

UNSATURATED FATTY ACIDS INDUCED CHANGES IN SURFACE CHARGE DENSITY AND LIGHT - SCATTERING IN PEA THYLAKOIDS

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Summary. The influence of the unsaturated fatty acids (oleic, linoleic, linolenic and arachidonic) on the electron-transport processes in thylakoid membranes were investigated.

UFA caused an inhibition of the fast ion-exchange processes with photosynthetically active light ($2950 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). A decrease in the dark relaxation processes produced by UFA after switching off the light followed the same order of effectiveness as the slow ion-exchange processes (arachidonic acid > linolenic acid > oleic acid > linoleic acid) and decreased the level of fatty acids unsaturation. A dark swelling of the stacked thylakoids in the presence of 2–55 μM concentrations of UFA was observed.

The addition of UFA to thylakoids produced various surface charge density and photoinduced scattering effects.

Key words: unsaturated fatty acids, light-scattering, surface charge, thylakoids, pea (*Pisum sativum* L.)

Abbreviations: UFA – unsaturated fatty acids; LS – light-scattering; EPM – electrophoretic mobility; Tricine – N-tris(hydroxymethyl)methylglycine; PMS – phenazine methosulphate; OIA – oleic acid; LIA – linoleic acid; LnA – linolenic acid; ArA – arachidonic acid.

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Introduction

Numerous and extensive studies have been reported on the mechanism of the chemical treatment on the structure and function of thylakoid membranes.

Thylakoid membranes bear a net negative surface charge in the physiological pH domain (Dilley and Rothstein, 1967). The corresponding negative electric surface potential may be considered as an entity that controls the ionic compensation in diffuse double layer adjacent to the membrane surface and thereby possessing intrinsic metabolic and structural membrane properties, including the translocation of ions (Gross and Prasher, 1974; Barber et al., 1977).

The light-scattering technique is a convenient tool for studying the characteristics of biological particles. The method of light-scattering (LS) can be used as a mean for detecting conformational changes and characterizing the energized state of the thylakoid membrane (Thorne et al., 1975; Yakovleva and Molotkovsky, 1982).

The associated conformational changes which take place in the membrane occur as a result of the protonation of the anionic groups and of osmotic shrinkage or swelling of thylakoids. There exists a dependence, although not a linear one between the increase of LS and the formation of the light-induced proton gradient. Moreover, the changes in LS at 535 nm can be correlated with the cation transport at physiological conditions (Krause, 1984). Coughlan and Schreiber (1984) conclude that the narrow angle 90° scattering component is a direct reflection of the light induced proton pumping across the membrane.

The aim of the present investigation was to study the effects of some unsaturated fatty acids on the surface charge density and light-scattering changes in pea thylakoid membranes.

Materials and Methods

The method proposed by Whatley and Arnon (1963) was used to isolate thylakoids from 14-day-old pea seedlings (*Pisum sativum* L.) which had been grown in a greenhouse under sunlight. The isolation medium contained 67 mM phosphate buffer pH 7.80, 330 mM sorbitol and 5 mM MgSO₄.

The thylakoids were stored in liquid nitrogen by the method of Goldfeld et al. (1978). Immediately prior to use the thylakoids were thawed and diluted with a buffered medium 10 mM Tricine (KOH), pH 7.80, 5 mM MgSO₄ to a chlorophyll concentration of 6 µg.ml⁻¹.

The dark swelling of thylakoids was estimated by the decrease in basal light-scattering level at $\lambda=550$ nm.

Photoinduced scattering kinetics of thylakoid membranes were evaluated by measurement of the LS increase at 90° ($\lambda=550$ nm) with a modified spectrophotom-

eter. The photo cell was protected with cut-off filters from actinic light ($\lambda > 640$ nm) with intensity $2950 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

The reaction medium used in the LS experiments contained $40 \mu\text{M}$ phenazine methosulphate (PMS) as one of the most effective mediators of photosynthetic electron transport processes.

