Vibrational Properties Calculation of Various Modeled Bacteriochlorophyll-a Molecules

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ABSTRACT

Density functional theory has been used to calculate normal mode infrared stretching vibrational frequencies and intensities of neutral as well as cationic modeled bacteriochlorophyll molecules. For bacteriochlorophyll-a, the calculated IR absorption band position for neutral and cation states of Bchl-a$_1$ and Bchl-a$_2$ structures have well matched with the published experimental results. Bacteriochlorophyll models having 13^3-ester C=O and 13^1-keto C=O have both symmetric as well as antisymmetric vibration in neutral state. Symmetric mode of vibration has higher frequency and lower intensity whereas antisymmetric mode has slightly lower in frequency and higher in intensity. However, in cation formation, the 13^1-keto and 13^3-ester vibrations decoupled and 13^1-keto mode has higher frequency having intensity nearly double than that of 13^3-ester C=O mode. This observation of frequency upshift and IR intensity change of various carbonyl groups of Bacteriochlorophyll-a$_4$ model molecule upon cation formation is a strong reasoning to explain the experimental difference spectra of bacteriochlorophyll-a dimer in purple bacteria reaction centre (Leonhard & Mantele, 1993, Breton *et al.* 1999).

Keywords: Bacteriochlorophyll-a, Keto vibrations, Ester vibrations, IR Spectroscopy

INTRODUCTION

Bacteriochlorophyll-a molecules are mainly found in the reaction center of purple bacteria and green sulfur bacteria. They play important role in electron and proton transfer processes that occur in anaerobic photosynthesis (Scheer 1991). In aerobic photosynthesis, there exist a coordination between both Photo System II (PS II) and Photo System I (PS I) and the processes occur serially one after another but in anaerobic photosynthesis either PS II or PS I is present (Barber 1992).

In purple bacteria, bacteriochlorophyll-a molecules are bounded by groups of protein sub units. Thus, the fourier transform infrared (FTIR) absorption contains information not only from Bchl-a molecules but also from the rest of molecules present in the sample. Bacteriochlorophyll-a, pheophytin, Q$_A$ and Q$_B$ molecules undergo change in their electronic state upon cation formation but using experimental techniques for example, the FTIR difference spectroscopy, one can extract the vibrational information about Bchl-a molecule only (Masaki *et al.* 2015). FTIR difference spectra (DS) analysis gives rise to accurate information of the Bchl-a molecules (Lamichhane, 2011, Wang *et al.* 2007, Bandaranayake *et al.* 2006).

In the purple bacteria reaction center, the main pigment of the photosynthesis is the dimeric form of Bchl-a molecules. Being calculation of the vibrational properties of dimeric form is computationally expensive, calculations are first done in monomeric form and the results are compared with the experimentally observed data (O'Malley 2000). If the results match the trend of the dimeric form, then it can be generalized in dimeric form (Wang *et al.* 2007, Parameswaran *et al.* 2008).
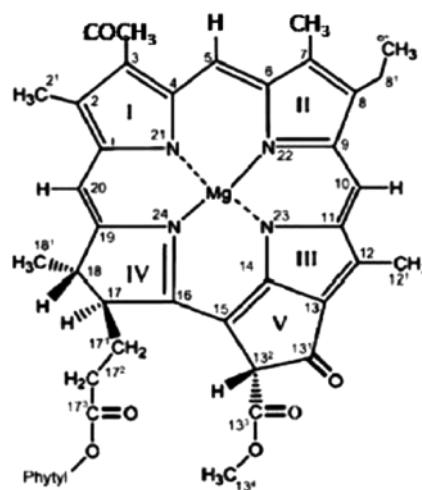

Fig. 1. Structure and IUPAC numbering scheme of bacteriochlorophyll-a

The molecular structure of Bchl-a is shown in Fig. 1 (Ke 2001). It contains Mg at the center of the molecules

surrounded by four nitrogen atoms and four carbonyl groups at the 3^1, 13^1, 13^3 and 17^3 positions. Magnesium ion at the center of the structure is hydrophilic in nature and make the atom ionic and side carbonyl are hydrophobic in nature and make the atom polar (Sumanta *et al.* 2014). Side groups and phytyl tail are removed for simplicity.

