Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 112 (2013) 403–409

Contents lists available at SciVerse ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

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

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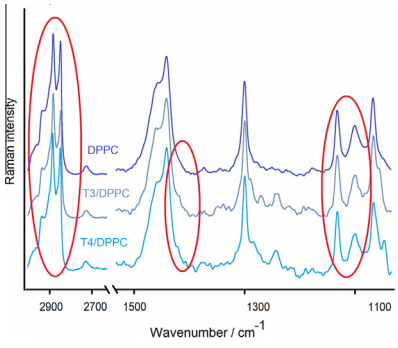
## HIGHLIGHTS

- 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




## ARTICLE INFO

*Article history:*
Received 15 November 2012
Received in revised form 18 April 2013
Accepted 24 April 2013
Available online 3 May 2013

*Keywords:*
DPPC liposomes
Lipid gel phase
Raman spectroscopy
Thyroid hormones

## 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.



## 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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http://dx.doi.org/10.1016/j.saa.2013.04.094

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 $L_\alpha$ 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 (p$K$ = 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

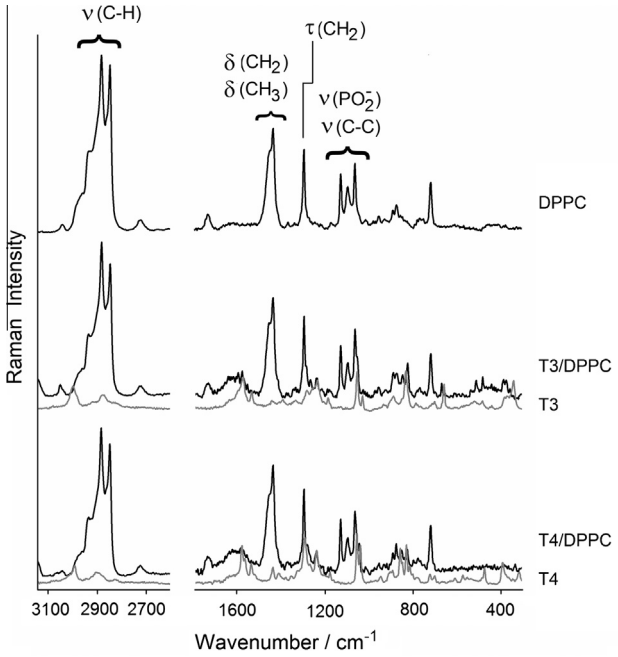


**Fig. 1.** Raman spectra of the pure DPPC multilamellar vesicles and the iodothyronine–DPPC complexes between 3200–2600 and 1800–300 $cm^{-1}$. Band assignment of the main lipid vibrations is included in the spectrum of DPPC. The Raman bands of the iodothyronines are easily identified in the complex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