

ANGLE RESOLVED DEPOLARISED RESONANCE RAMAN STUDIES
OF BETA-CAROTENE IN LIPID/WATER MIXTURES

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The orientational ordering of beta-carotene incorporated in lamellar model membranes above their main phase transitions has been studied by angle resolved depolarised Resonance Raman scattering. The ordering strongly depends on the chemical composition of the lipid bilayers: in di-oleoyl lecithin (DOPC) the carotenoid molecules lie parallel to the bilayer plane, while in di-galactosyl diglyceride (DGDG) a bimodal distribution was found with molecules lying both parallel and perpendicular to the membrane phase.

Although beta-carotene molecules have been used as a Resonance Raman probe to monitor membrane fluidity [1] little is known about their actual organisation in the membranes. The orientation of carotenoids in model membranes had been studied by linear dichroism techniques [2,3]. In one study a preferential orientation of molecular chains perpendicular to the plane of the membrane [2] was found, while another indicated an orientation parallel to that plane [3].

Reliable conclusions about the orientational statistics can however only be drawn from the knowledge of both the order parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$ [4,5,6]. Whereas linear dichroism studies [3] only yield $\langle P_2 \rangle$, angle-resolved depolarised Resonance Raman scattering allows the determination of both $\langle P_2 \rangle$ and $\langle P_4 \rangle$ [4,7].

We have therefore studied the orientational statistics of beta-carotene molecules embedded in macroscopically ordered lipid bilayers by Resonance Raman techniques [8]. The temperature of the samples was well above the phase

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transition of the lipid chains. On taking the absorption moment of the main optical transition to lie along the long molecular axis [9] we have been able to determine the order parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$. The orientational distribution of the molecular axes in the membrane system was calculated by an information theoretic approach [10].

Our results show that the orientation of beta-carotene in lipid bilayers strongly depends on the chemical composition of the lipids.

Materials and Methods

All-trans β -carotene (from carrots, type III), and di-oleoyl lecithine (DOPC) were obtained from Sigma. Digalactosyl diacylglycerol (DGDG) was prepared as described in [7,11].

Ethanol and hexane, 'AR', were obtained from Baker. Deionized and doubly distilled water was used for sample preparation.

The purity of the materials was checked chromatographically. The absence of any fluorescence background from solvents and water was confirmed by recording their Raman spectra. Stock solutions of 10^{-3} M β -carotene in hexane were freshly prepared.

Solutions of lecithin or DGDG in ethanol were mixed with a solution of β -carotene to yield a molecular ratio $10^3 : 1$. The samples were dried in the dark by flushing with nitrogen gas. Water was added gravimetrically to the dry material to form a 30% (w/w) mixture. The sample was then allowed to equilibrate for 5 hours at 37°C under a humidified nitrogen atmosphere in the dark.

The hydrated lipid mixture was sandwiched between two microscope glass cover slips (thickness $175 \pm 5 \mu\text{m}$). Macroscopically oriented multibilayers were prepared at room temperature by gently rubbing the lipid mixture between the coverslips. The alignment was monitored by a polarising microscope and samples well aligned over at least one cm^2 were sealed along their four rims with a two component epoxy resin. Sample thicknesses determined by dark field microscopy [12] were between 12 and $42 \mu\text{m}$ with an accuracy of $2 \mu\text{m}$.

The angle resolved depolarised Raman spectra were obtained under excitation with an Ar^+ -laser (Coherent model CR 18-UV). An Anaspec 300 S premonochromator was used to filter the plasma lines. A 90° detection geometry was used. The state of polarisation of the laser beam was varied by rotating a half-wave plate positioned between two Glan-Thompson prisms. HN-32 polaroids were used as analysers.

The scattered radiation was passed through a Jobin-Yvon double monochromator, Ramanor HG-2S, equipped with a polarisation scrambler and two concave holographic gratings with 1200 lines/mm, to a peltier-cooled red-sensitive RCA 31034 A-02 PM tube operating at -25°C and -1800V .

The collection angle of the scattered radiation was 0.05 sterad.

