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EFFECT OF pH ON THE ELECTROKINETIC AND LIGHT SCATTERING PROPERTIES OF PEA THYLAKOIDS IN THE PRESENCE OF PHYTOHEMAGGLUTININ

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Summary. We studied the effect of phytohemagglutinin on the surface charge density and 90° light scattering of pea thylakoids as a function of pH (from pH 6.0 to pH 7.8). These pH-dependent modifications were interpreted in terms of the membrane conformational changes. Lectin induced a decrease in LS level due to the increase in aggregation of the particles at all pH studied. These changes were accompanied by a reduction in the electrophoretic mobility of the particles after lectin treatment at pH 7–7.8 and had an optimum at pH 7.5. Light exposure stimulated the recovery of reduced electrokinetic potential of lectin treated thylakoids (pH 7.5) up to approximately the initial higher negative surface charge density. Acidification induced a smaller decrease in electrokinetic potential and energization of the membrane after lectin treatment and light exposure. Lectin plus light treatment increased the energization of the membrane. Phytohemagglutinin-Phaseolosaxin (PHA-P) influenced the enhancement of ΔpH gradient formation more effectively at alkaline than in acid pH. Screening of the charges of the surface exposed groups (in the presence of Mg^{2+}) and lectin binding decreased the primary ionic-exchange processes through the membrane at all pH tested and significantly activated them at pH 7.0. Alkaline pH determined the strong increase in dark relaxation of thylakoids pretreated with PHA and inhibition of this phase at pH 6.3. The pH-dependent phenomena in the membrane-membrane interactions and the lectin regulated processes of aggregation in the thylakoid membranes at pH range of 6.0 to 7.8 was discussed.

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Key words: Lectin (phytohemagglutinin), Electrophoretic mobility, Surface charge density, Light scattering, pH, pea thylakoids.

Abbreviations: EPM – electrophoretic mobility; LS – light scattering; PHA-P – Phytohemagglutinin-Phaseolosaxin; PMS – Phenazine methosulfate; HEPES – N-(2-Hydroxyethyl)piperazine-N´-(2-ethanesulfonic acid); MES – 2-(N-Morpholino)ethanesulfonic acid; PSI – Photosystem 1; PSII – Photosystem 2; I – ionic strength

Introduction

In recent years the effect of pH changes on several membrane-related phenomena, such as binding of external molecules to the membrane, was the subject of considerable interest. Current interpretations of these pH-influenced processes considered almost exclusively the role of acidification on the polar head of the negative charged phospholipids, or on the molecules interacting with the bilayer. Acidification modified the electrokinetic potential and light scattering of thylakoid particles pretreated with phytohemagglutinin. This was presumably as a consequence of modifications of lipid packing and non-specific electrostatic interaction of PHA-P with the surface components of thylakoid membrane.

Thylakoid membranes, like most biological particles carry a negative charge of about –0.0025 C/m^2 on their outer surface (Barber, 1980b; Barber, 1982). Investigation of the nature of the surface charge is important in order to analyse the surface components of the thylakoid membrane and to obtain information for the thylakoid stacking processes (Barber, 1989).

The net negative electrical charge on the outer surface of the thylakoid membrane is mainly due to the carboxyl groups of glutamic and aspartic acid residues of exposed portions of integral proteins. The galactosyldiglycerides monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are the basic lipid components of the membrane system of higher plant chloroplasts. They are slightly polar uncharged lipids. The negatively charged polar lipids such as phosphatidylglycerol or sulfolipid could also be responsible for the negative surface charges of intact chloroplasts (Neuburger et al., 1977; Nakatani et al., 1978; Nakatani and Barber, 1980). At neutral pH, thylakoid membrane surfaces carry excess negative electrical charge. Below pH 4.3–4.5 the surface becomes positively charged, which results from the guanidine group of exposed arginine residues (Nakatani and Barber, 1980; Goltsev et al., 1983, Doltchinkova, 1990).

The widespread distribution of lectins throughout the plant kingdom and their abundance in many plants suggest that these molecules are of physiological importance to the plants (Etzler, 1985). Both animal and plant cell surfaces possess exposed

glycosyl moieties bound to either proteins or lipids (Hoekstra and Duzgunes, 1989). Schroder and Petit (1992) reported for a minor binding of *Ricinus communis* agglutinin and peanut lectin to purified spinach thylakoid membranes.

