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Raman and infrared spectroscopic study of phytantriol and phytantriol-water cubic phases

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Phytantriol and phytantriol-water cubic phases were studied by vibrational spectroscopy (FT-IR and FT-Raman). Assignments of the bands were performed based on the OH/OD isotopic substitution, measurements of FT-Raman polarization spectra and temperature-dependent spectral changes. The conformation and H-bonding sensitive modes have been identified. It has been shown that the frequency of methylene deformation vibration increases in liquid-crystalline cubic phases as compared with melted phytantriol, indicating an alteration of the interactions between alkyl chains in the lipidic layer. Analysis of the high frequency FT-Raman spectral range revealed broadening of the CH_3 asymmetric stretching band of cubic phases due to an increased heterogeneity of the local environment of methyl groups.

Key words: phytantriol, cubic phases, FT-IR, FT-Raman, hydrogen bonding

INTRODUCTION

Amphiphilic molecules spontaneously form various self-assembled structures in water [1]. Among them, the three-dimensional bicontinuous cubic phases [1–3] appear most promising for the practical applications, including crystallization of membrane proteins [4], development of drug delivery arrays [5], bioelectrodes [6], enzyme-based biosensors [7], and as model matrices to mimic biological membranes [8–10].

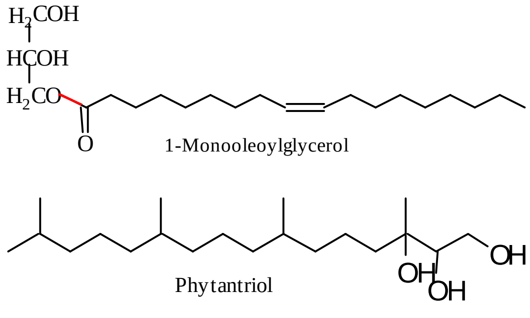

Fig. 1. The structures of monoolein and phytantriol

Monoolein (MO, 1-monooleylglycerol) (Fig. 1) is one of the lipids most frequently used for the preparation of cubic phases [1, 2]. However, because of ester linkage, this unsaturated monoglyceride suffers from a slow hydrolysis reaction. In addition, some ghosted biomolecules may interact with the double bond. Recently it has been demonstrated that chemically stable phytantriol (Phyt, 3, 7, 11, 15-tetramethylhexadecanthriol) (Fig. 1) forms the same liquid-crystalline cubic phases as monoolein [11]. We are interested in the molecular level characterization of the structure of lipids and ghosted molecules in the cubic phases [6, 9, 10]. The aim of this work was to elucidate the conformational and hydrogen bonding sensitive vibrational modes of phytantriole molecule and to determine alterations of the spectral bands induced by the formation of liquid-crystalline cubic phases. We have used vibrational spectroscopic techniques: FT-IR and FT-Raman spectroscopy.

MATERIALS AND METHODS

Phytantriol (96%) was obtained from Aldrich Chemical Co (Milwaukee, USA) and used without additional purification. Isotopic OH/OD substitution was performed by refluxing the solution of phytantriol in CD_3OD for 0.5 h, and evaporating the solution in vacuum. The procedure was repeated 4–5 times. The degree of isotopic exchange determined

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Table. **FT-Raman and FT-IR frequencies and vibrational assignments of phytantriol**

Frequencies, cm^{-1}	Assignment	Frequencies, cm^{-1}	Assignment
733	$\delta(CH_2)$	1148	$\nu(C\text{-}C)$
800	$\delta(COH)$*	1304	$\tau(CH_2)$
810	$\nu_s(C\text{-}CH_3)$, $\delta_s(COH)$	1336	$\delta_{as}(CH)$
866	$\delta_s(COH)$	1438	$\delta(CH_2)$
880	$\delta(COH)$*	1459	$\delta_{as}(CH_3)$, $\delta(CH_2)_{FR}$
929	$\nu(C\text{-}OH)$	2846	$\nu_s(CH_2)$
952	$\delta(CH_3)$	2869	$\nu_s(CH_3)$
1022	$\nu(C\text{-}OH)$	2892	$\nu_{as}(CH_2)$
1055	$\nu(C\text{-}C)$	2926	$\nu_s(CH_3)_{FR}$
1056	$\nu(C\text{-}OH)$*	2955	$\nu_{as}(CH_3)$