In purple bacteria difference spectra, neutral band and cation band of keto mode is found respectively at 1682 cm^{-1} and 1702 cm^{-1}. Similarly, neutral band and cation band of ester mode is found respectively at 1736 cm^{-1} and 1748 cm^{-1}. During cation formation, intensity of keto mode is downshifted and ester mode is upshifted (Leonhard & Mantele 1993). So, for the better understanding of these vibrational properties, we have carried out the computational method.

COMPUTATIONAL METHOD

Geometrical optimization and vibrational mode analysis of various modeled bacteriochlorophyll-a (Bchl-a) molecules were performed using hybrid density functional theory (DFT) method within Gaussian 03W (Frisch *et al.* 2004). The functional B3LYP along with basis set 6-31 G(d) were employed for all the calculations. The International Union of Pure and Applied Chemistry (IUPAC) numbering scheme is used throughout this manuscript. Bchl-a model molecules were constructed directly using the software Gauss View 4.1. The simplest of the Bchl-a molecules which has keto groups at 13^1C and 3^1C positions and all other substituents of the tetrapyrrole rings are replaced with hydrogen (H) atoms is called Bchl-a$_1$ (Fig. 2(A)).

To further investigate possible effects due to coupling of the C=O groups, we have also studied the second Bchl-a model, called Bchl-a$_2$ that contains the 13^1-keto C=O group as well as the 17^3-ester C=O group but lacks the 13^3-ester C=O group in addition to 3^1C keto group (Fig. 2(B)). The third Bchl-a model studied, Bchl-a$_3$ (Fig. 2(C)) contains the 13^1-keto as well as the 13^3-ester C=O groups but lacks the 17^3-ester C=O group. Bchl-a$_3$ allows the study of the coupling between the 13^1-keto and the 13^3-ester C=O groups without interference from the 17^3-ester C=O group. Finally, Bchl-a$_4$ (Fig. 2(D)) contains the 13^1-keto C=O as well as the 13^3 and 17^3-ester C=O groups.

We have first tabulated the complete set of calculated normal mode vibrational frequencies and intensities for Bchl-a$_1$. Then we have undertaken calculations using more realistic Bchl-a models that contain two or all three of the C= O groups. From the calculated harmonic vibrational mode frequencies and intensities, we have been able to construct IR spectra by convolving these stick spectra with a Gaussian function of 4 cm^{-1} bandwidth which are more realistic IR absorption spectra. Finally, we compute

cation minus neutral infra-red difference spectra (IR DS) for all the Bchl-a model molecules.

RESULTS AND DISCUSSION

Calculated vibrational properties of several model Bchl-a molecules are presented here. Among four modeled bacteriochlorophyll-a molecules the first model bacteriochlorophyl-a molecule, Bchl-a$_1$ contains only the 13^1-keto group attached directly to the tetrapyrole ring and hence its mode of vibration will be the free from the coupling with other ester groups presented in the bacteriochlorophyll-a molecule.

Calculation of vibrational properties of Bchl-a$_1$ and Bchl-a$_2$

Bchl-a$_1$ and Bchl-a$_2$ have 141 and 177 normal modes of vibration respectively. Most of these modes have very low infrared intensities and are undetectable in an FTIR absorbance spectrum. For this reason, we have discussed only the most intense vibrational modes lying between 1900-1500 cm^{-1}. Calculated vibrational mode frequencies and intensities for the most prominent modes of Bchl-a$_1$/ Bchl-a$_2$ and Bchl-a$_1^+$/Bchl-a$_2^+$ are presented in Table 1.

Fig. 3(A) shows calculated IR absorbance spectra for Bchl-a$_1$ and Bchl-a$_1^+$ as well as the corresponding cation minus neutral IR absorbance difference spectra (DS) in the 1900-1500 cm^{-1} region. The corresponding spectra for Bchl-a$_2$, Bchl-a$_2^+$ as well as the corresponding cation minus neutral IR absorbance DS in the 1900-1500 cm^{-1} region are also shown in Fig. 3(B). No negative frequency of bacteriochlorophyll-a is found while calculating frequency for both neutral and cation state indicating that the molecule studied is the true energy minimized structure.

