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Thermotropic phase behaviour of α-dipalmitoylphosphatidylcholine multibilayers is influenced to various extents by carotenoids containing different structural features – evidence from differential scanning calorimetry

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Abstract

Carotenoids are the effective modulators of physical properties of model and natural membranes. To demonstrate the relationship between the structure of carotenoids and their effect on the molecular dynamics of membranes, we have investigated the influence of five structurally different carotenoids: β-carotene, lycopene, lutein, violaxanthin, zeaxanthin and additionally carotane – a fully saturated derivative of β-carotene, on thermotropic phase behaviour of dipalmitoylphosphatidylcholine (DPPC) multilamellar vesicles by means of differential scanning calorimetry (DSC). The results obtained indicate that the carotenoids used modulated the thermotropic properties of multibilayers to various extents, broadening the pretransition and the main phase transition peaks and shifting them to lower temperatures. Pronounced decrease of pretransition enthalpy (ΔH_p) proves that carotenoids very strongly alter the membrane properties in its gel phase. Comparison of the influence of several carotenoids shows that a rigid, polyisoprenoid chain plays a basic role in altering the thermotropic properties of such membranes and the presence of rings without oxygen-containing groups has a minor significance for the observed interactions. Carotenoids containing epoxy and/or hydroxy groups attached to their rings modify the thermotropic phase behaviour of DPPC multilamellar vesicles stronger than carotenes – a result of their orientation in the DPPC bilayer.

Keywords: Carotenoid; DPPC; DSC; Lipid membrane; Liposome; Phase transition

1. Introduction

Carotenoids are generally known to play two main roles in photosynthetic organisms. Functioning in the light harvesting antennae they transfer excitation energy towards chlorophylls, extending the wavelength ranges used in photosynthesis [1–3]. Under excess of light, when the antenna system becomes overexcited, carotenoids protect the photosynthetic apparatus against photooxidative damage by quenching the triplet states of chlorophyll and also by scavenging free radicals and oxidising agents [4–6]. Carotenoids also play an important role in photoprotection against an excess of light, e.g. the xanthophyll cycle [7,8]. Although carotenoids are bound to proteins in the thylakoid membranes, some part of them may exist in a free form. They can modulate the thylakoid membrane fluidity [9] that, inclusive of the synthesis of lipid alkyl chains of proper structure, can be considered as an adaptive mechanism to unstable temperatures of plant environment. Antioxidative properties of carotenoids also seem to be important for organisms which do not photosynthesise. Animals, which do not have ability to synthesise carotenoids, receive them from food and incorporate it into their tissues: blood, muscles, liver, eye. There is growing evidence that carotenoids have anti-disease and health-promoting properties in

Abbreviations: DPPC, α-1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine; DSC, differential scanning calorimetry; ΔH_p, enthalpy change of lamellar-to-undullated-lamellar phase transition (pretransition); ΔH_m, enthalpy change of gel-to-liquid crystalline (main) phase transition; T_p, temperature of lamellar-to-undullated-lamellar phase transition (pretransition); T_m, temperature of gel-to-liquid crystalline phase transition; C_p, molar heat capacity of gel-to-liquid crystalline phase transition

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some kinds of cancer, e.g. breast [10] and prostate [11], coronary heart disease [12,13], age-related macular degeneration and cataract formation [12,14]; they may also stimulate the immune system [6]. Thus, carotenoids form an integral component of human diet. Molecular mechanisms responsible for the health-promoting action of carotenoids are not satisfactorily elucidated. It is highly probable that the factors involved are the antioxidative activities of these compounds, as well as their effect on the membrane physical properties.

Carotenoids are rigid, rod-shaped molecules that is a consequence of the presence of a polyisoprenoid chain with a conjugated double bond system, which is usually terminated with two ionone rings. The polyisoprenoid chain, being strongly hydrophobic, is localised inside the hydrophobic core of the membrane and its orientation seems to be rather casual [15–17]. Those carotenoids which have polar, oxygen-containing groups bound to their ionone rings (xanthophylls), may interact by means of these groups with the lipid heads on the opposite sides of the membrane. Therefore, xanthophylls are oriented nearly parallel to the fatty acid chains [15,18–22]. The role of carotenoids in the modulation of the physical properties of model and natural membranes has been studied for the past three decades (for recent reviews see: Refs. [18,23] and references therein). A general conclusion resulting from these studies is that carotenoids fluidise the membrane in its gel phase, and they exert a rigidifying effect on the membrane in its liquid crystalline phase. However, the papers published so far used casual combinations of carotenoids and lipid species, and up to now, no systematic studies on the dependence between the various structural elements of carotenoids and their effect on the physical properties of membranes have been performed. Additionally, the majority of these papers, especially the older ones, also suffer from the fact that carotenoid preparations were used for the experiments without rigorously checking their purity by high performance liquid chromatography (HPLC), and the high sensitivity of carotenoids to photooxidation was also often neglected.

