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Raman spectroscopy study of the interaction of 3,5,3′-triiodo-L-thyronine with phosphatidylglycerol lipid bilayers

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A Raman spectroscopic study of anionic model membranes and their structural alterations exerted by a relatively small biomolecule, such as the hormone 3,5,3′-triiodo-L-thyronine (T3), is presented. Spectral differences between pure dipalmitoylphosphatidylglycerol (DPPG) multilamellar vesicles and DPPG–T3 mixture and between pure dilauroylphosphatidylglycerol (DLPG) and DLPG–T3 mixture were evaluated in order to determine the response of lipid membranes in gel and liquid-crystalline phases to the hormone incorporation. Density functional theory (DFT) calculations support the band analysis of the complex 1150–1050 cm^{-1} Raman region. Geometry optimizations and vibrational behaviors of a model charged molecule that mimics the phosphatidylglycerol lipid moiety in solvated state were taken into account for the spectral interpretation of this specific region. The anionic nature of the lipid polar head plays an important role in the interaction with the hormone, as is evidenced by the C=O and PO_2^- stretching bands. In addition, the differential penetration of T3 into the hydrophobic region of the membranes shows to be dependent on the lipid phase. The spectral data were compared with those previously obtained for zwitterionic membranes. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: phospholipids; anionic membranes; vibrational analysis

Introduction

The basic support of biological membranes is a double layer of phospholipids formed by spontaneous aggregation. Phospholipids are usually composed by a glycerol backbone, two fatty acid chains, and a phosphorylated alcohol. Among the most frequently occurring alcohols are choline and glycerol. Due to the high complexity of the cellular membranes, model membranes composed by chemically different phospholipids, according to the nature of the polar head (zwitterionic and charged) and to the length and degree of unsaturation of the acyl chains are commonly used. Thus, a quite extensive literature accounts for the structural characteristics of bilayers and for their interactions with small biomolecules or peptides.[1–7] In particular, we are focused in contributing to elucidate the mechanisms by which thyroid hormones exert their so-called nongenomic or extranuclear effects by affecting the properties of various cellular[8–12] and model membranes.[13,14] Bilayers constituted by dilauroylphosphatidylcholine (DLPC) and dipalmitoylphosphatidylcholine (DPPC) in interaction with 3,5-diiodo-L-thyronine (T2), 3,5,3-triiodo-L-thyronine (T3), and thyroxine (T4) were previously studied by using Raman spectroscopy.[15–18] It is well known that this technique is useful to describe the changes that suffer model membranes by the action of different biomolecules.[19–23] Moreover, the use of a confocal microscope integrated to the Raman spectrometer improves the efficiency of this technique because Raman scattering is collected from a small volume of sample containing a relatively high local concentration of lipid and minimum contribution from the surroundings.[24,25] By monitoring specific bands known as sensors of the lipid packing, the number of *gauche* conformers in the hydrocarbon chains, and the degree of hydration of the polar heads,[26–30] we recently evaluated and interpreted the effect of hormones in phosphatidylcholine (PC) lipid bilayers. In addition, we were able to discriminate between the PO_2^- symmetric stretching and the C—C *gauche* stretching modes in the gel and the liquid crystalline phases of lipid membranes, such as in DPPC and DLPC, respectively, whose assignments had been ambiguous up to then.[17,18]

Our studies focus now on lipid systems with different chemical nature, by analyzing the interactions between T3 and bilayers composed by phosphatidylglycerol lipids (PG) in both the liquid-crystalline (L_α) and the gel ($L_{\beta'}$) phases. The main purpose of studying these systems is to inquire about the response that anionic membranes give when they interact with relatively small biomolecules.

Raman spectra of pure liposomes of dilauroylphosphatidylglycerol (DLPG) in the L_α phase, dipalmitoylphosphatidylglycerol (DPPG) in the $L_{\beta'}$ phase, and the corresponding DLPG–T3 and DPPG–T3 complexes were analyzed. In addition, quantum-chemical calculations were performed in order to predict the vibrational wavenumbers of the PO_2^- symmetric stretching mode of the phosphatidylglycerol group in different conformations. A small model system resembling the polar head of the PG lipids was used.

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Materials and methods

Sample preparation

DPPG and DLPG were obtained from Avanti Polar Lipids; T3 was purchased from Sigma-Aldrich. All substances were used without further purification. Appropriate amounts of lipid and lipid/T3 mixtures (5:1) were dissolved in chloroform/methanol solution and dried as a thin film under a nitrogen stream. The samples were suspended by vortexing in 50 mM acetate/acetic acid buffer (pH 5.0) at room temperature to give a final concentration of about 1 mM phospholipid. A minimum ionization degree of the phenolic hydroxyl substituent occurs at pH 5.0 (pK = 8.45 for T3)[31] yielding the maximum lipophilicity of thyroid hormones and a particularly high partition coefficient between the lipid and the aqueous phase (pK of PG ~ 1).[32]

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 50-μm slit 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, 80 expositions with an exposure time of 2 s were accumulated for all samples. The laser power used was 10 mW for the multilamellar vesicles of DLPG and DPPG in pure state and for the DLPG–T3 and DPPG–T3 mixtures. All spectroscopic experiments were carried out at ambient temperature.

