Structural Changes of Cephalopod Rhodopsin and β-Arrestin Measured by FTIR Difference Spectroscopy and Isotope Editing

Joel M Kralj[1], Erica Raber[1], Jose Sarmiento[2], David Shumate[2], Christie Stanzel[2], Carrie Maxwell[2], Javier Navarro[2] and Kenneth J Rothschild[1]

[1]Department of Physics, Molecular Biophysics Laboratory, Photonics Center, Boston University, Boston, Massachusetts 02215, USA
[2]Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, Texas 77555, USA

Abstract:

Invertebrate rhodopsin is the primary photoreceptor found in the eyes of cephalopods. Unlike vertebrate rhodopsin, invertebrate rhodopsins such as sepia rhodopsin (s-Rh) can be activated by light and then rapidly cycled back to the original state with a second red-shifted photon, thereby facilitating a variety of novel biophysical studies. Additionally, invertebrate rhodopsins can bind to the ubiquitous β-Arrestin2 which is involved in regulating signal transduction in many GCPRs. In this study, we used static and time-resolved FTIR difference spectroscopy to investigate the photocycle of s-Rh complexed to β-Arrestin2. In the spectrum of s-Rh alone, difference spectra obtained using two colors to cycle between the ground state (rho) and acid meta state show an 11-*cis* to all-*trans*-photocycle as previously described. Additionally, several bands between 1750-1700 cm^{-1} are assigned using D/H exchange induced shift to as yet unknown carboxyl groups. Other large bands are seen especially in the amide I and II regions which indicate significant backbone structural changes. Upon addition of β-Arrestin2, the difference spectrum is altered, especially in the amide I and II regions, reflecting additional structural changes occurring in β-Arrestin2 upon photoactivation. A negative band at 1742 cm^{-1} was shifted to higher frequency in the complex indicating the β-Arrestin2 is perturbing at least one carboxyl group in s-Rh. In order to assign these changes, total 15N isotope labeling of β-Arrestin2 was utilized. Comparison of difference spectra from s-Rh complexes containing unlabelled and 15N labeled β-Arrestin2 reveals ~3 cm^{-1} downshift of a negative/positive feature at 1668/1655 cm^{-1} indicating that these bands reflect at least partially conformational changes of the β–arrestin involving α-helical structure. These results offer a promising new tool to investigate the molecular mechanism of β-Arrestin interactions with GCPRs regulating downstream signaling.

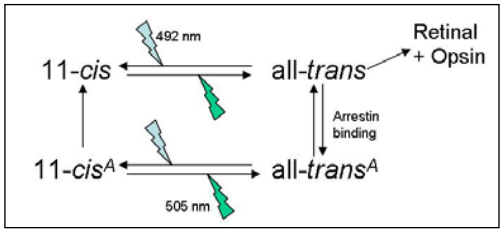


Figure 2 – Proposed scheme for activation and retinal binding. 11-*cis* sepia rhodopsin absorbs a photon at 492 nm and isomerizes to the all-*trans* signaling state. This protein can dissociate on the order of hours, or a second, 505 nm photon can reset to the ground state. The all-*trans* state can also bind a β-arrestin which undergoes a similar isomerization scheme.

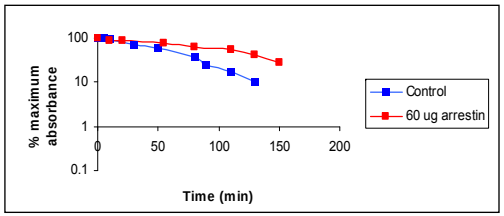


Figure 3 – Photobleaching dynamics of sepia rhodopsin and rhodopsin arrestin complexes. Mixtures of the two protein result in significantly less photobleaching indicting that a more stable complex is formed between the rhodopsin and arrestin.

