The Raman and infrared spectra of all-trans-astaxanthin (AXT) in dimethyl sulfoxide (DMSO) solvent were investigated experimentally and theoretically. Density functional calculations of the Raman spectra predict the splitting of the $\nu_1$ band into $\nu_{1,1}$ and $\nu_{1,2}$ components. The absence of splitting in Raman experimental spectra is ascribed to the competition between the two symmetric C=C stretching vibrations of the backbone chain. The $\nu_1$ band is very sensitive to the excitation wavelength: resonance excitation stimulates the higher-frequency $\nu_{1,2}$ mode, and off-resonance excitation corresponds to the lower-frequency $\nu_{1,1}$ mode. Analyses of the intramolecular hydrogen bonding between C=O and O\textsubscript{H} in the AXT/DMSO system reveal that the C4=O1\textsubscript{H1}O3 and C4\textsuperscript{'}=O2\textsubscript{H2}O4 bonds are strengthened and weakened, respectively, in the electronically excited state compared with those in the ground state. This result reveals significant variations of the AXT molecular structure in different electronic states.

Key words: C=C stretching vibration, Resonance excitation, Intramolecular hydrogen bonding, Molecular structure

I. INTRODUCTION

Carotenoids, which have a significant role in biological systems, have been intensively investigated because of their rich photochemistry [1, 2], photophysics [3, 4], and photobiology properties [5]. Accordingly, numerous theoretical [6–12] and experimental [3, 12–20] studies have investigated the excited electronic structures, molecular configurations, and environmental effects for different carotenoids. Astaxanthin (AXT), which exists in the all-trans configuration, is a member of the carotenoid family containing C=O, O–H, and terminal β-ionone ring groups [13]. AXT is a strong antioxidant in comparison with other carotenoids [13, 21, 22], and is thus increasingly used in biological and medical applications for its protective action [23–25].

Weesie et al. investigated the polarization mechanism of AXT by calculating the absorption spectra of a series of intermolecular hydrogen-bonded complexes (C=O···H–O or N–H···O) of the compound [26]. Eriksson’s group studied the bathochromic shift of the absorption by AXT in crustacyanin by quantum chemical methods [6], including configuration interaction with single excitations (CIS), time-dependent density functional theory (TDDFT), and Zerner’s intermediate neglect of differential overlap (ZINDO/S). Their results revealed that the bathochromic shift could be mostly attributed to the formation of a hydrogen bond between one of the C4-keto groups of AXT and a histidine residue of the surrounding protein. Although the study neglected to account for solvent effects, the predicted maximum absorption at 468 nm was consistent with experimental data (488 nm) for AXT in benzene [6]. Combining TDDFT and spectroscopy, van Wijk et al. showed that changes in the conformational twisting of the chromophore and the hydrogen bond interactions between AXT and the protein could only account for about one-third of the total bathochromic shift in α-crustacyanin [27]. Accordingly, it can be seen that the C=O and O–H groups have an essential role in the photochemical and photophysical properties of AXT [28, 29]. The intermolecular hydrogen bonding characteristics of AXT as both donor and acceptor, and in both the ground and excited states, have been extensively studied via the vibrational frequencies of the stretching modes of the C=O and O–H groups involved in hydrogen-bonded complexes [28, 30, 31]. However, the intramolecular hydrogen bonding interactions between C=O and O–H in AXT have not been studied. Intramolecular hydrogen bonding changes in the molecular structure of AXT, and the effect of these changes on the calculated infrared spectra in different electronic
FIG. 1 The optimized molecular structure (a) and important atom labels (b) for AXT.
B. Methods

The off-resonance Raman measurements were carried out using an i-Raman instrument (BWS415-7855, B & WteK, USA; excitation wavelength $\lambda_{ex}=785$ nm, laser power: $300 \text{ mW}$, interval time: $50 \text{ ms}$, integration time: $5000 \text{ ms}$). The resonance Raman spectra were obtained using an HR800 Raman spectrometer (Jobin Yvon, France; argon ion laser, excitation wavelength $\lambda_{ex}=458$ and $633$ nm, laser power: $10 \text{ mW}$, interval time: $10 \text{ s}$). The measurements were performed at room temperature in a dark room.

