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Iodothyronine–phospholipid interactions in the lipid gel phase probed by Raman spectral markers

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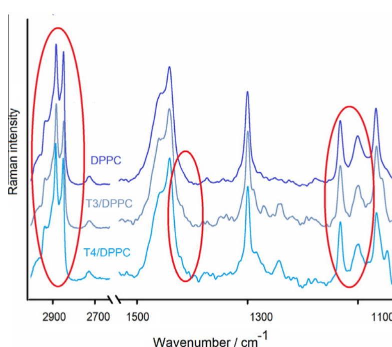
HIGHLIGHTS

- We studied the structural effects of T3 and T4 on a DPPC membrane in the gel phase.
- Spectral changes demonstrate that both hormones penetrate the ordered bilayer.
- Interdigitation and increase in the *gauche* content occur upon hormone incorporation.

GRAPHICAL ABSTRACT



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ABSTRACT

A better understanding of the structural effects induced by thyroid hormones in model membranes is attained by Raman spectroscopy. The interactions of T3 and T4 with multilamellar vesicles of dipalmytoylphosphatidylcholine (DPPC) in the gel phase are characterized by analyzing the spectral behavior of the C–H and C–C stretching vibrations of the acyl chains. The spectra evidence an increase in the relative number of *gauche* conformation, which indicates the hormones are able to penetrate into the hydrophobic region of the bilayer and partially alter the lipid structure. In addition, the density packing of the acyl chains appears increased and the rotational mobility of the terminal methylene groups is slightly reduced in the iodothyronine/DPPC mixtures. These effects are interpreted in terms of the transition to an interdigitated phase due to the hormone incorporation to the membrane. The polar heads of the lipids also interact with the hormone, as evidenced by the PO_2^- symmetric stretching band.

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Introduction

Phospholipid bilayers constitute the basic structure of cell membranes. For this reason, their structures and properties have been studied extensively. A particularly useful tool to probe the molecular organization of phospholipids, in relation to the structure of biomembranes, is the Raman spectroscopy [1–5]. Several studies have been focused on the thermotropic properties of different lipid bilayers in pure state [5–7] and influenced by proteins [8] or small molecules [9–11]. Penetration of these molecules into the lipid bilayer results in conformational changes of the alkyl chains. These changes are well characterized in terms of the wavenumbers and the relative intensities of specific Raman bands [10,12,13].

It is well known that the thyroid hormones 3,5,3′-triiodo-L-thyronine (T3) and L-thyroxine (T4), which give rise to a wide range of effects on metabolism, growth, and development [14] are able of affecting the membrane fluidity at different levels of mammalian cells by nongenomic actions [15–17]. Due to their hydrophobic nature, thyroid hormones can penetrate the lipid matrix of the various cellular membranes, normally rigidifying them by affecting their lipid composition [18–21].

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The passage of T3 and T4 across model membranes was previously evaluated in our group by the addition of physiological concentrations of the hormones to liposomes containing a fluorescent marker. The results indicated that while T3 was able to permeate phospholipid membranes in the liquid-ordered and gel phases, T4 could permeate the liposomal membrane in liquid–crystalline phase solely [22]. In a recent publication, we reported the interactions between thyroid hormones with phospholipids of dilauroylphosphatidylcholine (DLPC) in the liquid–crystalline L_α phase, studied by Raman spectroscopy [23]. A carefully analysis based on derivation, deconvolution, and peak fitting procedures of the spectral region comprised between 1000 and 1150 cm^{-1} of the pure DLPC and the iodothyronine/DLPC systems was presented there. Now, the analysis is extended to the interactions of T3 and T4 with phospholipids of dipalmitoylphosphatidylcholine (DPPC), which at room temperature are in the $L_{\beta'}$ phase. Although a significantly reduced incorporation, or even negligible, of the hormones into this ordered phase is expected, the spectral changes experienced by specific Raman bands of the lipid point out that, indeed, part of the hormone molecules are introduced in the hydrophobic region of the membrane.

The aim of the present report is to complement the analysis of the structural effects induced by thyroid hormones in model membranes and to contribute to the characterization of specific spectral changes that reflect these lipid–hormone interactions.