The preliminary measurements of interaction between carotenoids and phospholipids in the model membranes by means of differential scanning calorimetry (DSC) have been previously performed [24–27]. However, these studies were limited to β-carotene and lutein [24], β-carotene and astaxanthin [27], β-carotene and zeaxanthin [25] or β-carotene, lutein and zeaxanthin [26]. Another weak point is that only in one of these papers [26] was the purity of carotenoid preparations checked by HPLC, but even in this case, the exact information about the degree of purity of carotenoids used is missing. In this paper, we undertook a systematic study to elucidate the role different structural elements of the carotenoid molecules play in their interaction with phospholipids. For this purpose, five structurally different carotenoids: β-carotene, lycopene, lutein, zeaxanthin, violaxanthin and a fully saturated β-carotene derivative, carotane, were selected and their influence on the thermotropic properties of the multilamellar vesicles made of DPPC were studied. DSC is well-suited for this kind of study because it does not require any external substances as a probe that could influence the structure and dynamics of membranes. Comparison of the effects of β-carotene and lycopene allows us to estimate the role of β-ionone rings in carotenoid structure, while comparison of β-carotene and its fully saturated derivative, carotane, shows the significance of polyisoprenoid chain rigidity that is a feature common to all carotenoids. A comparative account of β-carotene and zeaxanthin brings information about the role of hydroxy groups at 3 and 3′ positions of the ionone rings, whereas the significance of epoxy groups at 5,6 and 5′,6′ positions of these rings can be elucidated by comparing the effects of zeaxanthin and violaxanthin. Over and above these comparative aspects summarised in the preceding pages, the comparison of the effects exerted by lutein and zeaxanthin permits us to assess whether structural difference between these compounds, which is limited only to the position of one double bond in one of the ionone rings, might play a significant role in interacting with membranes.

2. Materials and methods

2.1. Materials; isolation and purification of carotenoids

L-α-dipalmitoylphosphatidylcholine (DPPC) and β-carotene were purchased from Sigma Chemical Co. (St. Louis, MO, USA), zeaxanthin and carotane (perhydro-β-carotene) were gifted by Hoffmann-La Roche (Basle, Switzerland). Violaxanthin and lutein were isolated from lucerne (*Medicago sativa*) leaves by a pigment extraction with acetone and cold saponification followed by column chromatography on alkalised silica gel (F254, Merck, Darmstadt, Germany) in hexane/acetone (4:1, v/v). Before isolation, the leaves were stored in the dark for several hours. Lycopene was isolated from tomato paste by extraction with chloroform/methanol (1:1, v/v). Then water was added in a stepwise manner until a good phase separation was obtained. Pigments from the chloroform fraction were separated by chromatography on MgO in hexane/benzene (9:1, v/v). DPPC was of 99% purity and it was used without further purification. Carotenoids (except of carotane) were purified using HPLC on reversed phase semi-preparative column (Apex Prepsil Ods., Jones Chromatography, Mid Glamorgan, UK), using PU 980 isocratic pump and PU 970 UV/Vis detector (Jasco Corp., Tokyo, Japan) set on 440 nm. β-carotene was purified using a mixture of methanol and ethyl acetate (17:8, v/v) as an eluent, lycopene – acetonitrile/hexane/methanol (5:2:2, v/v/v), zeaxanthin and lutein – acetonitrile/methanol/deionised water (72:8:1, v/v/v) and violaxanthin – acetonitrile/methanol/deionised water (72:8:8, v/v/v) mixtures. To check the purity of obtained carotenoid samples, aliquots were additionally separated on the Nucleosil 100 C18 analytical

column (Teknokroma, Barcelona, Spain) with the detector set on 250 nm and absorption spectra in the range 300–550 nm were recorded using a SLM DW 2000 Aminco spectrophotometer (Urbana, IL, USA). The final carotenoid purities were estimated to be between 91% and 98%. Both DPPC and carotenoids were dark-stored at −35 °C in nitrogen atmosphere and used shortly after their purification.

Concentrations of carotenoids were determined spectrophotometrically using the molar extinction coefficients ε [28]. Concentration of colourless carotane was determined by weighing. During isolation and purification, the carotenoids were protected from light. HPLC solvents were obtained from Lab-Scan (Analytical Sciences, Dublin, Ireland). Other chemicals were of the highest purity available.

2.2. Preparation of carotenoid-containing liposomes

The appropriate amounts of lipid and of a carotenoid dissolved in double-distilled chloroform were mixed and the solvent was evaporated under a stream of N_2. Further evaporation was carried out by keeping the samples under vacuum for 1 h. Dry lipid films were suspended with 20 mM Hepes buffer pH 7.2 and the multilamellar liposomes were prepared by vortexing the samples for 15 min at the temperature above gel-to-liquid crystalline phase transition of the pure DPPC (~42 °C). The final phospholipid concentration in the buffer was 1 mM. The concentrations of carotenoids in liposome membranes were in the range 0.05–10 mol%. To check the repeatability of measurements, two to four samples of each concentration were prepared.