All of the quantum chemical calculations were performed using the Gaussian 09 suite of programs [35]. The optimized molecular structures (FIG. 1(a)) of AXT (containing intramolecular hydrogen-bonded complexes) in the ground and excited states were obtained using the DFT and TDDFT basis sets of B3LYP with the 6-31G(d,p) basis set. The Raman and infrared spectra of the ground and excited states for AXT were calculated at the same level of theory. A scaling factor of 0.972 was applied to the frequencies calculated by this method to obtain a good agreement with experimental data [10, 36]. The vibrational modes were assigned using the GaussView 4.1.2.0 package. The polarizable continuum model (PCM) implemented in the Gaussian 09 package was employed to model the influence of the DMSO solvent ($\varepsilon=46.826$), with the default parameters for the AXT/DMSO system [10].

III. RESULTS AND DISCUSSION

A. Off-resonance Raman spectra of AXT/DMSO

A comparison of the measured off-resonance and calculated Raman spectra of AXT and AXT/DMSO is shown in FIG. 2. The Raman spectra of the AXT-containing samples exhibit at least seven fundamental vibrational modes, located around 1513, 1445, 1276, 1216, 1194, 1157, and 1007 cm$^{-1}$. The three main bands are those at 1513 cm$^{-1}$ ($\nu_1$), 1157 cm$^{-1}$ ($\nu_2$), and 1007 cm$^{-1}$ ($\nu_3$), which are in agreement with data of other groups [16, 27]. The vibrational frequencies and assignments are listed in Table I for AXT and AXT/DMSO.

The $\nu_1$ band which arises from the symmetric C=C stretching of the polyene chain of AXT, is located roughly between 1470 and 1540 cm$^{-1}$ for AXT/DMSO. The $\nu_1$ mode corresponds to $\pi$-electron delocalization in the ground state of AXT, and its frequency has a linear dependence on the $S_0\rightarrow S_2$ electronic transition for carotenoids of different conjugation lengths [10]. It is noteworthy that the calculated $\nu_1$ bond of AXT/DMSO splits into $\nu_{1,1}$ (1503 cm$^{-1}$) and $\nu_{1,2}$ (1523 cm$^{-1}$) modes, which are assigned to the different symmetric one phase and two phase C=C stretching vibrations [11]. However, splitting of the $\nu_1$ Raman band is not distinguishable in the experimental spectra (as shown in FIG. 2), which may be attributed either to the competition between the two symmetric C=C stretching vibrations of the backbone chain, or to the altered Raman activity caused by the choice of excitation wavelength in the experiment. The areas under the peaks in the range from 1486 cm$^{-1}$ to 1545 cm$^{-1}$ in the Raman spectra are $15.32$ (A$^4$amu$^{-1}$cm$^{-1}$) in AXT (expt.), $15.45$ (A$^4$amu$^{-1}$cm$^{-1}$) in AXT/DMSO (expt.), and $17.44$ (A$^4$amu$^{-1}$cm$^{-1}$) in AXT/DMSO (theor.). Therefore, the same areas indicate that $\nu_{1,1}$ and $\nu_{1,2}$ modes are located in the same frequency range, and the theoretical Raman data are in good accordance with the experimental data for this region. In addition, the center of the vibrational mode at 1445 cm$^{-1}$ corresponds to the deformation vibration of CH$_3$ groups on the conjugated polyene chain.

