M.-E. Michel-Beyerle (Editor)

Reaction Centers of Photosynthetic Bacteria

Feldafing-II-Meeting

With 165 Figures

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The workshop on "Structure and Function of Bacterial Reaction Centers" was held from March 24-26, 1990, to a day five years after the first meeting, at the Hotel "Kaiserin Elisabeth" in Feldafing, Bavaria (F.R.G.). The '85 workshop, on international stage quoted as the "Feldafing-Meeting" (Vol.42, Springer Series in Chemical Physics), had been dominated by the fresh impression of the three-dimensional structure of the reaction center from the photosynthetic bacterium Rhodopseudomonas viridis. The main emphasis in the follow-up meetings, 1987 at Cadarache (Vol.149, NATO ASI Series A: Life Sciences, Plenum Press 1988) and this year's "Feldafing-II", was put on the central issue of reaction center function. The reaction center is not only a paradigm for one of the most important light-transforming machineries in biology. Due to its detailed structural characterization, it has also become the best-defined supramolecular electron donor/acceptor-system and offers unprecedented opportunities for the study of basic questions of electron transfer and protein dynamics.

Safely rooted in the crystal structure of the reaction center of meanwhile two bacterial organisms, the present compilation of invited papers points in a self-evident way at the different lines of developments of a still vividly expanding field. The abundance of open questions initiated novel methodological approaches bridging the gap from biochemistry, genetic engineering and complex spectroscopies to chemical modelling, molecular dynamics calculations and electron transfer theory. The material is organized into four Parts:

I. Native Reaction Centers : Structure and Spectroscopy
II. Native Reaction Centers : Electron Transfer Dynamics
III. Modified Reaction Centers : Effects of Mutagenic and Chemical Modifications
IV. Reaction Centers : Modelling of Structure/Function-Relationship

It should be noted that Part (II) gives a status-report on the still controversial problem of the primary charge separation mechanism. The ambiguities of the available dynamic data have stimulated innovative developments of spectroscopic techniques which are aiming at the combination of optimal time-resolution and highest sensitivity. External electric and magnetic
fields shifting energy levels of cofactors have become necessary parameters in time-resolved absorption and fluorescence spectroscopy. The riddles in the reaction center function have also stimulated an explosively fast progress towards modifications along two lines, mutagenic replacement of single or multiple amino acids in the protein matrix and thermally induced replacement of cofactors against chemically modified ones. The expansion of the field in this direction is clearly manifested by the accumulation of contributions on modified reaction centers compiled in Part (III).

Parts (I) to (IV) altogether reflect the diversity of access to and different angles of looking at the same central object - the reaction center. This very employment of widely divergent and largely innovative methods is certainly a trade-mark of the fundamental contributions which Professor George Feher made to the area of elementary processes in photosynthesis. Thus, for the present level of our understanding of how the reaction center works, the community of researchers and "big game hunters in the field" owes him a great debt, and I am glad to dedicate this volume to him.

We like to express our gratitude to all the participants in the workshop for their contributions, and to the authors for the timely preparation of their manuscripts. I am especially indebted to my coworkers for their most valuable assistance and advice. As the main load and heavy responsibility for the success of the meeting was carried by Petra Kahlfuß, we have to thank her in particular.

In line with the '85 meeting, this workshop was organized under the auspices of the two Munich universities, the Technical University and the Ludwig-Maximilians-University, and the Max-Planck-Society. It was supported by the "Deutsche Forschungsgemeinschaft" within the frame of the "Sonderforschungsbereich 143". In this context we would like to thank especially Dr. Klaus Genius for his effective and continuous cooperation in helping to make this "Sonderforschungsbereich", and all activities around it, a success.

Garching, October 1990

M.E. Michel-Beyerle
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1. Introduction

Tryptophan M252 is located between the bacteriopheophytin H_A and the quinone Q_A in the photosynthetic reaction center of Rhodobacter (Rb.) sphaeroides (1-4). The indole ring of the tryptophan M252 is in van der Waals contact with both the bacteriopheophytin H_A and the quinone Q_A and was suspected from this unique position to participate as a (super-exchange) mediator in electron transfer (5). At the same time tryptophan M252 may contribute via a charge transfer interaction to the binding of quinone to the Q_A site (6).