Vibrational modes frequencies are assigned by visual inspection of vibration of the molecular groups that most prominently contribute to the vibration using Gauss View 4.1 are considered. We are primarily interested in vibrational frequencies changes that occur upon radical cation. Such frequencies differences are accurately calculated without scaling. Thus, frequencies presented here are also unscaled. The most prominent band in the calculated IR absorbance spectra of Bchl-a$_1$ and Bchl-a$_1^+$ is the 13^1-keto C=O mode. This mode occurs at 1803 cm^{-1} and up-shifts 30 cm^{-1} to 1833 cm^{-1} upon cation formation (Table 1, Fig. 3(A)). The 13^1-keto C=O mode decreases in intensity by 40 percent upon cation formation (750 to 449 km/mol, Table 1). This result is good in agreement with the published experimental difference spectra of bacteriochlorophyll in Rb. spheroids reaction center in which intense band is found at 1682/1704 cm^{-1} (Breton *et al.* 1999) and 1682/1702 cm^{-1} (Leonhard & Mantele 1993).
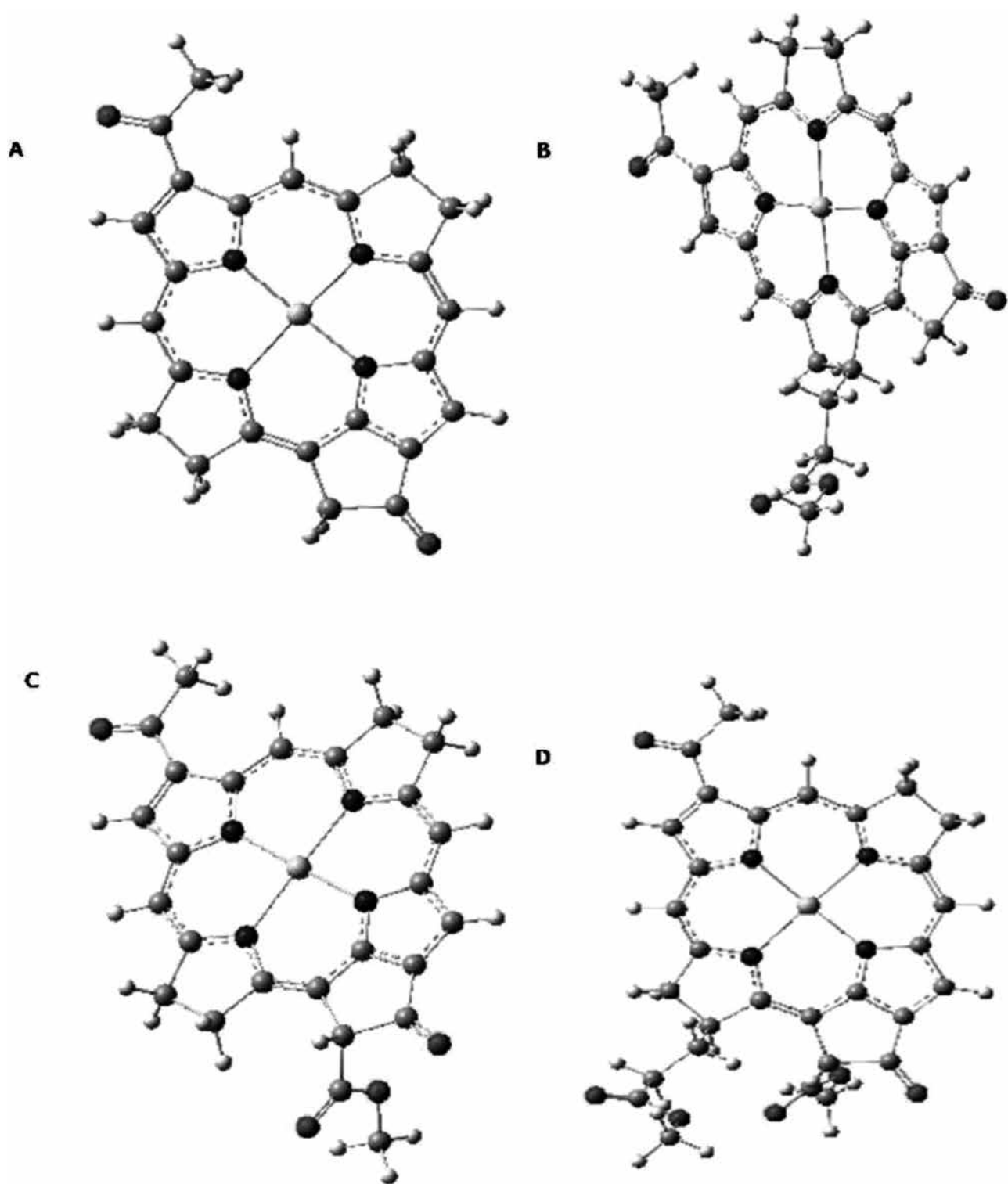
Fig. 2. Geometrically optimized (energy minimized) molecular structure of (A) BChl-a$_1$, (B) Bchl-a$_2$, (C) Bchl-a$_3$ and (D) Bchl-a$_4$. The structures were optimized using B3LYP/6- 31 G(d) method in gas phase