The frequency range of the $\nu_2$ band is located from 1110 cm$^{-1}$ to 1300 cm$^{-1}$, and this range contains four clear peaks in the experimental and theoretical Raman spectra (as shown in FIG. 2). The peak at 1276 cm$^{-1}$ in AXT/DMSO (expt.) is assigned to the in-plane C=C bending mode coupled to C=C and C=C stretching vibrations. The very weak 1216 cm$^{-1}$ mode is assigned to the C=C stretching vibration coupled to the adjacent C=C in-plane bending mode and the C=C stretching vibration of the C1–C2–C3–C4–C5–C6 ion one ring. The 1194 cm$^{-1}$ mode corresponds to the C=C stretching vibration coupled to the adjacent C=C in-plane bending mode and the C=C stretching vibration of the two ionone rings. The most intense mode, at about 1157 cm$^{-1}$, is assigned to the C=C stretching of the conjugated polyene chain. In this region, the theoretical Raman data are in good accordance with the
The bands in the ν3 region, which is located in the range from 990 cm\(^{-1}\) to 1025 cm\(^{-1}\), can be ascribed to modes caused by the coupling of the in-plane rocking vibrations of the methyl groups attached to the conjugated chain and the adjacent C–H in-plane bending modes. The central frequencies of this region are located at 1007 cm\(^{-1}\) in AXT/DMSO (expt.), 1006 cm\(^{-1}\) in AXT (expt.), and 999 cm\(^{-1}\) in AXT/DMSO (theor.). Therefore, the calculated Raman spectra of AXT/DMSO in this region are in good agreement with the experimental data and provide accurate information on the molecular vibrations of AXT in the ground state.

### B. Dependence of Raman spectra on the excitation wavelength for pure AXT

To characterize the molecular structural changes in the ground state of pure AXT in more detail, the dependence of the Raman spectra on the excitation wavelengths was investigated by experimental and theoretical methods. FIG. 3 shows the measured Raman spectra at different excitation wavelengths, together with the calculated Raman spectrum for pure AXT in vacuum. As the excitation wavelength decreases (from off-resonance to resonance Raman), the full width at half maximum (FWHM) values of the three main Raman bands decrease (as shown in Table II). This shows that the excitation wavelength influences the shape of the Raman spectrum of the molecule. However, in the experimental spectra, the three main Raman bands display different trends of peak position with respect to the excitation wavelength. For example, at 458 nm, the ν1 mode is shifted to the higher frequency of 1522 cm\(^{-1}\), a blue-shift of about 10 cm\(^{-1}\) with respect to the same mode at 633 nm. In contrast, the ν2 and ν3 modes remain almost unmoved upon changing the excitation wavelength. This difference between ν1 and ν2/ν3 is also evident in the areas under the curves of the Raman spectra (as shown in Table II). The area under the ν1 mode varies greatly with the excitation wavelength, taking values of 16.96 (Å\(^2\)amu\(^{-1}\)cm\(^{-1}\)) (785 nm), 38.34 (Å\(^2\)amu\(^{-1}\)cm\(^{-1}\)) (633 nm), and 76.05 (Å\(^2\)amu\(^{-1}\)cm\(^{-1}\)) (458 nm). In contrast, the ν2 and ν3 band areas are unchanged with respect to the three excitation wavelengths, and have values of about 17.42–20.89 (Å\(^2\)amu\(^{-1}\)cm\(^{-1}\)) and 5.75–8.58 (Å\(^2\)amu\(^{-1}\)cm\(^{-1}\)) in each case. Thus, the Raman spectra in FIG. 3 were normalized to the intensities of the ν2 mode (the 1156 cm\(^{-1}\) mode) of each spectrum. These results show that the excitation wavelength has an essential effect on both the frequency and the shape of the Raman spectrum for the AXT molecule. Specifically, resonance excitation at the wavelength of 458 nm leads to a blue-shift of the C=C stretching mode of the polyene chain of AXT. In contrast, off-resonance exci-

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**FIG. 3** Experimental Raman spectra at different excitation wavelength for pure AXT in the ground state, and theoretical Raman spectrum (frequencies multiplied by a scaling factor of 0.972) for pure AXT in a vacuum.