Data analysis

The overlapping components in a characteristic spectral region of DPPG and DLPG were mathematically decomposed by using an iterative curve-fitting process.[33,34] This process has been applied to decompose the complex bands in previous works.[17,18] Briefly, the number and position of components bands were obtained through deconvolutions and derivations. These, together with the band shape (a combination of Lorentzian and Gaussian functions),[35] 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.

Computational calculations

Quantum-chemistry calculations were performed with the Gaussian(R)03 Program Package.[36] Density functional theory (DFT) calculations were carried out with the B3LYP hybrid functional.[37–39] The standard split valence basis set 6-31 + G(d),[40] including single diffuse functions,[41] was used. In order to represent the polar heads of the PG lipids, the following charged model system was built with the GaussView 5.0 graphical interface[42]: $[CH_3—O—P(O)_2—O—CH_2—CHOH—CH_2OH]^-$. Geometry optimizations for different isolated conformers in vacuum were performed. The subsequent wavenumbers calculation, performed at the same level of theory, allowed to ensure that the structures were true minima since no imaginary wavenumbers were obtained and to determine the corresponding zero-point vibrational energies (ZPVEs). Latter, hydrogen-bonded dimmers composed by one of the optimized conformers and one water molecule in different positions/orientations with respect to the P=O bonds were also optimized and their vibrational wavenumbers calculated.

Results and discussion

The main transition temperatures (T_m) for DPPG and DLPG lipids are 41 °C and −5 °C, respectively.[43] Then, it was expected that the multilamellar vesicles of DPPG were in the ordered, gel phase at room temperature (20 °C), while those of DLPG were in the liquid-crystalline state.

The Raman spectra in the wavenumbers range 3100–800 cm^{-1} for both DPPG and DLPG samples are shown in Fig. 1. The significant spectral differences evidence the structural characteristics of the two lipid phases: wavenumbers, relative intensities, and band shapes of specific Raman signals account for the acyl chain conformational order including the extent of chain coupling, intramolecular motion, relative population of *gauche* and *trans* conformers, and chain twisting and bending.[19–22,25–30] Particularly useful is the evaluation of the band contours associated to the C—H and C—C stretching modes, such that a quick look at these spectral regions allows to conclude that the DPPG system is in the $L_{\beta'}$ phase while DLPG is in and the L_α lipid state at the experimental conditions used here.

Figure 1 also shows the Raman spectrum of T3, which is overlaid to each lipid spectrum. Previous spectroscopy studies focused on the structure of thyroid hormones in interaction with PC lipid membranes have suggested that all the observed spectral changes involve the phenolic ring (ring β) and the ether bridge of the hormone molecules (see inset on Fig. 1). Conversely, the vibrational modes of the double iodine-substituted aromatic ring (ring α) as well as those of the alanyl group remain unaffected when the hormone inserts into the lipid bilayer. Then, it has been concluded that thyroid hormones interact with PC lipid membranes by introducing the non-ionic ring β and the ether group into the aliphatic chains of the lipids via hydrophobic interactions, while the alanyl group and part of the ring α remain anchored in the polar region of the membrane.[15,16,44] Now, the analysis and interpretation of the effects induced by T3 on the structural properties of anionic membranes were based on the evaluation of the three Raman spectral regions that are highlighted in Fig. 1: (1) the 3100–2800 cm^{-1} usually depicts the C—H stretching modes; (2) the C—C and PO_2^- stretching vibrations corresponding to the hydrocarbon chains and the polar head groups, respectively, are observed in the 1200–1000 cm^{-1} region; (3) the C=O stretching band of the acyl linkage of the hydrocarbon chains is found in the 1780–1710 cm^{-1} interval. Although the spectral differences shown by the bands associated to the methylene scissoring mode (δCH_2), at ~1440 cm^{-1}, and the methylene twist mode (τCH_2), at ~1300 cm^{-1}, also evidenced the effect of hormone incorporation to the membranes, they are not discussed here.

