

Rosa M.S. Álvarez · Carlos O. Della Védova
Hans-Georg Mack · Ricardo N. Fariás
Peter Hildebrandt

Raman spectroscopic study of the conformational changes of thyroxine induced by interactions with phospholipid

Received: 8 March 2002 / Revised: 24 May 2002 / Accepted: 24 May 2002 / Published online: 3 July 2002
© EBSA 2002

Abstract Comparison of the Raman spectra of thyroxine (L-3,3',5,5'-tetraiodothyronine) in the pure state and in a 1:5 mixture with phosphatidylcholine reveals spectral differences that reflect structural changes of thyroxine induced by interactions with the phospholipid. These structural changes could be localized in specific parts of the thyroxine molecule on the basis of a vibrational analysis that was carried out by density functional calculations with the B3LYP hybrid functional applying the SDD effective core potential basis set. The calculated (and subsequently scaled) frequencies reveal a good agreement with the experimental data, which together with calculated IR and Raman intensities allow a plausible assignment of most of the IR and Raman bands. Thus, it is found that modes localized in the aromatic β -ring and in the ether group as well as the C-I stretching modes of ring α are affected upon lipid interactions, indicating that thyroxine interacts with the phosphatidylcholine bilayer via penetration of the hydrophobic part of the molecule.

Keywords Thyroxine · Phospholipid · Conformational changes · Raman spectroscopy · Density functional theory

Abbreviations *DFT*: density functional theory · *PC*: phosphatidylcholine · *T3*: 3,3',5-triiodothyronine · *T4*: 3,3',5,5'-tetraiodothyronine

Introduction

Thyroxine (T4, L-3,3',5,5'-tetraiodothyronine) is the principal secretor product of the thyroid gland (Fig. 1). T4 is considered as a prohormone that acquires biological activity only after the intracellular conversion into 3,3',5-triiodothyronine (T3). The intracellular T3 mainly originates from the cellular 5'-deiodination of T4 and acts at the level of its specific nuclear receptor (Oppenheimer 1991; Chin 1992; Tsai and O'Malley 1994). In order to reach the intracellular compartment, T4 has to cross the cell membrane, presumably via a flip-flop motion. However, details of the mechanisms by which mammalian cells take up thyroid hormones remain poorly understood (Chehin et al. 1999).

There is evidence that the thyroid hormones (mainly T4) are normal constituents of the biological membrane in vertebrates, as was reviewed by Hulbert (2000). The hormones are strongly associated with membranes in tissues and normally rigidify these membranes, affecting their lipid composition. It is suggested that both the alterations of the physical state of the membrane and the changes in membrane composition result in several other thyroid hormone effects (Hulbert 2000). Previous reports from our laboratory have shown that thyroid hormones induce changes of the fluidity and permeability of phospholipid bilayers, which in the gel state incorporate thyroxine only to a low extent (Chehin et al. 1995, 1999; Fariás et al. 1995). In order to analyse these interactions on a molecular level, we have employed Raman spectroscopy. This technique provides information about the molecular structures of thyroid hormones

P. Hildebrandt (✉)
Instituto de Tecnologia Química e Biológica,
Universidade Nova de Lisboa, Apartado 127,
Av. da República, 2781-901 Oeiras, Portugal
E-mail: hildebrandt@itqb.unl.pt
Tel.: +351-21-4469741
Fax: +351-21-4411277

R.M.S. Álvarez · R.N. Fariás
Departamento de Bioquímica de la Nutrición,
Instituto Superior de Investigaciones Biológicas (INSIBIO),
CONICET and Universidad Nacional de Tucumán,
Chacabuco 461, 4000 San Miguel de Tucumán,
Tucumán, Argentina

C.O. Della Védova
CEQUINOR (CONICET),
Universidad Nacional de La Plata,
45 esq. 115, 1900 La Plata, Argentina

H.-G. Mack
Institute of Physical and Theoretical Chemistry,
University of Tübingen, Auf der Morgenstelle 8,
72076 Tübingen, Germany

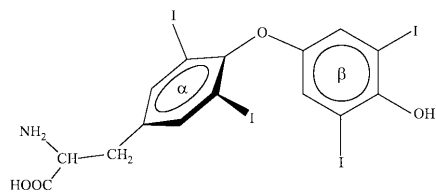


Fig. 1. Structure of thyroxine (T4) optimized by density functional calculations

and, hence, allows determination of their conformational changes induced upon binding to phospholipid. However, extracting structural information from the vibrational spectra requires a reliable assignment of the vibrational bands, which is not yet available for iodothyronine compounds. Thus, we have carried out normal mode analyses on the basis of density functional theory (DFT) calculations. This approach allows a plausible assignment for most of the observed IR and Raman bands and, eventually, the identification of spectral markers for localized structural perturbations of iodothyronines that may provide insight into the modes of interactions with phospholipid membranes. In the first step, we have focused on thyroxine and its complex formation with phosphatidylcholine (PC), which exists in the fluid state at ambient temperature.

