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ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY A · AUGUST 2011

Impact Factor: 2.69 · DOI: 10.1021/jp2028142 · Source: PubMed

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Carotenoids and β-Cyclodextrin Inclusion Complexes: Raman Spectroscopy and Theoretical Investigation

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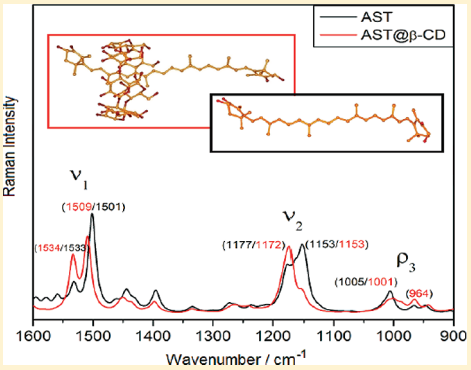
ABSTRACT: In the present study, the inclusion processes of β-carotene, astaxanthin, lycopene, and norbixin (NOR) into the β-cyclodextrin (β-CD) cavity were investigated by means of Raman spectroscopy and quantum mechanics calculations. The Raman ν_1 band assigned to C═C stretching was sensitive to the host−guest interaction and in general undergoes a blue shift (3−13 cm^{-1}) after inclusion takes place, which is the consequence of the localization of single and double bonds. This is supported by the molecular modeling prediction, which inclusion complexes show the ν_1 band blue shifted by 1−8 cm^{-1}. The calculated complexation energies was small for most of derivatives and was found to be −11.1 kcal mol^{-1} for inclusion of AST and +0.27 kcal mol^{-1} for NOR. The stability order was qualitatively correlated to topological parameters accounting for the opening angle of the chain. This means that after inclusion the guest molecules assume a slightly more extended conformation, which enhances the host−guest contact, improving the interaction energy. The results discussed here clearly demonstrate the matrix effect on the carotenes' spectroscopic profile and should contribute to fully characterize the raw samples.



1. INTRODUCTION

Cyclodextrins (CD, Figure 1) are cyclic oligomers of α-1,4-D-glucose and are efficient in the complexation of a variety of molecules, especially water-insoluble organic compounds.[1,2] The ability of CD and their derivatives to generate inclusion complexes with organic molecules is well-known in which they act as "host" and confer interesting and important electronic effects and/or specific properties to the product; in contrast, the "guest" molecules can be inserted inside the CD cavity without the formation of chemical bonds with subtle but demonstrable changes occurring in their molecular structures.[3−6]

The most studied CD hosts are α, β, and γ-cyclodextrin, which comprise oligomers of six to eight glucose units, respectively. The monomers adopt a chair conformation giving rise to structures resembling "truncated cones"; the wider side is formed by secondary hydroxyls (C2 and C3 positions) and the narrower side by the primary hydroxyls at the C6 position[7] (see Figure 1 for the numbering scheme). The oxygen atoms at the C1 and C4 form the glycosidic bonds and confer the hydrophobic character in the internal region of the cavity, unlike the external region, which has clearly hydrophilic behavior due to the free hydroxyl groups. The cavity dimension is determined by the number of glucose units, the main molecular feature in the manufacture of inclusion complexes with hydrophobic compounds.[7−11]

Carotenoids are very common pigments that are found in a large number of living organisms; they are used by most microorganisms to achieve important biological functions and processes.[12−16] Several plant's carotenoids are responsible for the exhibition of preventive metabolic effects and in animal species they can act protecting cellular disorder processes such as cancer, cardiovascular disease, arteriosclerosis, vision impairment, and other age-related diseases.[17] Carotenoids are hydrophobic compounds and very reactive to light, oxygen, and free-radical species; these aspects reinforce the necessity of the development of methods for increasing their bioavailability and stability toward irradiation, for example. In this sense, a study of the association between carotenoids and other chemicals is very important, since this could provide a better chemical system in terms of stability without loss of bioavailability.

The association of CD with β-carotene or lycopene has been studied by some authors.[18,19] Blanch and collaborators[18] studied the encapsulation of all-*trans*-lycopene from tomato using the conventional method and a supercritical fluid extraction process obtaining a good yield to the first process. Some possibilities are discussed from the Raman spectra analysis such as the isomerization (trans→cis) and the double inclusion hypothesis (1 lycopene/2 CD). In another work, Mele and collaborators[19] made a NMR spectroscopic study of aggregates between

Received: March 25, 2011
Revised: July 1, 2011
Published: July 05, 2011

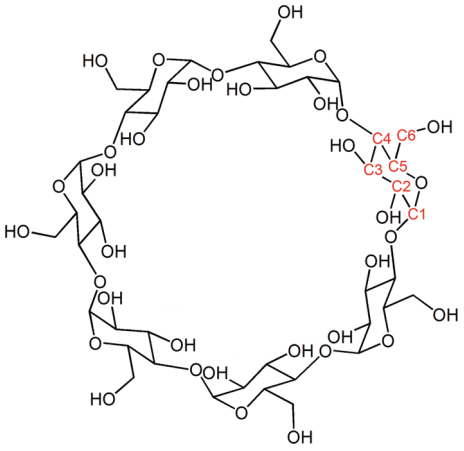


Figure 1. Structural representation of β-CD. The numbering scheme for the basic glucose unit is shown.

