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Rotational deviation of 3-acetyl group from cyclic tetrapyrrole π-plane in synthetic bacteriochlorophyll-*a* analogs by 20-substitution

Hitoshi Tamiaki *, Yuki Kotegawa, Keisuke Mizutani

Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan

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ABSTRACT

The 3-acetyl groups of synthetic methyl pyropheophorbides were rotated around the 3–3[1] bond and the rotational conformers were obtained in a dichloromethane solution of 20-bromo- and methyl-substituted compounds, based on their electronic and vibrational absorption spectra. Such a rotational deviation of the 3-acetyl group from the cyclic tetrapyrrole plane induced less π-conjugation to affect the redmost Q_y band, which has been observed in natural photosynthetic antenna systems, bacteriochlorophyll-*a* molecules in oligopeptides.

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Electronic absorption spectra of naturally occurring chlorophylls (Chls) and bacteriochlorophylls (BChls) are one of the key factors in photosynthetic light-harvesting (LH) and energy-migrating antennas as well as electron-transferring reaction centers.[1–3] Especially, the absorption bands at the longest wavelength, redmost (Q_y) bands, determine the singlet excited energy and the absorption maxima of (B)Chls in the natural apparatus are called 'site energy'. The Q_y maxima were partially affected by their surrounding environments and mainly determined by their molecular structures including peripheral substituents. In the same π-conjugated systems (porphyrin, chlorin, and bacteriochlorin), the 3-substituents primarily regulated the Q_y maxima of photosynthetically active (B)Chls (Fig. 1):[1] for example, Chls-*a*/*d* possessing the 3-vinyl/formyl groups gave their maxima at 662/689 nm in acetone.[4] In natural antenna systems, the same (B)Chl molecules gave different site energies, which provided an energetic gradient for efficient energy migration. The energy difference is created by environmental proteins: coordination with the central magnesium, hydrogen-bond with the carbonyl group at the 3- and 13-positions, and so on.[5]

Purple photosynthetic bacteria usually have LH2 as their main peripheral antenna.[5] LH2 had the most intense absorption band at 850 nm (B850), which was ascribable to the Q_y band of oligomeric BChls-*a* (see left of Fig. 1). Some purple bacteria produce LH3 instead of LH2.[5] The alternative LH3 gave a Q_y maximum at 820 nm (B820), although the two were almost the same supramolecular structures of proteins with BChls-*a*. The difference in

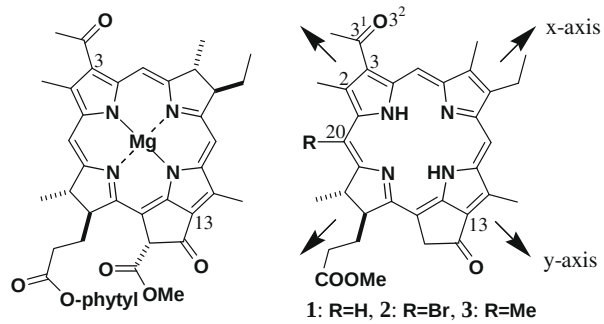

Figure 1. Molecular structures of natural bacteriochlorophyll-*a* (left) and the synthetic analogs **1**–**3** (right).

absorption bands was due to the conformation of the 3-acetyl group. X-ray crystallographic analyses clearly indicated all the acetyl groups in B850 were more planar to the cyclic tetrapyrrole π-systems in a molecule than those in B820: their deviations were about 20° and 50° for B850 and B820, respectively.[5,6] It is theoretically and experimentally known that such a larger and smaller π-conjugation of the 3-substituents shifted Q_y bands to a longer and shorter wavelength, respectively.[7–11] In FMO proteins as minor antenna systems of green sulfur bacteria, site energies of composite BChls-*a* were proposed to be similarly regulated by rotational conformation of the 3-acetyl groups.[11,12] Here, we report on a similar regulation of Q_y bands of methyl pyropheophorbides **1**–**3** (see right of Fig. 1) possessing the 3-acetyl group as synthetic BChl-*a* analogs

* Corresponding author. Fax: +81 77 561 2659.
E-mail address: tamiaki@se.ritsumei.ac.jp (H. Tamiaki).