Table 1. Prominent harmonic vibrational mode frequencies in cm^{-1} and intensities in km/mol in parenthesis calculated for Bchl-a$_1$, Bchl-a$_1^+$, Bchl-a$_2$ and Bchl-a$_2^+$

Modes	Bchl-a$_1$		Bchl-a$_2$	
	Neutral	Cation	Neutral	Cation
17^3-ester C=O vibration	---	----	1830(232)	1841(290)
13^1-keto C=O vibration	1803(750)	1833(449)	1802(740)	1843(690)
3^1-keto C=O vibration	1751(312)	1781(171)	1752(303)	1788(279)
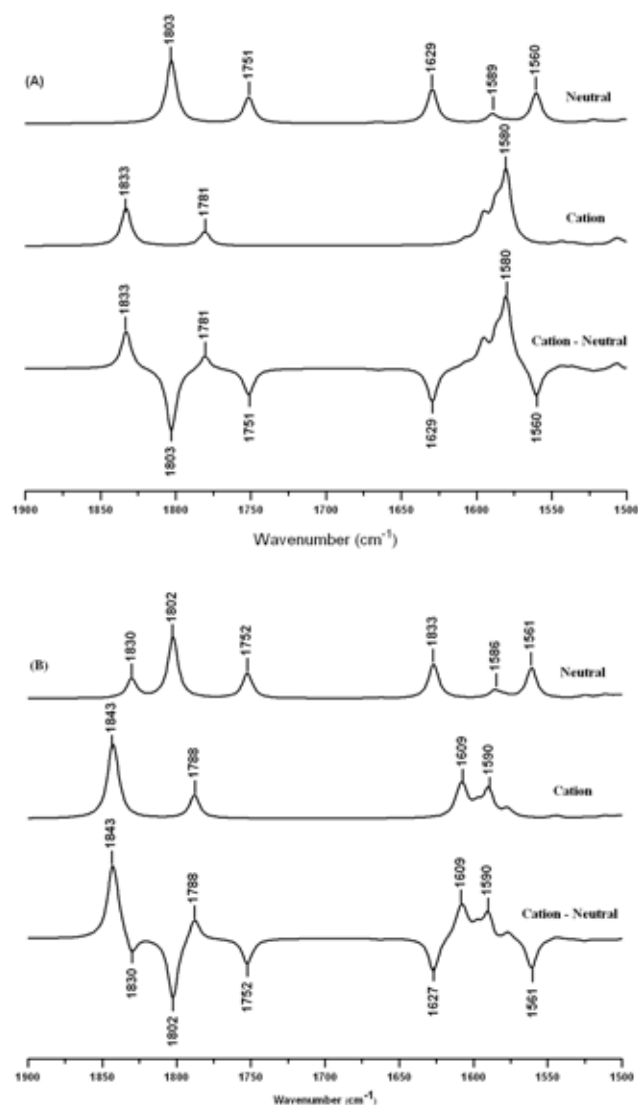
Fig. 3. Calculated IR absorption spectra of (A) Bchl-a$_1$ and (B) Bchl-a$_2$ in the gas phase.