β-carotene and CD, suggesting the presence of 1:1 complex in aqueous solution. The use of inclusion compounds of CD (β-CD in particular) with several guest molecules has been approved in the food industry and this application has generated a rather large number of reports in the literature involving β-CD and their complexes with natural products.[18−22] One of the big problems in the structural chemistry of such complexes is the final product characterization; in this respect, Raman spectroscopy emerges as an important technique for verifying the presence of the carotenoids inside the CD cavity giving rise to an inclusion compound, since the guest molecule is a very good Raman scatterer.[23]

Carotenes have characteristic Raman bands which can be assigned to three main vibrational modes, namely the −C═C− stretching, asymmetric angular deformation of −C═C−H in plane and the $-C-CH_3$ deformation, represented by ν_1, ν_2, and ρ_3, respectively.[24−26] The first set of bands is due to the carbon−carbon stretching vibrations of the main polyene chain involving single and double bonds. The band assigned to the ν_1 mode (observed in the range between 1400 and 1600 cm^{-1}) is highly sensitive to the chemical environment and thus provides a very good probe to monitor the inclusion structure.

In an earlier report,[27] we investigated in detail the influence of the matrix in the analysis of several carotenoids (in situ samples) and its relationship with the spectroscopic data. Similar studies have also been performed by research groups such as Andreeva, Velitchkova, and co-workers[28] in addressing the effect caused by proximity of pigments located in the plant photosystem I (in this case, the PSI of spinach). The authors claim that the lutein/chlorophyll interaction is an important complex responsible for the photoprotection function of the carotenoid. The location of carotenoids in the biological environment may fundamentally affect the positioning of their vibrational bands, rendering the quantitative analysis of the carotenoid ambiguous.[27−29] In this context, the verification of a direct interference of a controlled environment (namely, β-cyclodextrin in the present study) in the vibrational profile obtained for standard carotenoids will be very useful for further identification and characterization of such molecules in complex matrices.

The present study describes four inclusion compounds formed by the interaction between β-cyclodextrin (β-CD) and carotenoids. The β-CD was chosen since its cavity dimension has an appropriate size for the accommodation of the species concerned.[7,8] The purpose of this paper is to describe an experimental and theoretical vibrational analysis of the inclusion complexes of four carotenes (Figure 2) with β-CD in which we aim to understand the relationships involved in the formation of the compounds through a survey of the vibrational Raman modes of the carotenoids that are disturbed by the inclusion process.

2. METHODOLOGY

Experimental Section. β-CD and three carotenoids (β-carotene (BCT) type II of synthetic purity ≥95%, lycopene (LYC) from tomato, astaxanthin (AST) ≥ 92%) were purchased from Sigma-Aldrich. Norbixin (NOR) was obtained from extracts of urucum seeds (*Bixa orellana L.*), based on literature methods.[30] All organic solvents were used without previous treatment and deionized water was used for the inclusion compound preparations.

The inclusion compounds were obtained as follows: an excess of hydrated β-CD (360 mg or 0.32 mmol) was dissolved in deionized water (20 mL). Acetone solution of carotenoid (1.0 mg of BCT standard ≥ 95%; 1.0 mg of LYC standard; 1.0 mg of AST ≥ 92% standard, and 5.0 mg of NOR) was added to the β-CD solution; the mixture was purged with nitrogen and maintained under constant stirring and protected from light for 5 days. The resulting solid was filtered, washed with small portions of cold acetone, dried, and kept under vacuum.

Fourier-transform Raman spectroscopic measurements were carried out using a Bruker RFS 100 instrument and a Nd^{3+}/YAG laser operating at 1064 nm in the near-infrared with a CCD detector cooled with liquid nitrogen. Good signal-to-noise ratios were obtained with 3000 scans accumulated for the samples, using a range of laser powers at the sample from 20 to 100 mW with a 2 cm^{-1} spectral resolution. All spectra were recorded at least twice for each sampling position to demonstrate reproducibility and no changes in band positions and intensities were observed, consistent with the preservation of the sample integrity under laser illumination.

Calculations. The initial geometries of the carotenes and their inclusion complexes were generated in the Maestro environment[31] with the β-CD structure taken from X-ray diffraction data.[32] All structures were submitted to classical molecular dynamics (MD) simulations using the Macromodel software[31] following the appropriate MD protocol for inclusion systems containing CD.[33] MD runs were carried out employing the AMBER* force field.[34,35] Cutoff radii for van der Waals and Coulombic electrostatic interactions were 8 and 20 Å, respectively. All the C−H and O−H bond lengths were held fixed using the SHAKE algorithm.[36] The MD final structures were selected and submitted to density functional theory (DFT) calculations at SVWN[37]/6-31G level using the GAUSSIAN 03[38] software. The optimized geometries of all molecules were then used for the vibrational harmonic frequency calculations to derive theoretical vibrational Raman spectra. The sets of frequencies and Raman activities were adjusted by the application of a Lorentzian type function[39] to obtain the band envelope spectrum (bandwidth was set to 15 cm^{-1}), which allows a direct comparison with the experimental data.

The complexation energies (E_{comp} in eq. 1) were calculated using the DFT electronic plus nuclear repulsion energy

$$E_{comp} = E_{Car@\beta\text{-}CD} - (E_{\beta\text{-}CD} + E_{Car}) \quad (1)$$