Lectin (*Phaseolus vulgaris* agglutinin) activates the thylakoid aggregation processes and is used to study the stack formation and aggregation (Doltchinkova et al., 1995). Phytohemagglutinin (PHA) is a vacuolar storage glycoprotein of *Phaseolus vulgaris*. It is a protein of molecular weight of 126 kDa and requires metal ions (Mn^{2+}, Ca^{2+}) for binding to a saccharide (Liener, 1986). PHA (Sharon and Lis, 1972) is a tetrameric molecule with four sugar-binding sites. Due to their multivalent binding character lectins, when added exogenously, will cross-link membrane surface receptors, leading to lateral molecular reorganisation in the plane of the bilayer (Hoekstra and Duzgunes, 1989).

Phytohemagglutinin interaction with the lipid components of the membrane changed the electrostatic interactions on the outer membrane surfaces. This is because the lipid components could be involved in zeta potential formation due to interaction between the foreign molecules and various parts of the membrane.

Particle electrophoresis is a useful tool to estimate the particle stability, the influence of lectins on the thylakoid surface charge, the effect of pH of surrounding medium on the electrokinetic potential of thylakoids (Goltsev et al., 1985; Doltchinkova et al., 1995; Lambreva, 1998).

Our microelectrophoresis work had several aims: first, to investigate the changes in the effect of Phytohemagglutinin-Phaseolosaxin on the electrokinetic and light scattering properties of pea thylakoids at different pH. Second, we studied the relationship between the surface charge and the functional activity of thylakoid membranes. The effects of PHA-P on the level of aggregation, primary and secondary ionic-exchange processes, relaxation ability of the system were determined by measuring 90° LS at $\lambda = 550$ nm under near saturating intensity of light exposure.

Our results indicate that care should be taken in interpreting pH-dependent phenomena in membrane-membrane interactions which could be partially mediated through a recognition processes.

Materials and Methods

Thylakoid membranes were isolated from leaves of two-weeks old pea (*Pisum sativum,* L., cv. Ran 1) plants grown in full nutrient medium and in a controlled-environment chamber (2100 lx, 12 h photoperiod, day/night temperatures of 23 °C/20 °C) as described in (Doltchinkova and Lambreva, 2002). Two isolations of thylakoids at pH 7.8 (Doltchinkova and Lambreva, 2002) and pH 6.3 (Ford and Evans, 1983) were prepared. The thylakoid suspension (pH 7.8) was mixed with 10% (v/v) glycerol as a cryoprotectant and frozen in liquid nitrogen (Gold'feld et al., 1978). Pea thylakoids (pH

6.3) were stored in a liquid nitrogen plus 20% (v/v) glycerol (Doltchinkova et al., 1999). Chlorophyll content was determined in 80% acetone according to Lichtenthaler (1987). The chlorophyll concentration was 2.7 mg/ml in thylakoids, suspended at pH 7.8, and 1.7 mg/ml in the case of pH 6.3. The final chlorophyll concentration used in all the experiments was 6.6 μg/ml.

A cytopherometer "OPTON" was used for electrophoretic mobility (EPM) measurements (Doltchinkova et al., 1993). EPM of the thylakoid membranes were calculated as m^2/Vs (±SEM). Each independent value was obtained as an average of 20 to 35 particles pooled from three membrane preparations. Electrophoretic migration was measured in a rectangular chamber at a constant electric field of 3 to 5 mA in the case of different pH and 23°C. The movement of single particles over a known distance of 32 μm was timed using microprocessor equipment for both forward and backward (reversed field) runs. The light observation was provided by 16 V/15 W lamp (with intensity of 13 μmol/m^2 s) and a green (545 nm) interference filter.

Before suspending the thylakoids in the electrophoresis buffer they were incubated for 30 min in the absence or in the presence of various concentrations of PHA at 23°C. According to Schroder and Petit (1992), an incubation time of 30 min is the most effective for the binding of lectins. Illumination was performed on thylakoids already suspended at an appropriate final chlorophyll concentration in the electrophoresis chamber. Immediately before current application the thylakoids were illuminated for one minute at 23°C with light intensity of 920 μmol/m^2 s without green interference filter.