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### Table I Vibrational modes (in cm\(^{-1}\)) of the AXT and AXT/DMSO samples, and their assignments based on theoretical calculations.

<table>
<thead>
<tr>
<th>AXT</th>
<th>AXT/DMSO</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt.</td>
<td>Expt.</td>
<td>1006 1007.999 In-plane rocking vibration of CH(_3) groups attached to the conjugation polyene chain + the adjacent C–H in-plane bending mode</td>
</tr>
<tr>
<td></td>
<td>theor.</td>
<td>1156 1157.1153 C–C stretching of the conjugation polyene chain</td>
</tr>
<tr>
<td></td>
<td>theor.</td>
<td>1192 1194.1202 C–C stretching vibration coupled to the adjacent C–H in-plane bending mode + C–C stretching vibration of two ionone rings</td>
</tr>
<tr>
<td></td>
<td>theor.</td>
<td>1215 1216.1225 C–C stretching vibration coupled to the adjacent C–H in-plane bending mode + C–C stretching vibration of C1–C2–C3–C4–C5=C6 ionone ring</td>
</tr>
<tr>
<td></td>
<td>theor.</td>
<td>1277 1276.1267 In-plane C–H bending mode coupled to C=C and C–C stretching vibration</td>
</tr>
<tr>
<td></td>
<td>theor.</td>
<td>1446 1445.1449 Deformation mode of CH(_3) groups on the conjugation polyene chain</td>
</tr>
<tr>
<td></td>
<td>theor.</td>
<td>1514 1513.1503 Symmetric one phase C=C stretching vibration</td>
</tr>
<tr>
<td></td>
<td>theor.</td>
<td>1523 1523.1503 Symmetric two phase C=C stretching vibration</td>
</tr>
</tbody>
</table>

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tation induces a lower frequency of the C=O stretching mode of the backbone chain (1512 or 1514 cm$^{-1}$). The $\nu_2$ and $\nu_3$ bands, which mainly correspond to the C–C stretching of the conjugated polyene chain and the in-plane rocking vibrations of the methyl groups attached to the conjugated chain, are not sensitive to the excitation wavelength.

Importantly, the calculations of pure AXT in a vacuum predict that the $\nu_1$ band is split into $\nu_{1,1}$ (1514 cm$^{-1}$) and $\nu_{1,2}$ (1528 cm$^{-1}$), and that the intensity of the $\nu_{1,1}$ mode is much stronger than that of $\nu_{1,2}$. However, only one of these modes is typically observed in the experiment of resonant Raman spectra, usually the higher-frequency $\nu_{1,2}$ (1528 cm$^{-1}$) mode. This implies that resonance excitation leads to the higher-frequency $\nu_{1,2}$ mode [11, 16, 27], and off-resonance excitation corresponds to the lower-frequency $\nu_{1,1}$ mode, as the two modes arise from different, competing interactions of the two symmetric C=C stretching vibrations in the backbone chain. This result is good consistent with the suggestion supported by Hildebrandt’s group, i.e., the shift of the $\nu_1$ band is attributed to the overlap of the two ($\nu_{1,1}$ and $\nu_{1,2}$) modes [33].

**C. Intramolecular hydrogen bonding of AXT/DMSO**

In order to reveal the excited-state hydrogen bonding dynamics, numerous experimental and theoretical studies have monitored the infrared spectral shifts in the formation of intermolecular hydrogen bonds [28, 30, 31, 37, 38], such as the red-shift or blue-shift of the stretching frequencies of C=O and O–H groups. The calculated bond lengths, bond angles, and dihedral angles for the hydrogen-bonded AXT/DMSO system are listed in Table III.