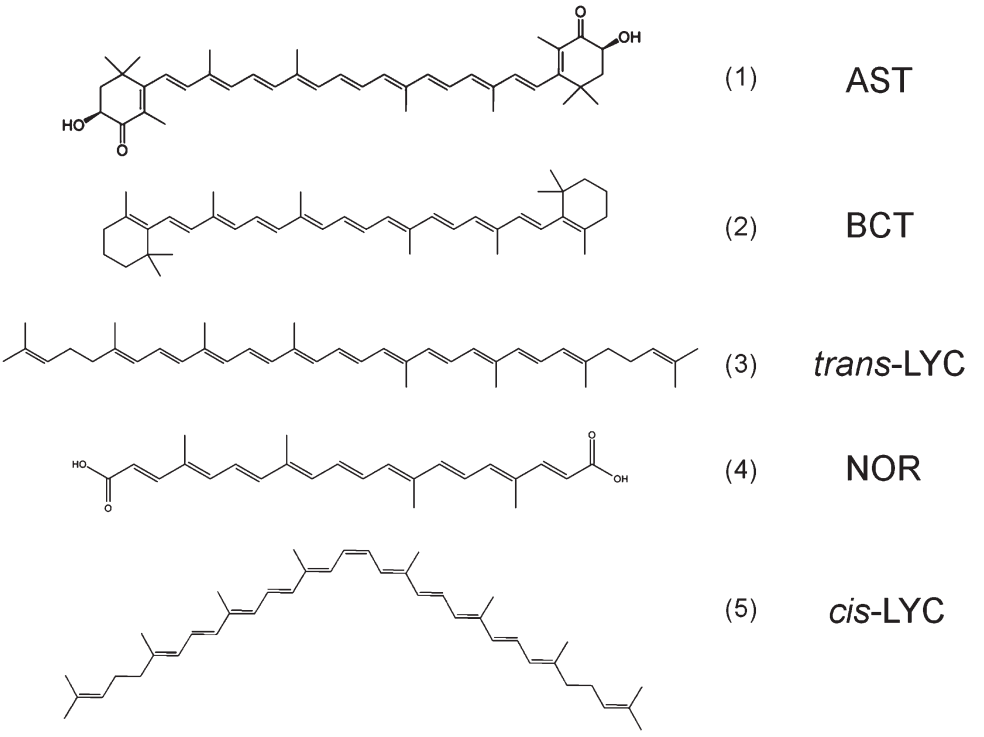


Figure 2. Structures of the naturally occurring carotenoids: BCT; *trans*- and *cis*-LYC; AST; and NOR.

where $E_{Car@\beta\text{-CD}}$, $E_{\beta\text{-CD}}$ and E_{Car} correspond to the total energies of the inclusion compound and the free species, β-cyclodextrin and carotenoids, respectively. In order to improve the complexation energy, a larger basis set (6-31+G(d,p)) was employed for energy calculation followed by basis set superposition error (BSSE) correction using the standard counterpoise approach.[40]

3. RESULTS AND DISCUSSION

The formation of inclusion compounds between β-CD and carotenoids is achieved by a quite complex mechanism, which involves, besides other steps, the water removal from CD cavity and accommodation of the guest molecule that decreases the total energy of the system, contributing to the spontaneity of the whole process.[41] Other factors, such as hydrogen bonding, change in the surface tension, van der Waals interactions and ring strain releasing will also influence the final complex formation. The inclusion phenomenon is usually a concentration-dependent process and the host/guest molar ratio is significant; it is possible that in solution the guest molecule interacts with other parts of the CD surface and thus generates agglomerates through self-association process.[42−44] In this study, an excess of β-CD (ca. 100:1, CD/carotenoid ratio) was used in order to maximize the inclusion availability. One day after the mixture, we note the cloudy of the final solution of carotene@β-CD complexes that indicates the inclusion complex formation. An excess of CD does not affect the complex characterization from the point of view of the Raman measurements since carotenoids are very good light scatterers and can be detected even in very small amounts.[27,29] This procedure is supported by the use of Raman spectroscopy for in situ identification of carotenoids in natural samples such as corals, fruit, vegetable,s and plants.[27,29] The β-CD and carotenoid interaction occurs mainly through the hydrophobic contact with the inner cavity of the host (carotenoids have a nonpolar character). In the carotenoids@β-CD compounds, the ease of the incorporation of the carotenoids may be attributed, in part, to a better adaptation into the cavity of the host (namely β-CD) in relation to other smaller or larger CD, which is the basis for the proposed size/shape concept.[7]

The experimental spectra of the four carotenes and their inclusion compounds have three main bands. They are as indicated and assigned to C═C stretching, ν(C═C), asymmetric angular deformation of C═C−H in plane, δ_{as}(C═C−H), and CH_3 rocking, $\rho(CH_3)$ (named ν_1, ν_2 and ρ_3, respectively).[45−47] For carotenoids in general, the ν_1 band (C═C stretching) ranges from 1510 for LYC (with eleven conjugated C═C bonds) to 1536 cm^{-1} for crocetin (containing seven conjugated C═C bonds) with the position of this band being chain-size dependent as well as sensitive to the matrix effects,[27,29] which makes possible to probe the inclusion process through vibrational spectroscopy. The observed Raman spectra obtained for the isolated carotenoid and their corresponding inclusion complexes are shown in Figure 3. For the LYC, the ν_1 mode shifts from 1509 to 1522 cm^{-1} upon inclusion, a shift ($\Delta\nu$) of ca. 13 cm^{-1} upward relative to the free guest molecule. For the other inclusion complexes studied here, the ν_1 mode was observed at 1517 cm^{-1} (BCT, $\Delta\nu$ = −8 cm^{-1}), 1515 cm^{-1} (AST, $\Delta\nu$ = 3 cm^{-1}) and 1526 cm^{-1} (NOR, $\Delta\nu$ = 9 cm^{-1}). The blue shift observed for ν_1 (the exception was BCT) suggests the localization of the single and double bonds upon inclusion, which may be due the conformational changes induced by the confinement process. In the regions of the transitions ν_2 and ρ_3, the changes in the band position are small (within 1−4 cm^{-1}). The wavenumbers and assignments (based on theoretical normal-mode analysis) are given in Table 1.