Values represent the mean of 3 replications (60–85 particles). The SEM was between 2 and 9%.

For electrophoresis and light scattering experiments the thylakoids were suspended in media containing: a) 25 mM HEPES, pH 7; pH 7.5; pH 7.8, 5 mM $MgCl_2$ (I = 0.015), b) 25 mM MES, pH 6.0; 6.3; 6.5, 5 mM $MgCl_2$ (I = 0.015) to a final concentration of 6.6 μg chlorophyll/ml. The results were expressed as a percent of electrophoretic mobility values of the control thylakoids.

The lectin-membrane effects on light-induced thylakoid membrane EPM shift were tested for phytohemagglutinin (PHA-P) in a concentration range of 2–40 ng/ml (higher concentrations of PHA-P induced a large aggregation of the thylakoid particles).

The behaviour of cells in an electric field allows measurement of electrophoretic mobility and characterisation of the surface properties (Bauer and Golovanov, 1999). Zeta potential (ζ), the electrostatic potential at the hydrodynamic plane of shear, is calculated from the measured value of electrophoretic mobility. Zeta potential is determined by the genotype of the cell and is a constant physiological parameter (Bauer and Golovanov, 1999). It could be used to estimate the stability and electrokinetic properties of colloidal-dispersed systems.

Zeta potential was calculated from the measured value of the EPM by the Helmholtz-Smoluchowski equation (Overbeek and Wiersema, 1967; Nakatani et al., 1978).

The surface charge density was estimated according to Barber (1989) and previously described (Doltchinkova et al., 1993; Doltchinkova et al., 1999).

PHA-induced changes in the light scattering of pea thylakoid membranes were measured at 550 nm by a laboratory-made apparatus using a Specol 10 Spectrophotometer (Carl Zeiss Jena) (Doltchinkova, 1990). Cut-off filters were used to protect the photocell from the actinic light ($\lambda \geq 640$ nm), providing 2950 μmol/m^2.s (near saturating intensity) at the position of the cuvette. Phenazine methosulfate (50 μM) was used as electron transport mediator. Pretreatment of the thylakoids by PHA was performed in the same way as described for the electrophoresis experiments.

We used the term "basal LS" to describe the stacking level of not-illuminated thylakoids. After illumination with active white light (intensity of 2950 μmol/m^2.s) the LS response of the thylakoid membrane consisted of two main phases: fast and slow with different signal amplitudes (Doltchinkova et al., 1999). The fast phase of LS reflects a membrane potential alteration. The slow phase of LS showed the transmembrane proton gradient formation and the related secondary ion exchange processes (Rottenberg, 1977). After switching off the light, the ionic gradients equilibrate as the influx of protons stops immediately (decay phase of LS) (Graber and Witt, 1974).

Results

Effect of pH on the electrophoretic mobility of thylakoid membranes

The electrophoretic mobility of untreated and PHA-P treated thylakoid membranes at different pH were monitored by particle electrophoresis. The relative mobility stimulation of EPM was estimated. The pH dependence of the effects of PHA-P on thylakoid EPM is presented in Fig. 1. A non-monotonous dependence of EPM on PHA concentrations was found for pea thylakoids. There was a significant decrease in the lectin induced EPM change at doses of 10 ng/ml up to 30 ng/ml (Fig. 1, pH 7.5) due to the maximal effectiveness of PHA binding to the membrane surface. The values changed from -0.92×10^{-8} m^2/Vs (without lectin, before illumination) to $-(0.75\div0.76)\times10^{-8}$ m^2/Vs (in the presence of 10 ng PHA/ml up to 30 ng PHA/ml, before illumination) at pH 7.5. This significant effect was expressed in the corresponding conformational state of its components, which increase the screening charges by magnesium cations. As a result more contacts between metal and polyvalent ions could be realised. Hence, the conformational changes during light exposure facilitate the lectin binding to the outer thylakoid membrane surface. Doses of 6 ng/ml and 20 ng/ml induced a slight increase in the EPM of stacked thylakoids at pH 7.0 (EPM varied from -0.78×10^{-8} m^2/Vs in the absence of lectin to -0.87×10^{-8} m^2/Vs after doses of 6 ng PHA/ml and 20 ng PHA/ml) and at pH 7.8 (EPM varied from -0.90×10^{-8} m^2/Vs in the absence of lectin to -1.02×10^{-8} m^2/Vs after lectin treatment).

