

# Raman excitation profiles of $\beta$ -carotene – novel insights into the nature of the $\nu_1$ -band

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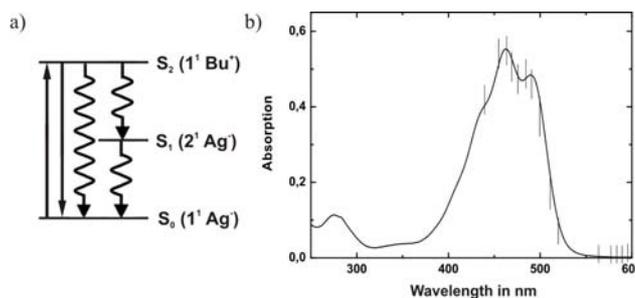
In the present work we have studied  $\beta$ -carotene dissolved in dichloromethane by means of resonance Raman spectroscopy. To obtain the Raman excitation profiles of  $\beta$ -carotene have been acquired at various wavelengths throughout the visible region.

It was found that the position of the prominent peak at ca.  $1524\text{ cm}^{-1}$  varies with the excitation wavelength indicating the involvement of two different modes. Possible origins of the different enhancement pattern of the two modes are discussed.

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**1 Introduction**  $\beta$ -Carotene is one of the most widespread natural molecules exhibiting a manifold of biological functions in a variety of organisms from bacteria and plants to animals. It plays a decisive role in photosynthesis due to its capability to absorb light in the region around 500 nm and to quench chlorophyll triplet states in photosystem I and II in order to prevent the formation of highly reactive singlet oxygen [1,2]. To understand the mechanisms of these processes it is essential to have a profound knowledge of the electronic properties of  $\beta$ -carotene, especially of the involved electronic states. It has been suggested that the electronic transition corresponding to the absorption band at around 480 nm (see Fig. 1 a) is the  $1^1\text{ Bu}^+ \leftarrow 1^1\text{ Ag}^-$  transition as the transition to the excited singlet state  $2^1\text{ Ag}^-$  is forbidden because of the pseudo  $C_{2h}$  symmetry of  $\beta$ -carotene [3,4]. To examine the excited electronic states particularly resonance Raman spectroscopy has been widely used.

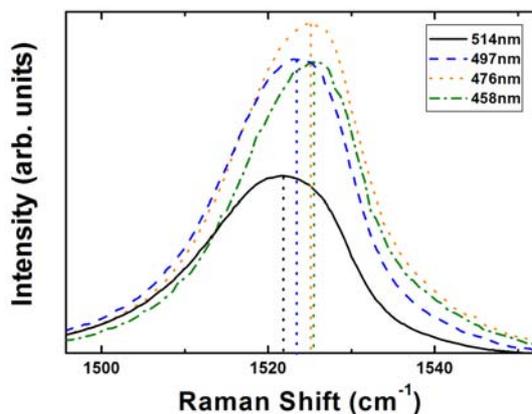
The Raman spectra of  $\beta$ -carotene shows three prominent vibrational bands named  $\nu_1$ ,  $\nu_2$  and  $\nu_3$  (at  $1524$ ,  $1157$  and  $1005\text{ cm}^{-1}$ , respectively), which are attributed to the double bond C=C stretching mode ( $1524\text{ cm}^{-1}$ ), the C-C in-plane single bond stretching mode ( $1157\text{ cm}^{-1}$ ), and the C-H bending mode ( $1008\text{ cm}^{-1}$ ), respectively [5–7].



**Figure 1** a) Schematic energy diagram of  $\beta$ -carotene. b) Absorption spectrum of  $\beta$ -carotene dissolved in dichloromethane; the excitation laser lines are indicated.

For all three bands resonance Raman excitation profiles of isolated  $\beta$ -carotene have been studied in the past [3, 8]. Interestingly these data showed a slight shift of the  $\nu_1$ -band depending on the excitation wavelength. The same observation was made in a resonance Raman study on photosystem II where the shift was attributed to a selective enhancement of two different carotene pools [9, 10].

In this work we report on the resonance Raman excitation profile of the  $\nu_1$ -band of  $\beta$ -carotene taking into



**Figure 2** Resonance Raman spectra of the  $\nu_1$ -region of  $\beta$ -carotene in DCM shown for four selected excitation wavelength. The data are normalised to a solvent Raman line

account quantum mechanical calculations of a  $\beta$ -carotene Raman spectrum [11]. These calculations show that the  $\nu_1$ -band consists of two Raman active modes. Different resonance behaviour of the two modes could lead to the observed apparent shift. The exact mechanism is still unknown, possible reasons will be discussed.