The SVWN/6-31G optimized geometries for the carotenes and their 1:1 inclusion complexes are depicted in Figure 4. The

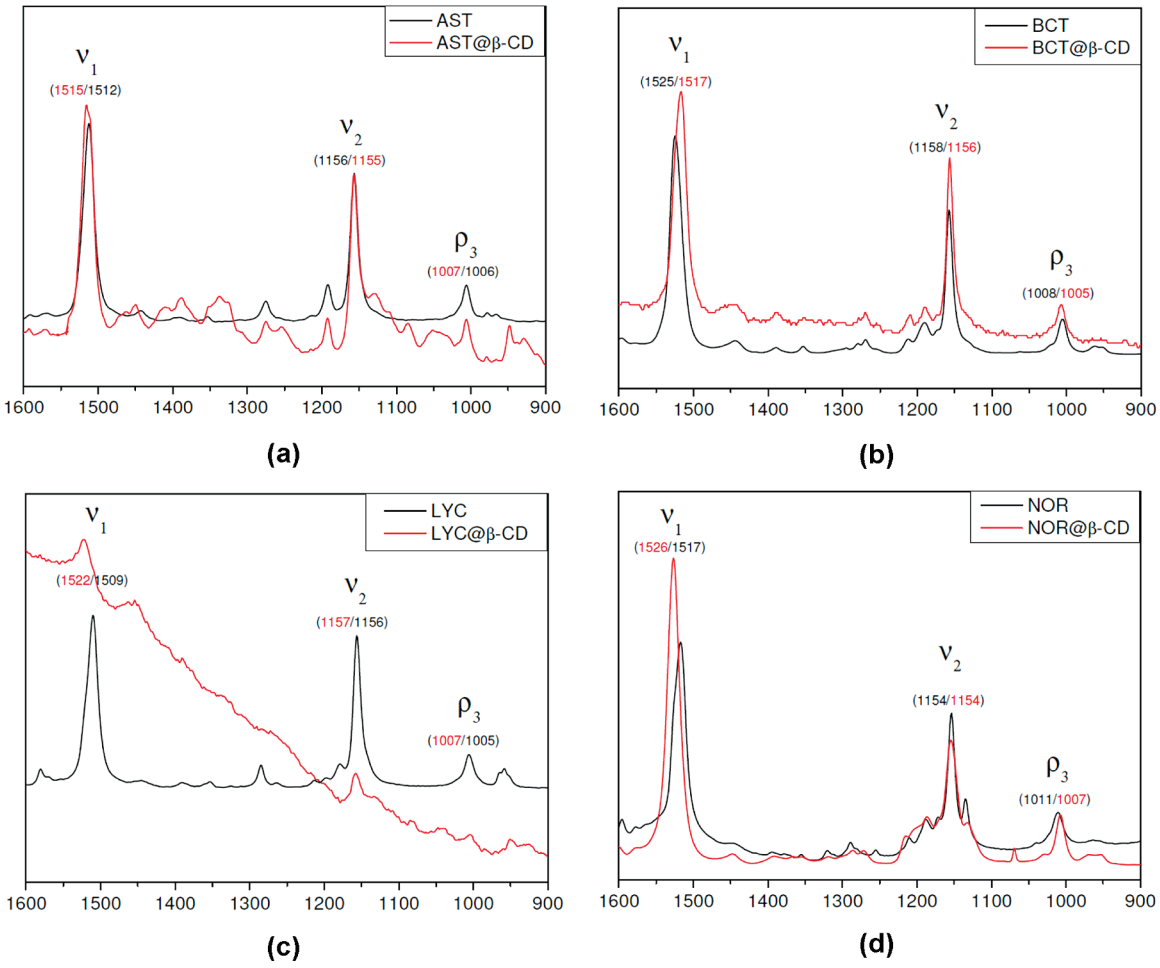


Figure 3. Experimental Raman spectra of carotenes and their corresponding inclusion complexes with β-CD. (a) AST, (b) BCT, (c) LYC, and (d) NOR.

initial guess used in the geometry optimization procedures came from the MD simulation, thus the geometries shown in Figure 4 represent the equilibrium arrangements found in the gas phase at 298 K. It can be noted that for long carotenes (BCT and AST) the CD migrates to the extremities through the secondary hydroxyl side. In the case of *trans*-LYC, which does not present the bulk terminal group, the CD migrates through the primary hydroxyl side. This findings suggests that more than one CD molecule would be likely to interact with such long carotenes. These calculations were not carried out once they are out of our current computational capability. The complexation energies are given in Table 2 as found at SVWN/6-31+G(d,p)//6-31G level of theory. The ZPE (calculated at SVWN/6-31G) and BSSE corrections were accounted for in the final values. The inclusion complex of AST was predicted to be quite stable compared to the other carotenes with the stability order predicted to be AST > *trans*-LYC > BCT > NOR. In order to correlate the complexation energies with structures, some topological parameters were computed. An interesting parameter to be analyzed is the overall distortion in the carotenes' backbone upon inclusion. In general, the carotenes assume a slightly more extended form in the inclusion complex. The variation of the distance between extremities ($\Delta d_e = d_e^{complex} - d_e^{carotene}$) for *trans*-LYC, AST, BCT and NOR were, respectively, 3.6, 2.3, 1.6, and −1.6 Å (in this case, a decrease of d_e is predicted), which are qualitatively correlated with the E_{comp}, that is, as larger the unfolding effect more stable is the inclusion complex. The only exception was the *trans*-LYC, which showed a large distortion upon inclusion (Δd_e = 3.6 Å). Looking at Figure 4, we note that the flexibility of the extremities of the molecule is mostly responsible to increase Δd_e. Therefore, the largest distortion predicted for *trans*-LYC is not solely due the unfolding of the backbone chain. According to ref 18, a trans→cis isomerization of LYC is supposed to occur in the presence of CD host. To check this hypothesis, we also analyzed the *cis*-LYC isomer (15-*cis*-Lycopene, in this case) and its complex with β-CD. The BSSE corrected complexation energy was −3.1 kcal·mol^{-1}, which is almost twice lower than the energy value for the *trans*-LYC form. This finding supports the proposal of Blanch and co-workers,[18] even though a molecular reason is not clear. For the *cis*-LYC the guest distortion was much larger (Δd_e = 13.9 Å) than for the *trans*-LYC (Δd_e = 3.6 Å, see Figure 4). Another useful parameter to investigate the distortion of the carotenes upon inclusion is the opening angle of the chain (θ_c). For all-trans structures, the optimal arrangement is for θ_c close to 180°, which favor the electronic delocalization and the host/guest contact. In our analysis the difference between the θ_c ($\Delta\theta_c$) of the carotene in the complex and the pure carotene was considered as a descriptor for relative stabilization of the inclusion complex. The $\Delta\theta_c$ values for AST, BCT, *trans*-LYC, and NOR were, respectively, 28.0, 16.0,

