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Interaction of anions and cations in regulating energy distribution between the two photosystems

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Abstract

Cations such as Mg^{2+} regulate spillover of absorbed excitation energy mainly in favour of photosystem (PS) 2. Effect of low concentration (<10 mM) of the monovalent cation Na^+ on chlorophyll (Chl) *a* fluorescence was completely overridden by divalent cation Mg^{2+} (5 mM). Based on Chl *a* fluorescence yield and 77 K emission measurements, we revealed the role and effectiveness of anions (Cl^-, SO_4^{2-}, PO_4^{3-}) in lowering the Mg^{2+}-induced PS2 fluorescence. The higher the valency of the anion, the lesser was the expression of Mg^{2+} effect. Anions may thus overcome Mg^{2+} effects up to certain extent in a valency dependent manner, thereby diverting more energy to PS1 even in the presence of $MgCl_2$. They may do so by reversing Mg^{2+}-induced changes.

Additional key words: chlorophyll fluorescence induction; $MgCl_2$; NaCl; Na_2HPO_4; Na_2PO_4; Na_2SO_4; spinach; *Spinacia oleracea*; state change; thylakoids.

Introduction

Cations affect several primary photoprocesses in thylakoids, including regulation of absorbed excitation energy distribution between photosystems 2 and 1 (Butler 1978, Wong *et al.* 1980). Cations modulate fluorescence emission of thylakoids resulting in the increase in yield of PS2 fluorescence and a concomitant decrease in that of PS1 (Homann 1969, Murata 1971). At low temperature (77 K), the F735/F695 ratio decreases (Mohanty *et al.* 1973). Cations induce a decrease in excitation energy

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Abbreviations: Chl - chlorophyll; DCMU - 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_0 - initial chlorophyll *a* fluorescence where all Q_A is oxidised; F_m - maximum chlorophyll *a* fluorescence, where all Q_A is reduced; F_v - variable chlorophyll *a* fluorescence; PS - photosystem; Q_A - bound plastoquinone.

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transfer from PS2 to PS1 (Wong *et al.* 1979, Barber 1982). Both spillover of energy and the absorption cross section of PS1 decrease in the presence of Mg^{2+} (Tsala and Strasser 1984).

Low concentrations (3-5 mM) of monovalent and divalent cations are antagonists of the excitation energy migration (Gross and Hess 1973, Vandermeulen and Govindjee 1974, Wong and Govindjee 1979). Na^+ causes less energy migration among PS2 units and more transfer to PS1 (Wydrzynski *et al.* 1975, Wong *et al.* 1981) and Mg^{2+} could reverse this effect. All these effects were interpreted in terms of Na^+ effects and no role was assigned to the counter anion associated with it.

Little work has been done to reveal the significance of the coexisting anion on the organisation and function of thylakoid membrane. Anions lower the Mg^{2+}-induced steady-state fluorescence of PS2 and increase the rate of PS1 electron transport even in the presence of Mg^{2+} (Jajoo and Bharti 1995). Anions function also in the reversal of cation induced functional and structural change in thylakoid membranes (Jajoo *et al.* 1994). Inorganic anions are important in the regulation of distribution of absorbed radiant energy in favour of PS1, by causing state changes (Jajoo *et al.* 1998). These studies encouraged us to explore the possible role of anions in overcoming the effects of Mg^{2+} on the radiant energy distribution. This is the first report explaining anion effects over Mg^{2+} in a valency dependent manner.

Materials and methods

Chloroplasts were isolated from market spinach (*Spinacia oleracea* L.) and suspended in a medium of low ionic strength (100 mM sucrose, 50 mM Hepes-KOH buffer, pH 7.6). Chl *a* fluorescence induction curves were monitored at room temperature on a fabricated set-up which included an excitation radiation source (*Kindermann*, 12 W m^{-2}), sample chamber with photodiode (*Hansatech*), *Hansatech* transient recorder TRI as a storage device, and an oscilloscope (*HP54603 B*) for replaying the signal. Two blue filters (both *Corning CS 4-72*) and red filters (*Corning CS 2-59, CS 2-64*) were used for the excitation and emission radiations, respectively. Samples received no other irradiation except the blue one. The F_m level obtained after 5 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) treatment was slightly higher than the 'P' level of our control, suggesting that the used irradiance was strong enough to reduce most of the primary electron acceptor Q_A even in the absence of DCMU, Q_A being a quencher of Chl *a* fluorescence (Duysens and Sweers 1963). Low temperature fluorescence emission spectra were monitored and autocorrected on a *SPEX 1680* double spectrofluorometer. Excitation radiation was 435 nm, 300 W m^{-2}.

