Light Harvesting Dynamics in Gloeobacter Rhodopsin (GR)

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Abstract: GR is directly shown by ultrafast pump-probe measurements to bind the carotenoid Salinixanthin, which acts as an efficient light harvesting antenna. Along with Xanthorhodopsin, This proves light harvesting to be a prevalent strategy in retinal proteins.
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1. Introduction

Retinal Proteins (RP) are thought of as single chromophore molecular machines where retinal functions both for light harvesting (LH) and processing. Recent studies reveal that, as in chlorophyll based photosynthetic complexes, the microbial proton pump Xanthorhodopsin (XR) includes an associated carotenoid antenna which efficiently harvests blue-green light. In addition, conservation of the carotenoid binding site in a variety of newly discovered microbial RPs suggests that this strategy for more efficient LH is not a curiosity but a general trend. To prove this GR, expressed from a sequence in *Gloeobacter violaceus*, was shown capable of binding salinixanthin (SX), and by fluorescence measurements suggested to act as an antenna assisted proton pump. Here we complete this investigation by directly measuring transient absorption spectra with ultrafast time resolution and photoselective probing, following excitation either of the carotenoid antenna, or the retinal moiety. Results show not only that GR acts as an antenna assisted proton pump, but that it does so with higher energy transfer efficiency than that in XR.

2. Experimental methods

GR was extracted from *G. violaceus* and expressed in *E. coli* using previously published methods and was reconstituted with Salinixanthin.[1] To ascertain the fraction of energy transfer from carotenoid to retinal, the latter was reduced in order to inhibit energy transfer. The energy transfer process was studied using visible pump and visible and near IR probe spectroscopy. The pump pulses were generated by a TOPAS (light conversion) and white light probe generated in 2 mm of Sapphire was read out on multichannel detection systems. Sub 10 fs time resolution experiments were performed using a pump and probe generated by a one stage non collinear parametric amplifier (NOPA). The 70 nm FWHM NOPA pulses were centered at ~ 560 nm and compressed with a deformable mirror - prism pair combo [2].

3. Results and Discussion

Without an associated carotenoid, GR exhibits photochemical dynamics reminiscent of proteorhodopsin involving bi-exponential internal conversion and all trans to 13 cis isomerization of retinal with 500 fs and 2.5 ps decay constants. The absorption band of GR peaks at 550 nm, partially overlapping the S_0-S_2 absorption band of SX. The S_0-S_1 absorption of the carotenoid occurs at 1150 nm, making S_2 the only state energetic enough to act as donor to the retinal. Accordingly, to determine the efficiency of energy transfer from XR to retinal, the S_2 lifetime was measured in GR reconstituted with SX (GRS), and in a GRS where the retinal conjugation length has been shortened by double bond reduction (RGR). This shifts the retinal absorption to the near UV, and prohibits energy transfer with minimal disruption of protein structure.

Figure 1A presents the transient absorption spectra following SX excitation for both samples in the form of evolution associated difference spectra (EADS) obtained by fitting to a sequential kinetic model. The short lived initial red EADS is assigned to S_2. It consists of carotenoid ground state bleach, its matching hot stimulated emission, and a rising excited state absorption at the red edge. The lifetime assigned to this state in GRS increases from ~80 to 120 fs upon retinal reduction, as expected if it blocks an effective route of energy transfer. In order to corroborate this, two additional experiments were done. In view of the intense S_2 absorption at ~1200 nm, the same kinetics were covered using near IR probing with InGaAs array spectrograph. The results are presented in panels B and C. B presents EADS as in A, but with fewer intermediates since later stages of evolution are not apparent in the NIR. Again the S_2 EADS shifts in lifetime from 83 to 133 upon retinal reduction. This is graphically depicted in Fig

1c, for both GRS and RGR. Finally, ~6 fsec NOPA pulses were used to measure the same dynamics with superior resolution, producing very similar S_2 lifetimes.

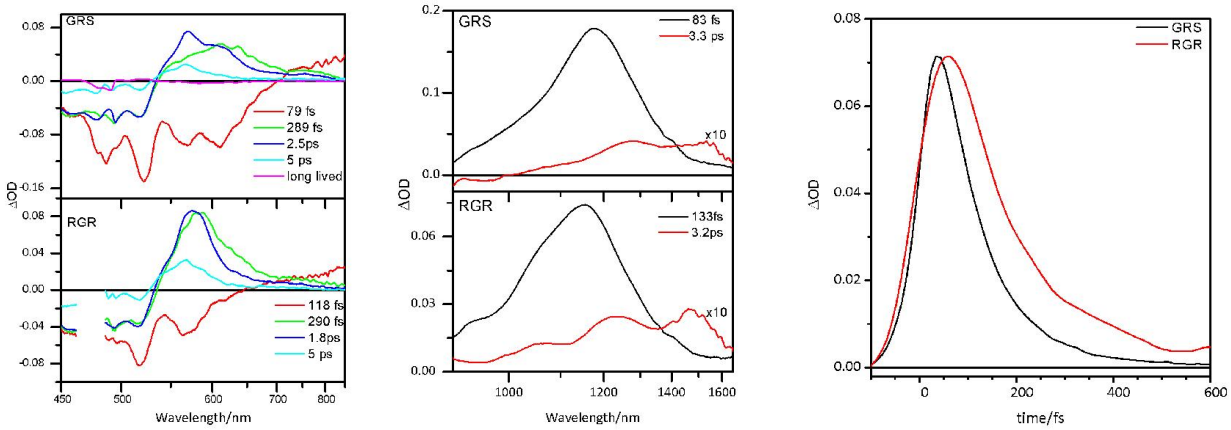

Fig 1: EADS of GR-salinixanthin conjugate with retinal (GRS) and reduced retinal (RGR) using (a) Visible probe and (b) IR probe measurements. The spectrum of the long lived S_1 state of salinixanthin in (b) magnified by 10 times for clarity. (c) Kinetic traces of GRS and RGR at 1150 nm.

The major conclusion of all these measurements is that efficient energy transfer takes place from carotenoid to the retinal in GR. From the lifetime of the S_2 state the quantum efficiency of energy transfer is evaluated to be 0.38, even higher than that in XR, and a rate of energy transfer is evaluated to be 4.4×10^{-3} fs^{-1}[2, 3]. This conclusion was also corroborated by measuring rhodopsin photocycle intermedates populations following excitation of the carotenoid leading to similar estimates for energy transfer efficiency. The importance of this finding is a realization that carotenoid light harvesting, prevalent in "green" photosynthetic complexes, is a substantial strategy in microbial RPs as well. This is likely not limited to the two proteins discussed here alone, since many proteorhodopsins for instance also have protein sequences which can anchor carotenoid antennae [4]. Finer details of our experiments point to additional similarities of XR and GRS dynamics, including an unexplained reduction of polarization ratio of the carotenoid absorption upon internal conversion of the SX from S_2 to S_1. The reduced ratio indicates the ultrafast development of an angle between the transition dipoles of the S_1 and S_2 during the radiationless relaxation. Future work will be required to fully reveal not only the prevalence of carotenoid light harvesting in other RPs, but to identify the organisms which harbor them and the degree of their expression in those hosts.

4. References

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