* FT-IR measurements; Abbreviations: δ, deformational; ν, stretching; τ, twist;
s, symmetric; as, asymmetric; FR, Fermi resonance.

by FT-IR spectra was 85%. All solutions were prepared from Millipore purified (18.2 MΩ cm) water.

Liquid-crystalline samples were prepared by weighing definite amounts of phytantriol in glass bottles, melting it at 26 °C, and then adding appropriate volumes of water required to obtain cubic phases with a *Pn3m* or *Im3m* symmetry. The mixture was immediately mixed, sealed and centrifuged for 1 h at 3000 ´ g and stored at room temperature for at least one week.

Infrared measurements were performed with a FT-IR spectrometer (Perkin Elmer, Model Spectrum GX) equipped with a DGTS detector. The sample was pressed between two ZnSe windows. The temperature of the sample was controlled with a water thermostat and was accurate within ±0.5 °C.

Raman measurements were carried out with a FT-Raman spectrometer (Perkin Elmer, Model Spectrum GX) equipped with an InGaAs detector operating at room temperature. The excitation was provided by an air-cooled diode-pumped Nd-YAG laser with an emission wavelength of 1064 nm. The laser beam was focused to a spot of area ~1 mm^2, and the laser power at the sample was set to 300 mW. In order to reduce photo/or thermo effects, the holder with the sample was moved linearly with respect to the laser beam (~20 mm s^{-1}) [12]. Polarized spectra were recorded in 90° geometry, while all other FT-Raman measurements were carried out in 180° geometry. The spectral resolution was set at 4 cm^{-1} and the wavenumber increment per data point was 1 cm^{-1}. All spectra were acquired by 200 scans. None of the spectra presented have been smoothed.

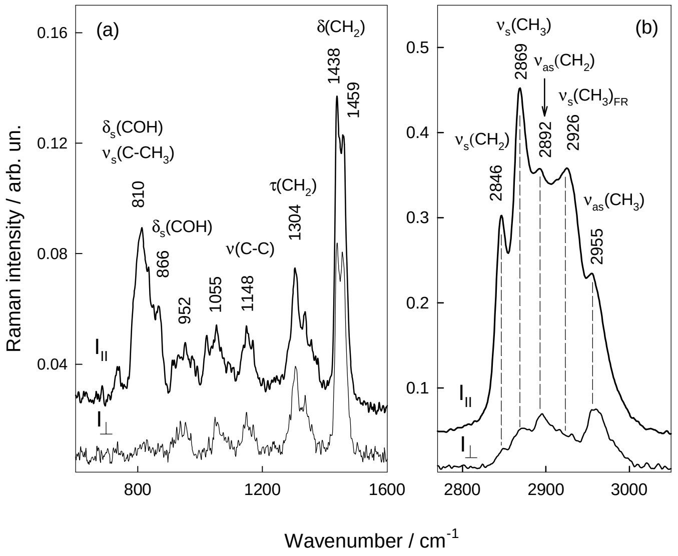

Fig. 2. Polarized FT-Raman spectra of melted phytantriol in the middle (*a*) and high (*b*) frequency spectral regions. Excitation wavelength is 1064 nm