2.3. Differential scanning calorimetry (DSC) measurements

The DSC measurements were performed using a Differential Scanning Calorimeter (CSC Model 6100 Nano 11, Calorimetry Sciences Corporation, Provo, UT, USA). The carotenoid-containing multilamellar vesicle suspension in buffer and the buffer used as reference were degassed before measurement. The scan range was 20 − 60 °C and heating/cooling scans rate were 1 °C per minute. The heating scans were carried out first. Obtained data were analysed using the original calorimeter software. The calorimeter accuracy for temperature of the main phase transition and for enthalpy were ±0.1 °C and ±0.2 kcal/mol, respectively.

3. Results

All heating profiles of the carotenoid-DPPC multilamellar vesicles were referred to those made of pure DPPC. It is known that the pure DPPC multibilayers exhibit three endotropic phase transitions: a subtransition occurring at about 20 °C, a pretransition (lamellar-to-undulled-lamellar phase transition) at 35 − 36 °C and a main (gel-to-liquid crystalline) phase transition at 41.8–42 °C. We did not record the subtransition peak because it was not observed for multibilayers prepared with the method we used. Presented endotherms of DPPC exhibit the pretransition with a mid-point temperature at 35.4 − 35.8 °C, an average enthalpy change (ΔH_p) of about 1.33 kcal/mol and the sharp, almost reversible peak of the main phase transition with a mid-point temperature at 41.8 − 42.0 °C and an average enthalpy change (ΔH_m) of about 8.22 kcal/mol (Table 1, Fig. 1). All thermal values obtained for the pure DPPC multilamellar vesicles are in agreement with the previously published data [29,30].

The effects of carotenoids on the phase transitions of DPPC liposomal membranes are summarised in Fig. 1. It

Table 1
The enthalpy and entropy values of the pretransition (ΔH_p, ΔS_p) and the main phase transition (ΔH_m, ΔS_m) of the DPPC multilamellar liposomes containing carotenoids or a carotenoid derivative – carotane

Carotenoid	Concentration (mol%)	The pretransition		The main phase transition	
		ΔH_p (kcal/mol)	ΔS_p (kcal/kmol)	ΔH_m (kcal/mol)	ΔS_m (kcal/kmol)
Carotane	0	1.1	0.004	7.8	0.025
	0.5	0.9	0.003	7.9	0.025
	1	0.9	0.003	7.5	0.024
	2.5	0.8	0.003	7.2	0.023
	5	0.7	0.002	6.8	0.022
	10	0.7	0.002	7.3	0.023
Lycopene	0	1.1	0.004	8.1	0.026
	0.5	0.9	0.003	7.9	0.025
	1	0.9	0.003	7.9	0.025
	2.5	1.0	0.003	8.1	0.026
	5	0.8	0.003	8.0	0.025
	10	0.6	0.002	8.1	0.026
β-carotene	0	1.6	0.005	8.6	0.027
	0.1	1.4	0.005	8.6	0.027
	0.25	1.1	0.004	8.7	0.027
	0.5	1.1	0.003	8.6	0.027
	1	1.0	0.003	9.1	0.029
	2.5	0.9	0.003	8.6	0.027
	5	1.0	0.003	8.6	0.027
Lutein	0	1.4	0.004	8.3	0.026
	0.1	1.1	0.004	8.4	0.027
	0.25	1.0	0.003	8.4	0.027
	0.5	1.2	0.004	8.7	0.028
	1	0.8	0.003	7.8	0.025
	2.5	–	–	–	–
	5	–	–	–	–
Zeaxanthin	0	1.4	0.005	8.4	0.027
	0.05	1.1	0.003	8.6	0.028
	0.1	1.3	0.004	8.8	0.028
	0.25	1.1	0.004	8.8	0.028
	0.5	1.3	0.004	8.7	0.028
	1	1.2	0.004	8.8	0.028
	2.5	–	–	–	–
	5	–	–	–	–
Violaxanthin	0	1.6	0.005	7.9	0.025
	0.1	0.9	0.003	8.1	0.026
	0.25	0.9	0.003	7.7	0.025
	0.5	0.8	0.003	8.1	0.026
	1	0.6	0.002	7.9	0.026
	2.5	–	–	–	–
	5	–	–	–	–

