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Biophysical Chemistry 67 (1997) 239–244

Biophysical Chemistry

Surface electric properties of thylakoid membranes from *Arabidopsis thaliana* mutants

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Received 5 September 1996; revised 25 February 1997; accepted 25 March 1997

Abstract

Electric light scattering measurements of thylakoid membranes from wild type and two mutant forms (JB67 and LK3) of *Arabidopsis thaliana* have shown that application of external electric pulses induces electric dipole moments of different origin. The asymmetric surface charge distribution and electric polarizability are significantly altered by the lipid modification. Mild trypsin treatment of *Arabidopsis* thylakoids leading to digestion of small polypeptides from the light-harvesting chlorophyll *a*/*b* protein complex of photosystem II (LHCP II) gives evidence for a lower content of LHCP II in the mutant forms. The results demonstrate the significance of the level of thylakoid lipid unsaturation in determining the surface charge distribution through changes either in the pigment–protein content and membrane appression induced by the lipid modification or in the exposure of charged polypeptides on the thylakoid membrane surface(s) arising from alteration of the lipid geometry. © 1997 Elsevier Science B.V.

Keywords: Thylakoids membranes; *Arabidopsis thaliana*; Mutants; Electric light scattering; Electric dipole moments

1. Introduction

The galactosyldiglycerides–monogalactosyldiglyceride (MGDG) and digalactosyldiglyceride (DGDG) are the basic lipid components of the membrane system of higher plant chloroplasts. These are slightly polar uncharged lipids characterized by a high level of unsaturation of the fatty acyl chains [1–3]. The remaining phospholipids and sulfolipids have a single negative charge head group [4]. Transversal and lateral asymmetry is characteristic for the lipid distribution in thylakoid membranes [5–7]. The unusual lipid composition of the chloroplast membrane and the high level of unsaturation of the fatty acids are supposed to favor the optimal photosynthetic activity and to maintain the structure of chloroplast membranes [8–13].

Convincing evidences, such as the shift in the liquid-to-gel phase transition temperature [3] and the dependence of the capability to the formation of bilayer or micellar structures [12,13] of the abundant chloroplast lipid MGDG upon small changes in the level of saturation, indicates the importance of the lipid unsaturation for the chloroplast structural organization.

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PII S0301-4622(97)00042-2

Among the variety of approaches to study the role of lipid saturation in the structural organization and function of thylakoid membranes [6,10,13–15], the genetic approach has been recently applied as well [16–20].

Chloroplasts carry high negative surface charge due to protein and lipid components [21–25] which is important for the segregation of the membrane chlorophyll protein complexes in appressed (grana) and non-appressed (stroma) domains. Electrostatic interactions of the pigment–protein complexes with the charged head groups of the acidic lipids and van der Waals interactions with the hydrophobic phase of the membrane stabilize the oligomer pigment–protein complexes and their tight packing in the membrane [13,26].

In this paper, we report on the study of surface electrical properties of two mutant lines (JB67 and LK3) and the wild-type of *Arabidopsis thaliana* by electric light scattering. The two *Arabidopsis* mutants are deficient in lipid fatty acid desaturases, which is due to a single nuclear mutation of locus, designated: *fad*B (JB67) and *fad*C (LK3). *Fad*B mutant contains a reduced level of unsaturation of the 16-carbon acyl chains and *fad*C contains high levels of palmitoleic ($C_{16:1}$) and oleic ($C_{18:1}$) fatty acyl residues [17,18]. The reduction of the level of lipid unsaturation is accompanied by: enhanced thermal stability of both mutant forms; decreased rate of the electron transport; decreased amount of appressed membranes, chlorophyll, protein and LHCP II content [19,20,27,28].

The observations we have made on the wild type and mutant forms are: (i) existence of a permanent dipole moment (asymmetric charge distribution) and electric polarizability of thylakoids from the wild type and both mutant forms; (ii) a strong effect of the reduced level of lipid unsaturation on both electric moments and anisodiametricity of mutant thylakoids; (iii) a reduced amount of the LHCP II in *fad*B and *fad*C mutants as compared to the wild type revealed by thylakoid trypsinization.

