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# Carotenoid binding to proteins: Modeling pigment transport to lipid membranes

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ABSTRACT

Carotenoid pigments play numerous important physiological functions in human organism. Very special is a role of lutein and zeaxanthin in the retina of an eye and in particular in its central part, the *macula lutea*. In the retina, carotenoids can be directly present in the lipid phase of the membranes or remain bound to the protein–pigment complexes. In this work we address a problem of binding of carotenoids to proteins and possible role of such structures in pigment transport to lipid membranes. Interaction of three carotenoids, beta-carotene, lutein and zeaxanthin with two proteins: bovine serum albumin and glutathione S-transferase (GST) was investigated with application of molecular spectroscopy techniques: UV–Vis absorption, circular dichroism and Fourier transform infrared spectroscopy (FTIR). Interaction of pigment–protein complexes with model lipid bilayers formed with egg yolk phosphatidylcholine was investigated with application of FTIR, Raman imaging of liposomes and electrophysiological technique, in the planar lipid bilayer models. The results show that in all the cases of protein and pigment studied, carotenoids bind to protein and that the complexes formed can interact with membranes. This means that protein–carotenoid complexes are capable of playing physiological role in pigment transport to biomembranes.



## 1. Introduction

Carotenoid pigments (see Fig. 1 for chemical structures) are ubiquitous in the biosphere and play several important physiological functions, among which protection against oxidative damage of biological structures seems to be one of the most important [1,2]. Very special is a role of carotenoids in the retina of an eye and in particular in its central part called the *macula lutea* [2]. It is generally accepted that macular carotenoids act as antioxidants and play additionally a role of blue light filter, protecting photoreceptors against photo-degradation [2–4]. It is a matter of ongoing debate whether a natural environment of physiological functioning of carotenoids in the retina is a lipid phase of the membranes or rather membrane-situated pigment–protein complexes [5,6]. Importantly, protein–carotenoid complexes have been isolated from the human *macula lutea* [5]. The complexes were composed of the Pi isoform of the glutathione S-transferase (GST) and zeaxanthin (Zea), one of the principal macular xanthophyll. It is possible that GST acts solely as a pigment transporter to the membrane but also that protein–xanthophyll complexes remain intact in the membrane environment, in where they can act as antioxidants and blue-light filters. Polar carotenoids, such as Zea and lutein (Lut), present directly in the lipid phase of the membranes are reported to influence considerably the physical properties of lipid bilayers and to protect lipids against oxidative degradation [3,7]. In the present work we address the problem of binding of carotenoid pigments to proteins and interaction of such complexes with model lipid membranes. Several carotenoid-binding proteins have been isolated and described in vertebrate and invertebrate tissues [8]. Two essentially different proteins have been selected in the present work, to study molecular interactions with carotenoids. One, with reported ability to form specific pigment–protein complexes, the Pi

*Abbreviations:* Zea, zeaxanthin; Lut, lutein; β-car, β-carotene; BSA, bovine serum albumin; HSA, human serum albumin; GST, glutathione s-transferase; FTIR, Fourier-transform infrared absorption spectroscopy; BLM, bimolecular lipid membrane; CD, circular dichroism; THF, tetrahydrofuran; EYPC, L-α-egg yolk phosphatidylcholine; PBS, phosphate-buffered saline; ATR, attenuated total reflection.

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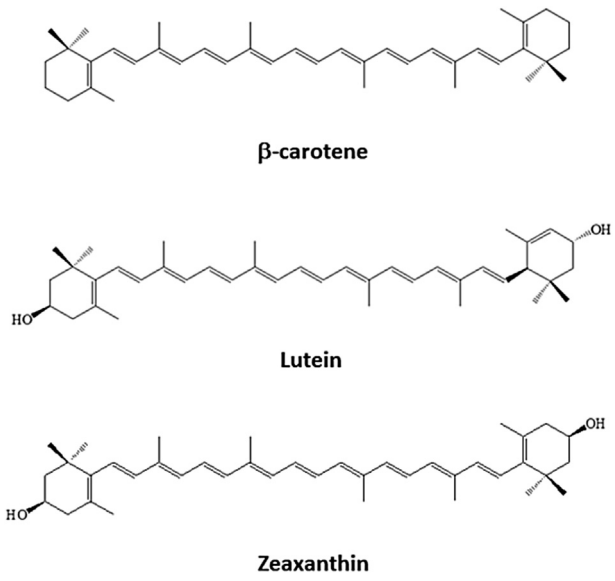