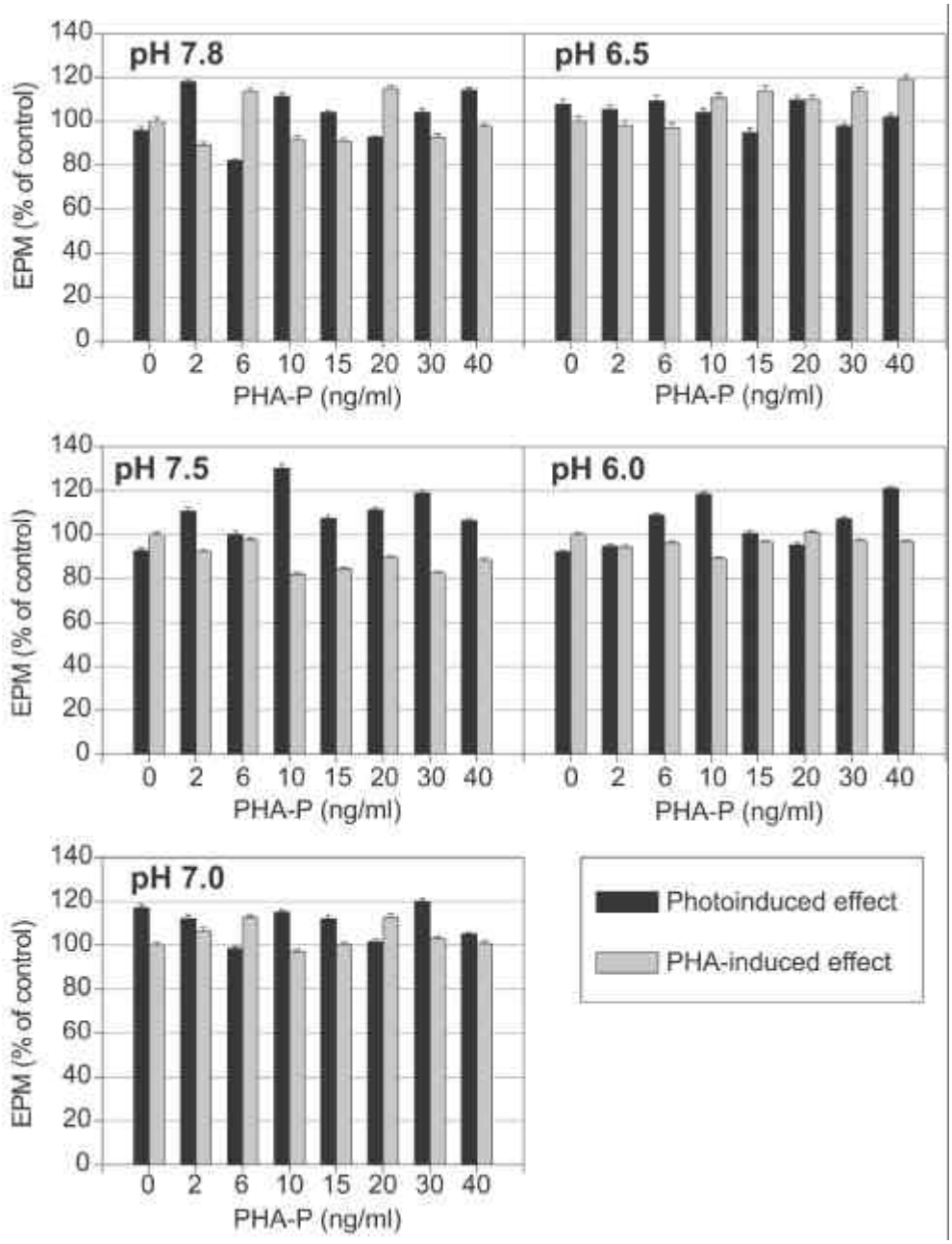

Figure 1. Effect of Phytohemagglutinin-Phaseolosaxin (PHA-P) on electrophoretic mobility (EPM) of pea thylakoid membranes at different pHs. Lectin was mixed with thylakoids at 23 °C for 30 min. Pretreated thylakoid membranes with different concentrations of PHA-P were dissolved in buffers of different pH and measured at 23 °C. The buffers (both containing 5 mM $MgCl_2$) used were 25 mM HEPES(KOH) for pH 7–7.8 and 25 mM MES(NaOH) for pH 6–6.5 and concentration of 6.6 µg chlorophyll/ml.