Materials and methods

Sample preparation

T4 and egg yolk phosphatidylcholine were purchased from Sigma and used without further purification. Methanolic solutions of T4 (100 mg/mL) and PC (40 mg/mL) were prepared and the appropriate amounts of PC or of a mixture of T4 with PC (molar ratio of 1:5) were dried under a nitrogen stream and suspended by vortex in 50 mM citrate-phosphate buffer (pH 5.0) at ambient temperature to give a final concentration of 1 mM PC. At pH 5.0, the ionization degree of the phenolic hydroxyl substituent ($pK_A = 6.73$; Gemmill 1955) is less than 2%, corresponding to a maximum lipophilicity of T4 and a particularly high partition coefficient between the lipid and the aqueous phase. The suspensions were centrifuged at 6000 rpm for 15 min and the pellets were used for spectroscopic measurements.

Infrared and Raman spectra

Infrared spectra of KBr pellets of T4 were measured with a FT-IR spectrometer (Perkin Elmer 1600, 4-cm^{-1} resolution). Raman spectra of T4, PC, and the T4/PC mixture were recorded with a BioRad FT Raman spectrometer (4-cm^{-1} resolution, 1064-nm excitation) using an 180° back-scattering geometry (Matysik et al. 1995). The spectra were obtained from the samples deposited in quartz tubes of 0.25 cm inner diameter. In order to achieve a sufficient signal-to-noise ratio, 8192, 42,584, and 69,188 scans were accumulated in the case of T4, PC, and the T4/PC mixture, respectively, corresponding to a total accumulation time between 5 and 48 h. All spectroscopic experiments were carried out at ambient temperature.

Quantum chemical calculations and normal mode analyses

Quantum chemical calculations were performed with the GAUSSIAN 98 program package (Frisch et al. 1998) on a personal

computer. DFT calculations were carried out with the B3LYP hybrid functional and the SDD effective core potential basis set suitable for heavy elements. This basis set had to be chosen owing to the presence of iodine atoms that could not be treated on the level of the more commonly used 6-31G* basis set. However, comparative calculations for small organic molecules demonstrated that the SDD basis provides results of nearly the same quality.

The X-ray structure of T4 (Camerman and Camerman 1974) was taken as the starting point for the geometry optimization, yielding a structure closely related to the experimental one: predicted bond lengths and angles agree with the experiment within 0.017 \AA and 1.8° , respectively. The most significant deviation is related to the orientation of the phenyl rings with respect to each other. Whereas the calculations predict a nearly perpendicular orientation (R.M.S. Álvarez et al., unpublished results), in the crystal structure the angle between the planes of rings α and β is 45° . This discrepancy is ascribed to the effect of intermolecular interactions in the crystal. A second problem inherent to the quantum chemical calculations refers to the α -amino-carboxyl function that in the solid state as well as in solution exists as a zwitterionic form. In the calculations the non-ionic form represents the structure of lowest energy, and any attempts to achieve a local energy minimum with a zwitterionic structure failed. Consequently, all experimental vibrational bands that originate from the zwitterionic moiety could not be reproduced by the calculations. These are particularly the relatively strong IR bands at ca. 1600 cm^{-1} that partially obscure the aromatic ring modes, which in turn are dominant in the Raman spectra (vide infra). Conversely, all calculated modes that originate from the non-ionic α -amino-carboxyl group were not considered for the vibrational assignments.