Figure 2 shows the spectral region comprised between 3100 and 2800 cm^{-1} of the pure membranes and the corresponding lipid–T3 complexes. The strongest three bands of the spectra correspond to the methylene symmetric stretching ($\nu_s CH_2$) observed at about 2850 cm^{-1}, the methylene asymmetric stretching ($\nu_{as} CH_2$) located at ~2880 cm^{-1}, and the terminal methyl symmetric stretching ($\nu_s CH_3$) at ~2930 cm^{-1}.[22,28–30] The peak intensity ratio $I[\nu_{as}CH_2]/I[\nu_s CH_2]$ has been largely considered as indicative of the conformational order and lateral packing density of the acyl chains.[18,26,29,30] Our Raman spectra show that this ratio increases about 6% upon hormone incorporation in both membranes. This fact indicates a

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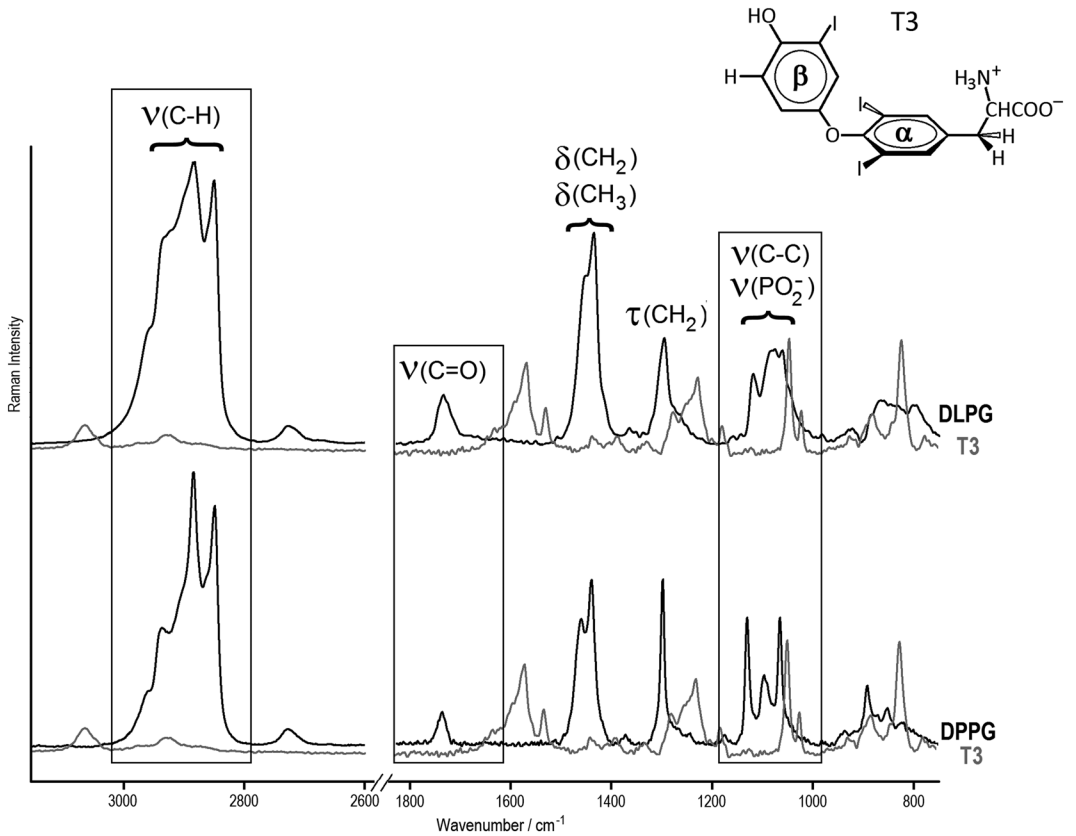

Figure 1. Room-temperature Raman spectra of multilamellar vesicles of DLPG and DPPG in the 3100–2700 and 1800–800 cm^{-1} regions. Band assignment of the main lipid vibrations is included. Raman spectrum of T3 in pure state is overlaid to each lipid spectrum (gray traces). Spectral areas revealing significant structural differences between the L_α and $L_{\beta'}$ phases and variation upon hormone incorporation are highlighted by frames.