Upon photoexcitation, the AXT/DMSO system is directly excited to the $S_2$ state. The bond lengths of C4=O1 and C4′=O2 are increased from 1.239 and 1.238 Å, in the ground state to 1.244 and 1.242 Å in the excited state, respectively. This is attributed to the substantial increase in the charge density of the C=O group after the orbital transition from the highest occupied molecular orbital (HOMO) to the third-lowest unoccupied molecular orbital (LUMO+2) (as shown in FIG. 4). The O3–H1 bond is slightly lengthened from 0.976 Å to 0.977 Å. In contrast, the length of O4–H2 is unchanged, being 0.970 Å in both electronic states. The bond angles of C4=O1⋯H1 and O3⋯H1⋯O1 increase after the transition, but the bond angles of C4′=O2⋯H2 and O4⋯H2⋯O2 decrease. Moreover, the dihedral angle between the AXT backbone plane and the O1⋯H1–O3 plane is only slightly changed, from 178.28° in the ground state to 179.15° in the excited state, which indicates that the AXT backbone atoms and the C4=O1⋯H1–O3 hydrogen bond remain in the same plane. However, the dihedral angle between the AXT backbone plane and the O2⋯H2–O4 plane increases markedly, from 6.98° in the ground state to 9.42° in the excited state, which clearly reveals that the AXT backbone atoms and the C4′=O2⋯H2–O4 hydrogen bond are not in the same plane. More importantly, the hydrogen bond lengths of O1⋯H1 in C4=O1⋯H1–O3 and of O2⋯H2 in C4′=O2⋯H2–O4 in the ground state are 1.991 and 2.419 Å, respectively. For comparison, these hydrogen bond lengths in the excited state are 1.971 and 2.485 Å, respectively. It can be seen that the structure of the excited state is very different from that of the ground state.

These values therefore demonstrate that the intramolecular hydrogen bond C4=O1⋯H1–O3 is strengthened in the excited state in comparison with the ground state, while C4′=O2⋯H2–O4 is weakened in the excited state. Thus, the electronic transition from S0 to S2 causes the changes of the structure in the excited state, and is strongly facilitated by the concerted strengthening and weakening of these intramolecular hydrogen bonds in the electronic excited state of the AXT/DMSO system [28].

**D. Infrared spectra of ground and excited states for the AXT/DMSO**

The strengthening and weakening of intramolecular hydrogen bonds in AXT/DMSO can be revealed by infrared spectral shifts in different electronic excited states.
TABLE III Calculated bond lengths, bond angles, and dihedral angles of the hydrogen-bonded AXT/DMSO system.

<table>
<thead>
<tr>
<th>AXT/DMSO</th>
<th>Bond length/Å</th>
<th>Bond angle/°</th>
<th>Dihedral angle/°</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C4=O1</td>
<td>O3–H1</td>
<td>O1⋯H1</td>
</tr>
<tr>
<td>Ground state</td>
<td>1.239</td>
<td>0.976</td>
<td>1.991</td>
</tr>
<tr>
<td>Excited state</td>
<td>1.244</td>
<td>0.977</td>
<td>1.971</td>
</tr>
</tbody>
</table>

FIG. 5 Calculated infrared C=O stretching (a) and O–H stretching (b) modes involved in the hydrogen-bonded AXT/DMSO system in different electronic states (the infrared frequencies are multiplied by a scaling factor of 0.972).

states. FIG. 5(a) shows the infrared vibrational spectra of the hydrogen-bonded AXT/DMSO system in the ground and excited states in the range from 1560 cm$^{-1}$ to 1660 cm$^{-1}$, corresponding to the C=O group. Upon electronic excitation, the C=O stretching mode ($\nu_{C=O}$), which corresponds to both the strengthened intramolecular hydrogen bond between C4=O1 and O3–H1 and the weakened intramolecular hydrogen bond between C4′=O2 and O4–H2, is red-shifted by 31 cm$^{-1}$, from 1641 cm$^{-1}$ in the ground state to 1610 cm$^{-1}$ in the excited state. This red-shift is consistent with the increased charge density of the carbonyl groups, which are displayed in FIG. 4. Thus, the concerted strengthening and weakening of hydrogen bonds occurs in the excited state, and mutually facilitates internal conversion of the AXT/DMSO system [28].