The theoretical frequencies are associated with a systematic error, mainly due to the insufficient consideration of electron-correlation effects and the neglect of anharmonicity. It has been shown previously that scaling of the force field can efficiently compensate these errors (Pulay et al. 1983; Magdó et al. 1999). In this way, accuracy in the frequency calculation of ca. 10 cm^{-1} can be achieved. At the B3LYP/6-31G* level the scaling factors that refer to different internal coordinates are very similar. Thus, we have simplified the procedure by adopting a uniform factor to scale the frequencies directly. Determination of this factor was based on the highest frequency ring modes $\alpha(1)$ and $\beta(1)$ that give rise to the strongest Raman bands between 1570 and 1600 cm^{-1} (Harada and Takeuchi 1986). The factor was chosen such that the best agreement was achieved between the calculated and the experimental frequencies for three iodothyronines (T4, T3, and 3,5-diiodothyronine). This simplified scaling procedure is associated with an average error of the frequency prediction that is estimated to be higher by a factor of 2 than in the case of force field scaling. Nevertheless, this reduced accuracy is considered to be sufficient for a consistent assignment of most of the observed bands, inasmuch as in the spectral region of interest the average density of modes per wavenumber interval is less than 1 per 20 cm^{-1} . Moreover, additional assignment criteria are provided by calculated Raman and IR intensities, which were classified semi-quantitatively in terms of very strong (vs), strong (s), medium (m), weak (w), and very weak (vw) intensities (Table 1). Whereas a detailed vibrational analysis of T4 as well as of T3 and 3,5-diiodothyronine will be presented elsewhere (R.M.S. Álvarez et al., in preparation), the present report focuses on the results obtained for those spectral regions which undergo changes upon T4 binding to PC.

Results

Raman spectra of T4, PC, and T4/PC complexes were recorded in the frequency range between 150 and 4000 cm^{-1} . Figure 2 presents the spectra of the T4/PC mixture (A) and PC (B). In order to determine the interactions of T4 with PC (C), the Raman spectrum of the

Table 1. Comparison of calculated and experimental Raman and IR bands in selected spectral regions^a

Calculated modes				Experimental bands					
ν (cm ⁻¹)	I_{IR}	I_{Ra}	Assignment	T4, IR		T4, Raman		T4/PC, Raman	
				ν (cm ⁻¹)	I_{IR}	ν (cm ⁻¹)	I_{Ra}	ν (cm ⁻¹)	I_{Ra}
1591	m	vs	$\beta(1)$	1582	m	1580	s	1584	m
1576	w	vs	$\alpha(1)$	–	–	–	–	–	–
1560	m	s	$\beta(2)$	–	–	1567	m	–	–
1537	m	m	$\alpha(2)$	1538	m	1538	m	–	–
1232	vw	s	$\beta(6)$	1229	w	1229	w	–	–
1227	vw	w	Residue	–	–	–	–	–	–
1223	s	s	C α -O	1242	s	1238	m	1243	m
1210	w	w	C _{sp2} -C _{sp3}	–	–	1215	vw	–	–
1205	vw	vw	$\alpha(6)$	1207	vw	1205	vw	–	–
1168	w	w	C β -O	1184	w	1177	m	1212	w
1092	w	m	C-N	1048	w	–	–	–	–
1038	w	s	$\alpha(7)$	1053	w	1053	s	1053	s
1028	vw	s	$\beta(7)$	1040	vw	1038	s	1040	s
2 \times 492	–	–	2 $\tau(\beta)$	–	–	857	s	–	–
881	w	w	$\alpha(9)$	882	m	–	–	–	–
864	vw	m	$\beta(9)$	850	m	847	w	–	–
805	w	w	Residue	–	–	–	–	–	–
801	m	s	Ring def	829	m	828	s	819	m
492	vw	w	$\tau(\beta)$	430	–	–	–	–	–
225	vw	w	C-1 β	–	–	221	vs	216	vs
214	vw	w	C-1 α	–	–	190	vs	186	vs

^aRaman (I_{Ra}) and IR intensities (I_{IR}) are classified as very strong (vs), strong (s), medium (m), weak (w), and very weak (vw). Assignments are given in terms of the principal mode character:

