Structures of photosynthetic pigment-protein complexes and probing electrostatic field inside them by Stark spectroscopy

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Contents

• A comparative look at the structural requirements for photosynthetic light-harvesting

• Structural study of the RC of purple photosynthetic bacteria

• Stark spectroscopy on the native and reconstituted pigment-protein complexes of purple photosynthetic bacteria
• The structure of all photosynthetic reaction centres is strongly conserved.

• This is because the structural constraints on electron transfer are very strict.

• A comparison of the structures of photosynthetic light-harvesting complexes shows that they form a very heterogeneous group. Why is this?
LHCII from spinach
Peridinin-Chlorophyll a complex
LH2 complexes
The physics of energy transfer is rather tolerant. This means that there are many structural solutions to the problem of building an efficient light-harvesting complex.
Absorption spectrum of the chromatophores of *Rb. sphaeroides* 2.4.1
Photosynthetic system of purple bacteria in the intracytoplasmic membrane

ATP synthase

ADP + P_i

ATP

H^+

Light

LH2

LH1

B850

B800

RC

Q

Q

Q

Q

Q

e-
e-

B880

Cytochrome b/c_1

Cyt c

Cytoplasm

Periplasm

System of pigments in RC

Fe

QA

QB

Q_B

Q_A
Contents

• A comparative look at the structural requirements for photosynthetic light-harvesting

• Structural study of the RC of purple photosynthetic bacteria

• Stark spectroscopy on the native and reconstituted pigment-protein complexes of purple photosynthetic bacteria
Electron transfer in the RC

Bchlα 'Primary donor' P

Accessory Bchl

Pheophytin

Spheroidene

Ubiquinone-10

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Our high-resolution (1.95 Å) X-ray crystal structure analysis of the wild-type RC from *Rba. sphaeroides* strain 2.4.1.
Electron densities of the surfactant molecules surrounding the RC
'Solvent' molecules surrounding the RC
Contents

• A comparative look at the structural requirements for photosynthetic light-harvesting

• Structural study of the RC of purple photosynthetic bacteria

• Stark spectroscopy on the native and reconstituted pigment-protein complexes of purple photosynthetic bacteria
Research Background

- B800 → B850: 1.2 ps
- B850 ring: 100 ~ 200 fs
- B800 → B800: ~500 fs
- LH2 → LH1: 3 ~ 5 ps
- LH1 → RC: 35 ps
- LH2 → LH1: 1.2 ps
- LH1 → RC: 35 ps
- LH2 → LH2
- RC
Research Background

Great success of X-ray crystallography on pigment-protein complexes of purple photosynthetic bacteria

Ultra-fast laser spectroscopy to understand each step of energy and electron transfer
Research Background

It is important to understand the exact mechanisms of electrostatic interaction between pigments and their surrounding proteins in order to clarify the true functional mechanisms of the pigments in photosynthesis.
Characteristics of electroabsorption (Stark effect) spectroscopy

• Pigments in pigment-protein complexes are under the influence of internal electric field produced by the electrostatic potential of apoprotein

• Electroabsorption spectroscopy is one of the most promising methods to probe the pigment-protein interaction modulated by the external electric field
Optical absorption spectra under the influence of external or internal electric field

Intensity change
Spectral shift
Broadening & Shrinkage

Absorbance
Photon energy

Absorbance
Photon energy

Absorbance
Photon energy

\[ \Delta A \]

\[ \Delta A \]
**Liptay equation**

\[
\Delta A(\nu) = A(\nu, E_{\text{int}}) - A(\nu, 0)
\]

\[
= (DA(\nu, 0) + F \nu \frac{d(A/\nu)}{h d\nu} + H \nu \frac{d^2(A/\nu)}{2h^2 d\nu^2})E_{\text{int}}^2 R(\chi, \zeta)
\]

- \[D = \frac{A^2}{M^2} + \frac{2B}{M},\]
- \[F = \frac{2A\Delta\mu}{M} + \frac{\Delta\alpha}{2},\]
- \[H = \Delta\mu^2\]

\[
R(\chi, \zeta) = (5 + (3 \cos^2 \zeta - 1)(3 \cos^2 \chi - 1))/15
\]

\[
M(F_{\text{int}}) = M + AE_{\text{int}} + E_{\text{int}}BE_{\text{int}}
\]

\[
h\nu_m(F_{\text{int}}) = h\nu_m(0) - \Delta\mu E_{\text{int}} - \frac{1}{2} E_{\text{int}} \Delta\alpha E_{\text{int}}
\]

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**Experimental setup for electroabsorption spectroscopy**