The neutral (top), cation (middle) and Difference (bottom) IR spectra are shown

For neutral Bchl-a$_2$, the 13^1-keto C=O mode occurs at 1802 cm^{-1} and upshifts 41 cm^{-1} to 1843 cm^{-1} upon cation formation. The intensity of this mode decreases by 7 percent upon cation formation (740 to 690 km/mol, Table 1). The band frequency is same but intensity and cation induced frequency shift of the 13^1-keto mode are not the same for both Bchl-a$_1$ and Bchl-a$_2$. This result shows that presence of 3^1-keto group in Bchl-a in the place of 3^1-vinyl of Chl-a alters the frequency and intensity of 13^1-keto group (Wang *et al.* 2007).

For Bchl-a$_2$, the 17^3-ester C=O mode occurs at 1830 cm^{-1} and up-shifts 11 cm^{-1} upon cation formation. Interestingly, its intensity is increased by 25 percent (232 to 290 km/mol, Table 1). It may explain the intense band at 1736/1748 cm^{-1} of experimental difference spectra of bacteriochlorophyll in Rb. spheroids reaction center (Leonhard & Mantele 1993). Experimentally it is seen that ester mode vibrational frequency is upshifted by 12 cm^{-1} and intensity is also upshifted during cation formation (Breton *et al.* 1999, Leonhard & Mantele 1993). For neutral Bchl-a$_2$, the 17^3-ester C=O mode intensity is about 3 times less than that found for the 13^1-keto C=O mode (Table 1).

The calculated 3^1-keto C=O stretching mode of vibration of Bchl-a$_1$ occurs at 1751 cm^{-1} and upshifts 30 cm^{-1} to 1781 cm^{-1} upon Bchl-a$_1^+$ formation for the case in which the 3^1-keto C=O lies in the plane of the macrocycle. Intensity of this mode decreases by 8 percent upon cation formation (312 to 171 km/mol, Table 1). The band associated with the 3^1-keto C=O stretch is clearly visible in the spectrum of Bchl-a$_1^+$ as well but it is still about 2.6 times less intense than the band associated with the 13^1-keto C=O mode (Table 1).

The calculated 3^1-keto C=O stretching mode of Bchl-a$_2$ occurs at 1752 cm^{-1} and upshifts 36 cm^{-1} to 1788 cm^{-1} upon Bchl-a$_2^+$ formation for the case in which the 3^1-keto C=O lies in the plane of the macrocycle. This mode is also infrared active for the neutral state but decreases in intensity by an amount of 8 percent upon cation formation (303 to 279 km/mol, Table 1). The band associated with the 3^1-keto C=O stretch is clearly visible in the spectrum of Bchl-a$_{2+}$ but it is still about 2.5 times less intense than the band associated with the 13^1-keto C=O mode (Table 1).

Table 2. Summary of calculated cation induced frequency shifts of the 13^1-keto C=O, 17^3- ester C=O and 3^1-keto C=O modes of Bchl-a_1 and Bchl-a_2

Molecules	13^1-keto mode		17^3-ester mode		3^1-keto mode		$13^1/17^3$ intensity (percent)
	frequency cm^{-1}	intensity (percent)	frequency cm^{-1}	intensity (percent)	frequency cm^{-1}	intensity (percent)	
Bchl-a_1	+30	-40	---	----	+30	-45	319
Bchl-a_2	+41	-7	+11	+25	+36	-8	

Calculation of the vibrational properties of Bchl-a_3

For Bchl-a_3, 13^3-ester C=O group points out of the plane of the macrocycle and slightly away from the 13^1-keto C=O group. In the detail visualization of modes of vibration of 13^1-keto C=O and 13^3-ester C=O group, we have found some coupling between them. Since their orientations are not exactly parallel to each other, coupling between them is also not strong. The presence of 3^1-keto C=O group does not have much contribution to carbonyl vibration although 3^1-keto C=O group lies in the plane of the macrocycle for both the neutral and cation species.