dx.doi.org/10.1021/jp2028142 |*J. Phys. Chem. A* 2011, 115, 8511–8519

Table 1. Experimental and Calculated Frequencies and Assignments for the Main Bands Observed in the Raman Spectra of the Carotenes AST, BCT, *trans*-LYC, NOR, and 15-*cis*-LYC and their corresponding inclusion complexes with β-CD

	wavenumber/cm^{-1}									
	ν_1 (0.9859)[a]			ν_2 (0.9602)[a]			ρ_3 (0.9906)[a]			
structure	expt	calc[b]	assignment[c]	expt	calc	assignment[c]	expt	calc	assignment[c]
astaxanthin (AST)	1512	1533/1501 (1512)	ν(C=C)$_{mc}$/ν(C=C)	1156	1177/1153	δ_{as}(C=C−H)$_{ip}$; ν(=C−C=)$_{inc}$	1006	1005	ρ(CH$_3$)$_{ip,mc}$
astaxanthin@β-CD	1515	1534/1509 (1519)	ν(C=C)$_{mc}$/ν(C=C)	1155	1172/1153	δ_{as}(C=C−H)$_{ip,ni}$; ν(=C−C=)$_{inc}$	1007	1001/964	ρ(CH$_3$)$_{ip,ni}$; γ(C=C−H$_{inc}$)$_{oop}$
betacarotene (BCT)	1525	1538/1520 (1525)	ν(C=C)$_{mc}$/ν(C=C)	1158	1174/1155	δ_{as}(C=C−H)$_{ip}$	1008	1007	ρ(CH$_3$)$_{ip,mc}$
betacarotene@β-CD	1517	1539/1518 (1527)	ν(C=C)$_{mc}$/ν(C=C)	1156	1180/1165	δ_{as}(C=C−H)$_{ip}$	1005	1005/972	ρ(CH$_3$)$_{ip}$; γ(C=C−H$_{inc}$)$_{oop}$
trans-lycopene (LYC)	1509	1532/1505 (1513)	ν(C=C)$_{mc}$/ν(C=C)	1156	1175/1154	δ_{as}(C=C−H)$_{ip}$; ν(=C−C=)$_{mc}$	1005	1006	ρ(CH$_3$)$_{ip,mc}$
trans-lycopene@β-CD	1522	1532/1510 (1515)	ν(C=C)$_{mc}$/ν(C=C)	1157	1173/1152	δ_{as}(C=C−H)$_{ip,ni}$; ν(=C−C=)$_{mc}$	1007	1007	ρ(CH$_3$)$_{ip,ni}$
norbixin (NOR)	1517	1591/1543 (1543)	ν(C=C)$_{lm}$/ν(C=C)$_{mc}$	1154	1191/1161	δ_{as}(C=C−H)$_{ip}$; δ(C−O−H)	1011	1016	ρ(CH$_3$)$_{ip,mc}$
norbixin@β-CD	1526	1583/1534 (1544)	ν(C=C)$_{lm}$/ν(C=C)$_{mc}$	1154	1164/1152	δ_{as}(C=C−H)$_{ip,ni}$; δ(C−O−H)	1007	1014	ρ(CH$_3$)$_{ip}$
cis-lycopene (cis-LYC)		1522/1508 (1511)	ν(C=C)$_{mc}$/ν(C=C)		1230/1168	δ_s(C=C−H)$_{cis}$; δ_{as}(C=C−H)$_{ip}$		1005	ρ(CH$_3$)$_{ip}$
cis-lycopene@β-CD		1525/1495 (1504)	ν(C=C)$_{mc}$/ν(C=C)		1244/1145	δ_s(C=C−H$_{inc}$)$_{cis}$; δ_{as}(C=C−H)$_{ip}$		1001/973	ρ(CH$_3$)$_{ip}$; γ(C=C−H$_{inc}$)$_{oop,cis}$

[a] The values in parentheses are the scaling factor applied for each spectral region. The use of three distinct scaling factors is justified in ref 49. [b] The values in parentheses correspond to the average frequency. [c] mc, middle of chain; lm, linked at methyl; ip, in plane; ni, not included; inc, included; cis, unsaturation of cis isomer.

Table 2. BSSE-Corrected Complexation Energy and Topological Parameters Calculated for the Carotenes@β-CD Inclusion Complexes[a]

		Topological parameters	
complexes	$E_{comp}{}^{cor}$/kcal mol^{-1}	Δd_c/Å	$\Delta\theta_c$/°
astaxanthin@β-CD	−11.1	2.3	28.0
betacarotene@β-CD	−1.11	1.6	16.0
trans-lycopene@β-CD	−1.65	3.6	1.3
norbixin@β-CD	0.27	1.6	−41.4
cis-lycopene@β-CD	−4.07	13.9	29.6

[a] The energies were obtained at the SVWN/6-31+G(d,p)//6-31G level of theory. The complexation energy was calculated as $E_{comp}{}^{cor} = E_{comp}$ + BSSE + ZPE.