**Fig. 1.** Chemical structure of β-carotene, lutein and zeaxanthin.

isoform of the glutathione S-transferase [5], referred to GST, and BSA (bovine serum albumin) which is a prominent representative of the transport protein class. BSA is an analog of human serum albumin (HSA), which is the most abundant protein in human blood plasma and constitutes ca. half of the blood serum protein fraction. HSA has been shown not to form specific pigment–protein complexes with Zea but to bind relatively efficiently Lut, with a dissociation constant $k_d = 0.54$ μM [5]. It has been concluded that the carotenoid may occupy 3 binding sites in albumin molecules. A specific binding of Lut and Zea has an inverse affinity in GST as compared to HSA. The dissociation constant for Zea binding to GST has been determined at the level $k_d = 0.14$ μM while relatively weak binding of Lut has been observed ($k_d = 1.30$ μM) [9]. It has to be noticed that carotenoid–protein complexes which bind pigments specifically have been examined exclusively in the studies referred to above. The pigment fractions bound nonspecifically to protein surface have been removed by washing with hexane [5,9]. On the other hand, nonspecific binding of carotenoids to protein molecules may be also expected, in particular in water environment, owing to the fact that dietary carotenoids found in human blood plasma (such as β-carotene, lycopene, lutein and zeaxanthin [10]) are water insoluble. Hybrid pigment–protein molecular organization forms may play a physiological role in transporting carotenoids within an organism. Binding of carotenoid pigments to proteins and possible activity of GST and BSA in carotenoid transport to lipid membranes are addressed in the present study.

## 2. Materials and methods

### 2.1. Chemicals

Zeaxanthin was isolated from fruits of *Lycium barabarum*, lutein was isolated from leaves of *Spinacia oleracea* while β-carotene was purchased from Sigma Aldrich Chem. Co. (USA). Zeaxanthin (Zea) and lutein (Lut) were purified chromatographically by HPLC technique (on the phase-reversed, C-18 column), flow velocity 0.8 mL/min, mobile phase: acetonitrile:methanol:water (72:8:3, v:v:v). Synthetic β-carotene (β-car) was dissolved in the HPLC mobile phase, methanol:hexane (4:1, v:v) and purified as in the case of zeaxanthin and lutein. The pigments were purified directly before experiments. More details regarding pigment isolation and purification in our laboratory were presented previously [11,12]. Carotenoid concentration, in organic solvent solutions, were evaluated based on the molar extinction coefficients listed by Britton [13]. Proteins, bovine serum albumin (BSA) and glutathione s-transferase from human placenta, containing the Pi isoform (GST) were purchased from Sigma Aldrich Chem. Co. (USA). Synthetic L-α-egg yolk phosphatidylcholine (EYPC) and Phosphate-buffered saline (PBS, pH adjusted to 7.4) were also purchased from Sigma Aldrich Chem. Co. (USA).

### 2.2. Sample preparation

Protein (BSA and GST) solutions were prepared in PBS (pH 7.4). The protein concentration in the solutions was ($1 \times 10^{-6}$ M). Carotenoid solutions were prepared in tetrahydrofuran (THF). In order to transfer carotenoids to THF, the solutions in other organic solvents were evaporated in darkness, under stream of gaseous Argon. Protein–carotenoid complexation was achieved via injecting of the pigment solutions, prepared in THF, into the protein solution in PBS at 37 °C. Final concentration of THF in the water phase was always 2% (by volume) and initial protein–pigment ratio was changed via variation in initial carotenoid concentration in a THF solution. THF has been selected as a solvent (following the protocol developed by Bhosale et al. [5]) owing to relatively high solubility threshold for carotenoids, very good miscibility with water and, importantly, because it does not cause any structural changes to proteins, at the concentrations applied. In the case of control samples carotenoid solutions in THF were injected into PBS (without protein). Following THF injection into the protein solutions in PBS, the samples were incubated for 1 h, at 37 °C, with continuous shaking. After incubation, the samples were transferred to refrigerator (4 °C) and incubated for 12 h. After that, possible aggregates and microcrystals of carotenoids, which were not bound to proteins and remained in the water phase, were removed from the samples by 20-min. centrifugation at 20 °C at 15 000× *g*. Samples were monitored at each step of preparation by means of absorption measurements in the UV–Vis spectral region.

Liposomes were formed from EYPC according to a general procedure described previously [14]. EYPC solution in chloroform was dried under gaseous nitrogen and was further kept under vacuum for 30 min in order to remove traces of an organic solvent. Large multilamellar vesicles were prepared by rehydration with the PBS buffer and vortex mixing: 3 cycles for 15 s separated by 5 min-incubation at 32 °C. After that, small unilamellar vesicles were prepared by sonication with VCX–130 ultrasonic processor (Sonics Inc., USA) for fifteen cycles of 3 s with 100% amplitude with a titanium probe. Sonication was carried out in the water bath, in equilibrium with ice (~0 °C). Final lipid concentration was 0.2 mg/mL.

### 2.3. Spectroscopic measurements

UV–Vis absorption spectra were recorded with Cary 50 spectrometer from Varian (Australia). Liquid samples were placed in quartz cells (1 cm optical path-length). The spectra were corrected by subtracting the Rayleigh-type light scattering background component proportional to $\lambda^{-4}$ and subjected to smoothing procedure (Savitzky–Golay, 2-nd order polynomial, 11 points).

Infrared absorption spectra were recorded by Vector 33 Fourier Transform Infrared (FTIR) absorption spectrometer (Bruker, Germany). The spectrometer was equipped with attenuated total reflection (ATR) attachment. The internal reflection element was a ZnSe crystal (45° cut) yielding 10 internal reflections. Typically, 10 scans were collected, Fourier transformed and averaged for each measurement. Absorption spectra at a resolution of one data point every 4 $cm^{-1}$ were recorded in the region between 4000 and