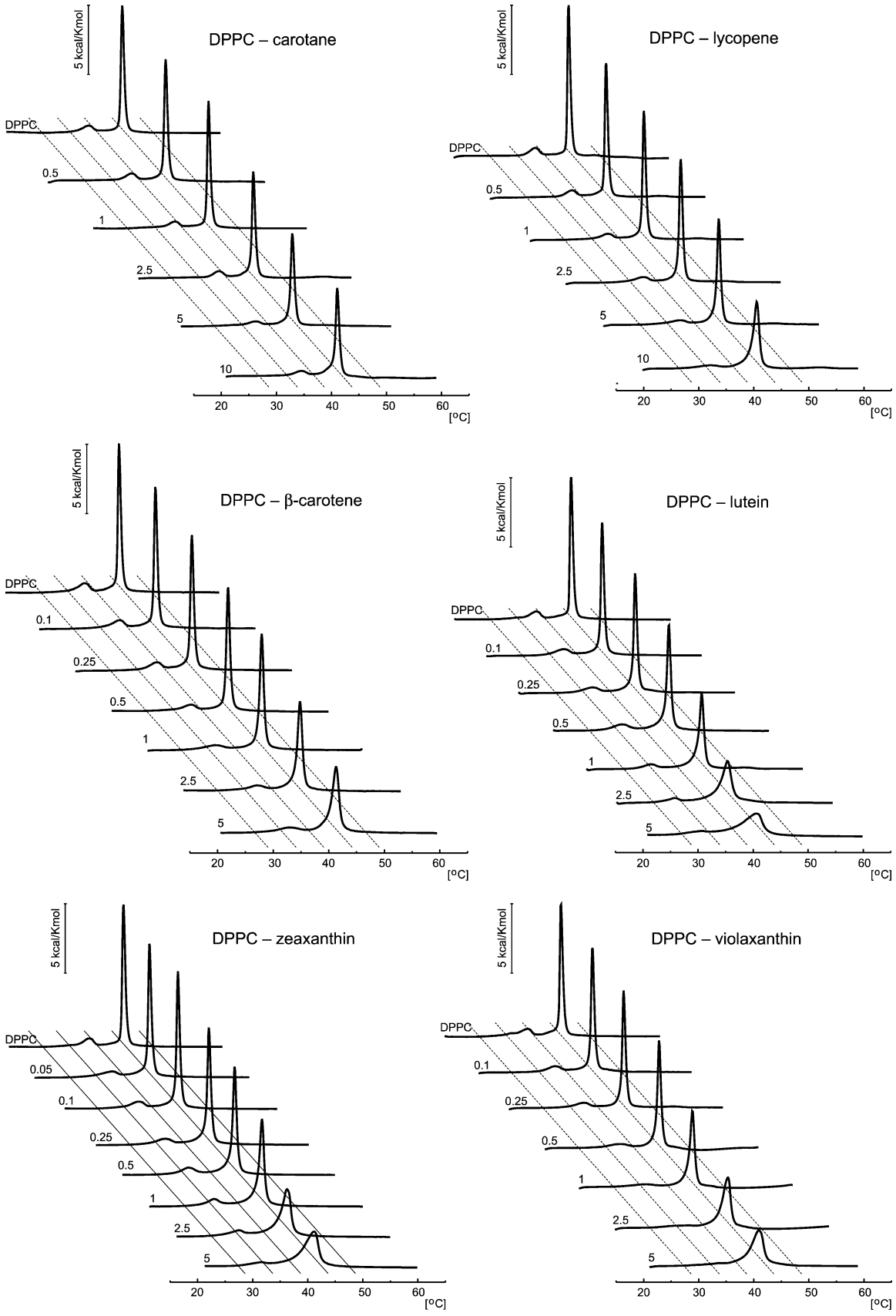

Fig. 1. The DSC heating thermograms of the DPPC–carotenoid multilamellar vesicles. Molar concentration of carotenoid is expressed on the curves.

shows that all carotenoids broaden the gel-to-liquid crystalline phase transition peak and the extent of this effect is strongly dependent on carotenoid species and concentration. The main phase transition peak becomes asymmetric and the pretransition peak becomes progressively smaller and more flat as the content of the carotenoid in the membrane rises. The decrease of molar heat capacity values for the samples containing carotenes and carotane is close to linear, whereas in the case of xanthophylls, the curve is rather hyperbolic (Fig. 2). The latter can be considered as a result of a possible aggregation of the xanthophyll molecules in the membrane at their higher concentrations (2.5 and 5 mol%). Therefore, to evaluate the influence of structurally different carotenoids on the DPPC multibilayers and to