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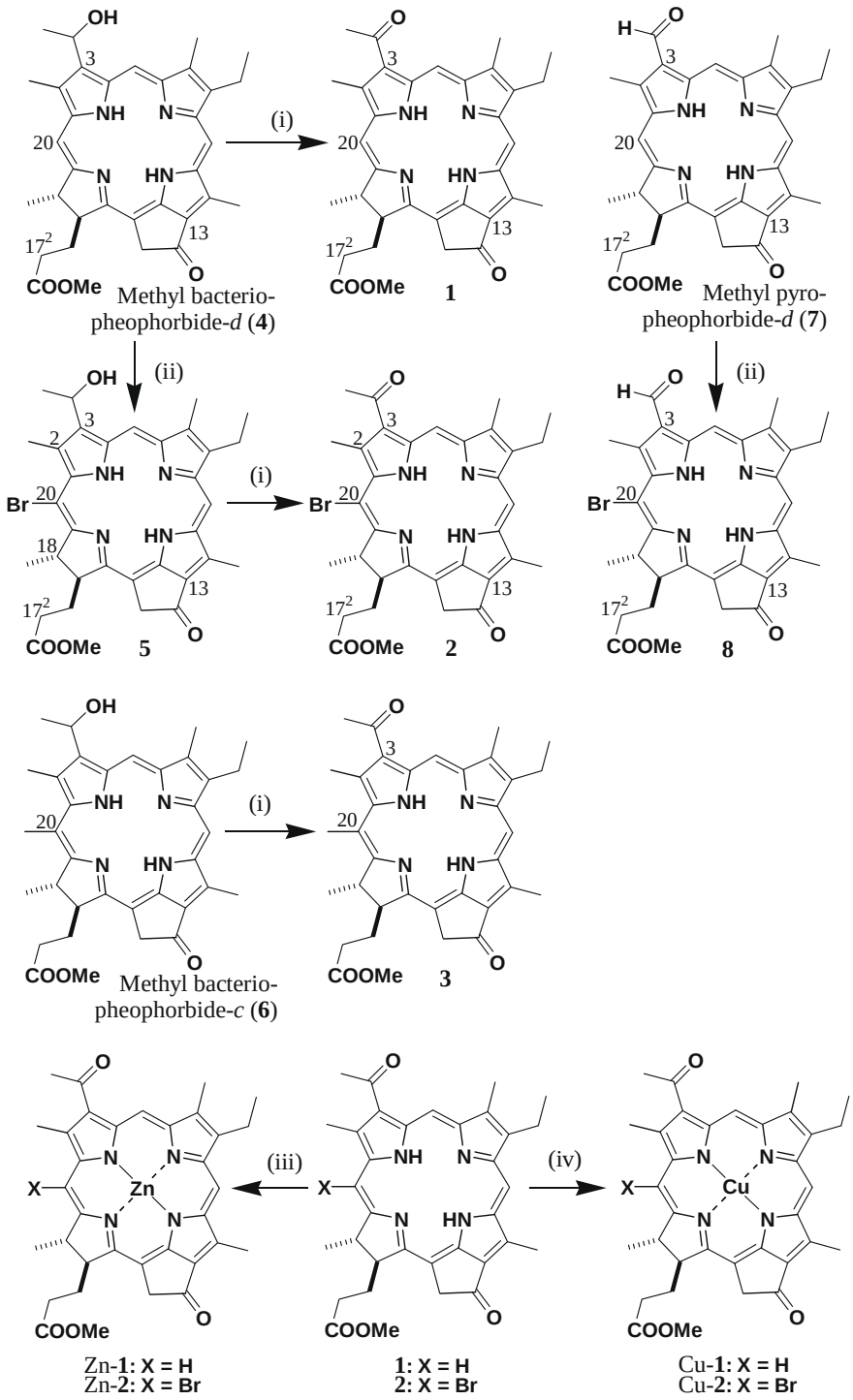
in dichloromethane. This is the first experimental evidence, to our best knowledge, of such rotational conformers of chlorophyllous pigments in a solution.

All compounds examined here were prepared by modifying Chl-*a* and BChl-*c* as shown in Scheme 1 and the experimental details are reported in Supplementary data.

Methyl bacteriopheophorbide-*d* (**4**) possessing 1-hydroxyethyl group at the 3-position was dissolved in dichloromethane to give electronic absorption bands at the full visible region (see the dotted line of Fig. 2b).[13] The most intense band at 410 nm was called Soret band and several bands at a region of longer wavelength than the Soret peak were termed *Q* bands. The four peaks at 505, 536, 604, and 660 nm were observed as the *Q* bands and assigned to $Q_x(0,1)$, $Q_x(0,0)$, $Q_y(0,1)$, and $Q_y(0,0)$, respectively (see Table 1). The subscription *x* and *y* indicate the directions of transition dipole moments in the bands (see Fig. 1). The redmost $Q_y(0,0)$ band was more intense than any other *Q* bands, which was characterized in chlorin chromophores.[14] Its sharpness clearly indicates compound **4** to be monomeric in a diluted dichloromethane solution: the full width at a half maximum = 360 cm^{-1}.



