A Generalized Mechanism of the Primary Photoreaction in Microbial Rhodopsins as Revealed by Femtosecond Time-Resolved Spectroscopy

（フェムト秒時間分解分光による微生物型ロドプシンの

光反応初期過程の機構解明）

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The biological functions of microbial rhodopsins are activated by all-*trans* to *13-cis* photoisomerization of the protonated retinal Schiff base (PRSB) chromophore. Such photoisomerization proceeds on the femto-to-picosecond time scale, giving rise to the formation of the first ground-state intermediate having *13-cis* chromophore conformation. While this ultrafast photoisomerization process has been extensively studied, it was recognized that the excited-state relaxation dynamics differs from one rhodopsin to another. For example, bacteriorhodopsin (BR), the most prototypical proton (H^+)-pumping rhodopsin, shows nearly single exponential decay of the excited-state population with a ~500 fs time constant,[1] whereas the excited state of other rhodopsins such as halorhodopsin,[2] proteorhodopsin (PR)[3] and *Anabaena* sensory rhodopsin[4] exhibit complex multi-exponential decays consisting of the fast (<1 ps) and slow (>1 ps) decay components. So far, these multi-exponential decays of the excited-state chromophore have been considered to originate from the branching of the relaxation pathway on the S_1 excited-state potential energy surface (PES),[5] from which the isomerization occurs with different rates and efficiencies. However, such conventional understanding does not explain why the S_1 PES of different rhodopsins exhibits different branching and why such branching does not occur in BR. Recently, a time-resolved absorption study on sodium (Na^+)-pumping rhodopsin KR2 showed that the ratio of the fast and slow decays strongly depends on pH of the buffer, indicating that the molecular origin of the multi-exponential decay of the excited state is the acid-base

equilibrium of the PRSB counterion in the protein in the ground state.[6] This study provides a very different picture to explain the multi-exponential decay of the excited state of rhodopsins, but its generality is still unclear because KR2 is a rhodopsin that has a unique chromophore environment and exhibits a peculiar Na^+-pumping function.[7] Therefore, it is highly desirable to examine the importance of the acid-base equilibrium of the PRSB counterion for the excited-state dynamics of ordinary H^+-pumping rhodopsins. Based on this idea, ultrafast dynamics of several microbial rhodopsins were thoroughly studied by femtosecond time-resolved spectroscopy in order to obtain a generalized picture of the primary photoisomerization process in microbial rhodopsins.

First, the author describes the primary photoreaction dynamics of PR, its D97N mutant and bacteriorhodopsin (BR) having ordinary H^+-pumping function, which were investigated in a wide pH range by femtosecond time-resolved absorption spectroscopy. These samples were chosen not only because they are abundant in nature but also because their PRSB counterion characteristics are different (see Figure 1(a)–(c)). Quantitative analysis of the time-resolved absorption spectra confirms that the excited states of these rhodopsins exhibit multi-exponential decays, containing the fast (<1 ps) and slow (>1 ps) decay components. Furthermore, it was found that the excited-state relaxation dynamics and the retinal photoisomerization efficiency are decisively governed by the protonation state of the corresponding PRSB counterion (Asp97 for PR and Asp85 for BR, respectively): When the PRSB counterion is deprotonated, the excited state predominantly shows the fast (<1 ps) decay, and the photoisomerization proceeds efficiently. On the other hand, the excited state mainly exhibits the slow (>1 ps) decay, and the photoisomerization proceeds less efficient when the counterion is protonated (i.e., neutral). These results clearly indicate that the acid-base equilibrium of the PRSB counterion in the ground state is the major molecular origin of the multi-exponential excited-state decay in the primary photoreaction of microbial rhodopsins.
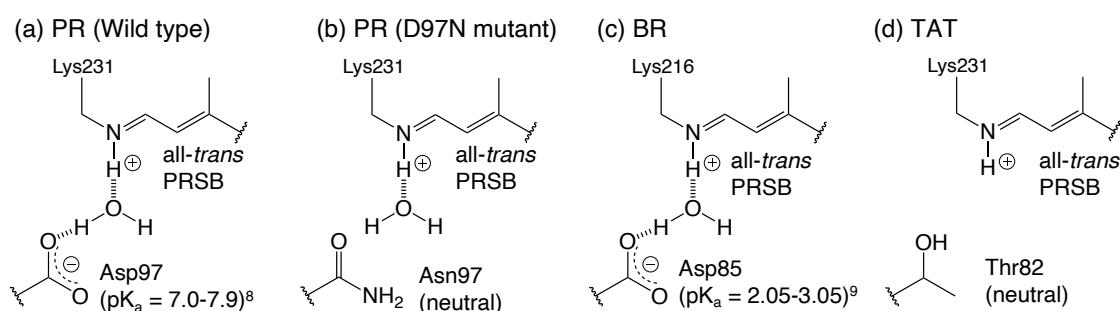

Figure 1. Chemical structure of the all-*trans* PRSB chromophore and its counterion residue of (a) PR, (b) the D97N mutant of PR, (c) BR and (d) TAT rhodopsin. The dashed lines represent hydrogen bonds.

Based on the understanding obtained with the H^+-pumping rhodopsins, the author studied the primary photoreaction dynamics of the recently discovered TAT rhodopsin that shows no biological function. In TAT rhodopsin, the analogue position of the PRSB counterion is neutral Thr82 residue (Figure 1(d)), which is considered

equivalent to that of PR and BR with the protonated PRSB counterion. As expected, the retinal photoisomerization is extremely inefficient and the excited-state population decays exclusively with the slow (>1 ps) decay components. Therefore, the argument proposed with the H$^+$-pumping rhodopsins is also applicable to the newly discovered TAT rhodopsin.

In addition, the author discusses the ground-state structural change of the PRSB chromophore in PR, which is associated with the change of the protonation state of the counterion Asp97. The author carried out impulsive stimulated Raman measurements, and the obtained Raman spectra revealed that the hydrogen-out-of-plane (HOOP) wagging modes in the 800–1000 cm^{-1} region is more pronounced when Asp97 is protonated, suggesting a more distorted retinal structure. Such a distorted chromophore structure is interpreted not be optimized for efficient retinal isomerization, giving rise to a lower photoisomerization reactivity when the counterion Asp97 is protonated.

In conclusion, this thesis study clarified that the protonation state of the PRSB counterion plays a decisive role in determining the excited-state dynamics and the photoisomerization reactivity of microbial rhodopsins in general. Although the excited-state relaxation dynamics in different rhodopsins have been explained with difference in the branching of the relaxation pathway on the S$_1$ PES, this study unambiguously showed that the difference, in fact, originates from the difference in the structure in the ground state of microbial rhodopsin, i.e., difference in the protonation state of the PRSB counterion. The present study provides a new, coherent view about the primary photoreaction in retinal proteins.

References
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