The statistical programme was a modification of the least-squares method (Doltchinkova et al., 1993).

Electrophoretic mobility studies were performed using an OPTON Cytophometer (FRG) as described in Doltchinkova et al. (1993). The total ionic strength of the suspending medium was $\mu = 0.0031 \text{ mol}\cdot\text{dm}^{-3}$. The surface charge density was estimated from EPM data according to Barber (1982).

Tricine was purchased from Fluka AG, Switzerland. MgSO_4 was obtained from VEB Jenapharm-Laborchemie Apolda and phenazine methosulphate was obtained from Serva (Germany).

The following list of unsaturated fatty acids – Sigma Chem.Co., USA was tested:

Oleic acid (9-octadecenoic), free acid;

Linoleic acid (9,12-octadecadienoic), free acid;

Linolenic acid (9,12,15-octadecatrienoic acid), free acid;

Arachidonic Acid (5,8,11,14-eicosatetraenoic acid), free acid dissolved in ethanol as 0.1 mM stock solutions.

The experiments were carried out at 20°C after 10 min incubation of thylakoids with UFA added to the definite quantities of incubation medium and then diluted to the final volume (2 ml for LS and 20 ml for the EPM measurements).

The viscosity of the different media, including the thylakoids, was evaluated by measurement with a viscoelastometer (sample volume of 1 ml) constructed in the Technical University (Bulgaria).

Results and Discussion

UFA can influence several structural parameters and photochemical functions of chloroplast *in vitro*. It was experimentally verified that fatty acids modified the physicochemical properties of the membrane, namely the osmotic properties in such a way that the thylakoid membranes swelled (Siegenthaler, 1973). Fatty acids damaged the membrane integrity in such a way that the subtle equilibrium between the factors controlling the electron flow activity: proton pump (buffer capacity), ΔpH , pH_i and pH_o , was disturbed (Siegenthaler and Depery, 1976).

The kinetic curves of photoinduced scattering of thylakoids after 10 min dark adaptation consisted of two main phases: fast and slow with different signal amplitude and characteristic time of enhancement (Doltchinkova et al., 1993). The inten-

sity of photoinduced scattering exponentially decreased after turning off the actinic light, but not always reached the initial state as in the presence of UFA in thylakoid suspension. Latter, we used the term decay phase of LS describing the dark relaxation of the system.

The dark scattering level at 90° represents the degree of stacking (Coughlan and Schreiber, 1984). The changes in scattering intensity of thylakoids under illumination had been caused by changes in selective dispersion induced by conformational changes of membrane components as a result of protonation of intrathylakoid luminal space (Thorne et al., 1975).