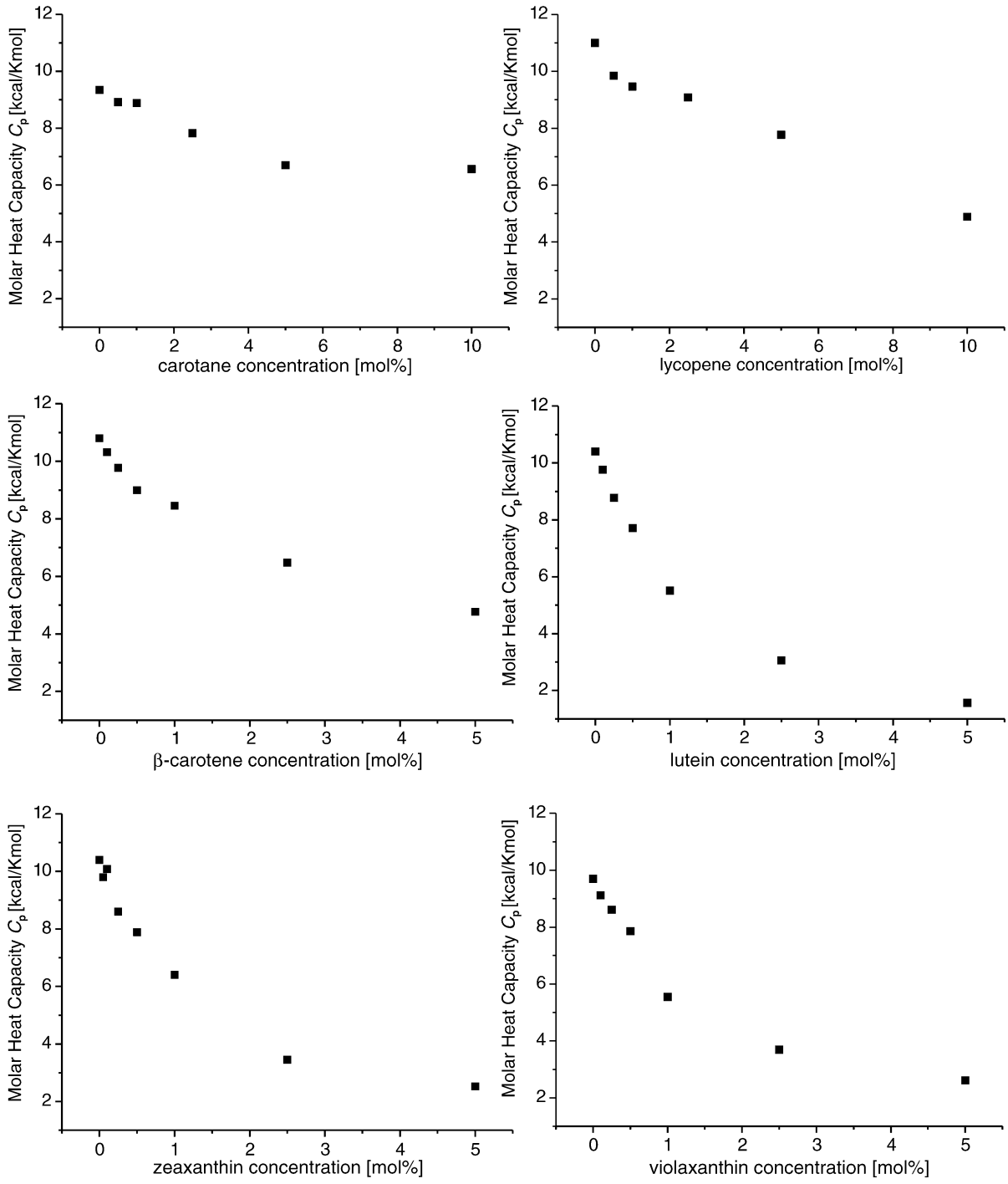

Fig. 2. The molar heat capacity (C_p) of the main phase transition as a function of carotenoid concentration in DPPC multilamellar vesicles.

Table 2
Selected thermal parameters of DPPC multilamellar liposomes containing different carotenoids at the concentration of 1 mol%

Compound	The molar heat capacity difference (ΔC_m)	The pretransition temperature shift (ΔT_p)	The main phase transition temperature shift (ΔT_m)	The width of main phase transition ($\Delta T_{1/2}$) [°C]
(1) DPPC	–	–	–	0.709
(2) Carotane	0.46	0.1	0	0.689
(3) Lycopene	1.56	0.5	0.1	0.721
(4) β-carotene	2.34	2.3	0.1	0.882
(5) Lutein	4.89	2.2	0.3	1.757
(6) Zeaxanthin	4.15	2.8	0.4	1.051
(7) Violaxanthin	4	3.2	0.3	1.067

avoid the effects which might result from the possible aggregation, we used samples containing 1 mol% of the respective carotenoid (Fig. 3, Table 2).

Increasing the amount of a carotenoid in multibilayers results in a decrease of the both phase transition temperatures. The analysis comparison of pretransition temperatures shows that the effect of zeaxanthin and violaxanthin is the strongest when the DPPC multibilayer is in the gel phase. These carotenoids in concentration of 1 mol% decrease the pretransition mid-point temperature by 3.2 and 2.8 °C, respectively (Table 2). Carotane and lycopene exert the weakest influence on the pretransition temperature (0.1 and 0.5 °C, respectively), while that exerted by β-carotene is close to the effect of xanthophylls. The strongest decrease of the temperature of the gel-to-liquid crystalline phase transition was found for the DPPC-xanthophyll multibilayers (0.3–0.4 °C), whereas the shifts exerted by incorporation of carotenes did not exceed 0.1 °C.

The presence of xanthophylls significantly broadens the main phase transition peak as can be seen in Fig. 3. The effect of carotenes is less pronounced and that of carotane is almost negligible, as compared to the control. The analysis of the main phase transition peak width ($\Delta T_{1/2}$) (Fig. 3, Table 2) shows the differences in the extent of perturbation of lipid alkyl chain cooperativity during the main phase transition caused by different carotenoid species. Figs. 1 and 3 show that all carotenoids have stronger influence on the DPPC membranes being in the gel phase than in the liquid crystalline phase.