RESULTS AND DISCUSSION

Figure 2 shows the polarized FT-Raman spectra of phytantriol in the middle (600–1600 cm^{-1}) and high (2700–3100 cm^{-1}) frequency ranges. Polarization measurements provide the possibility to distinguish between the symmetric and asymmetric vibrational modes and help in making the assignments of the bands. The depolarization ratio $\rho = I_{I} / I_{II}$ (where I_{I} and I_{II} is the Raman intensity of the particular mode in perpendicular and parallel polarization with respect to the incident laser beam, respectively) is between 0 and 0.75 for symmetric vibrations, while it equals to 0.75 for asymmetric vibrational modes. As one can see from Fig. 2, vibrations at 810, 866, 2846, 2869, and 2926 cm^{-1} clearly correspond to the symmetric vibrational modes. The intense band at 1438 cm^{-1} has been assigned to CH_2 deformation vibration, while the higher frequency component at 1459 cm^{-1} has been attributed to the asymmetric deformation vibration of CH_3 groups with some contribution from methylene deformation vibrations in Fermi resonance (FR) with methylene rocking obertone [13]. Assignments of the phytantriol vibrational bands are summarized in Table.

In order to unambiguously identify vibrations associated with OH groups and thus sensitive to hydrogen bonding interactions, we have performed isotopic OH/

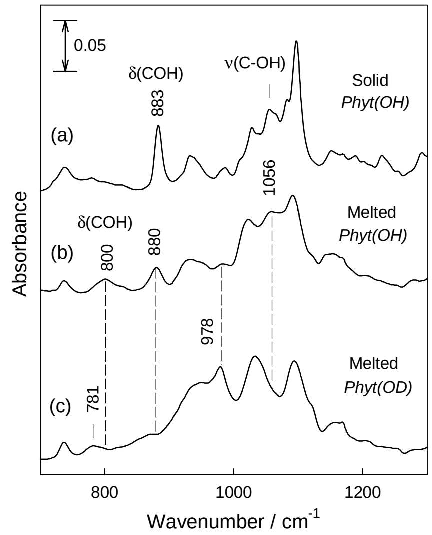

Fig. 3. Comparison of FTIR spectra of solid Phyt(OH), melted Phyt(OH), and melted Phyt(OD)

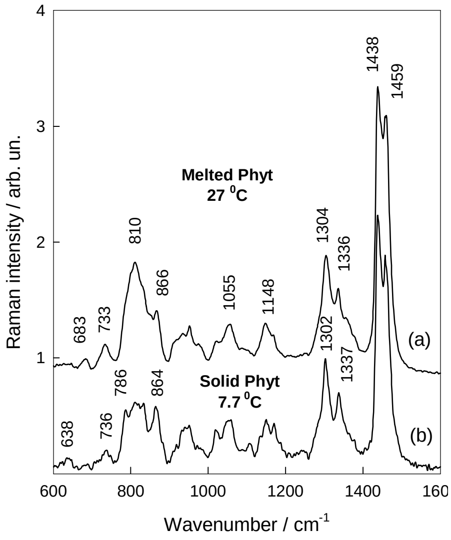

Fig. 4. Comparison of FT-Raman spectra of melted (*a*) and solid (*b*) phytantriol. Excitation wavelength is 1064 nm