Results and discussion

Organic salts such as Na-acetate, Na-succinate, and Na-citrate have no significant effect on the F_v/F_m ratio. PS1 and PS2 electron transport rates are also not affected by these organic anions, suggesting thereby that they do regulate radiant energy distribution (Jajoo *et al.* 1998). Inorganic sodium salts with counter anion having different valences (Cl^-, SO_4^{2-}, PO_4^{3-}) were selected against the divalent cation Mg^{2+}. For further experiments, we chose subsaturating concentrations of NaCl (10 mM), Na_2SO_4 (5 mM), and Na_2HPO_4 (5 mM) to keep the Na^+ concentration constant (10 mM) in order to specifically compare the effects of anions.

Table 1. Effects of anions on chlorophyll (Chl) *a* fluorescence yield (F_v/F_m ratio) in the presence of non-saturating (2 mM) and saturating (5 mM) concentrations of $MgCl_2$. Both the anions and $MgCl_2$ were added together, incubated for 5 min, and recorded. Reaction mixture contained 100 mM sucrose, 50 mM Hepes-KOH buffer, pH 7.6, 7 g(Chl) m^{-3}. Numbers in parentheses give the normalised values.

	F_v/F_m		
	$-Mg^{2+}$	$+Mg^{2+}$ (2 mM)	$+Mg^{2+}$ (5 mM)
Control	0.62 ± 0.02 (100)	0.68 ± 0.00 (110)	0.75 ± 0.01 (121)
10 mM NaCl	0.55 ± 0.01 (89)	0.63 ± 0.01 (102)	0.73 ± 0.01 (118)
5 mM Na_2SO_4	0.50 ± 0.01 (81)	0.59 ± 0.01 (95)	0.72 ± 0.01 (116)
5 mM Na_2HPO_4	0.46 ± 0.00 (74)	0.56 ± 0.01 (90)	0.70 ± 0.02 (115)

In the first set of experiments, 5 mM $MgCl_2$ was added to the thylakoid suspension prior to the addition of anions. An increase in F_v/F_m ratio was observed at this concentration of $MgCl_2$. Since a change in F_v/F_m could be either due to change in F_0, F_v, or both, we studied the effects on F_0 and F_v because F_m equals F_v+F_0. $MgCl_2$ caused a slight decrease in F_0 and a much larger increase in F_v. A decrease in F_0 may indicate, among other things, an excitation energy transfer towards reaction centre of PS1 and/or decrease in the redistribution of excitation energy towards PS1 (PS1 has a much lower fluorescence yield than PS2). On the other hand, the Mg^{2+}-induced increase in F_v/F_m reflects an increase in the quantum yield of photochemistry of PS2. No effects of any of the three anions on the F_v/F_m ratio were observed (values not shown). The Mg^{2+}-induced stacking of thylakoid membranes probably masked the anion binding sites, as a result of which anions were unable to express their effects and the effect of $MgCl_2$ thus predominated.

In the second set of experiments, $MgCl_2$ and inorganic anions were added together to the thylakoid suspension. Saturating and non-saturating concentrations of $MgCl_2$ were used in order to observe anion effects more precisely (Table 1). In the absence of Mg^{2+} a remarkable decrease in the 'P' level and a slight increase in F_0 (the minimum Chl *a* fluorescence level) was observed when the valency of the anion was increased. Thus the salts gradually decreased the F_v/F_m ratio with increase in the valency of their inorganic anions. This suggests an increased distribution of

Table 2. Effects of anions on chlorophyll (Chl) *a* fluorescence yield (F_v/F_m ratio) in the presence and absence of $MgCl_2$ (5 mM). Samples were first incubated with anion and then 5 mM $MgCl_2$ was added to it. The sample was again incubated for 5 min before recording. Reaction mixture as in legend of Table 1. Numbers in parenthesis give the normalised values.