Materials and methods

Sample preparation

T3, T4, and DPPC were purchased from Sigma and used without further purification. Methanol solutions of each thyroid hormone (100 mg/ml) and the phospholipid (40 mg/ml) were prepared. Appropriate amounts of phospholipids or hormone/phospholipid mixtures (molar ratio of 1:5) were dried under a nitrogen stream and suspended by vortexing in 50 nM acetate/acetic acid buffer (pH 5.0) at ambient temperature to give a final concentration of 1 mM phospholipid. A minimum ionization degree of the phenolic hydroxyl substituent occurs at pH 5.0 (pK = 8.45, and 6.73 for T3, and T4, respectively) [24] yielding the maximum lipophilicity of thyroid hormones and a particularly high partition coefficient between the lipid and the aqueous phase. In order to facilitate the penetration of the iodothyronines into the hydrophobic region of the lipid, the suspensions were heated up 50 °C and then cooled down to 10 °C. Three cycles of heating–cooling processes were performed for each sample.

Raman spectra

Raman spectra between 3500 and 50 cm^{-1} were collected using a DXR Raman Microscope (Thermo Fisher Scientific). Data were collected using a diode-pump, solid state laser of 532 nm (5 cm^{-1} spectral resolution). A confocal aperture of 25 μm pinhole was used. A 10× objective was used when collecting Raman data. A single drop of each sample solution was placed on gold-coated sample slides. In order to achieve a sufficient signal-to-noise ratio, 60 expositions with exposure time of 10 s were accumulated for all samples. To avoid photodegradation, the laser power was maintained at 4 mW when collecting data from the iodothyronines in pure state. 10 mW was the laser power used for the multilamellar vesicles of DPPC in pure state and the T3/DPPC and T4/DPPC mixtures. All spectroscopic experiments were carried out at ambient temperature.

Data analysis

The overlapping components in a characteristic spectral region of DPPC were mathematically decomposed by using an iterative curve-fitting process. This process has been widely applied to decompose the complex bands in proteins and is described elsewhere [25,26]. Briefly, the number and position of component bands were obtained through deconvolutions and derivations. These, together with the band shape (a combination of Lorentzian and Gaussian functions), were fixed during the first 500 iterations. The fitting was further refined by allowing the band position to vary for 50 additional iterations. Finally, the fitting result was visually evaluated by overlapping the reconstituted overall curve on the original spectrum.

Results and discussion

Raman spectra of DPPC, T3/DPPC, and T4/DPPC multilamellar vesicle samples were recorded in the range between 3500 and 50 cm^{-1}. At ambient temperature (25 °C), the DPPC bilayer is in the $L_{\beta'}$ phase (Tm ~ 41 °C). The regions comprised between 3200–2600 cm^{-1} and 1800–300 cm^{-1} of all these spectra are depicted in Fig. 1. The Raman spectra of pure T3 and T4 are superimposed in order to facilitate the identification of the iodothyronine bands.

The study of the effects produced by T3 and T4 on the membrane properties was focused on the evaluation of two specific spectral regions that are informative about the intermolecular order and conformation of the acyl chains: the methylene C–H stretching (~2800–3000 cm^{-1}) and the methylene C–C stretching (1000–1150 cm^{-1}) [4,10,12,27–30]. Other signals that resulted particularly valuable to support the spectral interpretation presented here are those associated to the methylene scissoring mode $\delta(CH_2)$, at ~1440 cm^{-1} and the methylene twist mode $\tau(CH_2)$, at

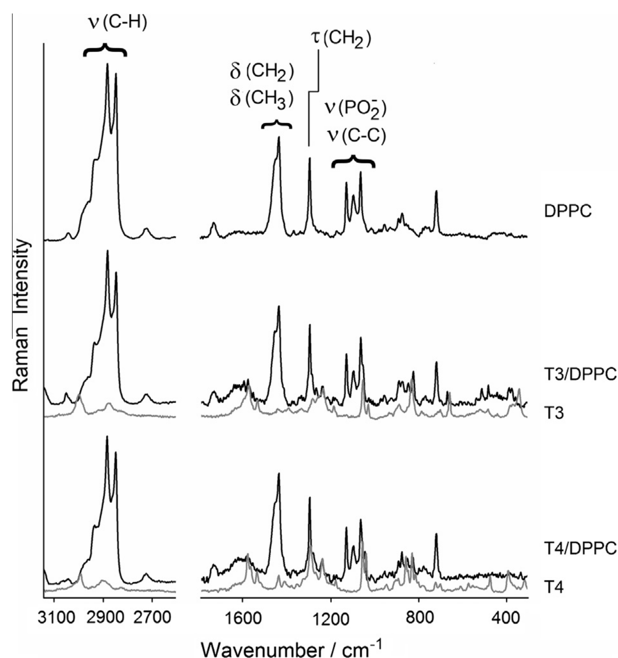

Fig. 1. Raman spectra of the pure DPPC multilamellar vesicles and the iodothyronine–DPPC complexes between 3200–2600 and 1800–300 cm^{-1}. Band assignment of the main lipid vibrations is included in the spectrum of DPPC. The Raman bands of the iodothyronines are easily identified in the complex spectra by overlaying the respective Raman spectrum (gray traces).