The laser beam was focussed on the sample with a 101 mm focal length lens. The laser power at the sample position was 50 mW as measured with a CR 210 power meter.

For each of the four settings of polariser and analyser (horizontal or vertical) 4 spectra were accumulated in the multiple scan mode (scan speed $200 \text{ cm}^{-1}/\text{min}$., at a slitwidth of 6.5 cm^{-1} at $18,000 \text{ cm}^{-1}$, 556 nm).

Experiments were carried out using a random sequence of 8-10 different angles of incidence measured in air. Results were reproducible within experimental error (15%) indicating that a systematic photodegradation of the molecules was negligible at the laser power level used.

Integrated line intensities were calculated after background subtraction.

The refractive indices of the birefringent samples were determined with an accuracy of 0.5 0/00 with a thermostated Abbe refractometer (Bleeker) and a greatly expanded and therefore low intensity 514 nm laser beam in order to prevent eye damage.

Results

Typical angle resolved depolarised Raman spectra of β -carotene in lipid bilayers at a temperature of 18°C well above the phase transition of the carbon-carbon chains are shown in Fig. 1.

The resonance enhanced peaks at 1158 cm^{-1} and 1525 cm^{-1} (accuracy 2 cm^{-1}) have been assigned [1,13,14] to the single- and double bond stretching modes, ν_2 (=C-C=) and ν_1 (-C=C-) respectively.

No photo-isomerisation processes were observed at this laser power level (50 mW) since a 1250 cm^{-1} line assigned to cis-isomers [15] was not observed.

The angular dependence of the depolarisation ratios $R_o (=I_{oe}/I_{oo})$ and $R_e (=I_{eo}/I_{ee})$ for a DGDG sample is shown in Fig. 2. Indices o and e

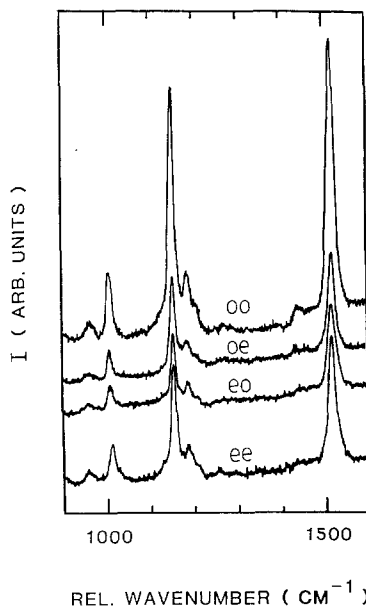


Fig. 1 Resonance Raman spectra of beta-carotene in a lamellar lipid multibilayer system: DGDG/water 70/30 (w/w) with 10^{-3} M beta-carotene. Sample temperature 18°C. Laser wavelength 514 nm. Laser power at the sample position 50 mW. Measurement angle $\phi = 60^\circ$. Indices o en e indicate ordinary and extraordinary beams (see text for details).

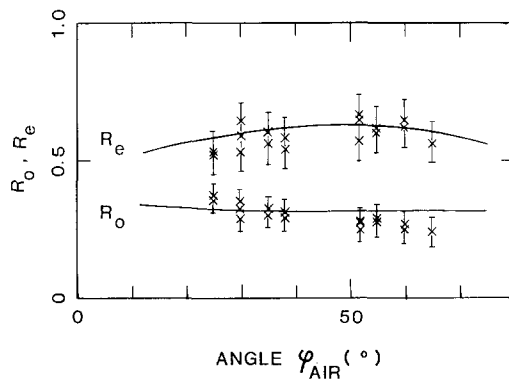


Fig. 2 The angular dependence of the ordinary and extraordinary depolarisation ratios R_o and R_e versus the angle ϕ in air. Sample: DGDG/water 70/30 (w/w) with 10^{-3} M beta-carotene. Laser wavelength 514 nm. Laser power at the sample position 50 mW. Lines: best fits for R_o and R_e .

indicate the ordinary and extraordinary experiment. The directions of polarisation of the incident beam (first index) and that of the scattered beam (second index) are respectively perpendicular (o) or parallel (e) to the plane of incidence.

