An Active Photoreceptor Intermediate Revealed by In Situ Photoirradiated Solid-State NMR Spectroscopy

Yuya Tomonaga,* Tetsurou Hidaka,* Izuru Kawamura,* Takudo Nishio,* Kazuhiro Ohsawa,* Takashi Okitsu,[†] Akimori Wada, [†] Yuki Sudo,[‡⊥] Naoki Kamo,[¶] Ayyalusamy Ramamoorthy,[§] and Akira Naito*

*Graduate School of Engineering, Yokohama National University, 79-5 Tokiwadai, Hodogaya-ku, Yokohama 240-8501, Japan; [†]Department of Organic Chemistry for Life Science, Kobe Pharmaceutical University, 4-19-1, Motoyamakita-machi, Higashinada, Kobe 658-8558, Japan; [‡]Graduate School of Science, Nagoya University, Chikusa-ku, Nagoya 464-8602, Japan; ⊥PRESTO, Japan Science Technology Agency (JST), 4-1-8 Honcho Kawaguchi, Saitama 332-0012, Japan, [¶]College of Pharmaceutical Science, Matsuyama University, 4-2 Bunkyo-cho, Matsuyama 790-8578, Japan; [§]Biophysics and Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1055, USA

Supporting Material

Materials and Method

[15-^{13}C, 20-^{13}C]retinal-*p*pR with a His-Tag (6xHis) at the C-terminal was expressed in *Escherichia coli* BL21(DE3) strain in M9 medium by induction with 1 mM isopropyl-1-thio-β-D-galactoside (IPTG) and 10 μM [15-^{13}C, 20-^{13}C] retinal. This protein was solubilized by using n-dodecyl-β-D-maltoside (DDM) and purified with a Ni-NTA column (QIAGEN, Hilden, Germany) as described previously (S1). The truncated transducer, *p*HtrII(1-159), was prepared by using the above method. Purified proteins in DDM micelles were incorporated into a lipid film of egg PC (*p*pR:eggPC molar ratio of 1:30), followed by gently stirring at 4 ºC for overnight. DDM was removed using Bio-Beads (Bio-RAD, Hercules, CA) to yield egg PC bilayers. Reconstituted suspensions were concentrated by centrifugation and suspended in 5 mM 2-[4-(2-Hydroxyethyl-1-piperizinyl) ethanesulfonic acid (HEPES), 10 mM NaCl buffer solution (pH 7). The pelleted samples of *p*pR and a complex of 1:1 molar ratio of *p*pR:*p*HtrII embedded in egg PC bilayers were placed in a 5.0 mm outer diameter (o.d.) zirconia pencil-type rotor for MAS solid-state NMR experiments. To prepare the sample with a long lived M-intermediate, solubilized *p*pR in DDM was concentrated using Amicon Ultra and was exchanged into OG buffer solution (50 mM KCl, 10 mM citrate, 1% non-detergent Sulfo-Betaine (NDSB)-195 and 1% OG) (S2).

We have significantly improved an in situ photo-irradiation system for solid-state NMR measurements under MAS. In situ continuous photo-irradiation was made by an optical fiber from outside the magnet through a tightly sealed piece of cap made of glass rod glued to a zirconia rotor (Fig. S1). Photo-irradiation was performed from the top of the spinner without touching the optical-fiber to the cap of the spinner. In this system, fast MAS experiment is possible under photo-illumination, leading to a high resolution solid-state NMR signal. Thus the MAS frequency was set to 4 kHz. Tip of the glass was ground to irradiate the light to random directions including the perpendicular direction to the glass rod. This is important for light to penetrate into the sample whose absorbance is quite large. Using CMX-400 infinity NMR spectrometer equipped with this photo-irradiation system, we could successfully irradiate green laser light (532 nm) of 5 mW to the sample in the rotor.

In the in situ solid state NMR measurements, 100 μL (50 mg) of *p*pR-Egg PC and *p*pR/*p*HtrII-Egg PC samples were put into the zirconia rotor and sealed with the glass rod as a cap. CP pulse sequence was used with the 1 ms of contact time followed by the acquisition with 50 kHz of TPPM proton decoupling pulses (S3). MAS frequency was

set to 4 kHz and the temperature was set to 0 and -20 ºC by using gas flow system. Typically, 20000 transients were accumulated for the dark and light experiments.

Photo irradiated NMR experiments have been reported by several groups. Most of the experiments have been done the photo illumination outside of the probe and freeze the sample to measure the trapped photo activated state in the probe (S4,S5). In situ photo illuminated solid state NMR system has been reported by illuminating the light by leading the light through photo fiber to the probe and illuminate the light from outside the rotor tube (S6,S7). On the other hand, in this present system, light illumination has been done from the inside the rotor tube though glass rod inserted into the rotor. This illumination system has been developed in the first time and allows us to illuminate the light to the sample with extremely high efficiency.