The calculated O–H vibrational modes ($\nu_{O-H}$) involved in the AXT/DMSO system in different electronic states in the range from 3490 cm$^{-1}$ to 3690 cm$^{-1}$ are presented in FIG. 5(b). Upon photoexcitation, the $\nu_{O3–H1}$ stretching mode is red-shifted by 18 cm$^{-1}$, from 3544 cm$^{-1}$ in the ground state to 3526 cm$^{-1}$ in the excited state, because of the strengthening of the C4=O1⋯H1–O3 hydrogen bond in the excited state. In contrast, the $\nu_{O4–H2}$ stretching mode is noticeably blue-shifted by 6 cm$^{-1}$, from 3660 cm$^{-1}$ in the ground state to 3666 cm$^{-1}$ in the excited state, which can be ascribed to the weakening of the C4′=O2⋯H2–O4 hydrogen bond in the excited state. Therefore, the spectral shifts (in opposite directions) of the $\nu_{O-H}$ and $\nu_{O4–H2}$ modes, induced by the concerted strengthening and weakening of hydrogen bonds, reveal significant changes of the AXT molecular structure in the different electronic states.

IV. CONCLUSION

The off-resonance Raman spectra of the AXT and AXT/DMSO systems were measured experimentally, and exhibited three main bonds, denoted $\nu_1$, $\nu_2$, and $\nu_3$. The spectra were also calculated by DFT and TDDFT at the B3LYP/6-31G(d,p) level, and good agreement was found between the calculated and measured data. The splitting of the $\nu_1$ mode into $\nu_{1-1}$ and $\nu_{1-2}$ components was predicted by the calculations, but was not distinguishable in the experimental Raman spectra, because of the competition between the two symmetric C=C stretching vibrations of the backbone chain. Furthermore, in the Raman spectra for pure AXT, the $\nu_1$ mode is significantly dependent on the excitation wavelength. The $S_0 \rightarrow S_2$ electronic transition induced by resonance excitation leads to a blue-shift of the C=C stretching mode of the polyene chain of AXT, corresponding to the $\nu_{1-2}$ mode, while off-resonance excitation induces a lower frequency (1512 or 1514 cm$^{-1}$) of the C=C stretching mode, corresponding to the
\(v_{1-1}\) mode. However, the \(v_2\) and \(v_3\) bands, which correspond to the C–C stretching of the conjugated polycyane chain and the in-plane rocking vibrations of the methyl groups attached to the conjugated chain, are not sensitive to the excitation wavelength. More importantly, the intramolecular hydrogen bond between C=O and O–H in AXT/DMSO was investigated theoretically. It was found that the intramolecular hydrogen bonds of C4=O1⋯H1–O3 and C4′=O2⋯H2–O4 were strengthened and weakened, respectively, in the electronically excited state. Frequency analysis indicated that irrespective of whether the intramolecular hydrogen bond was strengthened (C4=O1⋯H1–O3) or weakened (C4′=O2⋯H2–O4) in the excited state, the corresponding C=O groups were significantly red-shifted relative to the ground state, which is consistent with the increased charge density of the carbonyl groups (as shown in FIG. 4). However, the calculated O–H vibrational modes showed that the strengthened hydrogen bond makes the \(v_{O3-H1}\) stretching mode red-shift, while the weakened hydrogen bond makes the \(v_{O4-H2}\) stretching mode blue-shift. As a result, this concerted strengthening and weakening of hydrogen bonds in the excited state reveals significant changes of the AXT molecular structure in the different electronic states, and facilitates internal conversion for the AXT/DMSO system.

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