- **Monochromater**
- **Sample cell**
- **Bipolar power amplifier**
- **Photo-diode**
- **Pre-amplifier**
- **Dual-phase Lock-in amp**
- **AC**
- **DC**
- **PC**

*Physics of Biological Materials Lab.*
Contents of this presentation

1. Local electrostatic field induced by carotenoid in the reaction centre of purple photosynthetic bacteria

2. Stark spectroscopy on the LH2 complex from *Rhodobacter sphaeroides* strain G1C; frequency and temperature dependence
Local electrostatic field induced by carotenoid in the reaction centre of purple photosynthetic bacteria

Objective

Evaluation of electrostatic effect of Car on Bchls (especially P) in RC

- Electroabsorption (EA) spectra of two reaction centres were recorded
- One is prepared from *Rb. sphaeroides* strain R26.1 (*R26.1-RC*), which lacks carotenoid
Objective

Evaluation of electrostatic effect of Car on Bchls (especially P) in RC

- Electroabsorption (EA) spectra of two reaction centres were recorded
- The other one is a reconstituted RC (R26.1-RC+ Car) which was prepared by re-incorporating synthetic Car (3,4-dihydrospheroidene) into R26.1-RC.
Objective

Evaluation of electrostatic effect of Car on Bchls (especially P) in RC

The electrostatic effect of Car on P was evaluated from the difference of these two spectra

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Gatekeeper (Phe M162) and locking (Trp M75) amino acid residues in the site of RC where Car is re-incorporated

Influence of static-electric field on the excited state of Bchls caused by the presence of Car

Electrostatic field F around Bchls in RC

\[ F = F_a + F_p + F_c \]

- \( F_a \): applied electric field
- \( F_p \): pocket field
- \( F_c \): static-electric field due to Car
Absorption change due to applied electric field

Absorption change ($\Delta A$) due to applied electric field can be written as,

$$\Delta A = \left( \frac{1}{3} DA + \frac{1}{6} F \frac{d A}{d \nu} + \frac{1}{6} |\Delta \mu^*|^2 \frac{d^2 A}{d \nu^2} \right) |F_a|^2$$

$$D = \frac{1}{\mu_{eg}^2} (X^2 + 2\mu_{eg} Y) \approx 0 \quad F = Tr[\Delta \alpha] + 2 \frac{\mu_{eg} X}{|\mu_{eg}|^2} \Delta \mu^* \approx Tr[\Delta \alpha]$$

$$\Delta \mu^* = \Delta \mu_{\text{int}} + \Delta \alpha \cdot F_p + \Delta \alpha \cdot F_c$$
Influence of Car on Bchl

Change of $\Delta \mu$ value by static-electric field due to the presence of Car

$$\Delta \mu_c = \Delta \alpha \cdot F_c$$

This change is expected to be observed by EA spectroscopy

The contribution of $\Delta \mu$ is detected as the second-order derivative waveform of absorption spectra in EA spectra. Therefore, the second derivative waveform should be observed in the difference EA spectra between $R26.1-RC$ and $R26.1-RC+Car$. 

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Electroabsorption spectra of RC’s from *Rb. sphaeroides* R26.1 at 79 K

**In-phase**

![In-phase spectra](image1)

**Quadrature-phase**

![Quadrature-phase spectra](image2)

(a) *Rb. sphaeroides* R26.1  
(b) *Rb. sphaeroides* R26.1 + Car  
(c) (a)–(b)  
(d) *Rb. sphaeroides* R26.1  
(e) *Rb. sphaeroides* R26.1 + Car  
(f) (d)–(e)
Electroabsorption spectra of RC’s from *Rb. sphaeroides* R26.1 at 293 K

**In-phase**

(a) *Rb. sphaeroides* R26.1

(b) *Rb. sphaeroides* R26.1 + Car

(c) (a) – (b)