Scheme 1. Synthesis of methyl pyropheophorbides by modifying methyl bacteriopheophorbides-*c* and *d* as well as methyl pyropheophorbide-*d*: (i) Pr_4RuO_4–$O(CH_2CH_2)_2N(O)Me/CH_2Cl_2$; (ii) $C_5H_5NH^+Br_3^-/CH_2Cl_2$; (iii) $Zn(OAc)_2{\cdot}2H_2O/MeOH–CH_2Cl_2$; (iv) $Cu(OAc)_2{\cdot}H_2O/MeOH–CH_2Cl_2$.

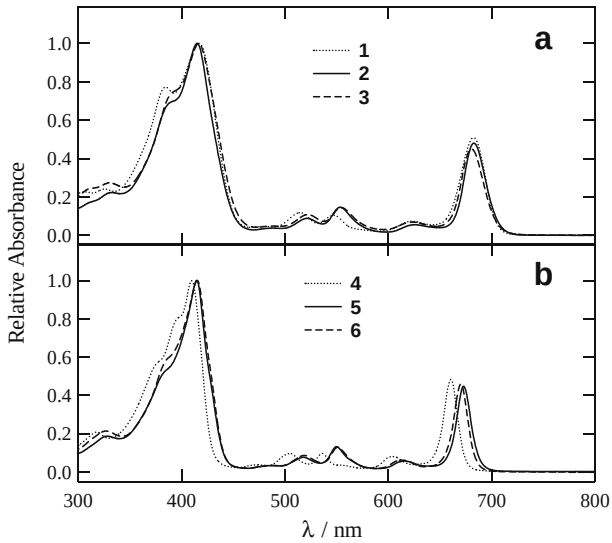


Figure 2. Visible spectra of methyl pyropheophorbides in CH_2Cl_2 (ca. 10 μM); (a) 3-acetyl-chlorins **1**–**3** and (b) 3-(1-hydroxyethyl)-chlorins **4**–**6**; 20-unsubstituted (dotted, **1** and **4**), brominated (solid, **2** and **5**) and methylated compounds (broken, **3** and **6**). All the spectra were normalized at the most intense Soret maxima.

Mono-bromination of **4** at the 20-position shifted all the absorption bands to longer wavelengths as shown by the dotted to solid lines of Figure 2b. The red-shifts are partially ascribable to electronic effect of the 20-bromo atom and largely explained by the following steric factor, which has been discussed previously.[15] Substitution of a bulky bromo substituent at the 20-position as in **5** distorts the chlorin π-plane through its steric repulsion with neighbor 2- and 18-methyl groups to give red-shifts in all the maxima. As expected, the 20-methylation of **4** gave similar red-shifts in the absorption bands of **6** (Fig. 2b) because a methyl group was comparable to a bromo substituent in size.

When 3-acetyl-chlorin **1**, the oxidation product of secondary alcohol **4**, was brominated at the 20-position as in **2**, surprisingly, no shift was observed in their $Q_y(0,0)$ bands at 683 nm (see Fig. 2a and Table 1). The Q_x bands were red-shifted by the 20-bromination and the values were smaller than those in **4** to **5**. Similar situations were observed in Soret bands: the main Soret (B_y) band (at >400 nm) was affected little and its side (B_x) band (at <400 nm) was red-shifted by bromination of **1** to **2**. These results indicate that any substituents on the *y*-axis were conformationally altered by the 20-bromination. Since the 13-carbonyl group was conformationally fixed on the exo-five membered ring, the alternative 3-acetyl group must be affected. The 3-acetyl group of **1** can be accommodated in the same plane as the chlorin π-system in a molecule and significantly π-conjugated to the chlorin chromophore to give Q_y (and B_y) bands at longer wavelengths than those of **4** possessing the 3-(1-hydroxyethyl) group (see dotted lines of Fig. 2a and b and Table 1). In 3-acetyl-20-bromo-chlorin **2**, the 20-bromo atom remotely affected the 3-acetyl group through the 2-methyl group and the coplanar conformer as seen in **1** could not be taken, thus less conjugation of the acetyl group with the chlorin π-system would afford no shift of the redmost Q_y peak positions.