$\alpha(i)$, $\beta(i)$, ring stretching; $\tau(\alpha)$, $\tau(\beta)$, ring deformation; the notation for further stretching coordinates is derived from Fig. 1

pure PC (B) was subtracted from the T4/PC mixture (A), such that the characteristic PC bands disappeared in the difference spectrum. No obvious subtraction artefacts, such as negative peaks, were observed in the difference spectrum (Fig. 2C). Note that this subtraction procedure was based on several bands in the entire frequency range from 150 to 4000 cm⁻¹. The underlying assumption of this approach is that the structure of PC and, thus, the Raman bands of the lipid are not affected upon T4 binding. Structural changes of the PC would be most sensitively reflected between 2800 and 3200 cm⁻¹ (not shown here) (Verma and Wallach 1984). However, owing to the relatively low signal-to-noise ratio and the broad and poorly structured peaks in this region, the possibility of structural changes in the PC can neither be ruled out nor confirmed. In the region between 150 and 1800 cm⁻¹ the PC bands partly overlap with some of the T4 bands. Thus, for these T4 bands, determination of possible spectral changes is associated with a significant uncertainty since even small variations of PC Raman bands may cause artificial frequency or intensity changes of the adjacent T4 bands in the difference spectrum. Consequently, the analysis of the membrane-induced spectral changes of T4 will be restricted to those spectral regions that do not include PC bands.

The strongest Raman bands of thyroxine are observed at 190 and 221 cm⁻¹ (Fig. 3) and originate from the symmetric C-I stretching vibrations of rings α and β , respectively. In the membrane bound state, both modes shift to lower frequencies by 4 and 5 cm⁻¹, respectively.

In the region between 400 and 1000 cm⁻¹ (Fig. 4), the only band that can reliably be detected in the difference Raman spectrum (T4/PC – PC) (A), is found at 819 cm⁻¹, which corresponds to the 828 cm⁻¹ band in the Raman spectrum of pure T4 (Fig. 4B). This band also exhibits a considerable IR intensity (Fig. 4C) and, hence, it is assigned to an aromatic ring deformation mode in view of the agreement with the calculated frequency (801 cm⁻¹) and the predicted IR and Raman intensities (Table 1). A second prominent IR band is found at 850 cm⁻¹, which in the Raman spectrum of T4 is only detectable as a shoulder of the substantially more intense 857 cm⁻¹ band. Conversely, this latter band has no counterpart in the IR spectrum. The only fundamental calculated in this spectral region is the symmetric ring mode $\beta(9)$. The corresponding mode of ring α is predicted at 881 cm⁻¹ and readily attributed to the IR band at 882 cm⁻¹. On the basis of this assignment, there is no other fundamental mode that could plausibly be ascribed to the 857 cm⁻¹ Raman band. This situation is reminiscent of the vibrational band pattern of tyrosine. In this case, two relatively intense Raman bands between 830 and 850 cm⁻¹ are assigned to a Fermi doublet of a totally symmetric ring vibration and an overtone of a ring out-of-plane mode (Siamwiza et al. 1975; McHale 1982). Adopting the same interpretation to the 850/857 cm⁻¹ doublet in thyroxine, the only candidate for the overtone is the second harmonic of the out-of-plane deformation mode of ring β , $\tau(\beta)$. The fundamental of this mode is assigned to the 430 cm⁻¹ IR band, although the difference to the theoretical frequency (492 cm⁻¹) is relatively

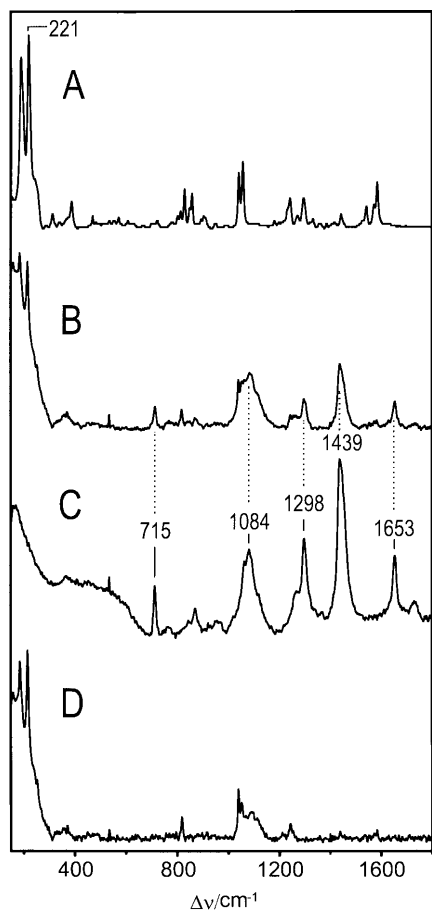


Fig. 2. Raman spectra of **A** a T4/PC mixture and **B** of PC. The difference spectrum (‘‘A minus B’’) that is shown in **C** represents the spectrum of T4 bound to PC.