Bchl-a_3 has 159 normal modes of vibration. Again, most of these modes are undetectable in an IR absorbance spectrum. Fig. 4 shows calculated absorbance spectra of (A) Bchl-a_3 and (B) Bchl-a_3^+ in the 1900-1500 cm^{-1} region. The cation minus neutral IR DS is also shown in Fig. 4(C). Table 3 has summary of several harmonic vibrational mode frequencies and intensities and their approximate mode assignments for Bchl-a_3 and Bchl-a_3^+.

For neutral Bchl-a_3, the mode compositions are considerably more complex than that of the previous two models. In this case, 13^1-keto and 13^3-ester C=O mode vibrations get mixed and the 13^1-keto C=O mode has major contribution to the anti-symmetric mode of vibration at 1805 cm^{-1} whereas the 13^3-ester mode has major contribution to the symmetric mode of vibration at 1814 cm^{-1} (Table 3). From above result, it is seen that coupling between 13^1-keto and 13^3-ester can not be decoupled by changing side group i.e. 3^1-keto in the place of 3^1-vinyl group (Wang *et al.* 2007).

Table 3. Prominent harmonic vibrational mode frequencies in cm^{-1} and intensities in km/mol in parenthesis for Bchl-a_3 and Bchl-a_3^+

Modes	Bchl-a_3	Bchl-a_3^+
13^1-keto C=O only	-----	1835(450)
(C=O)S	1814(473)	-----
(C=O)As	1805(570)	-----
13^3-ester C=O only	-----	1819(227)
3^1-keto C=O	1752(306)	1780(170)

The antisymmetric stretching of C=O mode of IR intensity is higher than symmetric mode at 1814 cm^{-1} mode (Table 3). Antisymmetric C=O mode of neutral Bchl-a_3 occurs at 1805 cm^{-1}. Upon Bchl-a_3^+ formation, the C=O modes are clearly separated (Table 3). However, the higher frequency vibration at 1835 cm^{-1} is due to the 13^1-keto C=O group while the lower mode at 1819 cm^{-1} is due to the 13^3 ester C=O group. 13^1-keto C=O mode frequency and intensity of Bchl-a_3^+ are nearly equal to frequency and intensity of Bchl-a_1^+ but less 8 cm^{-1} in frequency and 35 percent in intensity than that of Bchl-a_2^+.
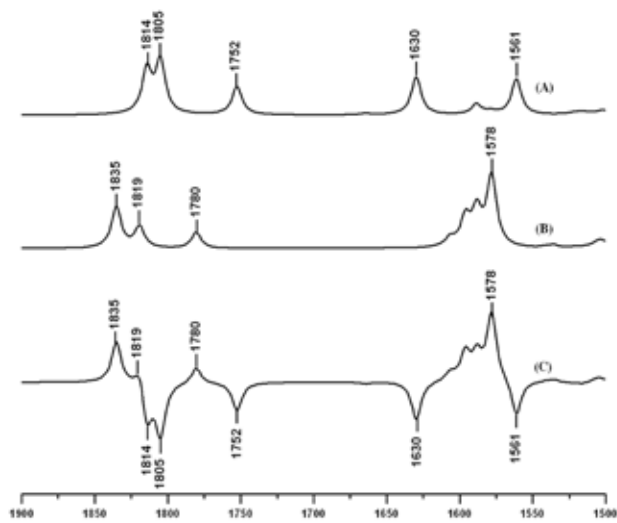

Fig. 4. Calculated IR absorption spectra for Bchl-a_3 in the gas phase. The neutral (A), cation (B) and cation minus neutral (C) IR spectra are shown

Calculation of the vibrational properties of Bchl-a_4

This model, called Bchl-a_4 (Fig. 2(D)) is most representative of Bchl-a. Fig. 5 shows calculated absorbance spectra for (A) Bchl-a_4 and (B) Bchl-a_4^+. The cation minus neutral IR DS is also shown in Fig. 5(C). For Bchl-a_4, the band at 1828 cm^{-1} is due only to the 17^3-ester C=O group. This mode upshifts 8 cm^{-1} upon cation formation and intensity changes a little. This behavior is virtually the same as that found for the same mode of Bchl-a_2. This upshift is nearly equal to the 12 cm^{-1} upshift in the published experimental difference

spectra of bacteriochlorophyll in Rb. spheroids reaction center of the purple bacteria (Leonhard & Mantele, 1993). The 17^3-ester group is therefore a little affected by the presence of the 13^3-ester and the 13^1-keto groups.