**2 Methods** All-trans  $\beta$ -carotene (reported purity >97%) was purchased from Fluka and used without further purification. The concentration of  $\beta$ -carotene dissolved in dichloromethane used for Raman measurements was either 9.2 M or 73 M depending on the excitation wavelength. Raman Spectra were recorded in backscattering geometry on a Dilor XY800 triple monochromator Raman spectrophotometer, using a  $N_2$ -cooled charge-coupled-device detector. Laser excitations in the range between 457.9 nm and 568.2 nm and at 647 nm (12 lines) were provided by Argon and Krypton lasers (Coherent). For wavelengths between 590 - 615 nm (7 lines) a dye laser (Radiant Dyes) running with rhodamin 6G and for 442 nm excitation a HeCd-Laser (Omnichrome) was used. The laser power at the sample was between 1 to 2 mW for all measurements. The measurements were performed at room temperature, all spectra were calibrated by neon and argon lines.

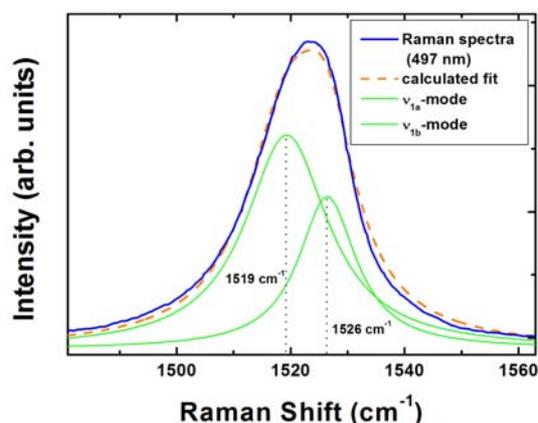
DFT calculations of the vibrational frequencies of  $\beta$ -carotene were done using the Gaussian03 program package [12] with the B3LYP functional and 6-31G\* basis sets. Raman intensities were calculated numerically by means of the finite electric field method [13].

**3 Results and discussion** Figure 2 shows the Raman spectrum of all-trans  $\beta$ -carotene excited at different wavelengths in the  $\nu_1$ -region. Intensities have been normalized using the Raman peak of dichloromethane at  $1423\text{ cm}^{-1}$  as an internal standard. From these intensities the

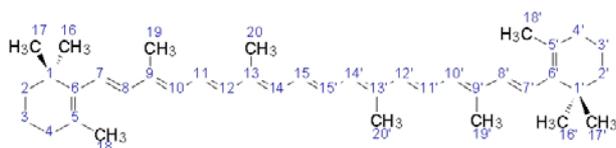
resonance excitation profiles can be derived as reported previously [5,6]. The band position seems to show a reproducible dependence on the excitation wavelength varying for example from  $1521\text{ cm}^{-1}$  at 413 nm excitation to  $1525\text{ cm}^{-1}$  at 476 nm excitation. A possible explanation for the observed shift could be the existence of different carotene isomers within the solution. Koyama et al. [14] showed that these isomers would cause a change in the shape of the  $\nu_2$ -band as well, which, however, was not observed in our experiments. This fact and the very good reproducibility allow ruling out any heterogeneity in the sample composition.

On the other hand, quantum mechanical calculations of the Raman spectrum of  $\beta$ -carotene predict two closely spaced modes at  $1528$  and  $1535\text{ cm}^{-1}$ , i.e. in the region of the  $\nu_1$ -band. The  $\nu_{1a}$ -mode at  $1535\text{ cm}^{-1}$  exhibits there twice the intensity of the  $\nu_{1b}$ -mode at  $1528\text{ cm}^{-1}$ . Considering this two-mode nature of the  $\nu_1$ -band we applied a global analysis to the experimental peak at around  $1524\text{ cm}^{-1}$  for all excitation wavelengths. Two Lorentzian functions were fitted to the experimental data. The position and the bandwidth of the two bands were iteratively adjusted to yield an optimum agreement with all experimental data on the basis of two Lorentzians with variable amplitudes but fixed frequencies and half widths. Figure 3 shows an example for excitation at 497 nm. Our analysis results in experimental peak positions at  $1519\text{ cm}^{-1}$  ( $\nu_{1b}$ ) and at  $1525\text{ cm}^{-1}$  ( $\nu_{1a}$ ) for the underlying modes, which is in good agreement with the calculated values. According to the DFT calculations both the  $\nu_{1a}$ - and the  $\nu_{1b}$ -mode originate from double bond C=C stretching of the polyene chain, although different C=C stretching coordinates are involved (Table 1).