2. Materials and methods

Seeds of wild type, JB67 and LK3 *Arabidopsis thaliana* (L.) Heynh mutants were obtained from Nottingham Arabidopsis Stock Centre. Chloroplasts were isolated as in Ref. [29] and osmotically shocked in ice-cold H_2O for 60 s. Then double strength buffer (20 mM Tricine, 0.2 M sorbitol, 20 mM NaCl, pH 7.8) was added. The thylakoid membranes were precipitated by centrifugation at $10000 \times g$ for 10 min. The pellet was resuspended in 10 mM Tricine, 0.1 M sorbitol, 10 mM NaCl, pH 7.8 for proteolytic treatment. The membranes were treated with different trypsin concentrations for 5 min as described in Refs. [30,31]. Membranes for electrooptical measurements were washed and resuspended in 0.25 M sorbitol, chlorophyll (chl) concentration was 5 μg chl/ml, pH 6.0 and conductivity 7 μS cm^{-1}.

The steady state electrooptic effect $\alpha = (I_E - I_0)/I_0$, defined as a relative change in the intensity of the scattered light, is caused by the interaction between the electric moments of the membranes and the orienting electric field. I_E and I_0 are the intensities of the light scattered by the membrane suspension when an external electric field of strength E is applied and without field, respectively. At low degree of orientation (i.e. $U/kT \ll 1$, U is the energy of orientation, kT is the thermal energy) α is expressed by [32]:

$$\alpha = \frac{A(Kl, Kd)}{I_0(Kl, Kd)}(\beta^2 + \gamma)E^2 \qquad (1)$$

where $A(Kl,Kd)$ and $I_0(Kl,Kd)$ are optical functions dependent on the dimensions of the particles; $K = (2\pi/\lambda')\sin^2(\theta/2)$; λ' is the wavelength of the light in the suspension, θ is the angle of observation (90° in this case); l and d are the semiaxis of an ellipsoid. $\beta = p/kT$, $\gamma = (\gamma_{\parallel} - \gamma_{\perp})/kT$, where p is the permanent dipole moment arising from the asymmetric surface charge density of the membrane particles, i.e. along the particle symmetry axis; $\gamma_{\parallel}$ and $\gamma_{\perp}$ are the electric polarizabilities along the symmetry and transversal axis, respectively; k and T have their usual meaning.

The dependence of the electrooptic effect α on the frequency (ν) of the applied a.c. field is related to different polarization mechanisms. This allows us to determine the contribution of permanent and induced dipole moments to the orientation of thylakoid membranes. The induced dipole moment is estimated from the initial slope of the electric field strength

dependence $\alpha(E^2)_{a.c.}$, measured at a frequency where the permanent dipole does not contribute to the alignment of the particles ($\beta = 0$) [33]:

$$\gamma_{\parallel} = \frac{I_0(Kl, Kd)}{A(Kl, Kd)} kT \left(\frac{\partial \alpha}{\partial E^2} \right)_{E \to 0} \quad (2)$$

where $(\partial\alpha/\partial E^2)_{E \to 0}$ is the initial slope of the a.c. electric field dependence.

Using the value of electric polarizability $\gamma_{\parallel}$, the permanent dipole moment p can be evaluated from the initial slope of d.c. field dependence of α or from the low frequency part of the frequency dependence $\alpha(lg\nu)$ using Eq. (1).

The dimensions of the thylakoids are estimated from the relaxation time of disorientation of the particles τ (at full orientation of the particles) after switching off the electric field [34,35].

3. Results

3.1. Electric light scattering of wild type and mutants of Arabidopsis thaliana

The electrooptic effect α arising from the orientation of dispersed particles upon application of an external a.c. electric pulses depends on the frequency and intensity of the pulses. The frequency dependence (Fig. 1A, Fig. 2A, Fig. 3A) was measured at low field strength ($E^2 = 0{,}15 \times 10^8$ V^2/m^2) where the electrooptic effect depends linearly on the square of the externally applied electric field, i.e. at a low degree of orientation. In the kHz range (called a plateau) the orientation of the thylakoids is determined by the charge(s) motion in the diffuse electric double layer (i.e. by the induced dipole moment). At low frequencies (in the Hz region) α is usually related to a permanent dipole moment along the minor axis of the particles [32]. Thus the frequency dependence allows to distinguish polarization mechanisms (permanent and induced electric dipole moments) with different relaxation times. Complementary data on both dipole moments are obtained by measurements of the electrooptic effect α as a function of the electric field strength (a.c. and d.c. electric fields). The wild type and two mutant forms of *Arabidopsis* thylakoid membranes exhibit similar orientational behavior, i.e. the electrooptic effect is