Table 4. Prominent harmonic vibrational mode frequencies in cm^{-1} and intensities in km/mol in parenthesis for Bchl-a$_4$ and Bchl-a$_4^+$

Modes	Bchl-a$_4$	Bchl-a$_4^+$
17^3-ester C=O	1828(236)	1836(207)
13^1-keto C=O only	-----	1834(461)
(C=O)S	1808(338)	-----
(C=O)As	1803(401))	-----
13^3-ester C=O only	-----	1814(254)
3^1-keto C=O	1753(298)	1781(166)

As was found for Bchl-a$_3$, the 13^1-keto and 13^3-ester groups are strongly coupled and unique vibrations of either C=O groups do not exist for the neutral state. For Bchl-a$_3$, the antisymmetric vibration of C=O groups is found to occur at lower frequency than the symmetric vibration. The intensity ratio of these two modes is also very similar to that found for Bchl-a$_3$. Being the symmetric and asymmetric vibrations are right angle to each others, they do not share much of their energy during coupling. Clearly, the 17^3-ester group plays no role in uncoupling the 13^1-keto and 13^3-ester C=O vibrations.
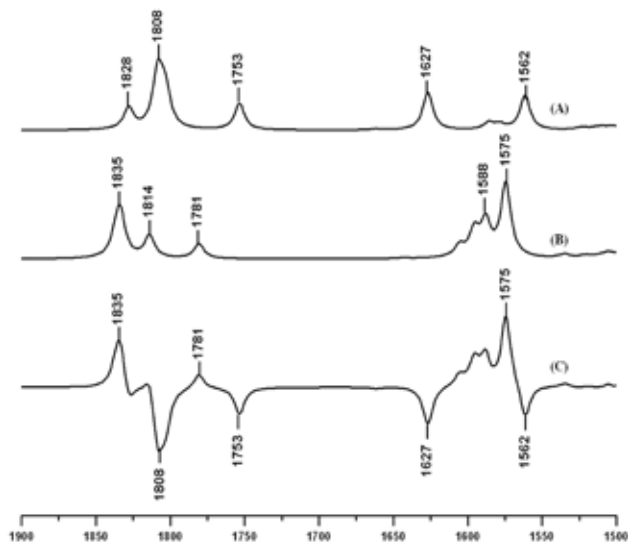

Fig. 5. Calculated IR absorption spectra for Bchl-a$_4$ in the gas phase. The neutral (A), cation (B) and cation minus neutral (C) IR spectra are shown

For Bchl-a$_4^+$, the 13^1-keto C=O vibration is separated from the 13^3-ester C=O vibration. Similar to Bchl-a$_3^+$, the 13^1-keto C=O group vibrates with higher frequency i.e.

1834 cm^{-1} compared to the 13^3-ester C=O group which vibrates with the lower frequency i.e. 1814 cm^{-1}.

CONCLUSIONS

We have studied the vibrational frequency band position and IR intensities of 13^1-keto group of bacteriochlorophyll in the presence or absence of 17^3 and 13^3-ester groups. Presence of 17^3-ester has the little effect on 13^1-keto while coupling is seen between 13^3-ester and 13^1-keto C=O group when 13^3-ester group lie near to the 13^1-keto group. It has also been seen that 13^1-keto group has higher intensity among other modes. Upon cation formation, both 13^1-keto mode and 13^3-ester mode upshifted. The upshift of 13^1-keto group is more prominent than that of 13^3-ester group. The calculated frequency upshift and IR intensity change upon cation formation of Bacteriochlorophyll-a$_4$ model molecule is affirmative to explain the experimental difference spectra of bacteriochlorophyll-a dimer in purple bacteria reaction centre (Leonhard & Mantele 1993, Breton *et al.* 1999).

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