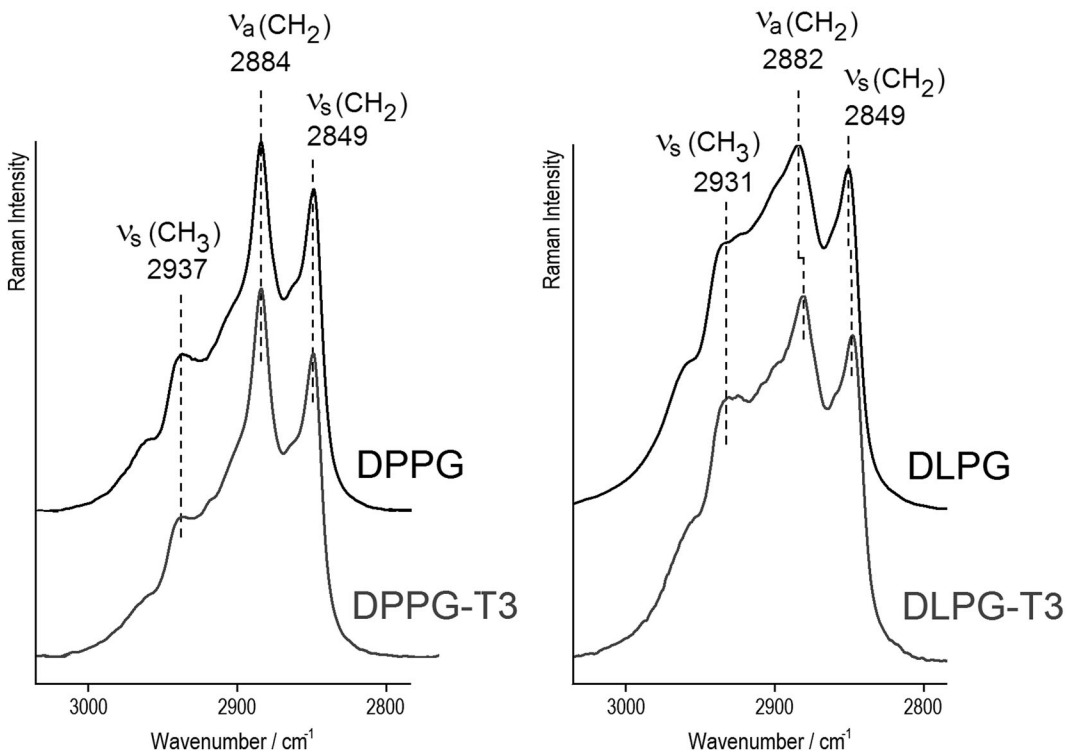

Figure 2. Raman spectra of pure DPPG and DLPG multilamellar vesicles and the respective lipid–T3 complex, in the region corresponding to methylene and methyl C—H stretching vibrations.

greater interchain packing in the T3-containing systems, compared with the corresponding pure membranes of DPPG and DLPG. In addition, a subtle downshift of the $\nu_{as}CH_2$ band is observed in the DLPG–T3 complex, which is also associated with an increased intermolecular coupling.[29] On the other hand, the intensity ratio of ν_sCH_3 to ν_sCH_2 is commonly used as indicator of the vibrational and rotational freedom of the terminal CH_3 groups of the hydrocarbon chains. It has been shown that the $I[\nu_sCH_3]/I[\nu_sCH_2]$ ratio increases as the interchain interactions decrease.[27,29,30] In the Raman spectra of DPPG-containing samples, the ratio decreases about 6% upon T3 incorporation. This fact, together with the above mentioned increased intermolecular packing points out that, in the gel phase, the hormone induces a decreased rotational disorder all along the hydrocarbon chains region. The same behavior has been observed in the DPPC($L_{\beta'}$)–T3 system.[18] Conversely, in DLPG–T3 Raman spectrum, the $I[\nu_sCH_3]/I[\nu_sCH_2]$ ratio is ~4% higher than in DLPG, indicating that the vibrational motion of the terminal CH_3 groups increases when T3 is incorporated to a bilayer in the liquid-crystalline state. This observation suggests that the increased density packing is restricted to a portion of the hydrocarbon chains without affecting the deep core of the membrane.