Other thermodynamical data: the enthalpy and entropy changes of the pretransition (ΔH_p, ΔS_p) and such parameters of the main phase transition (ΔH_m, ΔS_m) are displayed in Table 1. The incorporation of carotenoids does not significantly influence the ΔH_m and ΔS_m values (i.e. only carotane shows rather slight decreasing effect). In contrast to the

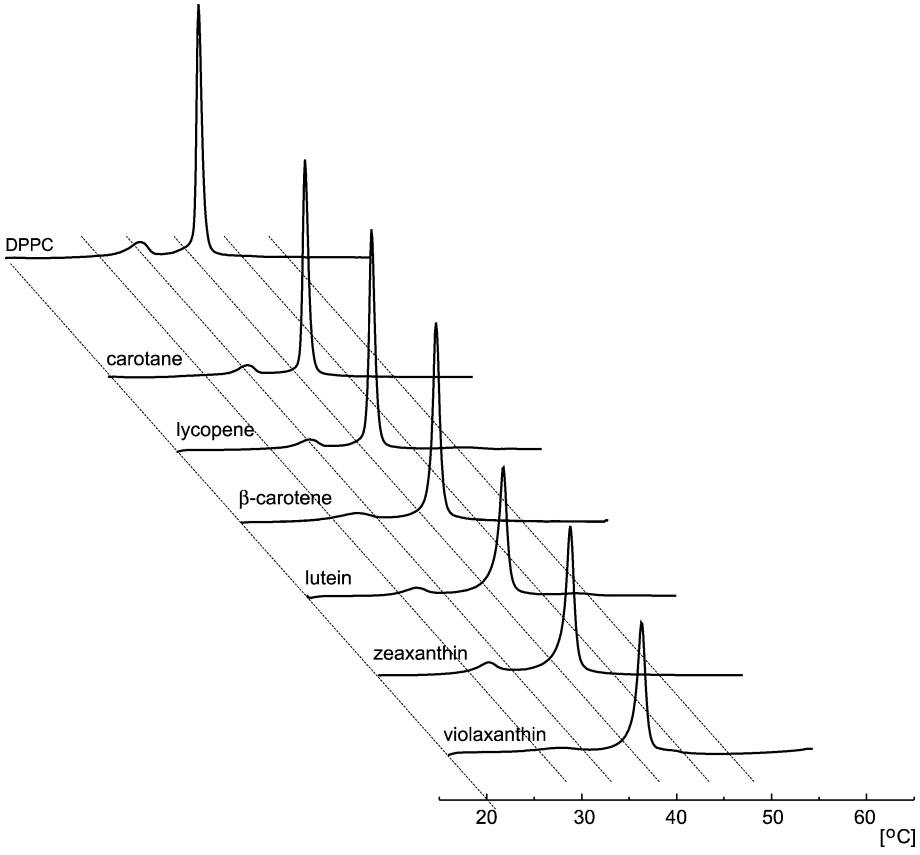

Fig. 3. The DSC heating thermograms of the DPPC multilamellar vesicles containing carotenoids at 1 mol% concentration.

ΔH_m and ΔS_m, the ΔH_p and ΔS_p values markedly decrease for all carotenoids studied. As can be noticed in Fig. 1, the presence of 2.5 or 5 mol% of xanthophyll in the bilayer causes strong decrease in the pretransition and the main phase transition cooperativity, so separation of the transition peak areas is not possible. Therefore, the enthalpy and entropy change values were not extracted for these samples.

4. Discussion

The carotenoids used in this study were selected on the basis of their structural differences to evaluate the significance of different structural elements of their molecules on thermotropic properties of DPPC multibilayers such as molar heat capacity, cooperativity and temperature shift of the pretransition and the main phase transition as well as changes of enthalpy and entropy. A characteristic feature of both carotenes and xanthophylls is their conjugated double bond system making them rigid, rod-like structures. As it was learned from our studies, the rigidity is the most significant feature of carotenoid molecule in their modifying effects on the membrane physical properties. When the rigidity is lost, the membrane-perturbing effect is also lost. This is clearly evidenced by a comparison of the effects on DPPC multibilayers of β-carotene molecule which is rigid, and its fully saturated derivative—carotane (perhydro-β-carotene). Due to the absence of the conjugated double bond system, carotane is a flexible molecule and its modifying effect on the measured thermal parameters of DPPC vesicles is negligible, even at its high concentration (10 mol%). Being flexible, carotane can adopt such conformations in the membrane which do not disturb the regular arrangement of the lipid molecules.

Among carotenoids, two structurally different groups can be distinguished: acyclic (e.g. lycopene) and cyclic. The latter contains carotenoids with ionone rings or their derivatives substituted with polar (oxygen-containing) groups. To evaluate the significance of ionone rings of carotenes for their interaction with the membrane lipids, the influence of lycopene (acyclic carotenoid) was compared to that of β-carotene (cyclic carotenoid with two β-ionone rings). We found that β-carotene was more effective than lycopene in modifying some thermal parameters of DPPC multibilayers. The most pronounced difference concerned the shift of the pretransition temperature, at a concentration of 1 mol%, β-carotene was almost five times more effective than lycopene (Fig. 4). The significantly stronger effect of β-carotene was also manifested in its influence on the molar heat capacity and cooperativity of the pretransition and the cooperativity of the main phase transition. On the other hand, there was no significant difference between the influence of β-carotene and lycopene on the shift of the main phase transition temperature.

The data presented indicate that, due to the presence of the ionone rings inserted among the lipid molecules, β-carotene disturbs the regular arrangement of phospholipid bilayer stronger than acyclic lycopene. The difference between the effects of β-carotene and lycopene is more pronounced when the membrane is in the gel phase, hence it manifests more distinctly in modification of the pretransition than of the main phase transition.