The changes in EPM after the light were presented in Fig. 1 as photoinduced effect for pea thylakoids in the presence of magnesium cations at different pH. Our results showed that light stimulated an increase in EPM of thylakoid membranes, especially at pH7.5, pH 7.8 and pH 7.0. There was 12–20% enhancement of the negative electrokinetic potential at 2–40 ng PHA/ml at pH 7.0. We observed a significant increase in the net negative surface electrical charge of thylakoids at pH 7.5. It was maximal at 10 ng PHA/ml (30%) and to a lower extent at other doses. There was an increase (11–17%) in EPM of PHA treated thylakoids (plus 2 ng PHA/ml, 10 ng PHA/ml and 40 ng PHA/ml, respectively) after light induction at pH 7.8.

There was an enhancement of the net negative surface electrical charge after light exposure at alkaline pH 7.0 (σ varied from $-0.0044\ C/m^2$ without lectin, to $-0.0045\ C/m^2$ in the presence of 2 ng PHA/ml and $-0.0046\ C/m^2$ in the presence of 30 ng PHA/ml), at pH 7.5 (σ varied from $-0.0041\ C/m^2$ without lectin, to $-0.0045\ C/m^2$ in the presence of 2 ng PHA/ml) and at pH 7.8 (σ varied from $-0.0041\ C/m^2$ without lectin, to $-0.0049\ C/m^2$ in the presence of 40 ng PHA/ml).

There was no significant alteration on the EPM of particles in the presence of lectin at pH 6.0, 6.3 (data not shown) and pH 6.5. (Fig. 1, PHA-induced effect). The lack of electrostatic effect at acidic pH was due to a smaller value of the net negative surface exposed groups, diminishing the binding of magnesium cations with the lectin molecule.

Light induction did not alter the electrokinetic properties of thylakoids, suspended at pH 6.5, i.e., the isoelectric point of the PHA-P. Lower pH of 6.3 determined the effect of 11% at 20 ng/ml (EPM varied from $-0.66\times10^{-8}\ m^2/Vs$ in the absence of lectin to $-0.73\times10^{-8}\ m^2/Vs$ after lectin treatment). The stronger enhancement of surface electrical charge in the presence of 6 ng PHA/ml ($\sigma = -0.0036\ C/m^2$), 10 ng PHA/ml ($\sigma = -0.0036\ C/m^2$) and 40 ng PHA/ml ($\sigma = -0.0040\ C/m^2$), pH 6.0, in comparison to EPM value of thylakoids before lectin treatment ($\sigma = -0.0031\ C/m^2$) was observed. This effect was accompanied by a strong aggregation of thylakoids in the presence of 6, 10 and 40 ng PHA/ml before and after light treatment.

Illumination altered the surface electrical properties of pea thylakoids after lectin treatment because of the enhancement in the polarisation and energization of the membranes. Light induced a recovery of the lectin-altered decrease in the EPM of pea thylakoids up to approximately the initial EPM values only at alkaline pH in comparison with the slight changes at acidic pH.

PHA-P binding was strongly exhibited at alkaline pH and in a smaller extent – at acidic pH. Only pH 6.5 did not change significantly the effect of PHA-P binding to pea thylakoids, as well as the EPM of the particles after illumination (Fig. 1, pH 6.5). The significantly pronounced PHA-induced changes in the surface charge density of pea thylakoids were established at pH values of 7.5 and 6.0.