Threonine M222, on the other hand, presumably forms a hydrogen bridge to the nitrogen atom of the indole ring (7). This hydrogen bridge could help to hold the tryptophan parallel to Q_A.

Oligodeoxynucleotide-directed mutagenesis was employed to elucidate the influence of this aromatic amino acid on the binding of Q_A and on the electron transfer between H_A and Q_A. For this, tryptophan M252 was changed to tyrosine or phenylalanine, and threonine M222 to valine.

2. Results

Mutant Rb. sphaeroides strains were constructed which contain the mutated reaction center genes in the chromosome (Stilz et al., submitted for publication). These strains
grow under photosynthetic conditions with a rate comparable to the wild type strain. In addition, photobleaching experiments at 860 nm on membrane bound reaction centers demonstrate that approximately an equal concentration of functional reaction centers are present in the membranes of the wild type and of the mutant cells.

In contrast to the membrane bound reaction centers, the isolated reaction centers lose quinone QA partially. However, QA could be reconstituted into the mutated proteins by addition of a 4.5 molar excess of ubiquinone-10 (UQ10). The QA content in the various reaction center samples was determined either by HPLC analysis or from the transient absorption data at 665 nm (Fig. 1). The values are summarized in Table I.

The time-resolved absorption measurements were performed on QA-reconstituted reaction centers. The data obtained at the probing wavelength of 665 nm reveal the consequences of the mutations for the binding of the quinone and for the electron transfer kinetics in the reaction centers.

Fig. 1a shows the results of measurements on the wild type sample. The instantaneous part of the absorption increase originates from the population of the excited electronic state P* of the special pair P.

It was shown recently that the subsequent slower absorption increase with a time constant of 0.9 +/- 0.3 ps may be related to the transfer of an electron from the accessory bacteriochlorophyll BA to the bacteriopheophytin HA and that the weak absorption decrease with time constant of 3.5 +/- 0.4 ps is due to the electron transfer from P* to BA (8-10).
Fig. 1: Time-resolved absorption data (points) at the probing wavelength 665 nm for reaction centers of wild type \textit{Rb. sphaeroides} ATCC 17023 and of the mutants Thr\textsuperscript{M222} \rightarrow Val, Trp\textsuperscript{M252} \rightarrow Tyr, and Trp\textsuperscript{M252} \rightarrow Phe. The reaction centers were excited at 860 nm. The delay time scale is linear between -1 and +1 ps and logarithmic for $t_D > 1$ ps. The dashed curves are based on the assumption that the electron transfer from $H_A^{-}$ to $Q_A$ proceeds within 220 ps.

At later delay times a pronounced absorption decrease with a time constant of 220 +/- 40 ps is found. This absorbance change reflects the electron transfer from the reduced bacteriopheophytin $H_A^{-}$, which absorbs strongly at 665 nm, to the primary quinone acceptor $Q_A$. 
Table I. Summary of mutations
Line 1, type of reaction center. Line 2, ability to phototrophic growth. Line 3, $Q_A$ content in membrane bound reaction centers. Line 4, total ubiquinone content of isolated reaction centers (HPLC analysis). Since $Q_B$ is quantitatively lost during the isolation procedure the total ubiquinone content corresponds to the $Q_A$ content (wild type taken to be 100%). Line 5, $Q_A$ content in the reconstituted reaction centers determined from the transient absorption changes remaining at a delay time of $t_D = 5$ ns at the probing wavelength 665 nm (Fig. 1). Lines 6-8, time constants assigned to particular electron transfer steps.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>None</th>
<th>TM222 $\rightarrow$ V</th>
<th>WM252 $\rightarrow$ Y</th>
<th>WM252 $\rightarrow$ F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phototrophic growth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>$Q_A$ content (%) in membrane</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>$Q_A$ content (%) isolated RC</td>
<td>100</td>
<td>80</td>
<td>13</td>
<td>58</td>
</tr>
<tr>
<td>$Q_A$ content (%) reconstituted RC</td>
<td>100</td>
<td>80</td>
<td>65</td>
<td>80</td>
</tr>
<tr>
<td>$t$ (ps) $P^* \rightarrow B_A$</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>$t$ (ps) $B_A^- \rightarrow H_A$</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>$t$ (ps) $H_A^- \rightarrow Q_A$</td>
<td>220</td>
<td>220</td>
<td>600</td>
<td>900</td>
</tr>
</tbody>
</table>
At early delay times the transient absorption curves of the mutants Thr^{M222} -> Val, Trp^{M252} -> Tyr, and Trp^{M252} -> Phe resemble that of the wild type. The time constants of the corresponding electron transfer steps are unchanged with 3.5 ps and 0.9 ps, respectively. In addition, these time constants have also been observed at probing wavelengths of 920 nm (3.5 ps) and 785 nm (3.5 ps und 0.9 ps).