Similar observation as in **1** → **2** was seen in copper and zinc complexes: 430/668 to 429/672 nm in Cu-**1** to Cu-**2** and 429/669 to 428/669 nm in Zn-**1** to Zn-**2** (see Fig. S1a and b). Moreover, 20-methylation of **1** to **3** shifted the peak positions of Q_y (and B_y) bands slightly and 20-methyl-chlorin **3** gave a similar absorption spectrum as the corresponding 20-bromo-chlorin **2** (see Fig. 2a). In contrast, significant red-shifts were observed in all the absorption bands by 20-bromination of 3-formyl-chlorin **7** to **8** (see Table 1 and Fig. S1c). Since a formyl group was less sterically hindered than an acetyl group, the 3-formyl group can be largely coplanar and conjugatable to the chlorin π-system even after the 20-bromination. Therefore, the bulky 3-acetyl groups of **2** and **3** were rotated around the 3–3[1] bond and conjugated less with the chlorin π-system to form rotational conformers.

To confirm the above conformers, vibrational spectra were measured in dichloromethane.[13] Two vibrational bands of **4** were observed at 1689 and 1734 cm^{-1} (see Table 1 and Fig. S2b) which were assigned to the 13-keto and 17^2-ester carbonyl stretching bands, respectively.[16] 20-Bromination of **4** to **5** shifted the former band to a 4 cm^{-1} larger wavenumber and induced no shift of the latter band. The difference in the shifts is explained by the 13-C=O being conjugated to the chlorin π-system while the 17^2-C=O was not. 3-Acetyl-chlorin **1** gave three carbonyl vibrational bands at 1669, 1695, and 1734 cm^{-1} (see Table 1 and Fig. S2a).[17] Comparing the data of **1** with that of **4**, the new band at 1669 cm^{-1} was assigned to the 3-acetyl carbonyl stretching band. The 6-cm^{-1} larger wavenumber shift of 13-C=O band by oxidation of **4** to **1** is due to the substituent effect of an electron-withdrawing acetyl group at the 3-position, similar to the above shift in **4** → **5**. 20-Bromination of **1** to **2** induced no shift of 17^2-C=O band at 1734 cm^{-1} and only a slight shift of 13-C=O band (+4 cm^{-1}; 1695 → 1699 cm^{-1}) as expected. The 3-C=O vibrational band of **2** moved dramatically to 1682 cm^{-1} by 20-bromination. Considering that the 3-C=O vibrational band of 20-bromo-3-formyl-chlorin **8** was slightly shifted from that of the corresponding 20-unsubstituted **7** (+2 cm^{-1}; 1674 → 1676 cm^{-1}, see Table 1 and Fig. S2c), the significant shift in the 3-keto carbonyl of **1** → **2** (+13 cm^{-1}; 1669 → 1682 cm^{-1}) could be explained not by a simple 20-bromination but by a rotation of the acetyl group (vide supra). The 3-acetyl group in **1** fully conjugated with the chlorin π-system to give a 1669-cm^{-1} band similar to the other 3-acetyl-chlorophyll derivatives reported previously.[5] The expected rotational conformer of **2** afforded less conjugation of the 3-acetyl group with the chlorin π-system to give the largely up-shifted band of the 3-C=O considerably isolated from the chlorin chromophore at 1682 cm^{-1}.

Table 1
Q absorption maxima λ_{max} (nm) and stretching vibrational peaks ν (cm^{-1})[a] of methyl pyropheophorbides in CH_2Cl_2

Compound	λ_{max}				ν(C=O)		
	$Q_x(0,1)$	$Q_x(0,0)$	$Q_y(0,1)$	$Q_y(0,0)$	3-	13-	17^2-
1 (3-COMe, 20-H)	515	547	622	683	1669[b]	1695[b]	1734
2 (3-COMe, 20-Br)	521	553	625	683	1682[b]	1699[b]	1734
4 (3-CH(OH)Me, 20-H)	505	536	604	660	–	1689	1734
5 (3-CH(OH)Me, 20-Br)	518	550	616	673	–	1693	1734
7 (3-CHO, 20-H)	522	554	634	695	1674	1697	1734
8 (3-CHO, 20-Br)	534	568	645	704	1676	1701	1734

[a] Errors were within 1 cm^{-1}.
[b] Estimated values from curve-fitting analysis.