1.3, and −41.4° (in this case a decrease of $\Delta\theta_c$ is predicted). These values show that the more positive the $\Delta\theta_c$ the more stable is the complex, which might be attributed to the more effective host−guest contact. For NOR, the guest molecule is more twisted inside the cavity ($\Delta\theta_c < 0$), which contributes to decrease the complex stability. Recently it has been published the synthesis and characterization of curcumin@β-CD inclusion complex, a molecule of similar size as NOR.[48] The authors proposed a 1:2 complex that could also be the case for NOR derivative. For LYC, we also analyzed the process with the cis isomer (15-*cis*-Lycopene), which was previously suggested by Blanch and coworkers[18] to exist in the medium in the presence of cyclodextrin. For this form, the complexation energy was −4.1 kcal mol^{-1} and $\Delta\theta_c$ = 29.6°, which is in agreement with the expected behavior, that is, larger $\Delta\theta_c$ means greater stability.

The calculated Raman spectra for the carotenes and their inclusion complexes are shown in Figure 5. The frequencies were scaled using three distinct factors as shown on the heads of Table 1. This unusual procedure has been proposed in a recent paper published by one of us,[49] where several scaling schemes were tested. The overall profiles resemble those observed (Figure 3) with three sets of intense bands around 1500−1550 cm^{-1} (ν_1), 1150−1200 cm^{-1} (ν_2), and ∼1000 cm^{-1} (ρ_3). As found experimentally and given in Table 1, the ν_1 band is more sensitive to the environment effects than the others. For the pure carotenes, the ν_1 band was predicted at 1513 (*trans*-LYC), 1525 (BCT), 1512 (AST), 1543 (NOR), and 1511 cm^{-1} (*cis*-LYC), which agree with the experiment within 4 cm^{-1} (except for NOR), considered excellent for the level of theory used here, namely SVWN/6-31G. For the inclusion complexes, the corresponding ν_1 band was centered at 1515 (*trans*-LYC@β-CD), 1527 (BCT@β-CD), 1519 (AST@β-CD), 1544 (NOR@β-CD), and 1504 cm^{-1} (*cys*-LYC@β-CD) that also agree well with the measured values (1522, 1517, 1515, and 1526 cm^{-1}, respectively) with the error lower than 10 cm^{-1} (except for NOR@β-CD).

The frequencies given previously were obtained as intensity weighted average of the two intense transitions in the ν_1 region, named ν_1' and ν_1'' as represented in Figure 6 and eq. 2

$$\nu = \frac{\sum_{i=1}^{n} I_i \cdot \nu_i}{\sum_{i=1}^{n} I_i} \qquad (2)$$

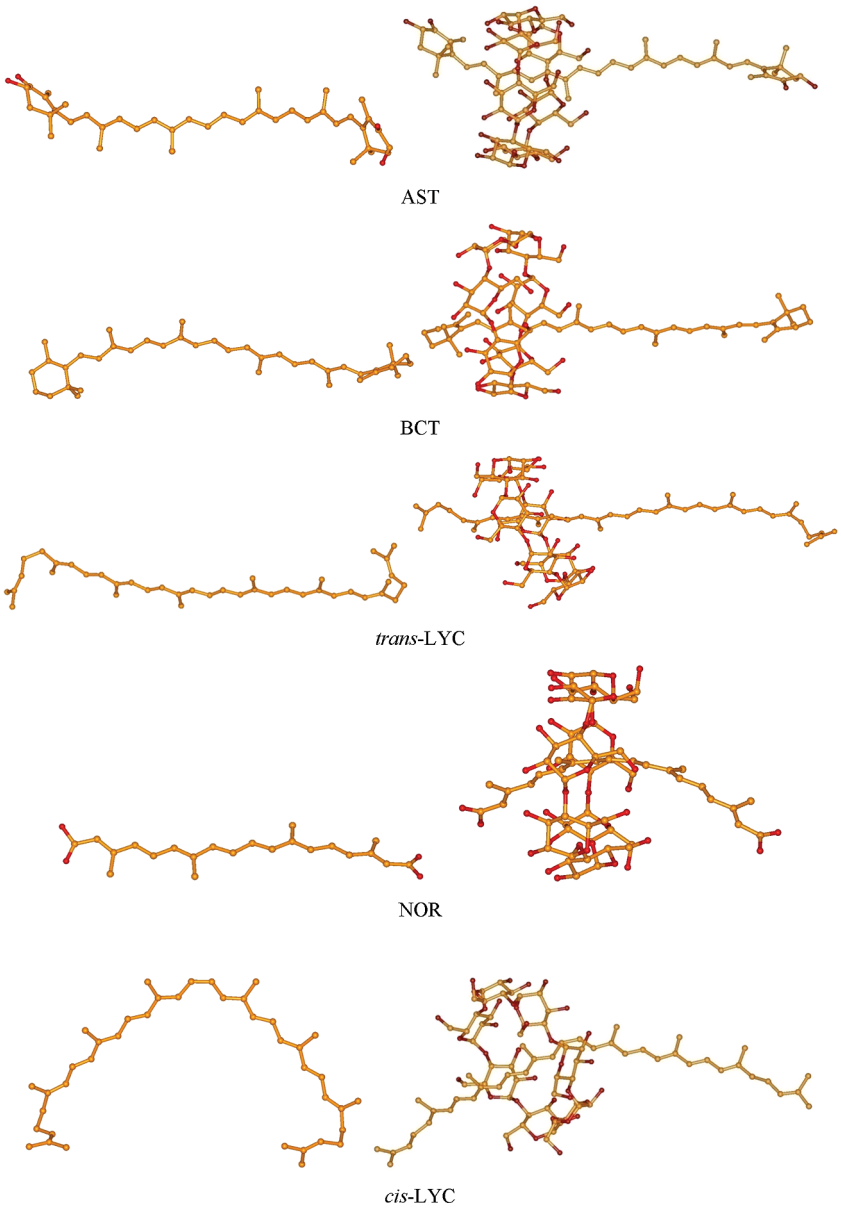


Figure 4. SVWN/6-31G optimized geometries for the carotenes studied in the present paper and their corresponding inclusion complexes.