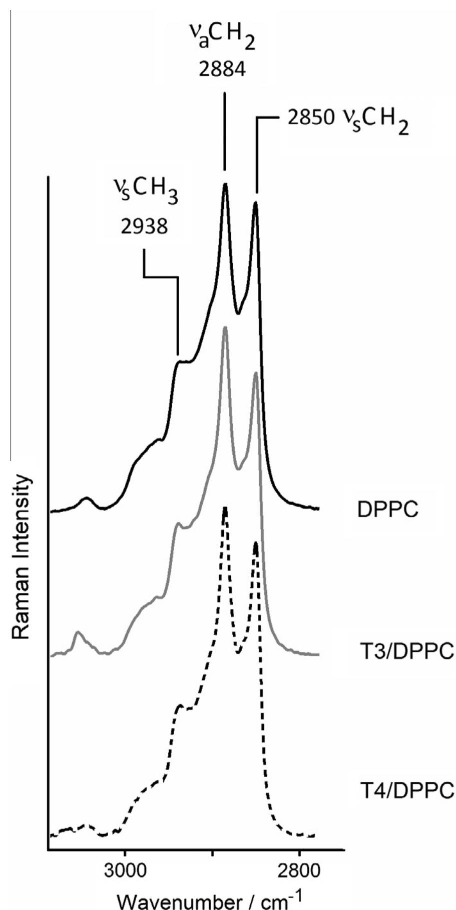

Fig. 2. Raman spectra of the pure DPPC multilamellar vesicles and the iodothyronine–DPPC complexes in the region corresponding to methylene and methyl C–H stretching vibrations.

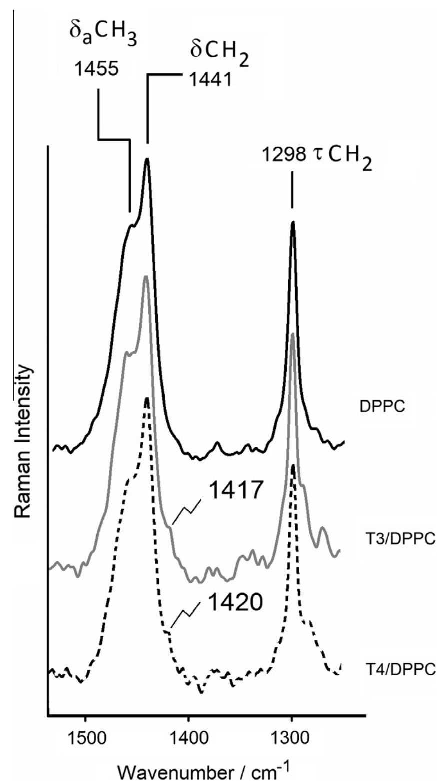

Fig. 3. Raman spectra of the pure DPPC multilamellar vesicles and the iodothyronine–DPPC complexes in the region corresponding to methylene and methyl H–C–H deformations.

~1300 cm^{-1} [5,7,10,29,31–36]. It has been shown that the strong $\delta(CH_2)$ band is sensitive to small change in lattice disorder [37].