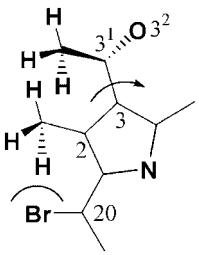


Figure 3. Partial stereodrawing of energy-minimized molecule **2** by MM+/PM3 calculation.

Resonance Raman (RR) spectra of **1** and **2** also supported the above explanation. Raman shift of the 3-C=O in **1** was observed at 1666 cm^{-1}, but the RR band in **2** had disappeared (see Fig. S3). Upon irradiation at 405 nm, the chlorin π-system was selectively excited and fully conjugated 3-C=O in **1** gave its RR band at 1666 cm^{-1}. In contrast, less conjugated acetyl group at the 3-position of **2** could hardly be resonated under the conditions and gave no distinguished RR band of the 3-C=O.[18]

Finally, to solidify the rotational conformers in **2** and **3**, molecular modeling was performed using MM+/PM3.[15] Energy-minimized structures of **1** as well as **7**/**8** showed that all 3-carbonyl groups were almost coplanar to the chlorin π-system: the dihedral angles of C2–C3–C3^1–O3^2 were estimated to be +0.1°, +0.1°, and −1.7° for **1**, **7**, and **8**, respectively. In the energetically minimized structure of **2**, the acetyl group at the 3-position was rotated around the C3–C3^1 axis (Fig. 3) and −9.2° was calculated as the dihedral angle.[19] The estimated model of **2** indicated that the 2-methyl group was conformationally restricted by the neighboring and bulky 20-bromo substituent, then one of the three hydrogen atoms on the 2^1-carbon was directed toward the 3^1-methyl group as shown in Figure 3. The hydrogen atom disturbed the coplanarity of 3-acetyl group with the chlorin π-system. 20-Methylated **3** also gave a similar conformer with **2** and the dihedral angle in **3** was calculated to be −7.9°. The absolute values (ca. 10°) of calculated dihedral angles for **2** and **3** seem to be smaller than those proposed from visible and vibrational absorption data (ca. 20–30°).[5,11] It is noteworthy that the underestimated values were still larger than those of **1**, **7**, and **8** and the 20-bromination and methylation in **2** and **3** remotely rotated the 3-acetyl group.

In summary, the 3-acetyl group in synthetic methyl pyropheophorbides was rotated around the 3–3^1 bond and deviated from its coplanar conformer by 20-bromination and methylation. Such rotational conformers possessing a less π-conjugated acetyl group were experimentally observed in a solution, while they are well-known for BChls-*a* possessing the 3-COMe rotationally fixed in some oligopeptides of photosynthetic bacteria including LH2 and FMO proteins.[5,6,11] Further investigation by synthetic models will be useful for determination of site energy of chlorophylls in photosynthetic light-harvesting and electron-transferring apparatus and for elucidation of their mechanisms at a molecular level.

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Supplementary data

Supplementary data (experimental details, spectral data for all new compounds Cu-**1**, **2**, Cu/Zn-**2**, **3**, and **8**, visible spectra of Cu-**1**/**2**, Zn-**1**/**2** and **7**/**8**, FT-IR spectra of **1**/**2**, **4**/**5**, and **7**/**8**, and RR spectra of **1**/**2**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.10.031.

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13. Visible, FT-IR, and RR spectra were measured with Hitachi U-3500 (a 1-cm quartz cell), Shimadzu FTIR-8600 (a 0.1-mm KBr cell), and Tokyo Instruments Nanofinder 30 spectrophotometers (excited by a Toptica iWave 405-nm diode laser), respectively.
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17. Tamiaki, H.; Hamada, K.; Kunieda, M. *Tetrahedron* **2008**, *64*, 5721.
18. The RR band of 3-C=O in **2** might be shifted to a larger wavenumber similarly to its IR band and be hidden by the RR band of 13-C=O in **2** at 1691 cm^{-1}.
19. The molecular models shows that 20-substitution in 3-acetyl-chlorins (**1** → **2**/**3**) induced a decrease in the bond lengths of 3-C=O and an increase of charge densities on both the C- and O-atoms, while no changes were estimated by 20-bromination of 3-formyl-chlorin (**7** → **8**): distances of the 3-carbonyl bond were 121.2, 121.1, 121.1, 120.8, and 120.8 pm and charge densities on the C/O-atoms were +0.33/−0.33, +0.35/−0.35, +0.35/−0.35, +0.34/−0.34, and +0.34/−0.34 for **1**, **2**, **3**, **7**, and **8**, respectively. The calculation results support the observed shifts in IR bands.