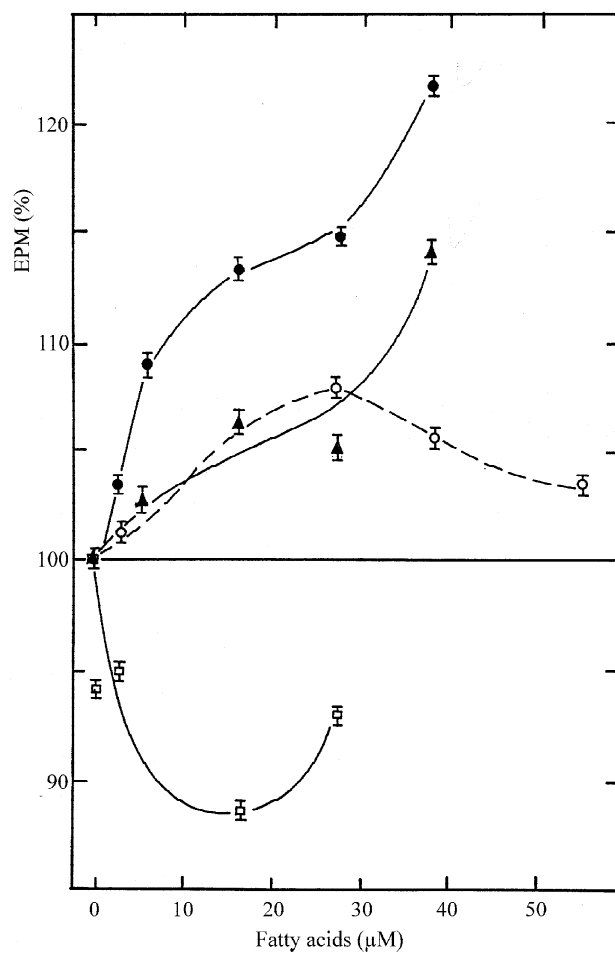


Fig. 1. Electrophoretic mobility of thylakoid membranes suspended in 10 mM Tricine (KOH) buffer, pH 7.80, 5 mM MgSO_4 treated with oleic (▲), linoleic (○), linolenic (●) and arachidonic (□) acids. Measurements were performed in the absence of PMS.

The surface charge density changes upon UFA treatment of thylakoid membranes were significantly less evident than the slow phase effects of LS. A 20% increase in EPM was detected of thylakoids treated with LnA and 15% – after OIA action (Fig.1). The enhancement of negative charges at the membrane surface was due to incorporation of the fatty acids into the membrane (Vernotte et al., 1983). A 5% effect on EPM was detected of thylakoids treated with LIA (Fig. 1). The EPM effect decreased only 10% post action of ArA. Similar dependences in the slopes of enhancement in photoinduced scattering of thylakoid suspension in the presence of UFA were ob-

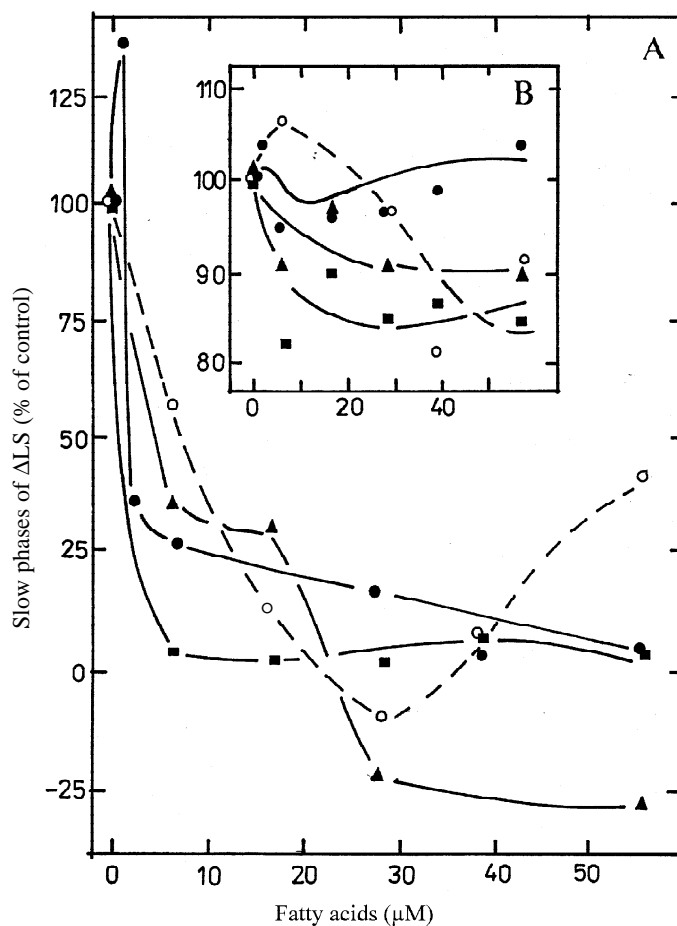


Fig. 2. The percent change in the basal LS (B) and in the slow phases of LS (A) as a function of oleic (▲), linoleic (○), linolenic (●) and arachidonic (■) acids. The standard reaction mixture contained 10 mM Tricine (KOH) buffer, pH 7.80, 5 mM MgSO₄, 40 μM PMS and various fatty acid concentrations.