The spectral region corresponding to the methylene C–H stretching modes is shown in Fig. 2. Three characteristic bands are observed in this region: the methylene symmetric stretching $\nu_s(CH_2)$ at 2850 cm^{-1}, the methylene asymmetric stretching $\nu_a(CH_2)$ at 2884 cm^{-1}, and the methyl symmetric stretching $\nu_s(CH_3)$ at 2938 cm^{-1}. The peak intensity ratios among these vibrations are indicative of the acyl chain rotational disorder and intermolecular chain coupling [29,36]. Both the wavenumbers and the band shapes observed in the DPPC and in the iodothyronine/DPPC spectra are typical of lipids in the gel phase. As it can be seen, the relative intensity of the 2850 cm^{-1} band to the 2884 cm^{-1} band is affected upon the incorporation of the thyroid hormones to the membrane: the $I[\nu_s(CH_2)]/I[\nu_a(CH_2)]$ intensity ratio in T3/DPPC and T4/DPPC is calculated to be approximately 9% and 5% lower, respectively, in relation to the ratio in DPPC. The intensity of the $\nu_s(CH_3)$ band appears slightly decreased in the spectra of the mixtures compared to the that in the DPPC Raman spectrum. These observations indicate that the hormones induce an increment in the intermolecular coupling of the acyl chains and a reduction in the freedom of CH_3 rotational motion when the lipids are in the gel phase. Such effects are opposite to those observed upon thyroid hormone incorporation to phospholipids in the liquid–crystalline state [23]. In the iodothyronine/DLPC systems, the $I[\nu_s(CH_3)]/I[\nu_s(CH_2)]$ peak intensity ratio increased of ca. 10% in relation to the ratio in the DLPC spectrum, indicating that T3 and T4 induced increase in the rotational and vibrational freedom of the terminal methyl groups. In addition, the $\nu_a(CH_2)$ band upshifted 7 and 5 cm^{-1}, according to the incorporation of T3 and T4, respectively, which evidenced increasing chain decoupling [23].

Fig. 3 shows the 1250–1500 cm^{-1} region of the DPPC, T3/DPPC, and T4/DPPC multilamellar vesicles. At the higher wavenumbers in this spectral region, several overlapping modes appear, but the strongest signal (1441 cm^{-1}) comes from the $\delta(CH_2)$ methylene deformation that reflects changes in lateral packing [10,34,38]. The asymmetric methyl bend $\delta_a(CH_3)$ also stands out, appearing like a well-defined shoulder at ~1455 cm^{-1}. The band at 1300 cm^{-1} is assigned to the $\tau(CH_2)$ mode [7,10,29]. It has been reported for alkane chains that an increase in the peak area ratio of the $\delta_a(CH_3)$ compared to the $\delta(CH_2)$ reveals increased intramolecular freedom of motion and chain decoupling [29]. The behavior of these deformation modes is in concordance with that observed for the C–H stretching bands upon hormone incorporation to DPPC in gel phase, i.e. there is a decreased disorder in the acyl chains. Our attention was specially called by the weak shoulder appearing at 1417 and 1420 cm^{-1} in T3/DPPC and T4/DPPC, respectively. Carrier and Pézolet reported a similar feature arising from the interaction of poly-L-lysine (PLL) with dipalmitoylphosphatidylglycerol (DPPG) in gel phase, in addition to the increased intermolecular coupling between adjacent acyl chains. This new band, appearing at 1422 cm^{-1} in the PLL/DPPG system enhanced from a weak shoulder to a medium-intensity well-defined band as the concentration of PLL was increased [8]. They inferred that there was a tighter packing and a modification of the molecular arrangement induced by the bound PLL. Both effects would result from interdigitation, which consists in the penetration of the acyl chains of one layer into those of the other layer [39,40]. It has been reported that glycerol, vinblastine and viscristine also favor the transition to the interdigitated phase of DPPC membranes [41,42]. Interdigitation

could also explain the increased lateral packing density of the chains and the decreased freedom of CH_3 rotation observed in the iodothyronine/DPPC complexes presented here.

The bibliography concerning the bands that characterize the 1000–1150 cm^{-1} region of phospholipids and their spectral changes accompanying the phase transitions and/or the interactions with proteins and other smaller molecules is rather abundant [8,10,11,43,44]. This complex spectral region is considered predominantly as an indicator of the *gauche*:*trans* conformers ratio in the acyl chains of lipids. Typically, the bands corresponding to the in-phase and out-of-phase C–C stretching modes of *trans* conformers ($\nu_{i.ph.}$(C–C)$_T$ and $\nu_{o.o.ph.}$(C–C)$_T$) appear at ~1130 and 1060 cm^{-1}, respectively [7,10,29]. Between these two bands, the valuable feature whose position, shape, and intensity are very informative represents both, the *gauch*e conformers (ν(C–C)$_G$) and the symmetric stretching of the PO_2^- groups ($\nu_s PO_2^-$). Most of the reported studies statement that for lipids in gel phase the band at ~1100 cm^{-1} corresponds to the ν(C–C)$_G$ of *gauche* conformers probably located at the end of the acyl chains ("end-*gauche*" conformation); a contribution from the $\nu_s PO_2^-$ mode to this band was also reported [30]. As the temperature increases, the number of *gauche* defects near the middle of the lipid acyl chains increases and the 1101 cm^{-1} band shifts to a broad feature centered around 1080 cm^{-1}, but with the highest intensity point at 1090 cm^{-1} [30]. However, this overall accepted behavior has not been fully analyzed yet and, in general, no further mentioning concerning the $\nu_s PO_2^-$ mode of lipids in the fluid phases is found in the bibliography.