Another line of division of carotenoids is related to the presence of oxygen-containing groups in their ionone rings. Carotenes are composed of carbon and hydrogen atoms only and xanthophylls are their oxygen derivatives. In commonly occurring xanthophylls, oxygen exists in the form of hydroxy groups (e.g. lutein, zeaxanthin) or, additionally, the epoxy groups (e.g. violaxanthin). While the totally hydrophobic molecules of carotenes like β-carotene and lycopene adopt less defined orientations in phospholipid bilayer [15–17]; oxygen-containing xanthophylls are localised more regularly, with their long axis being oriented almost perpendicularly to the plane of the membrane [15,18–22]. In such orientations, polar (oxygen-containing) groups attached to ionone rings of the carotenoid molecule may form hydrogen bonds with the polar headgroups of lipids. The different orientations of carotenes and xanthophylls in the membrane has a strong impact on their interaction with the membrane lipids. In general, all xanthophylls studied (lutein, violaxanthin and zeaxanthin) perturb the cooperativity of the main phase transition stronger than the carotenes (lycopene and β-carotene) (Fig. 3) and pronouncedly change its molar heat capacity value (Fig. 2). Also, their effect on the temperature shift of the main phase transition was more distinct as compared with the carotenes (Fig. 4). The effect of xanthophylls on the pretransition temperature shift was similar to that of β-carotene at lower concentration (1 mol%) but it was definitely stronger at higher concentrations (2.5 and 5 mol%). However, the differences between the values of the pretransition enthalpy change of β-carotene and its oxygen derivatives: lutein and zeaxanthin, seem to be insignificant for all concentrations used in our studies.

These results clearly show that the presence of oxygen-containing groups in the ionone rings increases the modifying properties of carotenoids on some thermal parameters of the phospholipid bilayer. In general, these studies confirm our previous findings, where the more pronounced effect of xanthophylls on DPPC headgroups has been detected using X-ray diffractometry [31]. We explain the differences obtained for carotenes and xanthophylls referring them to their different orientations in the bilayer, i.e. the xanthophylls being oriented more perpendicularly to the plane of the membrane than the carotenes. It is known that perturbants localised closer to the centre of the bilayer hydrophobic interior have a weaker influence on the pretransition and the main phase transition peaks [32]. In principle, the carotenoid aggregation process may also influence, to some extent, the DSC thermograms. However, the latter possibility can be excluded because in the obtained thermograms, apart from the main transition and pretransition peaks, no