**Quadrature-phase**

(d) *Rb. sphaeroides* R26.1

(e) *Rb. sphaeroides* R26.1 + Car

(f) (d) – (e)
## Nonlinear optical parameters of P band

<table>
<thead>
<tr>
<th>P band</th>
<th>$D \times 10^{-18}$ (m/$f_L V^2$)</th>
<th>$\text{Tr}[^\Delta \alpha]$ [Å$^3/f_L^2$]</th>
<th>$\Delta \mu^*$ [D/$f_L$]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>79 K</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R26.1-RC</td>
<td>-1.5 $\pm$ 0.4</td>
<td>(2.2 $\pm$ 0.3)$\times 10^2$</td>
<td>6.5 $\pm$ 0.3</td>
</tr>
<tr>
<td>R26.1+Car-RC</td>
<td>-1.8 $\pm$ 0.4</td>
<td>(2.4 $\pm$ 0.8)$\times 10^2$</td>
<td>6.5 $\pm$ 0.3</td>
</tr>
<tr>
<td><strong>293 K</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R26.1-RC</td>
<td>3.9 $\pm$ 0.4</td>
<td>-$(1.7 \pm 0.1)$\times 10^3</td>
<td><strong>10.8 $\pm$ 0.4</strong></td>
</tr>
<tr>
<td>R26.1+Car-RC</td>
<td>3.6 $\pm$ 0.4</td>
<td>-$(1.7 \pm 0.4)$\times 10^3</td>
<td><strong>9.0 $\pm$ 0.4</strong></td>
</tr>
</tbody>
</table>

$F_a = f_L \cdot F_{\text{ext}}$

$F_{\text{ext}}$ is externally applied electric field.

$f_L$ is a local field correction factor.

Even if the experimental errors are taken into account, the difference of $\Delta \mu^*$ between R26.1-RC and R26.1-RC+Car at 293 K is estimated to be $\sim 1.0$ [D].
Nonlinear optical parameters of B band

<table>
<thead>
<tr>
<th></th>
<th>B band</th>
<th>$D \ [10^{-18} \ (m/f_L V)^2]$</th>
<th>$\text{Tr}[\Delta \alpha] \ [\AA^3/f_L^2]$</th>
<th>$\Delta \mu^* \ [D/f_L]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>79 K</td>
<td>R26.1-RC</td>
<td>0.9 ± 0.2</td>
<td>-(1.2 ± 0.4)·10^2</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>R26.1+Car-RC</td>
<td>1.4 ± 0.5</td>
<td>-(1.1 ± 0.2)·10^2</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>293 K</td>
<td>R26.1-RC</td>
<td>-7.1 ± 0.5</td>
<td>-(5.3 ± 3.6)·10</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>R26.1+Car-RC</td>
<td>-7.0 ± 1.7</td>
<td>-(5.8 ± 2.4)·10</td>
<td>1.5 ± 0.2</td>
</tr>
</tbody>
</table>

$\Delta \mu^*$ of B band is different between R26.1-RC and R26.1-RC+Car at 293 K.
Nonlinear optical parameters of H band

<table>
<thead>
<tr>
<th>H band</th>
<th>$D$ [10^{-18} (m/f_L V)^2]</th>
<th>$\text{Tr}[\Delta \alpha]$ [Å³/f_L²]</th>
<th>$\Delta \mu^*$ [D/f_L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>79 K</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R26.1-RC</td>
<td>0.4 ± 0.2</td>
<td>-(8.4 ± 7.2)·10</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>R26.1+Car-RC</td>
<td>0.4 ± 0.1</td>
<td>-(0.9 ± 2.0)·10</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>293 K</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R26.1-RC</td>
<td>0.8 ± 0.2</td>
<td>(9.5 ± 2.3)·10</td>
<td>6.2 ± 0.4</td>
</tr>
<tr>
<td>R26.1+Car-RC</td>
<td>0.8 ± 0.3</td>
<td>(10.0 ± 4.0)·10</td>
<td>5.7 ± 0.5</td>
</tr>
</tbody>
</table>

No significant difference is observed between R26.1-RC and R26.1+Car-RC in the nonlinear optical parameters of H band.
Estimation of static-electric field induced by the presence of carotenoid

\[ \Delta \mu_c = \Delta \alpha \cdot F_c \approx 1.0 \left[ \frac{D}{f_L} \right] \]

\[ F_c \approx 1 \times 10^5 \text{ [V/cm]} \quad f_L = 2.2^b \]

Reported localized field around P is \( \sim 1 \times 10^6 \text{ [V/cm]} \).\(^a\)

The presence of Car causes \( \sim 10 \% \) change of the electrostatic environment around P. According to our results of X-ray crystallography of RC,\(^c\) carotenoid binding pocket becomes smaller when Car is absent. This may cause some rearrangements of amino acid residues around P. As a consequence, the above \( \sim 10 \% \) change should be occurred.

\[ [a] \text{ T.R. Middendorf et al., BBA, 1143, 1993, 223.} \]
\[ [b] \text{ M. Loche et. al., PNAS, 84, 1987, 7537.} \]
\[ [c] \text{ A.W. Roszak et al., Structure, 12, 2004, 765.} \]
Conclusions on RC

- Carotenoid bound to the RC was found to affect on the electrostatic environment around P and B (B_B).

- The static electric field on P due to the presence of carotenoid was estimated to be $\sim 1 \times 10^5$ [V/cm]. It corresponds to as high as 10% of the local electric field around P.