where I_i is the relative Raman intensity corresponding to the ν_i frequency and n is the number of frequencies with relative intensity higher than 50% of I_{max} of each band (see Figure 6). These are both assigned to the −C═C− stretching of distinct parts of the molecule. The low (ν_1') and high (ν_1'') frequency bands are related, respectively, to the terminal −C═C− moiety of the carotenes' chain and to the −C═C− bonds in the middle of the chain. It is worth noting that the predicted average values for AST (1512 cm^{-1}) and BCT (1525 cm^{-1}) are in excellent agreement with experiment (1512 and 1525 cm^{-1}). This carotene has bulky groups at the ends and consequently lower backbone flexibility, therefore, the actual structure in solution, which is an average of several possible equilibrium geometries, might be satisfactorily represented by one structure chosen from MD simulation.

When the inclusion complexes are addressed, we observe in Figure 5 that in addition to the shift in the band position, the overall spectrum profile is also changed when compared to those of the free carotene. Taking the average ν_1 frequency for analysis (Table 1), the calculated shifts are +7 (AST), +2 (BCT), +2 (*trans*-LYC), and +1 cm^{-1} (NOR) and the corresponding experimental values are +3, −8, +13, and +9 cm^{-1}. The discrepancies found can be justified first based on the small shifts (3−13 cm^{-1}), which are within the limit of confidence for the level of theory used here. A distinct argument is that the experimental environment is much more complex than the single molecule approach used to calculate the Raman spectra, thus a fine agreement is not always expected. Nonetheless, the theoretical analysis using a single molecule might be used to get some insights on the molecular way the host−guest interaction takes place. Focusing on the ν_1 band we found that the interaction with CD cavity intensifies the local C═C stretching. Thus, if the CD is on the middle of the carotene chain, ν_1'' is intensified and the resulting ν_1 band

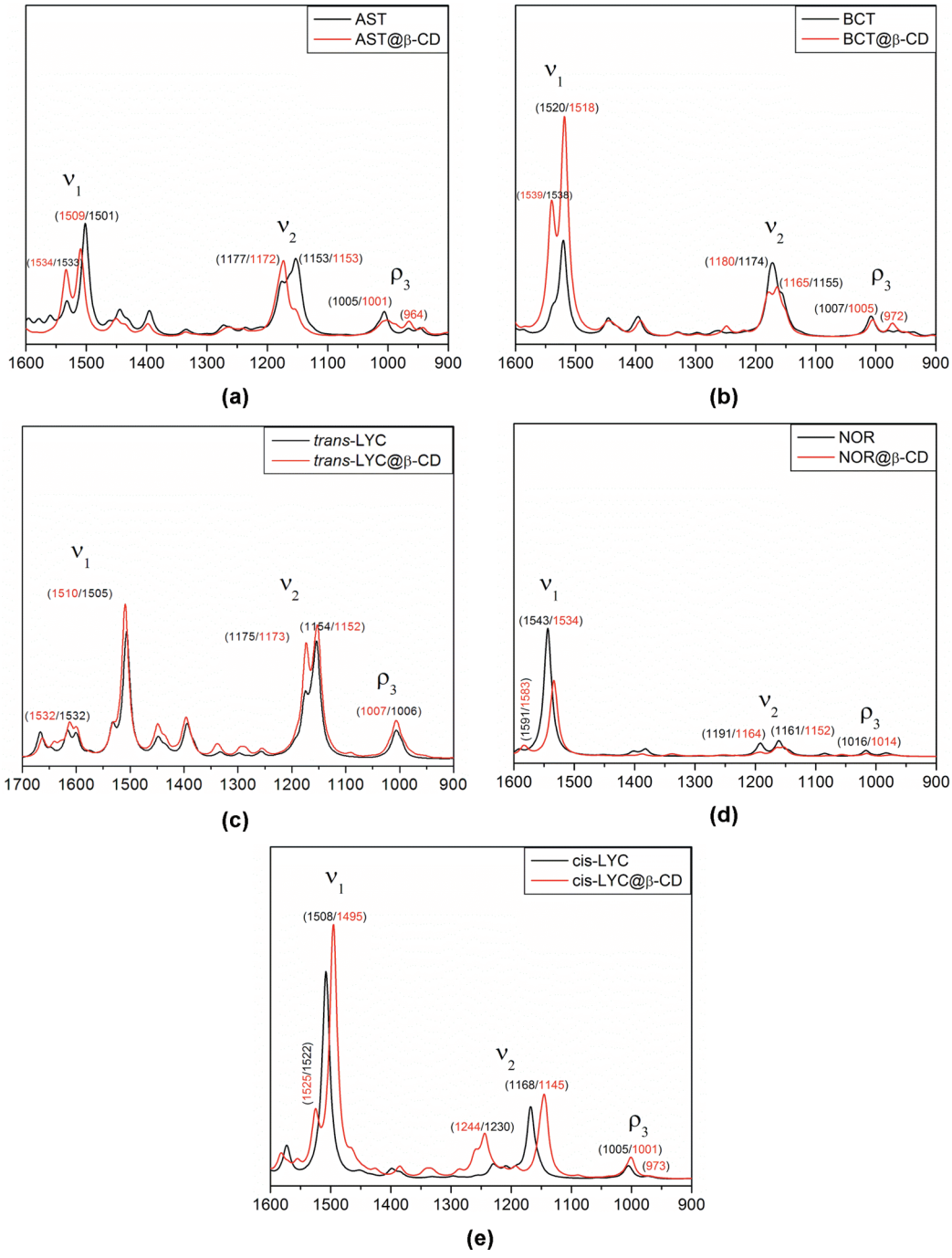


Figure 5. Calculated Raman spectra of carotenes and their corresponding inclusion complexes with β-CD. (a) AST, (b) BCT, (c) *trans*-LYC, (d) NOR, and (e) *cis*-LYC. The SVWN/6-31G level of theory was used.