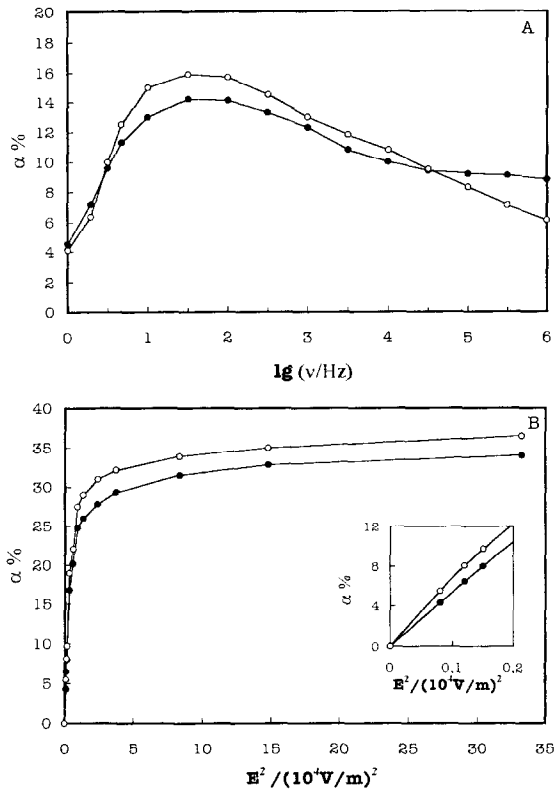

Fig. 1. Effect of trypsin treatment on the frequency dependence (A) and on the square of the applied a.c. electric field, $\nu = 100$ Hz, (B) of the electrooptic effect for wild type *Arabidopsis* thylakoids (●) and trypsinized thylakoids (○) with 20 μg trypsin/mg chl. Inset, initial part of the electric field dependence.

positive in the whole frequency range 0–1 MHz under study (Fig. 1A, Fig. 2A, Fig. 3A). Besides this similarity, however, some important differences are observed. A short plateau in the range of 10 to 500 Hz of the frequency dependence is observed for the wild type and *fad*C mutant, and a much longer plateau for the *fad*B mutant. The α effect has the highest magnitude in the plateau region for the wild type of *Arabidopsis* thylakoids, about twice as that for the mutant forms. Therefore, the wild type thylakoids have larger induced dipole moment (or electrokinetic charge density $\gamma_{\parallel}/S$) than both mutant forms (Table 1). The same conclusion follows from the initial linear part of the a.c. field dependence of the electrooptic effect (measured at low intensities of the applied electric pulses; Fig. 1B, Fig. 2B, Fig. 3B, inset).

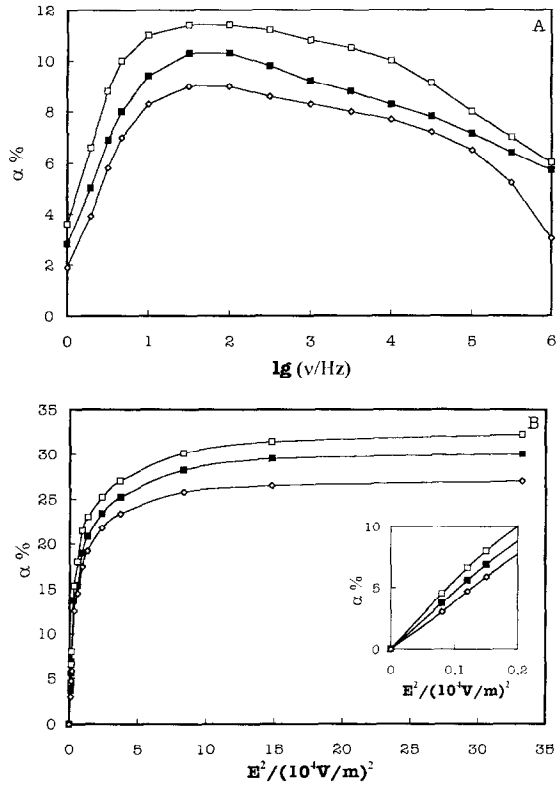

Fig. 2. Dependence of α on the frequency of the applied field (A) and on the square of the applied a.c. electric field (B) of *fad*B mutant thylakoids (■) and after proteolysis with trypsin, (□) 10 μg trypsin/mg chl and (◇) 20 μg trypsin/mg chl. Inset, initial part of the electric field dependence.