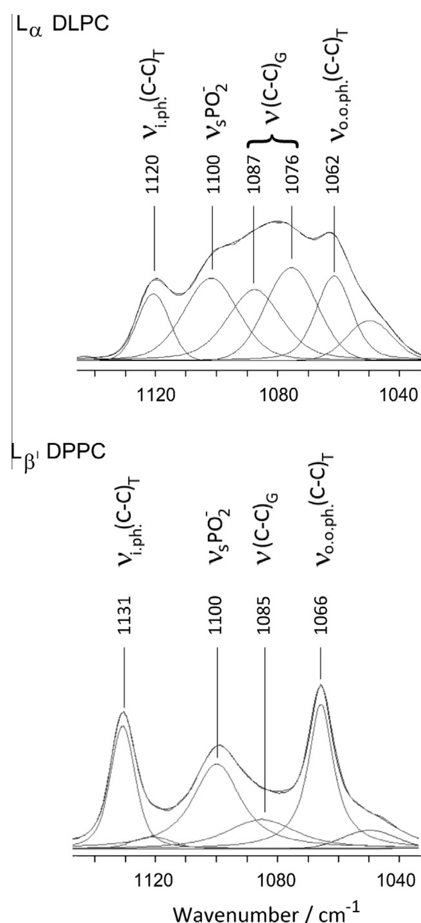

Fig. 4. Raman spectra of DLPC in liquid–crystalline phase and of DPPC in the gel phase in the region corresponding to the C–C stretchings, characteristic of the acyl chains. Decomposed bands after the curve-fitting procedure are shown. The reconstructed band is superimposed on the original spectrum in order to show the level of fit attained.

Our analysis of this 1000–1150 cm^{-1} spectral region initiates with a comparison between the spectra previously obtained for the L_α DLPC and the currently studied spectra of the $L_{\beta'}$ DPPC, following the corresponding decomposition into the contributing bands. In Fig. 4, the original spectra in the 1000–1150 cm^{-1} region are overlaid with the components bands obtained by a peak-fitting procedure [25,26]. For simplicity, the discussion is mainly focused on the features around 1100 cm^{-1}. In the spectrum of the L_α DLPC, the broad and complex band which highest point is located at 1080 cm^{-1} dominates the spectral region and reflexes the high population of *gauche* defects, characteristic of the fluid phase. The shoulder at ~1100 cm^{-1} is assigned to the $\nu_s PO_2^-$ mode. Upon decomposition of the L_α DLPC spectrum, three bands of similar intensities and centered at 1100, 1087, and 1076 cm^{-1} are observed; they are respectively assigned to $\nu_s PO_2^-$, ν(C–C)$_G$ of "end-*gauche*" conformers, and ν(C–C)$_G$ of *gauche* conformers in the body of the acyl chains, named "*gauche*–*trans*–*gauche*" (g–t–g) conformers [45–47]. The spectrum of $L_{\beta'}$ DPPC shows a neat, albeit broad band centered at 1100 cm^{-1}. Two bands are predicted as component of this feature: a main band at 1100 cm^{-1}, which is assigned to the $\nu_s PO_2^-$ mode; the other, considerably less intense and centered at 1085 cm^{-1}, which is associated with isolated "end-*gauche*" conformers that are expected to be present in DPPC in the gel phase [7]. Several publications support these assignments: (i) On the one hand, Orendorf et al. studied the conformations adopted by octadecane and polyethylene at different temperatures [29]. Their Raman spectra indicated that in the crystalline state no *gauche* conformation exists since solely the bands attributable to the C–C stretching of the *trans* form were observed (~1062 and 1128 cm^{-1}). As the temperature increased, a band at 1080 cm^{-1} raised and reached its maximum intensity when the alkanes were in the liquid phase. This band was associated with the *gauche* conformations. (ii) On the other hand, Kitagawa et al. studied the Raman spectra of barium dipentyl phosphate (DPP), which represents the shortest dialkyl phosphate that forms a liquid crystal, and then serves as a model of biomembranes. DPP in an aqueous solution, a liquid crystal, and the solid state were analyzed [48]. All the spectra showed two medium intensity bands at 1123 and 1058 cm^{-1} that were associated to the C–C stretching modes of the *trans* alkyl groups. In addition, both Raman spectra, the crystal and the monomolecular aqueous solution of DPP showed a very intense line at 1106 and 1075 cm^{-1}, respectively, which were assigned to the $\nu_s PO_2^-$ mode. In turn, the spectrum of the liquid crystal state showed two prominent bands, at 1075 and 1096 cm^{-1}, that were also assigned to the $\nu_s PO_2^-$ mode, since they seemed to be too intense and too narrow to be associated with the *gauche* conformers. Then, this assignment suggested the phosphate group of liquid crystal DPP participates in two different kinds of interactions [48]. (iii) More recently, the Raman spectra of the octadecylamine (ODA) in crystal structure, ODA Langmuir monolayers, and egg phosphatidylcholine/ODA – mixed monolayers at the air–water interface were analized [49]. In concordance with the lack of phosphate groups, the Raman spectra of solid ODA and ODA Langmuir monolayers showed the $\nu_{i.ph.}$(C–C)$_T$ and $\nu_{o.o.ph.}$(C–C)$_T$ bands at 1130 and 1060 cm^{-1}. Differences in their relative intensities, due to the selection rules associated with surface enhancement of metal substrates, were observed. In the spectrum of PC/ODA monolayers, a new band located at 1084 cm^{-1} appeared and it was assigned to the $\nu_s PO_2^-$ mode of the PC molecules.