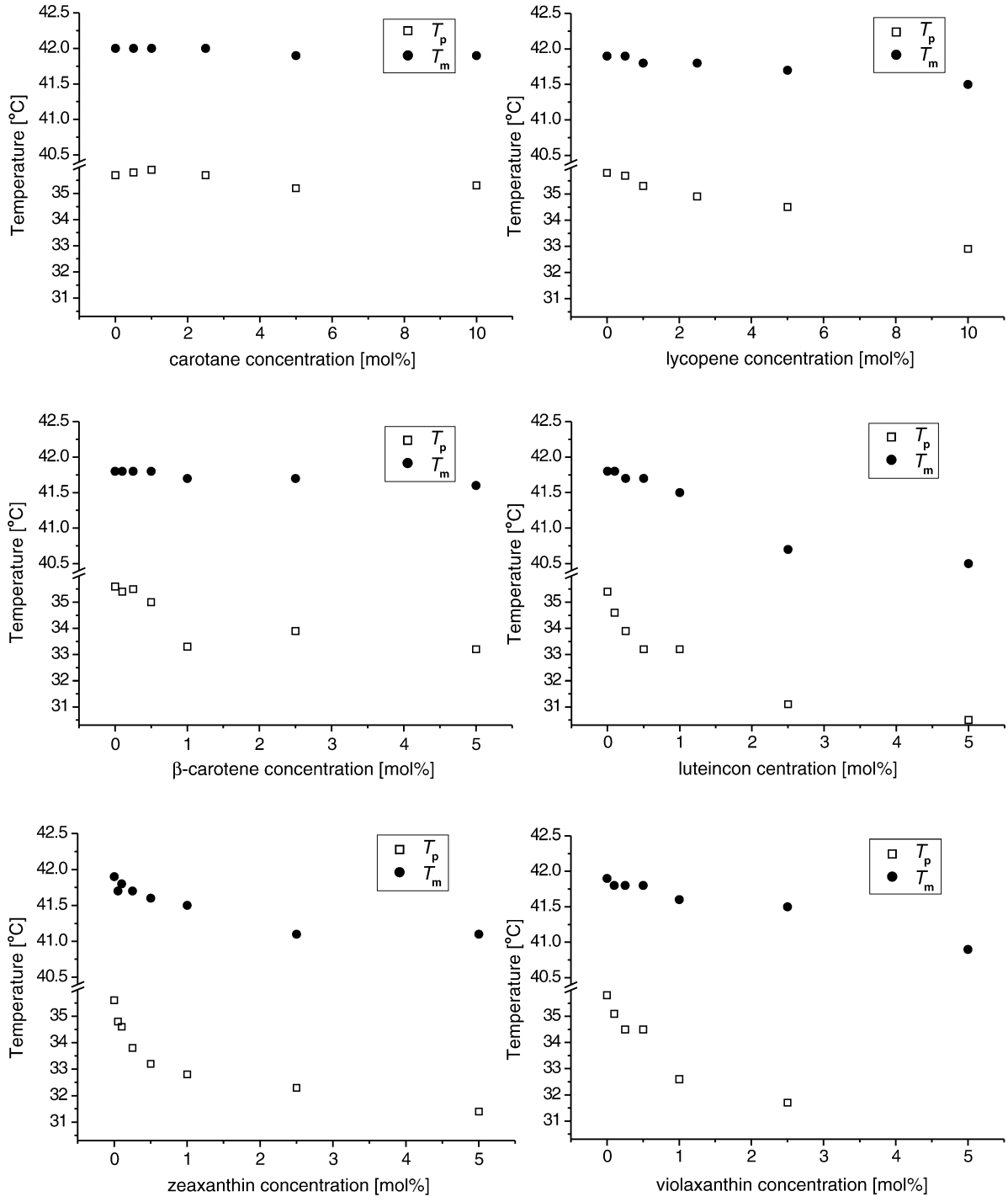

Fig. 4. The pretransition and main phase transition temperatures as functions of carotenoid concentration in DPPC multilamellar vesicles.

other additional peaks that could be attributed to melting of the carotenoid aggregates or phase separation were observed. It has been demonstrated that carotenoids aggregate easier at higher concentrations and the aggregation is enhanced in low (0–10 °C) temperature [33]. The temperature range of our measurements (20–60 °C) and low concentrations of pigments used in our studies suggest that the carotenoids and lipids are co-dispersed in the bilayer and the eventual aggregation process is negligible. Only at the highest concentrations of some xanthophylls (5 mol%) used in our studies did we notice slight asymmetry of the main transition peak, which could be a symptom of some aggregation or phase separation.

There are also differences among the three xanthophylls in their effect on thermal parameters of the DPPC multibilayer. Very interesting are the differences between lutein

and zeaxanthin. These two almost identical carotenoids are isomers differing in the position of one double bond only. However, this small structural difference has important consequences in dynamics. In the zeaxanthin molecule, the double bond in question is a part of the conjugated double bond system and, therefore, the adjacent ring does not have freedom of rotation. On the contrary, in the case of lutein, this double bond is localised in the ring in such a position that it is separated from the conjugated double bond system, the consequence of which is the rotational freedom of one ionone ring with respect to the rest of the molecule and the preferred ring-chain conformation is also different. As our data shows, among the three xanthophylls, it is the lutein that has the strongest impact on the molar heat capacity change as well as on the temperature shift of the pretransition and the main phase transition at higher concentrations of this carotenoid. The strong modifying property of lutein can be related to an increased repertoire of orientations in the membrane in comparison to other xanthophylls [34]. While zeaxanthin and violaxanthin molecules are oriented nearly perpendicular to the plane of the membrane, a significant proportion of the lutein molecules can adopt other orientations. Due to the rotational freedom of one of the two rings, these molecules can also orient rather parallel to the plane of the membrane. Since they are localised in the zone of lipid headgroups, they have strong influence on the molar heat capacity values and cooperativity of the main phase transition.

The effect of dihydroxycarotenoid, zeaxanthin on the DPPC thermal properties can also be compared to the effect of violaxanthin, which apart from hydroxy groups also has epoxy groups in its rings. The epoxy groups enhance the polar character of the violaxanthin molecule and they constitute an additional steric obstacle disturbing regular lipid arrangement in the gel phase. This is evidenced by the effect of violaxanthin on the broadening (Figs. 1 and 3) and the temperature shift (Fig. 4) of the pretransition. These results are also in good agreement with our X-ray diffraction studies [31]. On the other hand, the influence of violaxanthin on the molar heat capacity and temperature shift of the main phase transition peak is similar to that of zeaxanthin and lower than the effect of lutein on these thermal parameters (Figs. 2 and 4).

In conclusion, the presence of epoxy groups in addition to the hydroxy ones in the carotenoid molecule rings seems to predominantly affect the multibilayer in its gel phase. This is evidenced by changes in temperature, enthalpy and the molar heat capacity of pretransition. On the other hand, motional freedom of one of the two rings results in pronounced changes of thermotropic properties related to the main phase transition.

The results presented in this work clearly demonstrate that the interactions between carotenoids and lipids in the membrane strongly depend on the structure of the carotenoid, the rigidity of the molecule being the most important factor. These findings explain, to a greater extent, an evolutionary selection of some carotenoids to optimise their function in the membranes where they naturally occur [9]. On the other hand, they can be of importance for animal and human health. Supplementation of food, cosmetics and drugs with exogenous carotenoids has been gaining prominence in developed nations and is becoming a growing practice. When introduced into animal organism in higher amounts, the carotenoids may change the physical properties of some membrane systems with significant consequences for their functioning.

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