Valuable information can be extracted from the 1200–1000 cm^{-1} region of the Raman spectra (see Fig. 3). This region contains the methylene C—C stretchings, reported as indicators of the *gauche*: *trans* conformers ratio in the acyl chains of lipids.[29,30] In the Raman spectrum of DPPG, the sharp bands centered at 1130 and 1065 cm^{-1} are unambiguously attributed to the corresponding in-phase and out-of-phase C—C stretching modes of *trans* conformers, $\nu_{(i.ph)}(C—C)_T$ and $\nu_{(o.o.ph)}(C—C)_T$, respectively,[22,29,30] since they are characteristic of the $L_{\beta'}$ phase. These vibrations are observed at 1122 cm^{-1} ($\nu_{(i.ph)}(C—C)_T$) and at 1063 cm^{-1} ($\nu_{(o.o.ph)}(C—C)_T$) in the Raman spectrum of DLPG. Lipids in the liquid-crystalline phase typically show that these bands broader and less intense than in the gel phase. The band corresponding to the *gauche* defects in the lipid acyl chains ($\nu(C—C)_G$) is expected in between the *trans* C—C stretching bands. The shape and relative intensity of this band depend on the lipid phase,[22,29,30] yet the symmetric stretching of the PO_2^- groups ($\nu_sPO_2^-$) is expected in this Raman spectral region as well; therefore, the identification and assignment of the $\nu(C—C)_G$ vibration are not straightforward. Based on comparison with Raman spectra of DLPC and DPPC membranes at room temperature, quantum-chemical calculations of a PG model system, and a peak-fitting procedure that provides a better visualization of the possible bands components (see Fig. 3),[33,34] we propose a confident assignment of this complex spectral region of the DPPG and DLPG systems. DPPG Raman spectrum shows a medium intensity, broad band at 1096 cm^{-1} together with a prominent shoulder at approximately 1090 cm^{-1}. Upon band decomposition, three main components centered at 1102, 1096, and 1090 cm^{-1} and two less intense contributors at lower wavenumbers were obtained. Taking into account that only a few isolated C—C *gauche* conformers are expected in the gel phase[22] and according to the behavior observed in Raman spectrum of PC membranes,[17,18] the two weak components, at 1085 and 1079 cm^{-1}, are assigned to $\nu(C—C)_G$ of *gauche* conformers, located at different positions in the acyl chains. Then, the main component bands are associated to vibrations belonging to groups located in the polar region of the membrane (PO_2^- and glycerol —CH_2OH). This assignment is based on the consideration that the PO_2^- groups are involved in different inter and intramolecular associations.[17,18,22,45,46] Theoretical predictions concerning the conformations adopted by the isolated charged molecule $[CH_3—O—P(O)_2—O—CH_2—CHOH—CH_2OH]^-$ showed the preference for a geometry with one of the P=O bonds associated by hydrogen bond with the terminal OH group of the flexible glycerol chain.[47] In the presence of a nearby water molecule, three possible hydrogen-bonded dimmers were obtained. Subsequent wavenumber calculations were performed for all these optimized structures. Figure 4 shows the different optimized conformations of $[CH_3—O—P(O)_2—O—CH_2—CHOH—CH_2OH]^-$ in free state and hydrogen-bonded with a water molecule, together with the corresponding $\nu_sPO_2^-$ and νC—O theoretical wavenumbers obtained at the B3LYP/6-31 + G(d) level. These predictions support the assignment of the component bands obtained by deconvolution. Thus, in DPPG spectrum, the band at 1096 cm^{-1} is assigned to $\nu_sPO_2^-$ corresponding to P=O bonds in hydrogen-bonding interactions with the terminal OH group of the glycerol moiety, while the band at 1090 cm^{-1} is assigned to $\nu_sPO_2^-$ of groups associated with a water molecule. The 1101 cm^{-1} component band is assigned to the C—O stretching of the glycerol —CH_2OH group since this vibration was predicted by calculations at higher values than the $\nu_sPO_2^-$. Although the calculated wavenumbers differ from those experimentally observed, the overall trend supports our spectral interpretation.

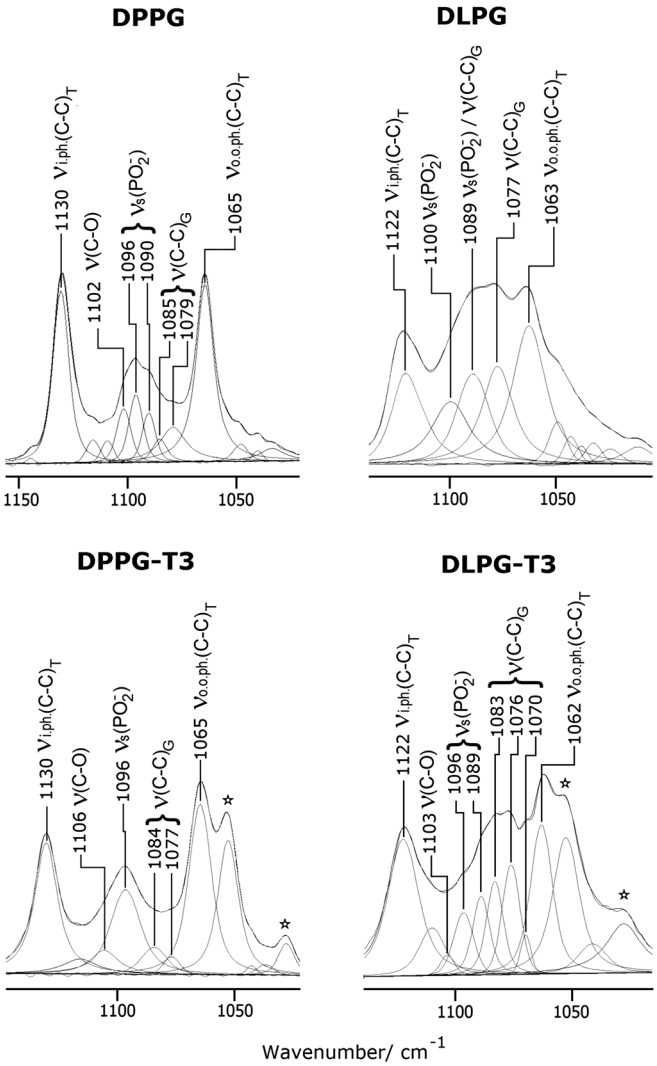

Figure 3. Raman spectra in the characteristic C—C stretching region of the acyl chains. Decomposed bands after the curve-fitting procedure are shown. The reconstructed band is superimposed on the original spectrum in order to show the level of fit attained. Features associated with vibrational modes of T3 are denoted with *.