- Carotenoid could be one of the important factors that regulate the function of P (vice versa).
Contents of this presentation

1. Local electrostatic field induced by carotenoid in the reaction centre of purple photosynthetic bacteria

2. Stark spectroscopy on the LH2 complex from Rhodobacter sphaeroides strain G1C; frequency and temperature dependence
Contents of this presentation

Stark spectroscopy on the LH2 complex from *Rhodobacter sphaeroides* strain G1C; frequency and temperature dependence

How can we evaluate electrostatic interaction between pigments and apoproteins?

• Most previous reports on the use of Stark spectroscopy to study photosynthetic pigment-protein complexes have only used *in-phase* signals.

• More detailed information on dynamic electrostatic interactions with the environment can be obtained from *out-of-phase (quadrature-phase)* signal.
How can we evaluate electrostatic interaction between pigments and apoproteins?

By studying the frequency and temperature dependence of the quadrature-phase signals the nature of the pigment-protein interaction can be investigated!
Absorption spectra of the LH2 complex from *Rb. sphaeroides G1C*

(a) in buffer solution

(b) in PVA matrix
Electro-absorption spectra of the LH2 complex from *Rb. sphaeroides* G1C

- in phase
- quadrature phase
Theoretical model to generate the phase-retarded signals

\[ \Delta A = \frac{(\Delta \mu^* \cdot \mathbf{E})}{\hbar} \frac{\partial A}{\partial \nu} + \frac{1}{2} \frac{\mathbf{E} : \Delta \alpha : \mathbf{E}}{\hbar} \frac{\partial A}{\partial \nu} + \frac{1}{2} \frac{(\Delta \mu^* \cdot \mathbf{E})^2}{\hbar^2} \frac{\partial^2 A}{\partial \nu^2} \]

\[ \Delta \mu^* = \Delta \mu_{\text{mol}} + \Delta \alpha : \mathbf{E}_{\text{pocket}} \]

\[ \mathbf{E}_{\text{pocket}}(\mathbf{x}) = \mathbf{E}_{\text{pocket}}(0) + \mathbf{C} \cdot \mathbf{x} \]

\( \Delta \mu^* \) is dipole-moment change upon photoexcitation
\( \Delta \alpha \) is polarizability change upon photoexcitation
Dependence of the phase-retarded signals on the modulation frequency of the applied electric field

\[ \frac{d^2x}{dt^2} = -\gamma \frac{dx}{dt} - \omega_0^2 x + \frac{q \cdot E}{m} \]

\[ E(t) = f_L \cdot E_{ext} \sin(\omega t) \]

\[ x(t) = \frac{q}{m} \frac{f_L \cdot E_{ext}}{\sqrt{\left(\omega_0^2 - \omega^2\right)^2 + \gamma^2 \omega^2}}^{1/2} \sin(\omega t + \phi_p) \]

\[ \tan \phi_p = \frac{\gamma \omega}{\omega^2 - \omega_0^2} \]
Absorption change modulated with $2\omega$ frequency

\[
\Delta A_{2\omega} = \frac{x_0C}{3h} \Delta \alpha \cdot \nu \frac{\partial A/\nu}{\partial \nu} \frac{f_L^2 E_{\text{ext}}^2}{2} \cos(2\omega t - \phi)
\]

\[
+ \frac{1}{6} \left( \frac{\Delta \alpha}{\nu} \frac{\partial A/\nu}{\partial \nu} + \frac{\Delta \mu^*}{h^2} \nu \frac{\partial^2 A/\nu}{\partial \nu^2} \right) f_L^2 E_{\text{ext}}^2 \cos(2\omega t)
\]
In-phase signals detected by a dual-phase lock-in amplifier

\[
\Delta A_{in} = \frac{1}{6} \left[ \left( \frac{\Delta \alpha}{h} + \frac{2x_0 \cdot C \cdot \Delta \alpha}{h} \cos \phi \right) \cdot \nu \frac{\partial A/\nu}{\partial \nu} + (\Delta \mu^*)^2 \nu \frac{\partial^2 A/\nu}{h^2 \partial \nu^2} \right] \cdot \frac{f_L^2 \cdot E_{ext}^2}{2}
\]

\[
= \frac{1}{6} \left( F_{in} \cdot \frac{\partial A/\nu}{\nu h \partial \nu} + (\Delta \mu^*)^2 \nu \frac{\partial^2 A/\nu}{h^2 \partial \nu^2} \right) \cdot \frac{f_L^2 \cdot E_{ext}^2}{2}
\]