large. However, this discrepancy can be understood in terms of the substantial deviation in the dihedral angles of the ring α /ring β linkage (see above), which may have a specifically strong impact on ring out-of-plane modes. Following this assignment, the $\tau(\beta)$ overtone is readily attributed to the band at 857 cm^{-1} . Thus, both requirements for a Fermi-type overtone enhancement are fulfilled: the energetic proximity to a fundamental [$\beta(9)$] and the localization of both modes in the same part of the molecule (ring β). In the difference Raman spectrum of the T4/PC complex this doublet cannot be observed (Fig. 4A). For this reason, it is concluded that the frequencies of either $\beta(9)$ or $\tau(\beta)$ or both are shifted upon membrane binding such that the energy gap to the $\beta(9)$ fundamental is too large for an enhancement of the $\tau(\beta)$ overtone. This band as well as the intrinsically weak $\beta(9)$ fundamental does not exceed the noise level in the difference Raman spectrum.

In the region between 1000 and 1800 cm^{-1} (Fig. 5) the frequencies of the most intense bands at 1038 and 1053 cm^{-1} are essentially unchanged upon binding to PC vesicles and only their relative intensities are altered. These bands are ascribed to stretching modes of ring α

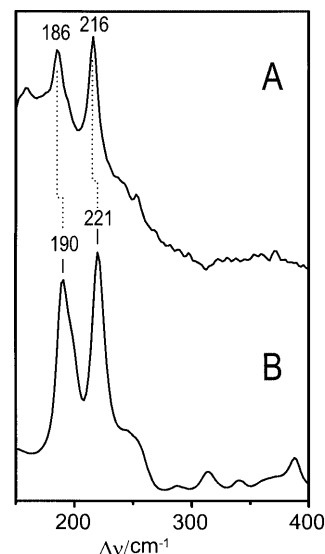


Fig. 3. Raman spectra of **A** T4 bound to PC and **B** pure T4 in the region between 150 and 400 cm^{-1} . The spectral contributions of PC are subtracted in **A**.

and of the C-N bond. On the other hand, there is a distinct frequency upshift by 6 cm^{-1} for the 1238 cm^{-1} band. This band is assigned to the C α -O stretching mode calculated at 1223 cm^{-1} and predicted to exhibit strong Raman and IR intensity, in agreement with the experimental findings (Fig. 5B and C). Close to the 1244 cm^{-1} band, the difference spectrum (Fig. 5A) displays a weak, albeit clearly detectable, band at 1212 cm^{-1} . In view of its similar relative intensity, this band may correspond to the 1177 cm^{-1} band of pure T4, which is attributed to C β -O stretching. This tentative assignment suggests that membrane binding induces a substantially larger frequency upshift of this mode as compared to C α -O stretching.

It cannot unambiguously be decided whether or not the band at 1291 cm^{-1} (ring α mode) is affected upon complex formation, since this band falls into a region of strong Raman PC bands. For this reason, the disappearance of this band in the difference spectrum may represent a subtraction artefact. Remarkable changes that cannot be attributed to such effects are noted for the aromatic ring mode $\beta(1)$, which for pure T4 is found at 1580 cm^{-1} . In the difference spectrum (Fig. 5A) the intensity of this band is lowered such that its frequency, which is evidently higher than in pure T4, cannot be determined precisely.

Discussion

Essentially all the spectral changes that are observed in the Raman spectrum of the T4/PC complex refer to modes that are localized in ring β and the ether bridge. It is therefore concluded that binding to membranes primarily occurs via this apolar part of the molecule.