The electrooptic effect decreases with the frequency decrease below 100 Hz, but remains positive for the all samples (Fig. 1A, Fig. 2A, Fig. 3A). This means a contribution of the permanent dipole moment in the orientation of the thylakoid membranes.

Thylakoids from the mutant forms have a lower relaxation time than the wild type as estimated from the relaxation process of disorientation at their full orientation (Table 1). The axial ratio $p_1 = 1/d$ decreases after the lipid modification as well.

3.2. Effect of trypsinization of Arabidopsis thylakoids on the electrooptic effect

Previous electrooptic studies of pea thylakoids has shown strong changes in the electric moments upon proteolytic treatment depending on the enzyme concentration and the time of treatment [31]. Larger values of the permanent dipole moment and electric polarizability have been estimated for pea thylakoids enzymatically-treated with 20 μg trypsin/mg chl for 5 min [31].

Changes of α in opposite directions appeared upon mild trypsin treatment of *Arabidopsis* thylakoids of wild type and mutant forms with an enzyme concentration of 20 μg trypsin/mg chl for 5 min. A pronounced increase of α for the wild type (Fig. 1) and a decrease for both mutants (Figs. 2 and 3) was observed after this treatment. This corresponds to an increase in the electrokinetic charge density $\gamma_{\|}/S$ for the wild type and almost no change for both mutants after proteolytic treatment performed at the above conditions (Table 1). This could

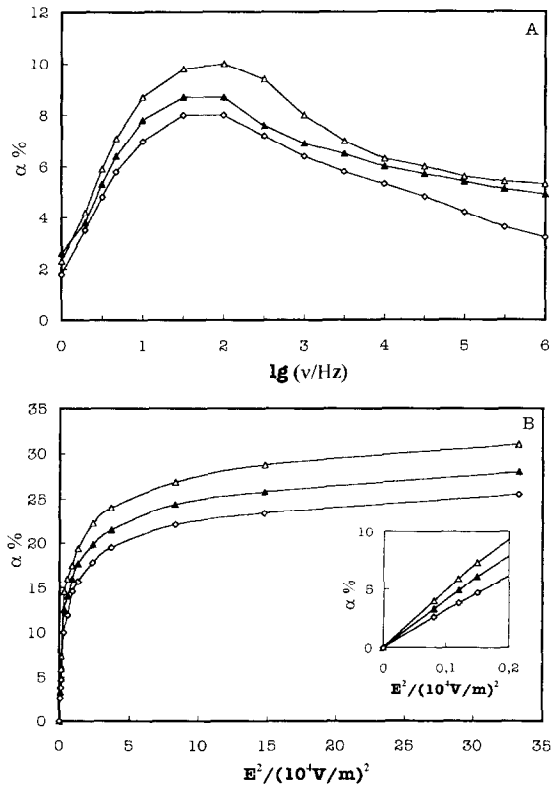

Fig. 3. Effect of trypsin treatment on the frequency dependence (A) and on the applied a.c. electric field dependence (B) of *fad*C mutant thylakoids. Untreated mutant thylakoids (▲), thylakoids trypsinized with 10 μg trypsin/mg chl (△) and 20 μg trypsin/mg chl (◇). Inset, initial part of the electric field dependence.

Table 1
The electrokinetic charge density (estimated to be proportional to the electric polarizability $\gamma_{\|}$ per unit surface area, S); permanent dipole moment (p); relaxation time of thylakoids disorientation (τ) and $p_1 = 1/d$ ratio of the long (l) and short (d) axis of the thylakoid membranes, considered as prolate ellipsoids, of wild type and mutant forms of *Arabidopsis thaliana* thylakoids before and after 5 min proteolytic digestion

Sample	$(\gamma_{\|}/S)\times 10^{16}$ (F)	$p\times 10^{24}$ (C m)	τ (m s)	$p_1 = 1/d$
Wild type	1.54	0.56	205	3.3
20 μg trypsin /mg chl	1.87	0.67	185	
*fad*B	1.27	0.48	170	2.5
10 μg trypsin /mg chl	1.46	0.51	160	
20 μg trypsin /mg chl	1.28	0.47	130	
*fad*C	1.25	0.52	160	2.5
10 μg trypsin /mg chl	1.64	0.54	140	
20 μg trypsin /mg chl	1.37	0.47	120	

be due to differences in the trypsin/polypeptide ratio because of altered protein content (i.e. a lower LHCP II content) in the mutants [19,20,28]. Thylakoid membranes were then treated with other concentrations of trypsin. Treatment of thylakoids from both mutant forms with a trypsin concentration of 10 μg trypsin/mg chl was found to produce changes in the frequency dependence of α in the same direction as 20 μg trypsin/mg chl does on the wild type thylakoids (Fig. 1A, Fig. 2A, Fig. 3A). Consequently the values of electric moments slightly increase after trypsinization of the mutant thylakoids (Table 1).