Resonance excitation profiles were obtained for both bands (see Fig. 5) and some distinct differences between



**Figure 3** Experimental and fitted spectra shown for 497 nm excitation. The position of the underlying  $\nu_{1a}$ - and  $\nu_{1b}$ -mode are shown.



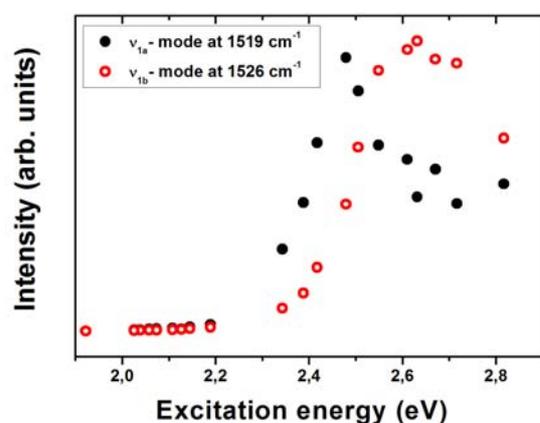
**Figure 4** Chemical structure of  $\beta$ -carotene.

$\nu$	I	PED [%]
1535 (1525)	100	C=C stretching: [20] 15=15', [12] 11=12, [12] 11'=12'
1528 (1519)	47	C=C stretching: [23] 13=14, [23] 13'=14'

**Table 1** Calculated frequencies (experimental results in parentheses) and intensities of the  $\nu_{1a}$ - and  $\nu_{1b}$ -band components. Potential energy distributions [PED] of the most intense modes are given in percentage.

the two profiles can be seen. The profile of the  $\nu_{1b}$ -band at  $1519\text{ cm}^{-1}$  shows a clear intensity gain from 2.3 to 2.5 eV compared to the  $\nu_{1a}$ -band excitation profile, which exhibits a maximum at 2.6 eV. Thus in the spectra from 2.3 to 2.5 eV nm the  $\nu_1$ -band seems to shift to lower wavenumbers due to the stronger contribution of the  $\nu_{1b}$ -band, whereas in the region around 488 nm excitation the apparent upshift is readily attributed to the stronger contribution of the  $\nu_{1a}$ -band.

According to Raman theory there are two main mechanisms for the resonance enhancement, the Franck-Condon and the Herzberg-Teller enhancement [15]. Franck-Condon enhancement involves shifting of the excited state potential curve with respect to the ground state potential curve due to displacements of the nuclei in the course of excitation. Herzberg-Teller enhancement arises from vibrational coupling of the resonant state with another excited state. As both vibrational modes are very similar in char-



**Figure 5** Resonance excitation profiles of the  $\nu_{1a}$ - and  $\nu_{1b}$ -band.

acter and symmetry it seems to be unlikely that they exhibit quite different enhancement mechanisms. Moreover, TD-DFT calculations reveal that the excited state displacements for both modes are essentially the same (data not shown). Since the displacements are quite large the main mechanism for resonance Raman enhancement of both the  $\nu_{1a}$ - and the  $\nu_{1b}$ -mode is attributed to Franck-Condon scattering.

Herzberg-Teller scattering, on the other hand, can be ruled out since vibronic coupling with the near-by  $S_1$  state is symmetry-forbidden for a one-photon excitation since the electronic states have same  $Ag^-$  symmetry and higher-lying excited states are too far away.

As an alternative explanation, we attribute the peculiar excitation profiles of the two modes to Duschinsky-rotation [16,17], implying mode mixing that arises from the off-diagonal elements of the quadratic vibronic coupling. The vibrational normal coordinates in the excited electronic state are rotated towards those in the electronic ground state. This effect could not be accounted for in the present quantum chemical calculations.

In summary, we reported resonance Raman spectra showing shifting of the  $\nu_1$ -peak of  $\beta$ -carotene due to two closely spaced modes that show different Raman excitation profiles. Both modes originate from C=C stretchings of the polyene chain. The difference Raman excitation profiles cannot be due to different enhancement mechanism (Franck-Condon vs. Herzberg-Teller) but may result from mode mixing in the excited state.

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