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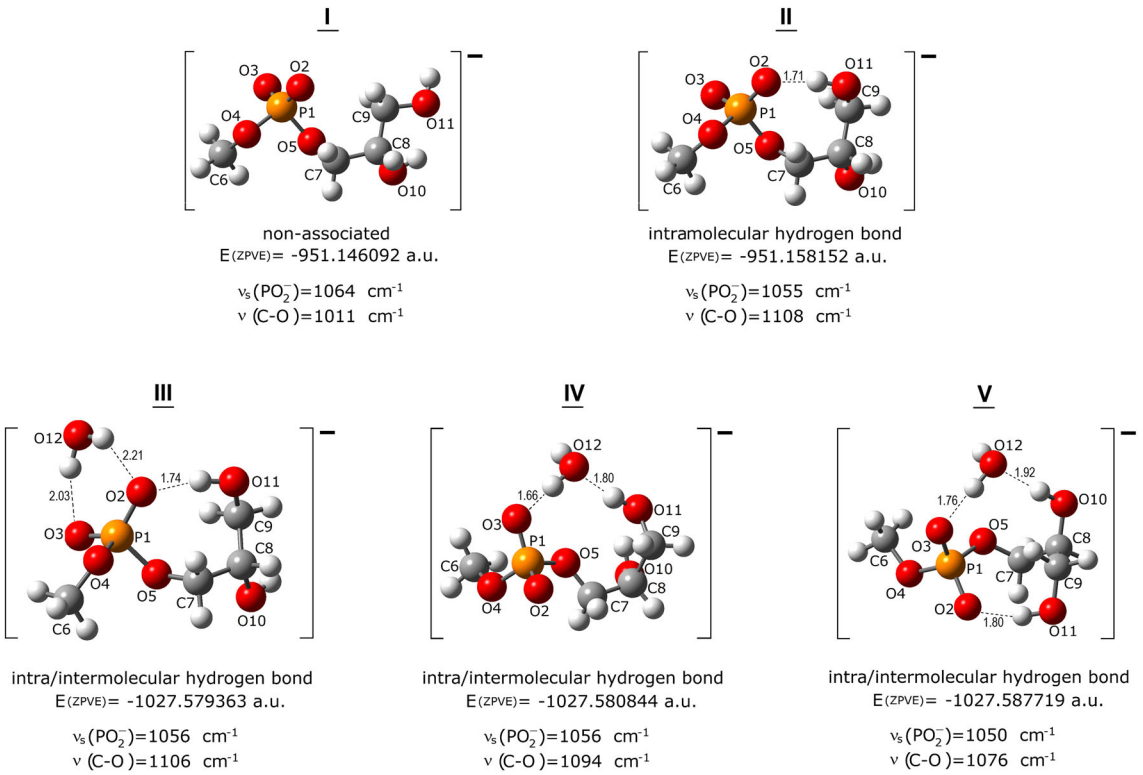

Figure 4. Optimized conformations of isolated $[CH_3—O—P(O)_2—O—CH_2—CHOH—CH_2OH]^-$ and water—$[CH_3—O—P(O)_2—O—CH_2—CHOH—CH_2OH]$—dimmers. Atom numbering and predicted hydrogen-bond distances are included. The calculated $\nu_s(PO_2^-)$ and ν(C—O) frequencies, corresponding to the O2—P1—O3 group and the C9—O11 bond, for each optimized structure are included. Zero-point corrected energies (ZPVE) are also included. Intramolecular hydrogen-bond association (II) is preferred over the non-associated conformation (I) ($\Delta E_{(ZPVE)} = 7.57$ Kcal/mol) for the single charged-molecule. The optimum water association is that with the water molecule linking one P=O bond with the H—O10—C8 group and the other P=O bond with the terminal H—O11—C9 group (V) ($\Delta E_{(ZPVE)} = 4.31/5.24$ Kcal/mol, with respect to (III) and (IV), respectively).

In the Raman spectrum of DLPG (Fig. 3), this central region is dominated by a broad band, strongest than the lateral $\nu_{(i,ph)}$(C—C)$_T$ and $\nu_{(o,o,ph)}$(C—C)$_T$ at 1122 and 1063 cm^{-1}, respectively. The band contour clearly suggests the presence of two components of similar intensities. Under decomposition, these appear centered at 1089 and 1077 cm^{-1}; a third contributing band, at 1100 cm^{-1}, is also observed. Since a high *gauche:trans* ratio is expected for the acyl chains in fluid phase, the lowest wavenumber component is assigned to the ν(C—C)$_G$ of the *gauche–trans–gauche* (*g–t–g*) conformations in the body of the chain, typical for the L_α phase.[17,48–51] The component at 1100 cm^{-1} is mainly assigned to $\nu_s PO_2^-$ of groups involved in hydrogen-bonding associations with the glycerol moiety; the νC—O band results obscured by the phosphate vibration. The broad 1089 cm^{-1} component is compatible with both, a high content of *gauche* conformers located at the chain ends (end–*gauche*) and a high population of PO_2^- groups associated by hydrogen bonds with water molecules.