References

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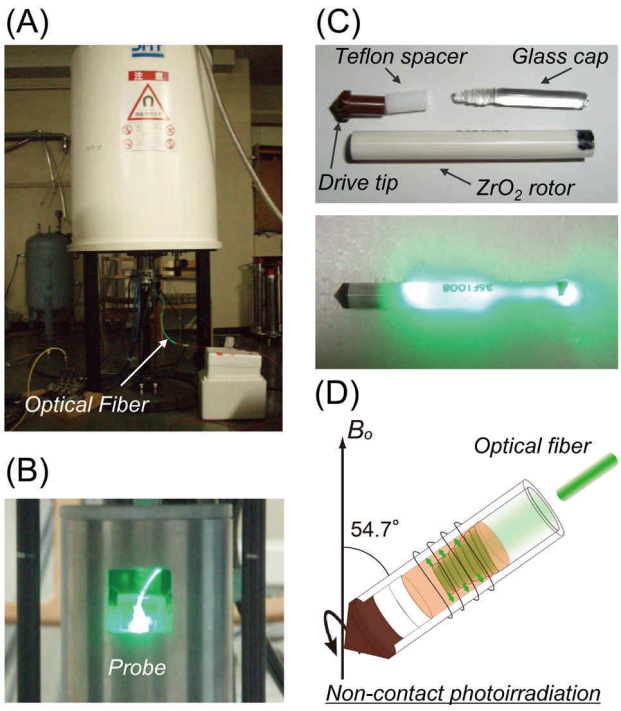

Figure S1. In situ photoirradiated solid-state NMR spectrometer. (A) An optical fiber is guided from outside of the magnet and connected to green laser (532 nm, 5 mW). (B) An optical fiber is guided from the bottom to the top of the probe head and accurately aligned to the top of the MAS rotor. (C) Top part of zirconia rotor is capped by a glass rod whose tip part is ground to irradiate the light perpendicularly to the spinner axis, so that the strong light is illuminated in the center part of the spinner. Specifically, light irradiates the sample from inside of the sample tube. (D) An optical fiber is guided from the outside of the magnet and illuminates the light to the top part of the spinning rotor. The light is penetrated from the top part of the spinner through an aligned optical fiber into the zirconia sample tube without any contact with the optical fiber, so that the rapid spinning was successfully established.

Results

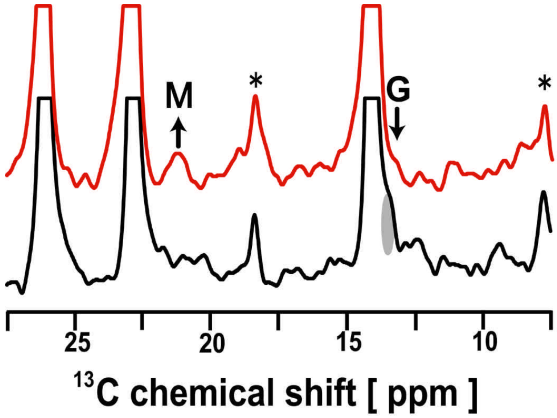

Figure S2. ^{13}C DD-MAS NMR spectra of [15-^{13}C, 20-^{13}C]retinal-*p*pR in OG. Photo irradiated state (top, red). Ground state (bottom, black). The ^{13}C NMR signals from NDSB are marked by *. The peaks M and G indicate ^{13}C NMR signals from [20-^{13}C]retinal in the M-intermediate and the G-state, respectively.

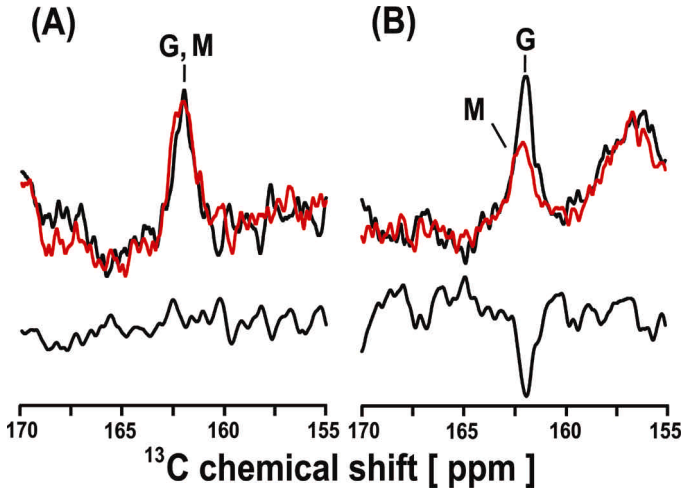

Figure S3 ^{13}C CP-MAS NMR spectra of [20-^{13}C, 15-^{13}C]retinal-*p*pR (A) and *p*pR/*p*HtrII complex (B) taken at -20 ºC. Top spectra were obtained from the light (red) and dark (black) states of *p*pR and *p*pR/*p*HtrII complex. The bottom spectra show the difference between those of light (red) and dark (black) spectra. The peaks M and G indicate the ^{13}C NMR signals of [15-^{13}C]retinal area in the M-intermediate and the G-state, respectively.

Table S1: Chemical shift values of [20-^{13}C, 15-^{13}C]]retinal-proteins

Retinal Proteins	G-state (ppm)[1)]		M-intermediate (ppm)[1)]	
	20-^{13}C	15-^{13}C	20-^{13}C	15-^{13}C
Retinylidenpropylamine[2)]	14.3 (all-*trans*)		22.2 (13-*cis*)	
*p*pR (0 ºC)	13.3		22.3	
*p*pR (-20 ºC)	13.5	162.0	24.1, 22.5, 21.7	162.0
*p*pR/*p*HtrII (0 ºC)	13.6		22.7	
*p*pR/*p*HtrII (-20 ºC)	13.5	162.0	23.5, 22.3, 21.3	162.0
*p*pR in OG	13.4		21.6	
T204A	14.0	160.7	21.3	161.6
BR[3)]	13.1 (all-*trans*)	160.0 (all-*trans*)	21.5	165.4

1) As referenced to TMS.

2) Harbison et al. *Biochemistry,* **1984**, *23*, 2662-2667.

3) Bajaj et al. *Proc. Natl Acad. Sci*. **2009**, *106*, 9244-9249.