The Raman spectra of the T3/DPPC and T4/DPPC multilamellar vesicles, particularly the irregular shape contour presented by the band at ~1100 cm^{-1}, evidence an effective interaction between thyroid hormones and lipids (Fig. 5). It has been shown that penetration of small molecules into the lipid bilayer may result in conformational changes (*trans*/*gauche*) of the alkyl chains as well as in changes of the interactions involving the polar head groups

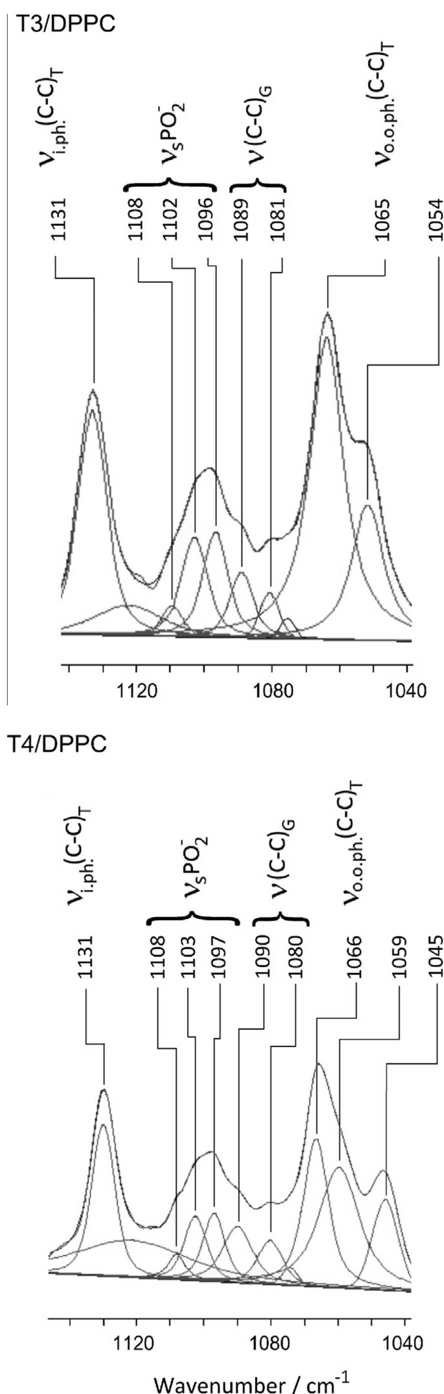

Fig. 5. Spectral region corresponding to the C–C stretchings of T3/DPPC and T4/DPPC multilamellar vesicles in the ordered phase. Decomposed bands after the curve-fitting procedure are shown. The reconstructed band is superimposed on the original spectrum in order to show the level of fit attained. The features appearing at low wavenumber correspond to the iodothyronine vibrations appearing in this region.

