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Abstract


The effects of static magnetic field (SMF, 2T) on pea thylakoid membranes were investigated under photosynthetic and actinic light exposure. The pea plants were subjected to light intensity of 2000 lx (HL, high light) and to 650 lx (IL, intermediate light), respectively in order to specify the effect of SMF (2T) on the electrokinetic potentials at the membrane surfaces. We determined the electrophoretic mobility, zeta potential and surface charge density of thylakoids in dependence on the iron content of the ‘Fe-sufficient’, ‘Fe-starved’ and ‘2 x Fe’ variants. The generation of light-induced increase in negative zeta potential of ‘Fe-sufficient’ SMF (2T) pre-exposed thylakoids (HL, IL) was due to an enhancement of net negative surface electric charge on the outer surface of the membranes.

The primary electron-transport processes across the thylakoid membrane was increased due to actinic light treatment to the ‘Fe-starved’ SMF pre-exposed thylakoids (HL) because of most densely packed thylakoids.

A decreased lipid peroxidation by SMF (2T) exposure before or after actinic illumination of thylakoids was observed. It could be due to a recombination of radical products as a consequence of an indirect action of magnetic field on thylakoids from ‘Fe-sufficient’ and ‘2 x Fe’ plants.

The electrokinetic potential changes in pea thylakoids upon SMF (2T) exposure depend on Fe content in growing medium as well as light intensity (HL, high light; IL, intermediate light) during pea plant growing.

Key words: Surface charge density; Static magnetic field; Light scattering; Thylakoid membranes; Lipid peroxidation

Introduction

The electric charge distributed over the surface of biological membranes plays an important role in the regulation of the molecular membrane processes, but also in the processes of interaction of biological cells with external influences such as magnetic fields. In pea leaves, 60% to 80% of cellular Fe content is in chloroplasts (Terry and Low, 1982). Iron excess in plants leads to a significant increase in Cyt b6/f content of thylakoids and could cause oxidative stress resulting from iron-dependent reactions that produce excessive amounts of highly reactive free radicals in cells as hydroxyl and alkoxy radicals, which in turn initiate various oxidation reactions of cellular components notably including lipid peroxidation (Halliwell and Gutteridge, 1989).

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The magnetic fields can interact directly with moving charges (ions, charges, proteins etc.) by the well-known Lorentz force deflection and magnetic materials with non-compensated spins in some specialized magneto-receptor system (complexes containing Fe, for instance) in membrane (Tenforde, 1992; Glaser, 2000).

In this study we report for the first time: i) the generation of light-induced increase in EPM, ζ potential and surface electrical charge of ‘Fe-sufficient’ thylakoids (HL; IL) because of strong complex aggregations of pea thylakoids upon SMF (2T) pre-treatment in non-equilibrium state and ii) using SMF (2T) exposure, as well as additional illumination with near saturated light intensity to decrease the lipid peroxidation products generated which depends on iron content in growing media of pea plants (HL).

Materials and Methods

Plant material, culture and preparation of thylakoids
Pea plants (Pisum sativum L., cv. Ran-1) were grown at three different iron concentrations in the Johnson’s nutrition medium: iron sufficient medium (‘Fe-sufficient’ variant, Control variant), iron free growing medium (‘Fe-starved’ variant) and in excess of iron supply (‘2 x Fe’ variant) as described in (Kim and Jung, 1993; Cohen et al., 1998).

Chloroplasts were isolated from the leaves of two-week-old seedlings (Pisum sativum L., cv. Ran 1) according to Whatley and Arnon (1963) with some modifications. The final pellet was resuspended to a concentration of 2 mg chlorophyll mL⁻¹ (Lihtenthaler, 1987). For experiments the following buffer containing 25 mM HEPES (KOH), 5 mM MgSO₄, pH 7.5 was used.

Microscopic (visual) microelectrophoresis
The electrophoretic mobility (EPM) measurements were performed using the particle electrophoresis technique with the OPTON Cytopherometer according to (Doltchinkova and Lambreva, 2002). Data are means of three independent experiments. The zeta potential (ζ, mV) and surface charge density (σ, C m⁻²) were calculated by (Barber, 1989).

Light scattering measurements
Experiments were conducted with a lab constructed apparatus and recorder. The ionic-exchange processes of LS of thylakoids by light-induced scattering were monitored at 550 nm at an angle of 90° in the presence of 50 μM phenazine methosulfate (a mediator of photosynthetic electron transport processes) (Doltchinkova et al., 2004). Cut-off filters were used to protect the photocell from the actinic light (λ ≥ 640 nm) with saturating intensity of 4750 μmol quanta m⁻² s⁻¹ at the position of the optical glass cuvette.

Magnetic fields treatments
All the preparations were treated with an external static magnetic field with varying magnetic exposure in the range 0.1; 0.5; 1.0; 1.5 and 2 T (Tesla unit). We used the dose of 2T. The treatment duration was selected to be 15 min. The experiments are carried out at a room temperature of 25°C in a static magnetic field generated by an electromagnet that was cooled with water. The magnetic field was perpendicular to the Eppendorf cups containing 0.5 ml of chloroplasts. At the end of exposure, the final thylakoid suspension (exposed and unexposed to SMF) were stored in ice at 0°C before measurements.

Detection of malondialdehyde (MDA)
Following the illumination with near saturated light the samples were subjected to the analysis of thiobarbituric acid – reacting substances (TBARS) products according to the method described by (Halliwell and Gutteridge, 1989) with slight modifications.

