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# Distribution of Violaxanthin Between Water and Lipid Phases upon Osmotic Swelling of Liposomes

Dystrybucja wiolaksantyny pomiędzy fazą wodną i lipidową podczas osmotycznego pęcznienia liposomów

## INTRODUCTION

The narrow changes of absorption spectra of carotenoid-pigmented liposomes one observes, when they are subjected to heating or cooling procedure [1,2]. The spectral changes observed, are directly related to phase transition of a lipid component of liposomes. Phase transition of a lipid bilayer from gel to liquid crystal state results in a decrease of the order parameter of acyl chains forming a hydrophobic core as well as in an increase of lateral diffusion [3]. The two different explanations of phase transition-related spectral changes are based, in principle, on these mechanisms. The first one consits in an aggregation and deaggregation of a pigment component inside a lipid core, following changes in the physical state of lipids [1]. Another explanation is based on a distribution of pigment molecules between liposomes and surrounding water [2]. This process depending on a fluidity of a lipid phase was postulated to be responsible for the spectral effect because of the different absorption of pigment depending on environmental conditions [2]. Such dependence is particularly strong when the molecular organization (monomeric, oligomeric) of a pigment could be affected by the polarity of a solvent.

In this research note the further experimental evidence is presented supporting the latter concept.

## MATERIALS AND METHODS

Violaxanthin (5,6,5′,6′-diepoxy,3,3′-diol,$\beta$,$\beta$-carotene) was extracted from fresh nettle leaves and purified chromatographically on silica gel plates (Merck). Benzene : ethyl acetate : methanol mixture (75:20:5, v:v:v) was applied as a developing phase [4]. All solvents (analytical grade) were distilled before use under reduced pressure. $L-\beta-\gamma$-dipalmitoyl$-\alpha$-lecithin (DPPC) was obtained from Fluka. Liposome suspension was prepared by the method of Batzri and Corn [5]. Volume of 0.5 ml of ethanolic solution of DPPC ($3 \times 10^{-2}$M) was injected to 10 ml of 1/15 M phosphate buffer, pH 7.5. Procedure of pigmentation of the liposomes with violaxanthin was carried out by an injection of a small amount of an ethanolic solution of the pigment directly into the liposome suspension in a buffer (20 $\mu$l per 2 ml of the suspension). The final lipid/pigment molar ratio was equal to 162.

The slow osmotic swelling of liposomes was achieved by the dialysis of a liposome suspension by its incubation in a dialysis tube submersed with the phosphate buffer, 10 mM, pH 7.5 (10 ml of the liposome suspension per 900 ml of incubation). The dialysis was carried out at 25°C, below the phase transition for DPPC.

Spectrophotometrical measurements were carried out with Specord UV--VIS apparatus (Carl-Zeiss, Jena).

## RESULTS AND DISCUSSION

Violaxanthin admixed to lipids demonstrates absorption spectra typical for its monomeric form with the main maximum at 449 nm [6]. It is generally accepted that carotenoid pigments dissolved with hydrated organic solvents appear in an aggregated form [7]. The aggregation of pigments is demonstrated in particular by the shift of their absorption spectra toward lower wavelengths. The aggregates of violaxanthin in water possess their main absorption maximum at 401 nm [2,8]. In the present experiment the ratio of the absorbance registered at the two wavelengths: I 449/401 corresponding to the absorption of violaxanthin as a monomer in lipid core (449 nm) and as an aggregate in water (401 nm) was employed to demonstrate the distribution of the pigment between these phases. The pigment was injected in a small amount of ethanol to a suspension of the DPPC liposomes. Absorption spectrum of liposomes pigmented in such manner does not show the picture related to a one, pure form, proving the same the distribution of the pigment between lipid and water [2]. After

pigmentation the liposomes were subjected to osmotic swelling affecting their structure by an increasing of an area occupied by one lipid molecule in a surface of the membrane. Liposome swelling increase as well a distance between acyl chains inside a lipid core. Such effect in liposome is analogical to that observed in the conditions of a lipid phase transition. The process

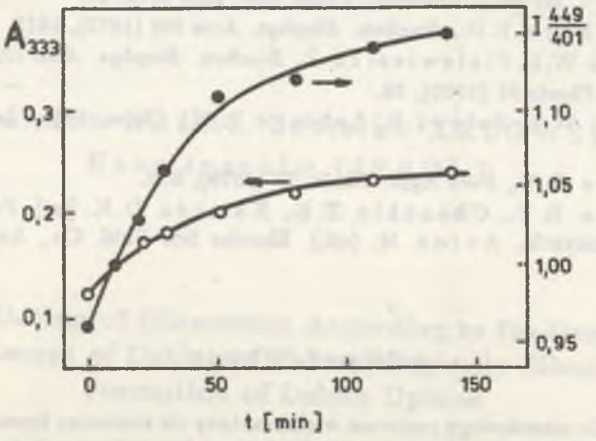


Fig. 1. Absorbance of unpigmented DPPC liposome suspension registered at 333 nm (– o – ) and ratio of the absorbances registered at 449 nm and 401 nm — I 449/401 of the pigmented liposome suspension (– • –) as a function of the time of dialysis

of swelling of liposomes was monitored by the scattering-related increase of an absorbance registered in the short-wavelength region of the spectrum of unpigmented liposomes at 333 nm (Fig. 1). Fig. 1 presents also the dependence of value of the parameter I 449/401 on the time of incubation. It clearly shows the swelling-related increase of the absorbance value measured at 449 nm as compared to that measured at 401 nm. Such result is an indication of the increase of a solubility of violaxanthin within lipid phase in course of the structural changes of liposomes. The fact, that miscibility of violaxanthin with lipids depends on a physical properties of a membrane suggests that this mechanism could be involved in the regulation of availability of the pigment to its enzymatic de-epoxidation [9]. The dependence of violaxanthin accessibility to de-epoxidation on a presence of the chloroplast lipids was clearly demonstrated by the *in vitro* studies [10]. Changes of the properties of thylakoid membranes, referred to above, one may relate to light-dependent alterations of the charged surface of a membrane as well as the surface of membrane proteins.

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## STRESZCZENIE

Wstrzyknięcie etanolowego roztworu wiolaksantyny do zawiesiny liposomów otrzymanych z lecytyny dwupalmitynowej powoduje rozdział barwnika pomiędzy fazę wodną i lipidową. Dystrybucja barwnika zmieniająca się podczas osmotycznego pęcznienia liposomów jest zjawiskiem popierającym jedną z hipotez na temat natury zmian spektralnych barwnikowanych liposomów. Postulowane jest fizjologiczne znaczenie badanych zjawisk.