[10,11]. In both Raman spectra, two shoulders at ~1090 and 1081 cm^{-1} are observed. Their wavenumbers are comparable with those assigned to the "end-*gauche*" and g–t–g conformers observed like strong features in the spectra of DLPC and iodothyronine/DLPC mixtures [23]. The appearance of these features in the iodothyronine/DPPC spectra is unquestionable evidence of the increased population of *gauche* defects, especially in the body of the acyl chains, induced by the incorporated hormones. However, due to their poor definition, a quantitative analysis of the I[ν(C–C)$_G$]/I[$\nu_{i.ph.}$(C–C)$_T$] intensity ratio is uncertain. In addition, the iodothyronines have two characteristic bands in this spectral region: the stretching mode νC–N (~1055 cm^{-1}) and the ring deformation α9 mode (1031 cm^{-1} in T3 and 1041 cm^{-1} in T4) [50–52]. Particularly, the strong band at 1055 cm^{-1} overlaps with the $\nu_{o.o.ph.}$(C–C)$_T$ band. Then, the evaluation of the conformational changes in the acyl chains was carried out by estimating the intensity decrease of the $\nu_{i.ph.}$(C–C)$_T$ in relation to the intensity of the band at ~1300 cm^{-1} (see Fig. 1). This strong band is associated to the methylene twist τCH_2, and its intensity has been considered to be independent of the chain conformation [10,11,53]. This intensity ratio indicates that the population of *trans* conformers decreases about 10% in the iodothyronine/DPPC systems relative to that in pure DPPC.

The irregular shape on the high-wavenumber side of the band at 1100 cm^{-1} in the iodothyronine/DPPC systems is indicative of alterations in the phosphate surroundings. Upon decomposition of this spectral region, several component bands showing identical wavenumbers in both iodothyronine–DPPC spectra are predicted (Fig. 5). The bands at 1108 and 1102 cm^{-1} are straightforward associated to the $\nu_s PO_2^-$ mode, since a similar behavior was observed in the spectrum of T4/DLPC mixture [23]. The interaction of T4 with the polar region of the membrane in the fluid phase was manifested by the presence of two bands, located at 1101 cm^{-1} (medium intensity) and at 1109 cm^{-1} (weak intensity). Due to hormone penetration into the hydrophobic region of the $L_{\beta'}$-DPPC is more constrained, larger interactions between the polar groups of the phospholipids and the iodothyronines are expected. These interactions not only are extended to T3, but also would be responsible of originating a third band associable to the $\nu_s PO_2^-$ mode: the band appearing at 1096 cm^{-1} [48]. All the component bands estimated for the 1000–1150 cm^{-1} spectral region are listed in Table 1 together with the proposed assignment.

In literature, there are numerous studies on the phospholipid conformations, whether alone or in lipid–drug complexes, focused on the lipid thermotropic properties monitored by vibrational spectroscopy. They show the temperature effects are first manifested by an increase in the rotational disorder and interchain decoupling, following an increase in the *gauche*/*trans* ratio in the acyl chains [6–9,11]. However, a few years ago, Csiszár et al. studied, by Raman spectroscopy, the phase transition behavior of DPPC influenced by 2,4-dichlorophenol (DCP). They found a decreased amount of *trans* conformations and a markedly more ordered acyl chains in the DCP/DPPC system below 25 °C, in relation to those parameters in the pure DPPC/water system, characterizing in this way the structural difference between the interdigitated DCP/DPPC and the $L_{\beta'}$DPPC systems [10].

Table 1
Experimental Raman bands (cm^{-1}) corresponding to the vibrations appearing in the 1000–1150 cm^{-1} spectral region of the DPPC, T3/DPPC, and T4/DPPC multilamellar vesicles are listed. Calculated band positions (cm^{-1}) of the components bands obtained after the curve-fitting procedure are shown in parentheses. Tentative assignments of the features are presented.