Statistical analysis
The experiments were performed in triplicate. The significant differences between means were determined by use of ANOVA. One-way analysis of variance was performed with Holm-Sidak method for comparison the significance of the treatment.

Results and Discussion

Effects of SMF on the electrophoretic mobility of thylakoids
The variation of the magnetic induction of the static magnetic field (0.1÷ 2T) resulted in the insignificant changes in the EPM of ‘Fe-sufficient’ thylakoids (HL). SMF (2T) didn’t cause a statistically significant influence on the EPM of ‘Fe-sufficient’ nor of ‘2 x Fe’ thylakoid membranes (HL, Figure 1). There was an effect of decrease in EPM values of SMF ‘Fe-starved’ pre-exposed thylakoids (with about 17%) in comparison to their thylakoid membranes without SMF treatment (p < 0.001). EPM of ‘Fe-starved’ thylakoids showed a 20% increase in its value comparatively to the value of ‘Fe-sufficient’ as well as of ‘2 x Fe’ thylakoid membranes before SMF exposure. It means that ‘Fe-starved’ thylakoids possess a significant enhancement in σ of their membranes. The photosynthetic light led to a decrease of EPM of SMF pre-exposed ‘Fe-starved’ thylakoid membranes (HL, Figure 2) which was due to a disorganized and swollen thylakoids upon starvation of pea plants (Lu et al., 1995). SMF (2T) led to less negatively charged groups to be exposed on the outer surface of the membrane because of a depletion of thylakoid
membrane proteins – especially pigment protein complexes (Lu et al., 1995). Figure 2 shows the effect of light–induced increase in EPM of ‘Fe-sufficient’ thylakoids (HL). We obtained a large increase in EPM of SMF pre-exposed ‘Fe-sufficient’ thylakoids upon light exposure in comparison with SMF pre-exposed thylakoids without light treatment (p < 0.001). A considerable enhancement in negative electrical charges on the outer surface of ‘Fe-sufficient’ thylakoid membranes (HL) of 30% was obtained with light and SMF of which 13% represents a contribution of the magnetic field.

It could be due to more negatively charged groups to be exposed on the outer surface of the ‘Fe-sufficient’ thylakoids (HL) upon photosynthetic light treatment. The main role of the changes in orientation of the thylakoid membranes in SMF is observed (i.e., a large ordered complexes formation of ‘Fe-sufficient’ thylakoids (HL) after photosynthetic light treatment of SMF (2T) pre-exposed thylakoids was viewed). The EPM of ‘Fe-sufficient’ (IL) thylakoids enhanced with 30.5% upon SMF (2T) treatment in comparison to unexposed in SMF control thylakoids (p < 0.001).

**Effects of SMF on the light scattering of thylakoids (HL)**

SMF exposure of ‘Fe-sufficient’, ‘Fe-starved’ as well as ‘2 x Fe’ thylakoids (HL) did not change significantly the aggregation of their thylakoids in comparison to the aggregation of thylakoids without SMF pretreatment. We described the LS of thylakoids before and after actinic illumination in terms of the three main parameters: fast, slow and decay phases as described in (Doltchinkova et al., 2004).

SMF (2T) did not alter significantly the light-induced scattering of ‘Fe-sufficient’ thylakoids (HL) in any phase. The slow phase of SMF pre – exposed ‘2 x Fe’ thylakoids (HL) was not significantly changed compared to the preliminary values without the SMF exposure. SMF (2T) did not change the decay phase of ‘Fe-sufficient’ as well as of ‘2 x Fe’ thylakoids without SMF.
Fe’ thylakoids after turning off the light. A maximal increase of the fast phase of LS of SMF pre-exposed ‘Fe-starved’ thylakoids was shown (p < 0.001). This enhancement of the primary ion-exchange processes after SMF treatment was accompanied by an increase in decay of SMF pre-exposed ‘Fe-starved’ thylakoids after turning off the light.

**Evaluation of lipid peroxidation in the slow phase of LS of thylakoid membranes (HL)**

The TBARS of ‘Fe-sufficient’, ‘Fe-starved’ as well as ‘2 x Fe’ thylakoids was significantly modified after SMF exposure without light treatment of the samples by a reduction of 39%, 27% and 23%, respectively in comparison to TBARS of the same thylakoids before SMF treatment (p < 0.001). However, light treatment induced a significant enhancement of 24, 6% in the TBARS of ‘Fe-sufficient’ thylakoids than the same preparations of Control thylakoids before illumination (p ≤ 0.001). A statistically significant increase in lipid peroxidation of ‘2 x Fe’ thylakoids upon light treatment approximately by 14.5% versus preparation without light treatment was observed (p = 0.001).

Similarly, light exposure of ‘Fe-starved’ thylakoids didn’t affect strongly TBARS comparing the values of thylakoids before actinic light illumination. Near saturated light treatment altered the lipid peroxidation of the SMF pre-exposed ‘Fe-sufficient’, ‘Fe-starved’ as well as ‘2 x Fe’ thylakoids (HL) by a reduction of 23%, 38% and 62%, respectively in comparison to unexposed samples without actinic light treatment (p ≤ 0.001). SMF influence on the thylakoid membrane is by generation of radical pair intermediates due to changes in conformation of lipids in the processes of free radical interactions with its macromolecules (Harris et al., 2009; Liu, 2008).

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**References**