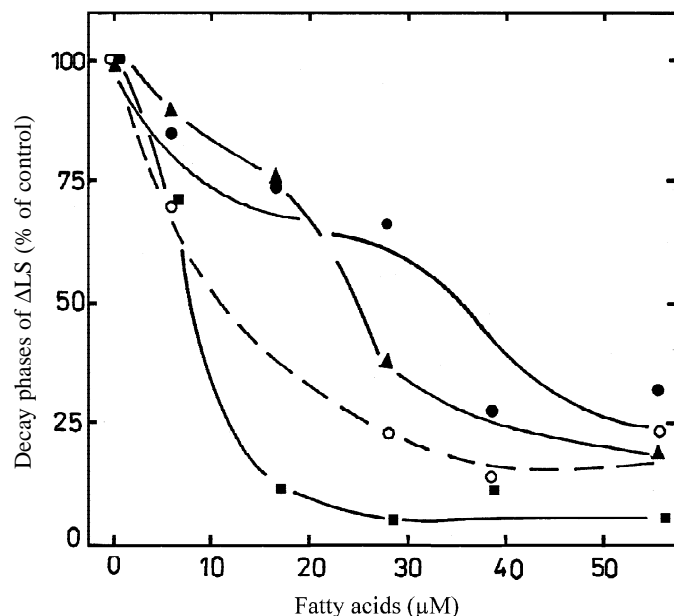


Fig. 3. The percent change in the decay phases of LS as a function of oleic (▲), linoleic (○), linolenic (●) and arachidonic (■) acids. The standard reaction mixture contained 10 mM Tricine (KOH) buffer, pH 7.80, 5 mM MgSO_4 , 40 μM PMS and various fatty acid concentrations.

served. The slope of the fast phases of LS after LnA, OIA or ArA treatment was strongly influenced (data not shown).

The slow phase of LS of thylakoids was altered much better even by the least concentrations of UFA investigated (Fig. 2). As known the oleic acid's anion possesses the highest inhibitory activity (Ljadsky, 1987). OIA altered the slow phases of LS up to -30% (Fig. 2).

Addition of LnA led to a small inhibition (up to 10%) of the secondary ionic-exchange processes characterized by the slow phase of the kinetic curve but in that case there was a concomitant slight decrease (up to 30 μM concentration range) and increase (at 40–55 μM LnA concentrations) in the basal LS level and in the EPM effect (Fig. 2, Fig. 1).

According to Vernotte et al. (1983) at alkaline pH an increase of negative charges in the membrane had been due to incorporation into the membrane of linolenic acid. We also detected 20% increase in EPM effect on thylakoids by LnA (Fig. 1)

As shown in Fig. 2 the slow phase increase induced by addition of 2 μM LnA was detected due to electrostatic effect of interaction in consequence of LnA-induced conformational change.

An inhibition of the processes concerning the relaxation and ΔpH dependent slow phases of LS in accordance to reduction in surface charge density effects of ArA

treated thylakoids was observed (Fig. 3, Fig. 2, Fig. 1). It could be explained by an ArA damage of membrane integrity accompanied by slight inhibition of the primary ionic-exchange processes (up to 29% decrease at all ArA concentrations used, Fig. 4). ArA was the most effective in decreasing the decay phase of LS at $\sim 15 \mu\text{M}$ inhibitory concentration (Fig. 3).

The ability of free fatty acid to inhibit stacking was previously reported by Shaw et al. (1976). UFA induced thylakoid swelling up to 17% in dark as shown by dark LS level (Fig. 4).

Negligible effects of OIA on photoinduced fast phase and basal LS of stacked thylakoids were detected (Fig. 4).