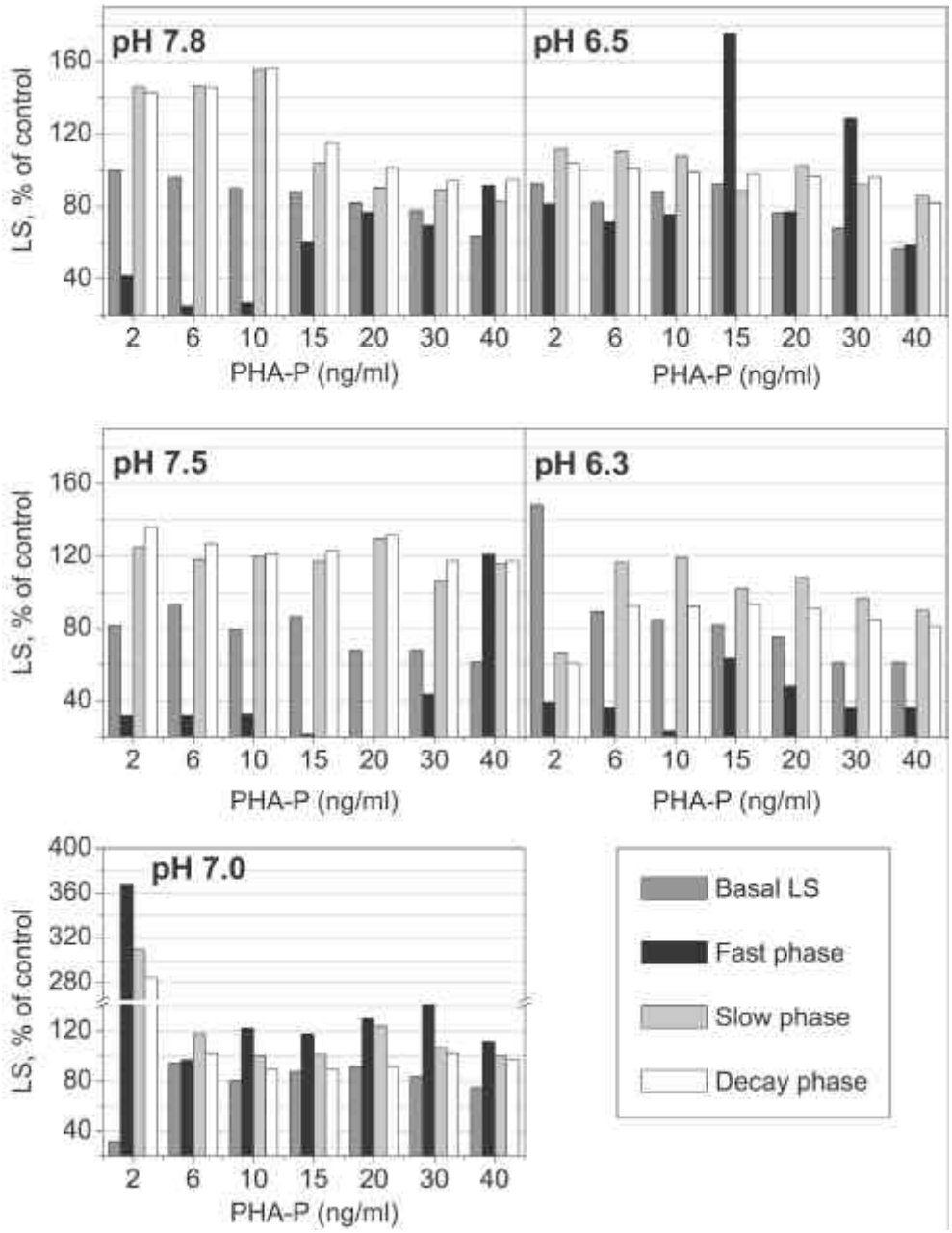

Figure 2. Effect of PHA-P on the light scattering (LS) of pea thylakoids suspended at different pH. Lectin was mixed with thylakoids (6.6 µg chlorophyll/ml) at 23 °C for 30 min. Pretreated thylakoid membranes with different concentrations of PHA-P were dissolved in buffers of different pH and measured at 25 °C. The buffers (both containing 5 mM $MgCl_2$ and 50 µM PMS) used were 25 mM HEPES(KOH) for pH 7–7.8 and 25 mM MES(NaOH) for pH 6.3 and pH 6.5

Effect of phytohemagglutinin on the light scattering of thylakoid membranes

We studied the pH-induced conformational changes of thylakoid membranes in the presence of 5 mM $MgCl_2$ and phytohemagglutinin on their LS properties at pH 6.3–7.8. Increasing the lectin concentration of treatment, a rapid change in volume of the thylakoids occurred at all the pH studied, as shown by the basal LS of the suspension (Fig. 2).