is found at higher frequency (positive shift). Conversely, if the CD is found at the ends of the chain, ν_1' intensifies and a red shift is expected. As a result, considering only the low frequency band (ν_1', more intense), the predicted shifts are +8 (AST), −2 (BCT), +5 (*trans*-LYC), and −9 (NOR) cm^{-1}, which agree better to the experimental trends. This result is in agreement with the optimized structures (Figure 4), since CDs are located at the end of the carotenes chain, except for NOR@β-CD, which complex has a different behavior, mainly due to the small chain of this carotene and the lack of bulky terminal groups.

Lastly, the two other regions of the Raman spectra, namely ν_2 and ρ_3, are not much affected by the interaction with CD, as also found experimentally (see Table 1). For the *cis*-LYC derivative, we note a similar behavior to the assignment of the bands. In the ν_1 region, the −C═C− stretching band appears with a red shift for the *cis*-LYC@β-CD complex. The presence of a quite intense band in the ν_1 region is also verified (1525 cm^{-1}). Differently of the trans carotenes, the *cis*-LYC shows great modifications in the ν_2 region. An intense band close to 1240 cm^{-1} appears in the spectrum of the *cis*-LYC@β-CD complex attributed to symmetric angular deformation of −C═C−H of the cis unsaturation.

dx.doi.org/10.1021/jp2028142 |*J. Phys. Chem. A* 2011, 115, 8511–8519

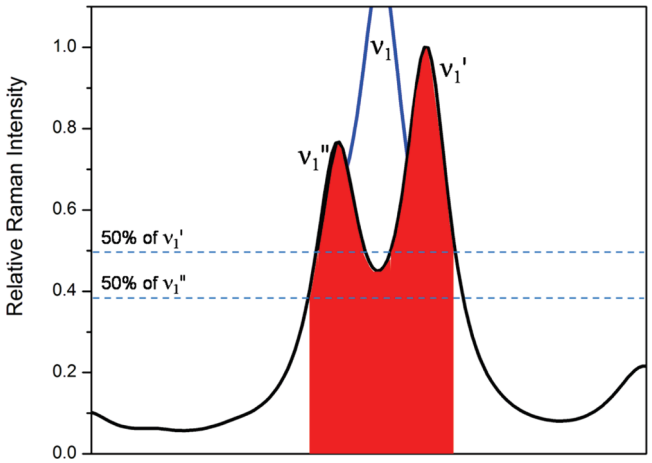


Figure 6. Scheme used for calculation of the average frequency in the ν_1 region. The value of ν_1 was predicted by averaging the intensity weighted frequencies in the shaded region.

In the same region, near 1150 cm^{-1} we can also note a shift corresponding to asymmetric angular deformation in plane for −C═C−H (trans).

4. CONCLUSIONS

In the present paper, the inclusion processes of four structurally related carotenes with β-CD were carefully analyzed by means of Raman spectroscopy with the aid of MD simulations and DFT spectroscopic analysis. The results clearly demonstrate the matrix effects on the band positions as a consequence of the inclusion complex formation. The most intense ν_1 band, assigned to −C═C− stretching, was found to be sensitive to the host−guest interaction. The band shift was more pronounced for LYC (13 cm^{-1}) and NOR (9 cm^{-1}) derivatives, which do not present bulky groups at the end of structures, allowing their deeper inclusion and closer contact inside the CD cavity. Interestingly, a negative shift was observed only for BCT (−8 cm^{-1}), which, according our molecular modeling, suggests a structure for the inclusion complex with the β-CD host found close to the ends of the guest molecule. The theoretical shifts obtained considering the average value of ν_1 band were +7, +2, +2, and +1 cm^{-1}, for AST, BCT, *trans*-LYC, and NOR, respectively, which are within the same range as observed experimentally, but do not follow it quantitatively due to the very small values to be achieved with SVWN/6-31G level of theory. Moreover, the changes in the Raman bands as consequence of the host−guest interaction can be promptly seen and pointed out to molecular features important to identify the overall matrix effect on the carotenes' structures. The complexes were found energetically favorable (except NOR) with the complexation energy ranging from −11.1 (for AST) to −1.1 kcal mol^{-1} (for BCT). For the NOR derivative a previous work suggests a 1:2 stoichiometric for the analogue curcumin guest,[48] which might be the reason for the positive complexation energy calculated here, once only 1:1 complex were attempted theoretically. Interesting, when the *cis*-LYC isomer was considered, the inclusion complex was found quite stable (−4.1 kcal·mol^{-1}), which reinforce the assumption of trans→cis isomerization induced by the inclusion process.

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ACKNOWLEDGMENT

The authors wish to thank CNPq, PRO-INFRA, and FAPEMIG financial support. This work was also partially supported by the project PRONEX-FAPEMIG/EDT-526/07. This work is a collaboration research project of members of the Rede Mineira de Química (RQ-MG) supported by FAPEMIG.

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