Upon OIA and LIA inhibitory alteration on the slow phase of LS only up to $50 \mu\text{M}$ concentration range the similar dependencies were registered. There were well pronounced differences in the course of the decay phase in the presence of OIA or LIA, consequently (Fig. 3).

According to the fast phase of LS level, we concluded that LnA and LIA ($55 \mu\text{M}$) induced a great inhibition of the primary ion-exchange processes whereas post OIA ($5\text{--}55 \mu\text{M}$) treatment practically no significant decrease of the fast phase of LS was occurred (Fig. 4). In thylakoids, we observed that ArA ($5\text{--}55 \mu\text{M}$) caused a detect-

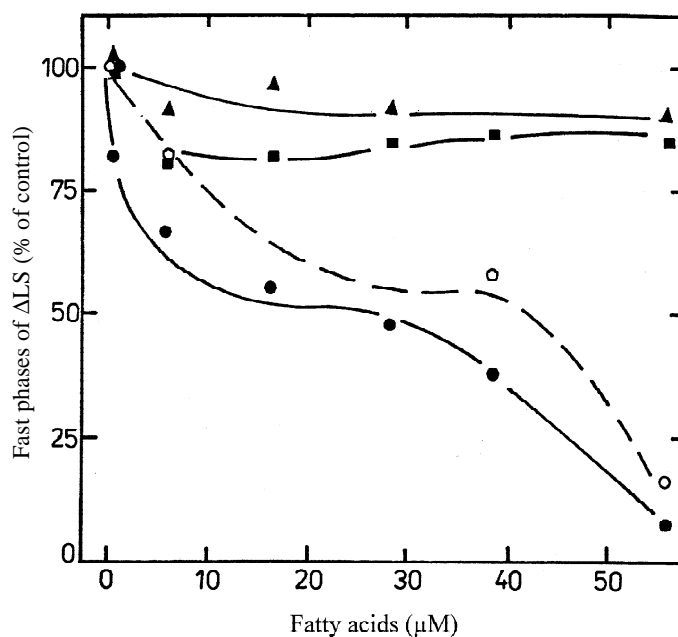


Fig. 4. The percent change in the fast phases of LS as a function of oleic (▲), linoleic (○), linolenic (●) and arachidonic (■) acids. The standard reaction mixture contained 10 mM Tricine (KOH) buffer, pH 7.80, 5 mM MgSO_4 , 40 μM PMS and various fatty acid concentrations.

able decrease in fast phase changes up to 30% (Fig. 4). These observations, however, open the possibility that the mechanism of ArA-induced action on fast ion-exchange processes might be analysed in relation to the structural differences in UFA.

The Δ LS kinetics of thylakoid membranes in the presence or without distinct concentrations of UFA were compared by statistical methods. Statistically significant variations in decay phases of LS were observed only in relation of LnA to ArA.

Higher negative surface charge density was expected in stacked thylakoids treated by OIA (σ varied from -0.0046 to -0.0054 C.m⁻²) and LIA (σ varied from -0.0048 to -0.0052 C.m⁻²). No significant changes in surface charge density effect by LIA (σ varied from -0.0046 to -0.0048 C.m⁻²) were produced. A slight reduction of the negative charges after involvement of ArA in thylakoid suspension was detected (σ varied from -0.0048 to -0.0044 C.m⁻²).

The present work demonstrated that linolenic acid induced significant enhancement of the net negative surface charge density of thylakoids at concentrations of 5–55 μ M of this study. Other fatty acid such as ArA was effective (although to a lower degree) in decreasing EPM values of thylakoid membranes.

A slight increase in EPM effect on thylakoids by OIA, LIA or LnA without any significant changes was detected in basal LS and a mild reduction in σ was observed with a great extent of inhibition in the Δ pH dependent ion-exchange processes (i.e. the slow phase of LS).

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