Fig. 2 illustrates LS parameters of pea thylakoids at different pH and concentrations of lectin. We found a maximal decrease of the primary ionic-exchange processes across the thylakoid membranes at all the pH tested in contrast to the strong increase of the same phase at pH 7.0. There was a maximal increase of the fast phase of LS at dose of 2 ng PHA/ml, pH 7.0, in comparison to the untreated thylakoids. The lectin influenced most strongly the secondary ionic-exchange processes at pH studied. At concentration of 2 ng/ml and neutral pH (7.0), the slow phase of LS increased 3 fold in comparison to the untreated thylakoids. We observed a strong increase in slow phase of LS at doses of 2–10 ng PHA/ml (pH 7.8) by 46–56%, at 2–40 ng PHA/ml (pH 7.5) by 15–30%. Consequently, there was a tendency of increase in secondary ionic-exchange processes across the membranes at pH 7–7.8. The relaxation ability of the thylakoids (Fig. 2, decay phase) significantly increased at doses of 2–10 ng PHA/ml, pH 7.8, by 43–56%. With increasing the doses of PHA-P treatment at pH 7.5, the level of activation of decay phase slightly decreased from 36% down to 17% at highest concentrations of lectin. Only at doses of 2 ng PHA/ml (pH 7.0), the decay phase of LS enhanced by 2.8 fold in comparison with the untreated thylakoids at neutral pH. Higher concentrations of PHA-P did not alter the relaxation ability of the membrane at pH 7.0.

The level of aggregation of thylakoids suspended at lower pH of 6.3 and pH 6.5 decreased with increasing concentration of lectin treatment. Only at dose of 2 ng PHA/ml (pH 6.3), the basal LS increased strongly (up to 48%) in comparison with the control value of LS. There was a tendency of decrease in fast phase of LS of thylakoids after lectin treatment at pH 6.5 and pH 6.3. PHA-P inhibited strongly the primary ionic exchange processes at dose of 10 ng PHA/ml with 76% (pH 6.3) in comparison with the untreated thylakoids. Lectin decreased thylakoids relaxation after turning off the actinic light with about 40% in the presence of 2 ng PHA/ml and with 15–20% at doses of 30 ng PHA/ml and 40 ng PHA/ml, respectively (pH 6.3). On the contrary, PHA-P did not alter the decay phase of LS of thylakoids at pH 6.5.

We observed a tendency of decrease in fast phase of LS of thylakoids at pH 6.5 and pH 6.3. Lectin decreased strongly the primary ionic-exchange processes at pH 6.3, as well as the relaxation of the thylakoids after turning off the actinic light.

Discussion

The effect of the surface charge on the organisation and functional activity of the thylakoid membrane is a result of conformational changes of thylakoid membrane com-

ponents and redistribution of energy between the two photosystems (Yerkes and Babcock, 1981). pH of the medium influences the integrity of the membrane by manipulating the intermolecular interactions via Coulomb's and van der Waals forces.

The EPM of thylakoid membranes in the presence of phytohemagglutinin depended on the pH and ionic strength of surrounding medium. Using the Gouy-Chapman theory we investigated the adsorption of Phytohemagglutinin-Phaseolosaxin (PHA-P) to the thylakoid membranes, facilitating the entropy-driven 'depletion attraction' between the adjacent membranes (Barber, 1980b; Chow, 1999).

Magnesium cations influenced the electrostatics of the pea thylakoid membrane surface by the lectin and transform the conformation of phytohemagglutinin. The screening of the surface negative charge of thylakoids by bivalent cations was completed by an electrostatic interaction with the phytohemagglutinin molecule.

We combined both techniques of particle electrophoresis and light scattering to investigate the biophysical properties of thylakoid preparations of pea at different pH of 6.0-7.8 in the presence of bivalent cations (Mg^{2+}) and polyvalent ion phytohemagglutinin without and post light exposure.