Treatment	F_v/F_m $-Mg^{2+}$	$+Mg^{2+}$
Control	0.62 ± 0.02 (100)	0.75 ± 0.02 (121)
10 mM NaCl	0.55 ± 0.01 (89)	0.64 ± 0.02 (103)
5 mM Na_2SO_4	0.50 ± 0.01 (81)	0.61 ± 0.00 (98)
5 mM Na_2HPO_4	0.46 ± 0.00 (74)	0.57 ± 0.00 (94)

excitation energy in favour of PS1, and a decrease in the quantum yield of PS2 photochemistry. Since concentration of sodium was kept constant in all the treatments, the effects were certainly due to anions. Anions showed larger effects in the presence of non-saturating concentration (2 mM) of $MgCl_2$. Hence in the presence of low concentration of $MgCl_2$, the thylakoids may remain fluid or partially stacked, exposing thereby a few anion binding sites. Anions may get access to these sites and thus show greater effects. It also suggests that anion action is somehow dependent on the conformational state of the membrane which may in turn regulate state changes. Anion effects are largely masked in the presence of higher (5 mM) concentration of $MgCl_2$. In other words, Mg^{2+} probably overrides anion-induced changes in a concentration-dependent manner.

In the third set of experiments, the thylakoids were first treated with the inorganic anions and $MgCl_2$ (5 mM) was added later. Anions seemed to bind with the unstacked membranes and the addition of $MgCl_2$ did not totally replace them. As a result, the effect of anions was observed upto a certain extent even in the presence of 5 mM $MgCl_2$ (Table 2). Thus in the absence of Mg^{2+}, anions may bind to thylakoid membranes and the addition of Mg^{2+} after anion binding does not allow Mg^{2+} to express its effect. In unstacked membranes anion binding sites may be expressed and accessible to anion binding.

The yield of fluorescence is regulated by the redox state of the PS2 acceptor, Q_A, which is a quencher of fluorescence when in oxidised state (Duysens and Sweers 1963). Fluorescence is also quenched if some state change occurs, *i.e.*, there is either a decrease in the ratio of concentration of highly fluorescent PS2 to weakly fluorescent PS1, or there is an increase in redistribution of more excitation energy into PS1 than into PS2 (Murata 1969, Govindjee 1995, Joshi and Mohanty 1995). To ascertain the reason for inorganic anion-induced fluorescence quenching over Mg^{2+}, we performed the experiments in the presence of DCMU, where all Q_A were converted to Q_A^-.

Inorganic anions quenched PS2 fluorescence even in the presence of DCMU (Table 3). The PS2 reaction centre exists in the Z^+ P680 Pheo Q_A^- state in the presence of DCMU. If $P680^+$ is present, PS2 would dissipate energy as heat and quench fluorescence (Shinkarev and Govindjee 1993). However, no $P680^+$ existed at

Table 3. Effects of various salts on chlorophyll (Chl) *a* fluorescence (F_v/F_m ratio) in the presence of 5 μM DCMU. Experiments were carried out at low irradiance, using a neutral density filter (25 % transmittance). Concentrations of salts were $MgCl_2$ (5 mM), NaCl (10 mM), Na_2S0_4 (5 mM), and Na_2HPO_4 (5 mM). Reaction mixture is shown in legend to Table 1.

Treatment	F_m	F_0	F_v/F_m
Control	70 ± 2 (100)	29 ± 2 (100)	0.580 ± 0.010 (100)
$MgCl_2$	78 ± 2 (111)	26 ± 1 (90)	0.660 + 0.010 (114)
NaCl	55 ± 2 (79)	26 ± 1 (90)	0.520 ± 0.010 (90)
Na_2SO_4	50 ± 1 (71)	26 ± 1 (90)	0.480 ± 0.005 (83)
Na_2HPO_4	47 ± 1 (67)	26 ± 1 (90)	0.450 ± 0.005 (78)
$MgCl_2$ + NaCl	75 ± 2 (107)	26 ± 1 (90)	0.650 ± 0.005 (112)
$MgCl_2$ + Na_2SO_4	73 ± 1 (104)	26 ± 1 (90)	0.640 ± 0.005 (110)
$MgCl_2$ + Na_2HPO_4	70 ± 1 (103)	26 ± 1 (90)	0.630 ± 0.005 (108)
NaCl + $MgCl_2$	65 ± 1 (93)	26 ± 1 (90)	0.600 ± 0.005 (103)
Na_2SO_4 + $MgCl_2$	62 ± 1 (89)	26 ± 1 (90)	0.580 ± 0.005 (100)
Na_2HPO_4 + $MgCl_2$	58 ± 1 (83)	26 ± 1 (90)	0.550 ± 0.010 (95)

the time of our measurement in the system after DCMU treatment. It suggests that the effects are unrelated to Q_A. Anions may reverse the state changes caused by Mg^{2+} to a certain extent. This is further supported by the fact that anions enhance PS1 rates and inhibit PS2 rate even in the presence of Mg^{2+} under low irradiances (Jajoo and Bharti 1995).