The 1150–1000 cm^{-1} spectral region of the DPPG–T3 complex (see Fig. 3) evidences that the hormone affects the polar head region of the membrane with little influence on the acyl chains conformations of the lipids when they are in the gel phase. The change in the band contour centered at 1097 cm^{-1} is interpreted as a decrease in the population of PO_2^- groups associated with water molecules. Indeed, the 1090 cm^{-1} component band observed in DPPG spectrum is absent in the decomposed DPPG–T3 spectrum; as consequence, the component at 1096 cm^{-1} gains in intensity relative to the band at 1130 cm^{-1} ($\nu_{(i,ph)}$(C—C)$_T$). In addition, the νC—O component appears significantly attenuated while negligible changes on the ν(C—C)$_G$ are observed. The general widening affecting the bands in this spectral region upon T3 incorporation is consistent on one hand, with the increased interchain packing and on the other with a tighter proximity between the polar head groups. Both T3-induced effects allow faster relaxation after vibrational excitations. In DLPG–T3 spectrum, the enveloping line that spans from 1150 to 1000cm^{-1} shows an even higher complexity than that for DLPG, which suggests that changes in the hydrophobic and the polar regions of the membrane are affected by T3 incorporation. Assuming that part of the hormone is inserted into the hydrocarbon chains and part is interacting with the C=O and PO_2^- groups, splitting and narrowing of the broad band observed in the DLPG spectrum are expectable in the DLPG–T3 spectrum upon the curve-fitting procedure. Then, the components obtained by band deconvolution are tentatively assigned according to the following interpretations: (1) the narrow components located at 1089 and 1083 cm^{-1} are assigned to $\nu_s PO_2^-$ and ν(C—C)$_G$, respectively. Alterations in the acyl chain conformations could lead to wavenumbers shifts of the C—C stretching modes and to the subsequent splitting of the broad band observed in the decomposed DLPG spectrum. (2) The new component at 1070 cm^{-1} is assigned to ν(C—C)$_G$ vibrations. Interestingly, a similar behavior in the C—C stretching region has been observed when studying interactions between thyroxine and DLPC membranes: the voluminous hormone molecule, partially inserted into the hydrophobic region, induced an increased population of end-*gauche* conformations (1089 cm^{-1}) and the split of the *g–t–g* band into signals at 1079 and 1071 cm^{-1}; the last feature was tentatively assigned to a third type of kinked-chain structure, the 'double *gauche*' (*g–g*) conformation.[17]

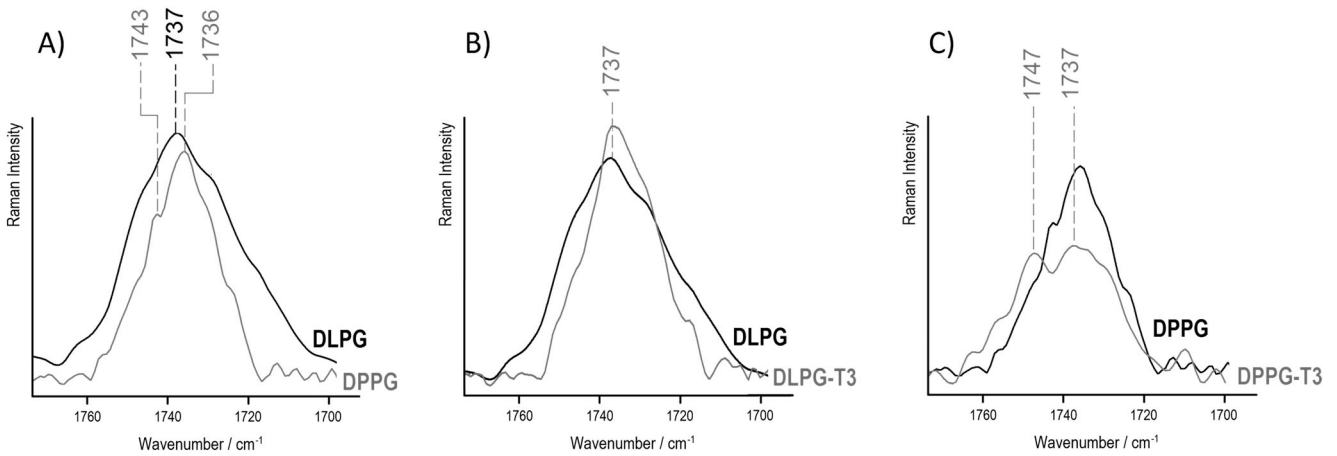

Figure 5. Raman spectra in the region corresponding to the carbonyl stretching modes of the acyl chains. A) Comparison between the νC=O band in pure DPPG and DLPG vesicles; B) comparison between pure DLPG and DLPG–T3 complex; C) comparison between pure DPPG and DPPG–T3 complex.