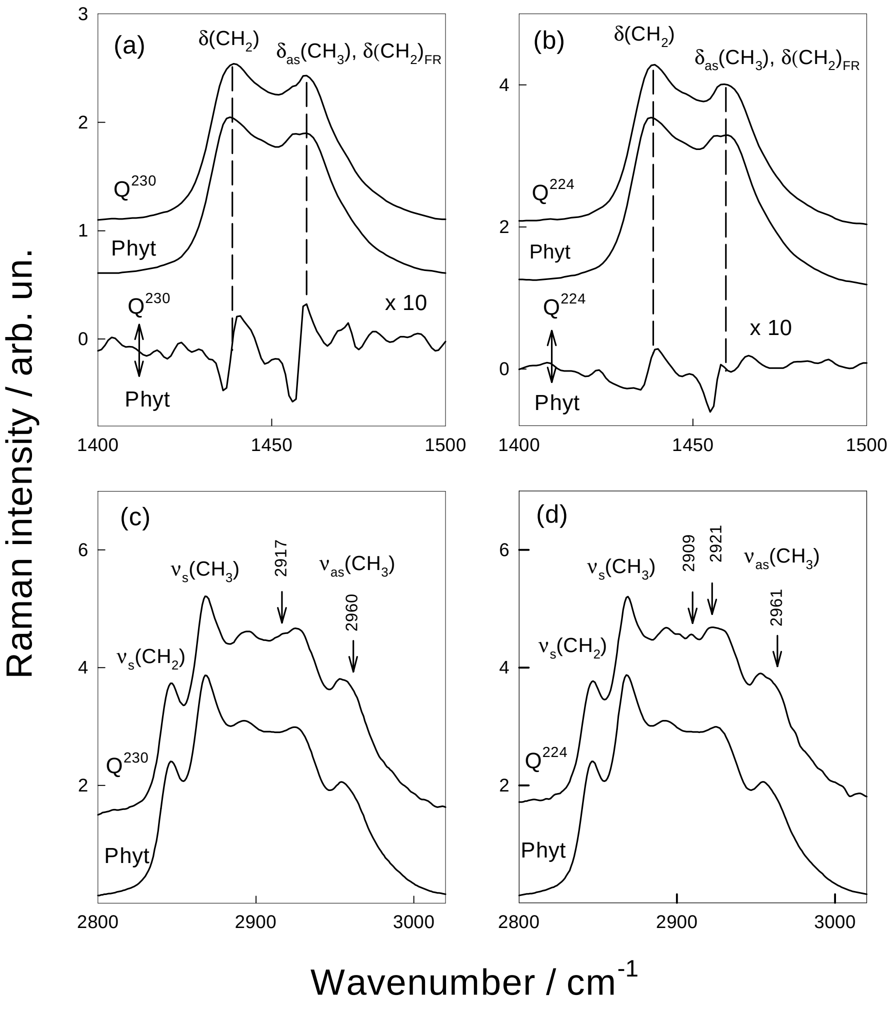

Fig. 5. Comparison of FT-Raman spectra of cubic phases Q^{230} (*a, c*) and Q^{224} (*b, d*) with spectra obtained from melted phytantriol in deformation (*a, b*) and stretching (*c, d*) vibrational spectral ranges of methylene and methyl groups. The difference spectra of deformation vibrational bands are also shown. Excitation wavelength is 1064 nm

OD substitution experiments. The deuterated and initial compounds were labeled in this work as Phyt(OD) and Phyt(OH), respectively. Figure 3 compares the FT-IR spectra of solid and melted Phyt(OH) with melted Phyt(OD). Isotopic substitution experiments indicate that the bands located at 800, 880, and 1056 cm^{-1} (melted Phyt(OH)) clearly shift due to OH/OD exchange and therefore must be assigned to the modes involving vibrations of the hydroxyl group. As can be expected, these bands are sensitive to the physical state of the compound (Fig. 3a,b) and can be used for the analysis of hydrogen bonding interactions. Especially noticeable is a decrease in intensity and frequency downshift by 3 cm^{-1} upon melting for the δ(COH) mode at 883 cm^{-1} (solid Phyt(OH)). Besides, a substantial broadening of the peak half-width (from 13.5 to 19.6 cm^{-1}) accompanies this transition. Thus the δ(COH) peak located at 880–883 cm^{-1} in the infrared spectrum may serve as a sensitive probe for the analysis of hydrogen bonding interactions in phytantriol-based systems. Figure 4 compares the FT-Raman spectra of solid and melted phytantriol in the 600–1600 cm^{-1} spectral region. Several bands show the sensitivity to the physical state of the compound. The peak at 810 cm^{-1} increases markedly on melting, while the frequency of other closely located feature shifts from 864 to 866 cm^{-1}. Both peaks have been assigned to the modes involving vibrations of hydroxyl group (Table 1). It is well known that methylene deformation bands in the 1400–1500 cm^{-1} spectral region are sensitive to the intermolecular interactions between the alkyl chains [13]. In the case of phytantriol we observed an increase in the relative intensity of the 1438 cm^{-1} component compared with 1459 cm^{-1} one going from the melted to the solid state (Fig. 4).

