to 0.85 at 72 h in dark control leaf thylakoids indicating inhibition of energy transfer from PS 2 to PS 1 as suggested by Butler (1978). However, the decrease was minimised by PAs treatments. A relative suppression of F_{735} peak in dark control by 40 % was marginalised by PAs (Fig. 1), suggesting the distribution of absorbed excitation energy more in favour of PS 1 due to PAs treatment. Spermine could more effectively restore the absorbed excitation energy transfer from PS 2 to PS 1 during dark ageing than spermidine or putrescine. These observations were in contrast to those obtained with isolated chloroplasts in the presence of inorganic cations where most of the absorbed excitation energy was distributed in favour of PS 2 (Murata 1969, Mohanty and Mohanty 1988) while in the presence of inorganic anions with increasing valency the energy distribution was more in favour of PS 1 (Jajoo *at al.* 1998). Thus PAs were suggested to stabilize thylakoid organization and delay the loss of photochemical activity during senescence probably by shielding negative charges on thylakoid membranes (Barber 1982).

In summary, PAs in a valency dependent manner minimised the loss of electron transport activities and restored absorbed excitation energy distribution in favour of PS 1 in wheat primary leaf segments during dark incubation.

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