A weak band corresponding to the carbonyl stretching modes (νC=O) of the acyl chains is observed between 1650 and 1780 cm^{-1} in the membrane Raman spectra. Information concerning the polar/apolar interfacial region of the lipid bilayers can be extracted from its analysis. Based on the fact that the ester carbonyl groups of lipids are potential participants in hydrogen-bonding interactions with interfacial water molecules or with other polar moieties of the lipids, it is expected that changes in the structure or hydration of the bilayer interface were evidenced by changes in the νC=O band contour.[52–54] An evaluation of the carbonyl spectral behavior in the different lipid phases is necessary prior to making the analysis of the effects exerted by the hormone incorporation on the interfacial region of the membrane. However, difficulties in the interpretation of the νC=O band contour arise due to possible differences in hydrogen-bond associations[54] are superimposed to different conformations about the acyl linkage in the lipids.[55] Figure 5 shows the νC=O Raman spectral region of DPPG and DLPG liposomes in free state and in the corresponding lipid–T3 complexes. A significant difference between the band shapes of the DPPG and DLPG is observed (see Fig. 5A). The broad signal centered at 1737 cm^{-1} in the spectrum of the liquid-crystalline phase evidences a considerable conformational freedom of the carbonyl groups around the link between the polar head group and the hydrophobic chain region. Moreover, a high population of strong hydrogen-bonded C=O groups is suggested by the asymmetry of the band that shows a long wing towards lower wavenumbers. On the contrary, a narrower νC=O band (1736 cm^{-1}) is observed in the DPPG spectrum. This signal, assigned to hydrogen-bonded carbonyl groups, shows a well-defined shoulder at 1043 cm^{-1} which is associated with conformations offering a few possibilities to form hydrogen bonds either with nearby water molecules or with the glycerol OH groups. Spectral changes in the νC=O band arise as consequence of the T3 incorporation to the membranes (see Fig. 5B and 5C). In DLPG–T3 Raman spectrum, the maximum of the band gains in intensity as it becomes narrower, which suggests that the components at higher and lower wavenumbers decrease. These changes are interpreted as (1) a displacement of water molecules from the interfacial region and (2) a reduction in the conformational freedom of the acyl linkage. The νC=O band in DPPG–T3 Raman spectrum shows an opposite behavior manifested by the attenuation of the 1736 cm^{-1} band which is accompanied with the appearance of a new signal at 1747 cm^{-1}. A possible explanation to these spectral differences for the lipid gel phase postulates that hormone-induced conformational changes in the polar heads of the lipids lead to disruption of hydrogen bond interactions between the C=O and the glycerol OH groups, and the consequent appearance of non-associated carbonyl groups.

Conclusions

In this paper, we present a Raman spectroscopy study of the interactions between the iodothyronine T3 and anionic membranes in two different phases.

The observed spectral changes indicate that the hormone is able to penetrate the hydrophobic region of the lipid bilayer in the fluid phase. Thus, interchain coupling increase and alteration in the C—C *gauche* conformation content are derived from comparative analysis between DLPG and DLPG–T3 spectra. However, the discreet increase in the rotational freedom of CH_3 groups suggests that the hormone achieves a restricted penetration into the hydrophobic region. Interaction of T3 with the zwitterionic PC membrane in fluid phase revealed a hormone-induced increase of ~10% in the rotational freedom of the terminal CH_3 groups.[17]

The interaction of the thyroid hormone with a membrane in the gel phase is significantly different. The negligible spectral changes induced by T3 in vibrations concerning the C—C stretching modes of the acyl chains of DPPG indicate that the hormone attains a minimal penetration into the hydrophobic region. However, structural distortions of the glycerol groups and/or displacements of water molecules from the surface and interfacial regions are evidenced from the vibrations of the C=O and PO_2^- groups in the DPPG–T3 complex spectrum. These effects suggest that the hormone remains anchored in the surface region of the bilayer due to the high density packing characteristic of the gel phase and the anionic nature of the membrane. The higher coupling between the alkyl chains and a decrease in the rotational freedom of the CH_3 groups in the DPPG–T3 spectrum is reasoned in terms of an interdigitation effect induced by the hormone.[56–58] The incipient shoulder observed at 1417 cm^{-1} confirms this assumption.[18,59]

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