In the following we will focus on the analysis of binary phytantriol-water systems. Depending on the temperature and water content, phytantriol forms two bicontinuous phases of cubic symmetry [11]: (i) the phase of the space group type *Ia3d* (Q^{230}), and (ii) the phase of the space group type *Pn3m* (Q^{224}). Figure 5 compares the FT-Raman spectra of cubic phases with the phytantriol spectrum. As one can see from the difference spectra (Fig. 5a,b), the derivative-like features in the vicinity of 1440 cm^{-1} reveal a slight upshift in the frequency of the deformation vibrational mode of methylene groups in the cubic phase as compared to melted phytantriol. Similar perturbations were also detected for the CH$_3$ deformation mode in the vicinity of 1460 cm^{-1}. As the CH$_2$ deformation mode is particularly sensitive to the interactions between the polymethylene chains [13], the observed frequency upshift reflects the subtle changes in the packing of CH$_2$ groups due to the formation of the cubic phase. The high frequency stretching vibrations of CH$_2$ and CH$_3$ groups are also affected by the formation of cubic phases (Fig. 5 c, d). First of all, low intensity features located in the vicinity of 2909–2921 cm^{-1} were observed in the spectra of cubic phases. Such spectral changes may be explained in terms of formation of gauche conformers leading to an increased intensity of the infrared-active asymmetric CH$_2$ stretching mode [13]. Secondly, broadening of the $\nu_{as}(CH_3)$ peak at ~2960 cm^{-1} accompanies formation of the cubic phases, indicating that the local environment of CH$_3$ groups is more heterogeneous in the liquid-crystalline Q^{230} and Q^{224} structures than in melted phytantriol.

To summarize, we have assigned phytantriol vibrational modes based on the measurements of polarized FT-Raman spectra, OH/OD isotopic substitution experiments, and temperature-dependent spectral changes. We have identified infrared and Raman bands sensitive to hydrogen bonding interactions. By comparing FT-Raman spectra of liquid-crystalline cubic phases and phytantriol we have shown that deformation methylene modes in cubic phases increase in frequency due to a modification of interactions between alkyl chains. In addition, we have demonstrated a broadening of the CH$_3$ asymmetric stretching band due to the increased heterogeneity of the local environment of methyl groups in the cubic phases.

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FITANTRIOLIO IR FITANTRIOLIO-VANDENS KUBINIØ FAZIØ TYRIMAS RAMANO IR INFRARAUDONOSIOS SPEKTROSKOPIJOS METODAIS

Santrauka

Fitantriolis ir fitantriolio-vandens kubinës fazës buvo tirtos virpesiø spektroskopijos (FT-IR ir FT-Ramano) metodais. Panaudojus izotopiná OH/OD pakeitimà, FT-Ramano poliarizacinius bei priklausomybës nuo temperatûros matavimus, spektrinës juostos priskirtos atitinkamiems virpesiams. Nustatyti virpesiai ypaè jautrûs konformaciniams pokyèiams bei vandeniliniams ryðiams. Nustatyta, kad metileno grupës deformaciniø virpesiø daþnis skystakristalinës kubinës fazës spektre padidëja, palyginus su iðlydyto fitantriolio spektru. Tai liudija kitokià fitantriolio grandinëliø sàveikà lipidiniame sluoksnyje. Analizuojant FT-Ramano spektrø aukðtø daþniø sritá pastebëta, kad kubinës fazës CH$_3$ grupiø asimetriniø virpesiø juosta yra platesnë, ir tai yra susijæ su padidëjusiu metilo grupiø lokalios apsupties heterogeniðkumu.