Lowering of Mg^{2+}-induced energy distribution in favour of PS2 by inorganic anions is supported by low temperature fluorescence emission spectra. The thylakoids showed a lower (normalized) F735/F685 and higher F695/F685 ratio in the presence of 5 mM $MgCl_2$, as compared to control (Table 4). Anions caused increase in the F735/F685 ratio in a valency dependent manner, the order of effectiveness being trivalent>divalent>monovalent anion. As evident from the results, different effects of anions were observed when they were added together with $MgCl_2$ or prior to the addition of the $MgCl_2$. An intermediate F735/F685 ratio was obtained over the individual effects of Mg^{2+} and anions. However, in the presence of $MgCl_2$, there was always a consistent increase in F695/F685 ratio over the control. This implies that Mg^{2+} directly affects the energy trap in PS2, and anions do not bring about any significant change in this effect of Mg^{2+}.

It may be argued that Cl^- should show its effect when it is present as 5 mM $MgCl_2$ (effective concentration of Cl^- in it being 10 mM). Effects of Cl^- (10 mM) were masked in the presence of Mg^{2+}, while 10 mM Cl^- exhibited its effect when it was present as NaCl. When both 5 mM $MgCl_2$ and 10 mM NaCl were present, then concentration of Cl^- became high enough (20 mM effectively) to exhibit at least some effects. Electrostatic changes caused by a divalent cation (even at low concentration of 5 mM) were much greater than the changes caused by the same concentration of monovalent cation (NaCl) (Murata 1969). This may be the reason why Cl^- shows its effects when present as NaCl, and not when present as $MgCl_2$. Thus at lower concentrations of salts having monovalent cation (Na^+, in this case), the anion effect

Table 4. Normalised emission peak ratio (F735/F685) and (F695/F685) measured at 77 K in sucrose washed chloroplasts after giving various treatments at Chl concentration 7 g m^{-3}. Concentration of salts used are $MgCl_2$ (5 mM), NaCl (10 mM), Na_2SO_4 (5 mM), and Na_2HPO_4 (5 mM). Each value represents an average of five different samples.

Treatment	F735/F685	F695/F685
Control	1.00 ± 0.02	1.00 ± 0.02
$MgCl_2$	0.86 ± 0.01	1.24 ± 0.02
NaCl	1.15 ± 0.01	1.18 ± 0.01
Na_2SO_4	1.24 ± 0.01	1.16 ± 0.01
Na_2HPO_4	1.42 ± 0.02	1.15 ± 0.01
A. anion and $MgCl_2$ added together		
$MgCl_2$ + NaCl	0.89 ± 0.02	1.23 ± 0.02
$MgCl_2$ + Na_2SO_4	0.93 ± 0.01	1.23 ± 0.02
$MgCl_2$ + Na_2HPO_4	0.95 ± 0.005	1.22 ± 0.01
B. anion added prior to the addition of $MgCl_2$		
NaCl + $MgCl_2$	0.98 ± 0.01	1.22 ± 0.01
Na_2SO_4 + $MgCl_2$	1.05 ± 0.02	1.20 ± 0.02
Na_2HPO_4 + $MgCl_2$	1.16 ± 0.02	1.19 ± 0.02

predominates. The effect varies with the change in the valency of the anion. The order of effectiveness of anions in quenching Chl *a* fluorescence and thereby diverting more energy to PS1, was trivalent>divalent>monovalent.

In this paper we show that, at low concentrations of salts (5-10 mM), besides monovalent cations also the coexisting anion regulates distribution of excitation energy between the two photosystems. Anions may function either by changing electrostatic repulsive/attractive interactions among the intrinsic protein complexes present in the membrane, which results in their reshuffling and as a consequence, reorientation of a few Chl *a* pigment molecules responsible for the regulation of energy transfer (Seely 1973, Barber and Chow 1979). Moreover, anions reverse Mg^{2+}-induced rigidity of thylakoid membrane which might help in the attainment of state 2 (Jajoo *et al.* 1998). The change in thylakoid membrane conformation by anions may result in a reversal of Mg^{2+}-induced state changes. Anions, depending on their valency, overcome cation-induced state changes to certain extent which results in transfer of more energy from PS2 towards PS1.

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