The analytical expressions for the dependence of the depolarisation ratios R_o and R_e on the scattering geometry and order parameters have been given in [7]. They can also be derived in a straightforward manner from the relations given in [5] for fluorescence depolarisation experiments on setting $\tau_F = 0$.

The values of the order parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$ were obtained by a non-linear least squares method from $R_{o,e}$ [7]. The results are given in Table I. The value of $\langle P_2 \rangle$ determined by linear dichroism measurements [3] is given for comparison and found to be in excellent agreement with our results for DOPC.

Finally orientational distribution functions were calculated from the values of $\langle P_2 \rangle$ and $\langle P_4 \rangle$ using information theory [10]. In this approach the functions take the form of a Boltzmann distribution with the angle-dependent potential

$$U(\beta) = -kT \{ \lambda_2 P_2(\cos\beta) + \lambda_4 P_4(\cos\beta) \}$$

TABLE I
ORDER PARAMETERS $\langle P_2 \rangle$ AND $\langle P_4 \rangle$ FOR BETA-CAROTENE INCORPORATED
IN LIPID/WATER MIXTURES

70/30 (w/w) lipid/water mixture with 10^{-3} M beta-carotene in	Resonance Raman exp.			
	1158 cm^{-1}		1525 cm^{-1}	
	$\langle P_2 \rangle$	$\langle P_4 \rangle$	$\langle P_2 \rangle$	$\langle P_4 \rangle$
DGDG	0.12	0.10	0.05	0.05
DOPC	-0.25	-0.13	-0.22	-0.13
	linear dichroism exp. [3]			
DOPC	-0.22			

where P_2 and P_4 are Legendre polynomials of order 2 and 4 and β is the angle between the bilayer normal and the long molecular axes; λ_2 and λ_4 are chosen so as to reproduce the experimental values of $\langle P_2 \rangle$ and $\langle P_4 \rangle$. Fig. 3 shows these distribution functions for typical DGDG and DOPC samples.

Discussion

The results presented above were corrected for Fresnel effects [5]. In the analysis of the experimental data an optically isotropic sample was used with a refractive index of 1.45 at 18°C . This is valid to a very good approximation since the measured optical anisotropy of the samples appeared to be less than 0.5% [7].

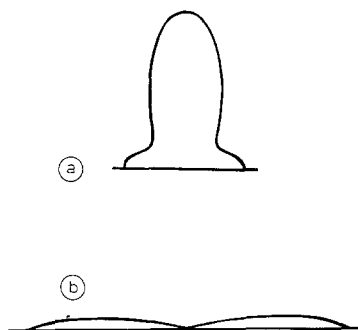


Fig. 3 Orientational distribution function for a) DGDG and b) DOPC. Samples: lipid/water 70/30 (w/w) containing 10^{-3} M beta-carotene.

Furthermore errors due to multiple reflections are smaller than 2% and can therefore be neglected with respect to other experimental errors.

The values obtained for $\langle P_2 \rangle$ and $\langle P_4 \rangle$ significantly deviate from the results predicted by a Maier-Saupe model [16] which uses a Gaussian orientational distribution of the molecular axes relative to the membrane plane. Thus orientational statistics based only on $\langle P_2 \rangle$ will give a misleading picture of the orientation of beta-carotene molecules in lipid bilayers.

The application of a technique such as angle-resolved Resonance Raman scattering, yielding both $\langle P_2 \rangle$ and $\langle P_4 \rangle$ is essential, in order to obtain a reliable description of the orientational distribution functions.

Inspection of Fig. 3 shows that carotenoid molecules embedded in DGDG bilayers give rise to a distribution function with two maxima: one parallel and one perpendicular to the bilayers.

The orientation of the long axes of the beta-carotene molecules in DOPC bilayers is however mainly along the plane of the membranes. We thus conclude that the orientation of beta-carotene in these lipid/water mixtures is sensitive to their chemical composition.

Furthermore we have here demonstrated that angle resolved Resonance Raman scattering can be used for determination of the orientational ordering of molecules in membrane systems.

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