The lectin recognition of membrane saccharides involves matching pairs of surfaces endowed with specific electrostatic properties modified by the interactions. The electrostatic properties determine the stability of the resulting aggregates. Our results showed that lectin (PHA-P) induced a reduction in the net negative surface electrical charge on the pea thylakoids with increasing concentration of lectin only at alkaline pH of 7.5 (Fig. 1, PHA-induced effect). The reduction of EPM was not due to specific electrostatic interaction between the lectin and membrane surface components at $I = 0.015$ of the surrounding medium. The effect could be related to the isoelectric point of both components – PHA molecules (pH 6.5) and chloroplasts (pH 4.5) (Kantcheva et al., 1983)

The PHA-P treatment of pea thylakoids induced a decrease in EPM values that was reversed after illumination so that the zeta potential recovered nearly to its initial values corresponding to a higher negative surface charge density (Doltchinkova and Lambreva, 2002). It could be due to an indirect influence on thylakoid membrane components and an additional negative charges exposure on the membrane surface (Schapendonk et al., 1980). The transverse motion of the intrinsic protein molecules could be associated with the increased exposure of the external surface charge groups, and subsequently increased σ of illuminated thylakoids. Consequently, PHA-P molecules increased the aggregation of the particles accompanied by a reorientation and rotation of thylakoids in the electric field applied.

Polyvalent ions did not alter the light-induced energization of stacked pea thylakoids at pH 6.5, the isoelectric point of PHA – molecules (Kantcheva et al., 1983). The strong enhancement of net negative surface electrical charge after lectin treatment and post light exposure of PHA pretreated thylakoids at pH 6.0 was observed (Fig. 1, pH 6.0). We related these effects to more extensive stacking by increasing the net space

charge density of ions in solution in a plane parallel to the membrane surface at an adequate distance. We suggested that the light stimulation effects were due to the energy-dependent rearrangements of the thylakoid membrane constituents and local variations in the viscosity and dielectric properties on the interface and ionic double layer.

The level of chlorophyll fluorescence (Barber, 1980a) as well as the level of light scattering (Wollman and Diner, 1980) correlated with thylakoid stacking. The chloroplasts largely maintained their initial structural state when diluted into the assay medium. The light scattering of pea thylakoid membranes indicated that lectin:

a) Decreased the level of aggregation between the thylakoid membranes, which could be a result of non-specific adsorption. It correlated with a significant decrease in the net negative surface charge density on the surface of the pea thylakoid membrane;
b) Increased the pH gradient formation across the membrane and the relaxation ability of thylakoids to reach the initial value after turning off the illumination at pH 7.8 and pH 7.5;
c) Inhibited the primary ionic exchange processes at all pH tested and significantly activated them at pH 7.0;
d) Decreased the dark relaxation of thylakoid membranes at pH 6.3.

In conclusion, lectin induced a reduction in net negative surface charge density of pea thylakoids. Light-induced energization of thylakoid membrane and the conformational rearrangement included changes in glycoprotein-lipid interaction and an increase in net negative surface charge density of the membrane. Light however did not induce an enhancement by itself but only after lectin treatment. Thus, in the case of lectin-treated and illuminated samples, there were no more negatively charged surface exposed groups than in the untreated control thylakoids taking into account that they were already reduced by the PHA binding. The role of light was to recover the electrokinetic charge reduced by lectin.

Phytohemagglutinin-Phaseolosaxin strongly influenced the electrokinetic and light scattering properties of pea thylakoid membranes at alkaline pH and in a smaller extent at acidic pH of treatment. Only at neutral pH of 7.0 screening of the charges of surface exposed groups increases the primary ionic-exchange processes through the membrane. It was shown that PHA-P altered the build up of transmembrane potential gradient observed in illuminated thylakoids at pH 7.5. Consequently, PHA-P was able to influence the charge separation across the pea thylakoid membrane, reflected by the ΔpH gradient formation. The light scattering change was due to some conformational changes in the reaction centres of thylakoid proteins after illumination with a near saturating intensity of light. The reduction of net negative surface charge density of stacked thylakoids caused a decrease in LS level due to aggregation of the complexes.

The study of the lectin effects on membrane electrokinetic properties using the method of particle microelectrophoresis showed that low PHA doses cause changes

in the membrane polarity and specific aggregate formation and cluster reorientation.

Future studies on lectin binding and the relationship of this structure to activity the lectin-induced electrokinetic potential is essential to determine if and how lectin activity may be regulated.

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