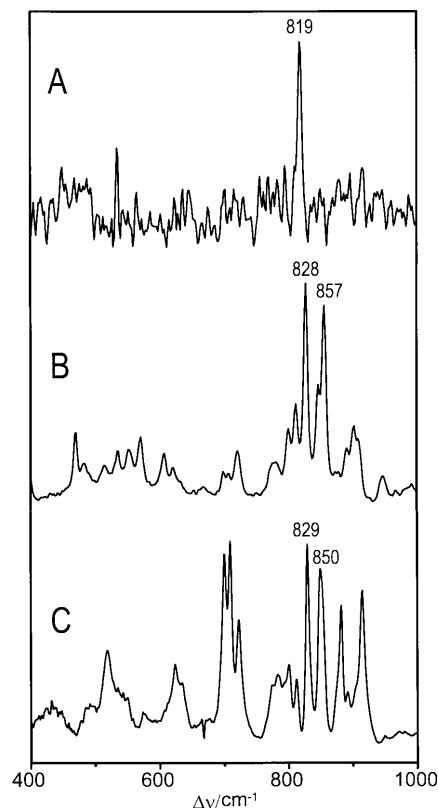


Fig. 4. Raman spectra of **A** T4 bound to PC compared to **B** the Raman spectrum and **C** the IR spectrum of pure T4 in the region between 400 and 1000 cm^{-1} . The spectral contributions of PC are subtracted in **A**

Conversely, except for the C-I stretching, ring α modes as well as those of the alanine residue remain unaffected. These findings imply that T4 inserts into the phospholipid bilayers in such a manner that ring β , the ether bridge, and possibly also a part of ring α are anchored between the aliphatic chains of the lipid via hydrophobic interactions. This conclusion is in agreement with Lai et al. (1985), who suggested that the nonpolar phenolic group of the hormone is close to the lipid core of the liposomal membrane. As a consequence, the hydroxyl substituent of ring β , which is not deprotonated owing to its $\text{p}K_{\text{A}}$ of 6.73 (Gemmill 1955), is transferred from a polar to a hydrophobic environment associated with the loss of hydrogen bonding interactions. In fact, the environmental changes experienced by this substituent may account for the disappearance of the Fermi doublet at 850/857 cm^{-1} . In the case of tyrosine, the intensity ratio of both band components, that reflect the degree of overtone intensity enhancement, sensitively varies with the type and strength of the hydrogen bonding interactions of the hydroxyl group (Siamwiza et al. 1975).

Furthermore, the insertion of T4 into the PC bilayer also affects the geometry of the ether bridge, as indicated by the upshifts of the C-O stretching modes.

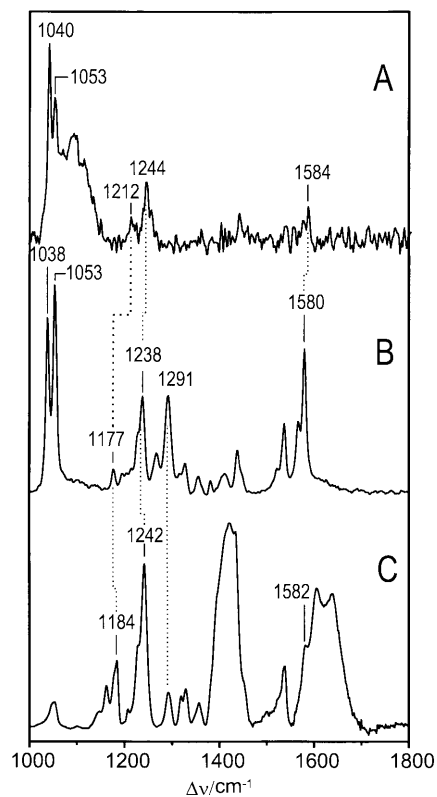


Fig. 5. Raman spectra of **A** T4 bound to PC compared with **B** the Raman spectrum and **C** the IR spectrum of pure T4 in the region between 400 and 1000 cm^{-1} . The spectral contributions of PC are subtracted in **A**

These frequency shifts may result from a reorientation of the phenyl ring planes relative to each other and from a distortion of the C-O-C bond angle. However, preliminary calculations for T4 at different C-O-C bond angles indicate that such a geometry change has only a relatively small effect on the C-O stretching frequencies. Thus, perturbations of the ring(α)-ring(β) dihedral angles are more likely. This reorientation of the phenyl rings probably affects also in-plane and out-of-plane deformation modes of ring β , which can be the origin of the large 9 cm^{-1} frequency shift of the band at 828 cm^{-1} and eventually (at least partly) for the loss of the $\tau(\beta)$ overtone enhancement.

Even though there are no spectral indications for changes of the ring α skeleton, the part of ring α carrying the iodine substituents is likely to be accommodated into the bilayer as well, which is reflected by the frequency shift of the corresponding C-I stretching mode.

Acknowledgements This work was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT). R.M.S.A., C.O.D.V., and R.N.F. are career researchers of CONICET.