\[
F_{in} = \Delta \alpha \cdot (1 + 2x_0 \cdot C \cos \phi) = \Delta \alpha \cdot \left( 1 + 2 \frac{qC}{m} \frac{(\omega_0^2 - \omega^2)}{(\omega_0^2 - \omega^2)^2 + \gamma^2 \omega^2} \right)
\]
Quadrature-phase signals detected by a dual-phase lock-in amplifier

\[ \Delta A_{\text{quad}} = \frac{1}{6} \left( 2x_0 \cdot C \cdot \Delta \alpha \sin \phi \right) \cdot \nu \frac{\partial A/\nu}{h\partial \nu} \cdot \frac{f_L^2 \cdot E_{\text{ext}}^2}{2} \]

\[ = \frac{1}{6} F_{\text{quad}} \cdot \nu \frac{\partial A/\nu}{h\partial \nu} \cdot \frac{f_L^2 \cdot E_{\text{ext}}^2}{2} \]

\[ F_{\text{quad}} = 2x_0 \cdot C \cdot \Delta \alpha \sin \phi = 2\Delta \alpha \frac{qC}{m} \frac{\gamma \omega}{\left( \omega_0^2 - \omega^2 \right)^2 + \gamma^2 \omega^2} \]
Temperature dependence of in-phase and quadrature-phase EA spectra

(a) Normalized \( \Delta A_{in} \) vs. Wavenumber [cm\(^{-1}\)]

(b) Normalized \( \Delta A_{quad} \) vs. Wavenumber [cm\(^{-1}\)]

Temperatures: 79K, 200K, 270K, 293K
Temperature dependence of $F_{\text{quad}} / F_{\text{in}}$ value

$$\frac{F_{\text{quad}}}{F_{\text{in}}} = \gamma \frac{1}{2} \frac{m}{q \cdot C} \cdot \left[ (\omega_0^2 - \omega^2)^2 + \gamma^2 \omega^2 \right] + \omega_0^2 - \omega^2$$

$$\propto \frac{1}{\tau_r} \propto \exp \left( - \frac{H}{RT} \right)$$

$H$ is the activation energy that characterizes the relaxation factor $\gamma$
Temperature dependence of $F_{\text{quad}} / F_{\text{in}}$ value

- Quite a number of molecular motions can be plausible candidates that generate the relaxation factor, $\gamma$.

For example, local mode relaxation, crankshaft and kink motion, rotation of methyl group, side chain motion, local intermolecular rearrangements etc.

$H \sim 12 \text{ kJ/mol}$
Temperature dependence of \( F_{\text{quad}} / F_{\text{in}} \) value

- Inspection of X-ray crystal structure of the LH2 complex from \textit{Rps. acidophila} strain 10050, suggests that a plausible candidate for the physical origin of \( \gamma \) could be either a local twisting motion of the apoprotein main chain, or rotational motion of apoprotein side chains.
Frequency dependence of $F_{\text{quad}}$ value

$$
F_{\text{quad}}(f) = 2\Delta \alpha\frac{qC}{m} \frac{2\pi f}{\left(\omega_0^2 - 4\pi^2 f^2\right)^2 + 4\pi^2 \gamma^2 f^2}
$$

$$
\omega = 2\pi f
$$
Dependence of the phase-retarded signals on the modulation frequency

\[ \omega_0 \sim 11 \text{ kHz} \]

Rotation of an aromatic side chain of an amino acid residue inside a protein

Deformation vibration

Stretching vibration
The X-ray crystal structure of the LH2 complex from *Rps. acidophila* strain 10050 clearly shows that the Mg$^{2+}$ ions at the centre of the B850 bacteriochlorin rings are liganded to histidine side chains.

These histidine residues are prime candidates for the strong electrostatic interaction suggested by the presence of the phase-retarded signals.

However, the resonance frequency of 11 kHz looks too low for a normal mode reflecting the Mg-His interaction (~1 THz).
The relevant normal mode could be an overall, coupled mode reflecting the total environment of pigment-protein interaction.
Conclusions on LH2

- Electroabsorption (Stark effect) signals due to electrostatic interactions between the B850 pigments and surrounding apoproteins were detected as a phase-retarded signal using dual-phase lock-in detection.

- The Arrhenius type activation energy ($H = 12$ kJ/mol) was determined from the temperature dependence of the phase-retarded signals. The resonance frequency ($\omega_0 = 11$ kHz) was determined from their frequency dependence.

- The calculated thermodynamic and kinetic parameters can be accounted for by assuming a strong electrostatic interaction of the B850 pigments with the dynamic environment provided by the apoproteins.
Thank you for your attention!