Short trypsin treatment (20 μg trypsin/mg chl, 5 min), on the other hand, results in ±4% decrease of the size (l) of wild type and 10% of mutant thylakoids.

4. Discussion

The present work proves that thylakoid membranes from wild type *Arabidopsis thaliana* possess transversal electric charge asymmetry (p) and electric polarizability along the membrane plane ($\gamma_{\|}$) like the pea thylakoids [31,36]. Higher relaxation time of disorientation (τ) was measured for the wild type *Arabidopsis* thylakoids than that previously observed for pea thylakoids [31] which points to a difference between the dimensions of the thylakoid membrane system from *Arabidopsis* and pea. The axial ratio $p_1 = 1/d$ is higher for the wild type than that estimated for pea thylakoids ($p_1 = 1.5$) [31] as well. Therefore, the geometry and anisodiametricity of thylakoid membranes from both higher plant species differ significantly.

The level of lipid unsaturation has a strong effect on the electric moments of *Arabidopsis* thylakoids (Table 1). The lower value of the electrokinetic charge density $\gamma_{\|}/S$ of *fad*B and *fad*C mutant thylakoids indicates a changed structure of the electric double layer of both mutants as compared to the wild type thylakoids. A presence of regular arrays of freeze-fracture particles associated with photosystem II core and LHCP II of chloroplasts of JB67 and LK3 mutants has been recently proved by Tzvetkova et al. [28]. The observed alteration of the electric double layer of the mutant thylakoids might be related to these specific structures.

The transversal charge asymmetry considerably decreases when the level of unsaturation of fatty acyl chains is reduced. This is more pronounced for the *fad*B mutant (Table 1). The interpretation of the changes in the electric dipole moments can not be limited to changes only in the lipid composition. The orientational behavior of the mutant forms may reflect a change in the exposure of charged polypeptides on the thylakoid membrane surface(s) arising from alteration of the lipid geometry induced by the different composition of the lipid acyl chain. A number of features of the mutant thylakoids like the reduced pigment–protein content, amount of appressed membranes, granal width and number of thylakoids per granum [19,28] may have strong influence on the surface electric properties. The smaller size and lower axial ratio of the mutant thylakoids as compared to the wild type might be related to the reduced granal width and appressed to non-appressed membrane ratio [19,28].

Hugly et al. [19] reported a significant loss of the pigment content (ca. 2 molecules chl *a* for one molecule of chl *b*) and LHCP II content associated with the lipid modification of *Arabidopsis thaliana*.

To verify the role of LHCP II, the effect of proteolysis on the electric moments of wild type and mutant forms was studied. Similar to trypsinization of pea thylakoids an increase in the values of the permanent dipole moment and electric polarizability is observed upon proteolytic digestion of wild type *Arabidopsis* thylakoids (Fig. 1, Table 1) performed at the same treatment conditions (trypsin concentration and time of incubation) as in Ref. [31]. Proteolysis at these conditions leads to opposite changes of the electric moments of thylakoids from both mutant forms (Figs. 2 and 3, Table 1). The removal of small positively charged polypeptides (2 kDa) from the LHCP II of the mutants results in a similar effect (on the electric properties) to that observed for the wild type at half the enzyme concentration. This is consistent with the previously found reduction in the amount of LHCP II in the mutants [19,20]. Thus, the difference between the trypsinization effect on the electric properties of mutant and wild type thylakoids might occur not only because of a change in the level of lipid unsaturation but because of the reduced level of LHCP [19,20], associated with the lipid modification.

The electrooptic measurements demonstrate the significance of the level of thylakoid lipid unsaturation in determining the surface electric charge distribution most probably through changes in the pigment–protein content and membrane appression arising from the lipid modification.

Acknowledgements

This work was supported by contract K-519 from the Bulgarian National Fund, Science Research.

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