Assignment[a]	DPPC	T3/DPPC[c]	T4/DPPC[c]
$\nu_{i.ph.}$ (C–C)$_{trans}$	1131 (1131)	1131 (1131)	1130 (1131)
		(1108)	(1108)
		1101sh (1102)	1101sh (1103)
($\nu_s PO_2^-$)	1099 (1100)	1098 (1096)	1098 (1097)
ν (C–C)$_{gauche}$	(1085)	1090sh (1089)	1090sh (1090)
		1080sh (1081)	1080sh (1080)
$\nu_{o.o.ph.}$ (C–C)$_{trans}$	1066 (1066)	1065 (1065)	1065 (1066)
ν (C–N)[b]		1055 (1054)	(1059)
α9[b]			1046 (1045)

[a] Stretching; s: symmetric; o.o.ph: out-of-phase; i.ph.: in-phase.
[b] Vibration belonging to the iodothyronine.
[c] sh: shoulder.

The overall spectral behavior observed in the present study shows significant similarities with those results reported by Csiszár. Thus, at room temperature, the incorporation of thyroid hormones to a DPPC suspension shows, on one hand an increased intermolecular chain coupling, as is derived from the C–H stretching bands, while on the other hand, according to the C–C stretching bands the relative number of *gauche* conformer increases. In addition, an incipient new signal at $\sim$1420 cm^{-1} indicates a slight modification of the chain molecular arrangement. All these facts can be consistently reasoned as follows. (I) As was described in Section 2.1, the partial insertion of the ring-β moiety of the iodothyronine into the hydrophobic region of the lipid was attained after heating the iodothyronine/DPPC mixture to a temperature approximately 10 °C above the gel to liquid–crystalline transition phase temperature. This procedure allowed the formation of *gauche* conformers and the partial incorporation of the hormone into the acyl chains. After cooling to return to the lipid gel phase not all the *trans* conformations were recovered, possibly due to steric interactions with the hormone. (II) The increased density packing of the acyl chains in the iodothyronine/DPPC mixtures, together with the slightly reduced rotational mobility of the terminal methylene groups are indicative of interdigitation occurring due to the interaction between the hormones and the phospholipids. (III) The spectral changes observed for the $\nu_s PO_2^-$ mode point out different kinds of interactions between the iodothyronines and the polar region of the membrane that, to a certain extent, allows the penetration of the ring-β moiety of the iodothyronines into the hydrophobic lipid region. This penetration could produce the spacing out of the polar head groups and lead to the interdigitation of the acyl chains.

The spectral data presented here and their interpretations differ from those previously obtained in our group by fluorescence assays [22]. As was mentioned above, those experiments showed thet T3 decreases the lipid order in vesicles of DPPC in the gel phase while no interactions were detected between T4 and the $L_{\beta'}$DPPC. The Raman spectra of T3/DPPC and T4/DPPC do not show such a different behavior between these two hormones; instead, T4 induces conformational changes in the lipid organization of similar magnitude of those induced by T3. This discrepancy is easily understood by taking into account that the vesicles used in the fluorescence assays were prepared at pH = 7.4 [22], which implied that a low concentration of T4 with its non-ionized OH group was present [24]. On the contrary, at pH 5.0 (see Section 2.1), the ionization degree of the phenolic OH substituent in T4 is less than 2% (p*K* = 6.73), corresponding to a maximum lipophilicity of T4 and a particularly high partition coefficient between the lipid and the aqueous phase. Lai et al. have reported the lateral diffusion of spin-labeled T3 and T4 by electronic spin–resonance techniques, and have suggested that the non-ionized phenolic-OH group was close to the lipid core of the membrane [54–56]. In addition, it is worthwhile to mention that, while the Raman spectra give direct information about intermolecular interactions, the fluorescence experiments are based on the hormone reaction with a pre-encapsulate amino-reactive compound (2,4,6-trinitrobenzene sulfonic acid) and not on a direct hormone–lipid interaction [22]. There are evidence that the presence of a third molecule in the membrane can disrupt the interdigitated L_I DPPC phase [42].

Further analysis of the iodothyronine–phospholipids interactions at physiological conditions by using these lipid Raman spectral markers are required.

Conclusions

In this paper, Raman spectroscopy is used to study the effects of iodothyronines T3 and T4 on a DPPC model membrane in the gel phase. Changes in the relative intensities of specific lipid spectral markers evince both iodothyronines are able of penetrating into the hydrophobic lipid region. Interdigitation induced by the partial hormone incorporation explains the increased lateral packing density of the chains and the decreased freedom of methyl rotation observed in the iodothyronine/DPPC complex spectra. By comparison with the spectral changes previously obtained for iodothyronine–phospholipid systems in the liquid–crystalline phase, a differential effect of the thyroid hormones on the membrane properties, according to the lipid state, is confirmed.

Acknowledgements

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