References

- Camerman A, Camerman N (1974) Thyroid hormone stereochemistry. I. The molecular structures of 3,5,3'-triiodo-L-thyronine (T₃) and L-thyroxine (T₄). *Acta Crystallogr Sect B* 30:1832–1840
- Cehin RN, Rintoul MR, Morero RD, Farias RN (1995) Differential effect of triiodothyronine and thyroxine on liposomes containing cholesterol: physiological speculations. *J Membr Biol* 147:217–221
- Cehin RN, Isse BG, Rintoul MR, Farias RN (1999) Differential transmembrane diffusion of triiodothyronine and thyroxine in liposomes: regulation by lipid composition. *J Membr Biol* 167:251–256
- Chin WW (1992) Current concepts of thyroid hormone action: progress notes for the clinician. *Thyroid* 15:1–19
- Farias RN, Cehin RN, Rintoul MR, Morero RD (1995) Differential effect of triiodothyronine and thyroxine on the liposomal membrane in liquid-crystalline and gel states. *J Membr Biol* 143:135–141
- Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Zakrzewski VG, Montgomery JA, Stratmann, RE, Burant JC, Dapprich S, Millam JM, Daniels AD, Kudin KN, Strain MC, Farkas O, Tomasi J, Barone V, Cossi M, Cammi R, Mennucci B, Pomelli C, Adamo C, Clifford S, Ochterski J, Petersson GA, Ayala PY, Cui Q, Morokuma K, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Cioslowski J, Ortiz JV, Baboul AG, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Gomperts R, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Gonzalez C, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Andres JL, Gonzalez C, Head-Gordon M, Replogle ES, Pople JA (1998) Gaussian 98, revision A.7. Gaussian, Pittsburgh
- Gemmill CL (1955) The apparent ionization constants of the phenolic hydroxyl groups of thyroxine and related compounds. *Arch Biochem Biophys* 54:359–367
- Harada I, Takeuchi H (1986) Raman and ultraviolet resonance Raman spectra of proteins and related compounds. In: Clark RJH, Hester RE (eds) *Spectroscopy of biological systems*, vol 13. Wiley, New York, pp 113–175
- Hulbert AJ (2000) Thyroid hormones and their effects: a new perspective. *Biol Rev* 75:519–631
- Lai G-S, Koritowsky W, Chien H-N, Cheng S-Y (1985) Transverse motion of spin labeled 3,5,3'-triiodo-L-thyronine in phospholipid bilayers. *Biochem Biophys Res Commun* 131:408–412
- Magdó I, Németh K, Mark F, Hildebrandt P, Schaffner K (1999) Calculation of vibrational spectra of linear tetrapyrroles. I. Global sets of scaling factors for force fields derived by ab initio and density functional theory methods. *J Phys Chem A* 103:289–303
- Matysik J, Hildebrandt P, Schlamann W, Braslavsky SE, Schaffner K (1995) Fourier-transform resonance Raman spectroscopy of intermediates of the phytochrome photocycle. *Biochemistry* 34:10497–10507
- McHale JL (1982) Fermi resonance of tyrosine and related compounds. Analysis of the Raman doublet. *J Raman Spectrosc* 13:21–24
- Oppenheimer JH (1991) Thyroid hormone action at the molecular level. In: Braverman LE, Utiger RD (eds) *Werner and Ingbars the thyroid: a fundamental and clinical text*, 6th edn. Lippincott, Philadelphia, pp 204–224
- Pulay P, Fogarasi G, Pongor G, Boggs J E, Vargha A (1983) Combination of theoretical ab initio and experimental information to obtain reliable harmonic force constants. Scaled quantum mechanical (QM) force fields for glyoxal, acrolein, butadiene, formaldehyde, and ethylene. *J Am Chem Soc* 105:7037–7047
- Siamwiza MN, Lord RC, Chen MC, Takamatsu T, Harada I, Matsuura H, Shimanouchi T (1975) Interpretation of the doublet at 850 and 830 cm⁻¹ in the Raman spectra of tyrosyl residues in proteins and certain model compounds. *Biochemistry* 14:4870–4876
- Tsai M-J, O'Malley BM (1994) Molecular mechanism of action of steroid/thyroid receptor superfamily members. *Annu Rev Biochem* 63:451–458
- Verma SP, Wallach DFH (1984) In: Chapman D (ed) *Biomembrane structure and function*. Verlag Chemie, Weinheim, pp 167–198