At later delay times, however, part of the induced absorption persists in the mutated reaction centers. This illustrates that the decay of the reduced bacterioopheophytin H_{A^-} is not complete in the mutant proteins emphasizing that the electron acceptor Q_{A} is partially missing.

The decay of H_{A^-} proceeds in the mutant Thr^{M222} -> Val as in the wild type sample with a time constant of 220 +/- 40 ps. In the mutants Trp^{M252} -> Tyr and Trp^{M252} -> Phe the electron is transferred more slowly from H_{A^-} to Q_{A}. The time constants are 600 +/- 200 ps and 900 +/- 200 ps for the mutants Trp^{M252} -> Tyr and Trp^{M252} -> Phe, respectively.

3. Discussion
The results demonstrate that the binding affinity of Q_{A} to its site is lowered in the mutated reaction centers compared to that of the wild type protein. The Q_{A} binding site of the mutants is only fully occupied in the photosynthetic membrane where an excess of quinone is present (11).

This clearly emphasizes an important function for threonine M222 and tryptophan M252 in the reaction center structure. Tryptophan M252 mediates binding of Q_{A} to its site presumably via a charge transfer interaction (6) between the electron donor tryptophan and the electron acceptor Q_{A} and thus optimizes reaction center function. This is especially evident in the crystal structure of \textit{Rps. viridis} were the \pi-electron rich C_{2}-C_{3} bond of the indole
ring system is located exactly above the p-benzoquinone ring system of the menaquinone-9 (12). It must be noted, however, that mutations at position M210 also causes loss of $Q_A$ (13).

Threonine M222, on the other hand, apparently holds the tryptophan in place via a hydrogen bond (7,12). The observation that the mutant Thr$^{M222}$ $\rightarrow$ Val partially loses its $Q_A$ indicates that the precise position of the indole ring is essential for a stable binding of $Q_A$.

In the mutants Trp$^{M252}$ $\rightarrow$ Tyr (600 ps), and Trp$^{M252}$ $\rightarrow$ Phe (900 ps) the electron transfer proceeds more slowly than in the wild type protein (220 ps). These slower transfer rates parallel the decrease in $\pi$-electron density of the corresponding aromatic ring systems: tryptophan (indole), tyrosine (phenol), and phenylalanine (benzene). This indicates that the $\pi$-electron system of the aromatic ring system contributes to the electronic coupling between $H_A$ and $Q_A$. In addition, it has been observed recently that replacing tryptophan M252 by leucine, where the $\pi$-electron system is completely missing reduces the electron transfer rate in the reaction center of Rb. capsulatus by a factor of 14 (14).

The weaker binding of $Q_A$ in the Thr$^{M222}$ $\rightarrow$ Val mutant does not influence the electron transfer. One may therefore assume that also in the mutants Trp$^{M252}$ $\rightarrow$ Tyr and Trp$^{M252}$ $\rightarrow$ Phe the weaker binding of $Q_A$ to its site does not considerably affect the electron transfer.

References