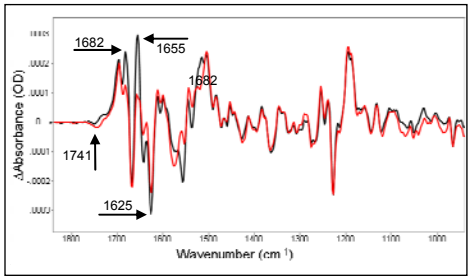
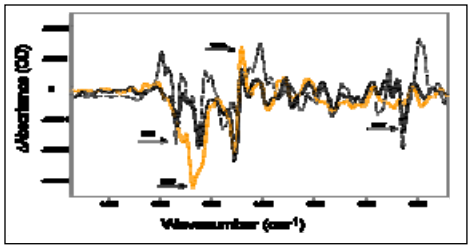


Figure 4 – Static FTIR difference spectrum of sepia rhodopsin (black) and sepia rhodopsin + arrestin (red). Overall the two are very similar as expected, yet there are major differences in the Amide I and II regions sensitive to protein backbone vibrations. Additionally, a carboxylic acid group undergoes a slight change in environment due to the effects of the arrestin.



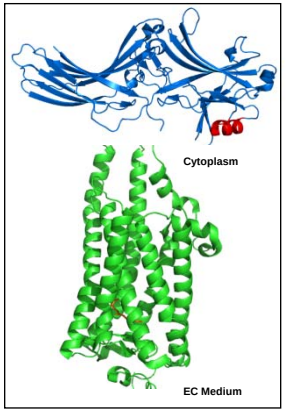


Figure 1 – Crystal structure of β-arrestin (1G4R, blue)[1] and squid rhodopsin (2Z73, green)[2]. The alpha helix of arrestin is highlighted in red. A photon is absorbed by the retinal in the rhodopsin and the signal is relayed through the cytoplasmic loops to activate the G-protein pathway. Bound arrestin inhibits the transmission of the signal.

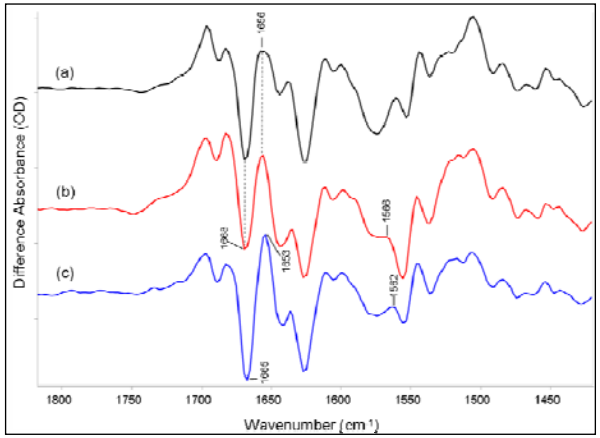


Figure 5 (above) – Time resolved rapid scan FTIR difference spectra averaged (5-35 ms – black) and (35-100 ms – orange). Structural changes are clearly evident as a function of time in invertebrate as can be deduced from changes in the difference spectra. This approach demonstrates the ability to apply time-resolved FTIR using a photocycle technique to invertebrate rhodopsin.

Figure 6 – FTIR difference spectra of sepia rhodopsin (black), sepia rhodopsin + arrestin (red), and sepia rhodopsin + ^{15}N total labeled arrestin (blue). Bands at 1668 and 1656 cm^{-1} shift by ~ 3 cm^{-1} in the labeled arrestin sample. This confirms that these bands are due at least in part to changes of arrestin during the photoactivation. Since it's known that different conformations of arrestin can trigger different pathways, this could be an important mechanism used by the sepia to control both visual feedback as well as other processes.

Conclusions:

- Sepia rhodopsin shows altered behavior in the presence of arrestin as evidenced by the rate of photobleaching.
- The presence of arrestin can be detected in the FTIR difference spectrum mainly in the amide I and II regions which are sensitive to protein backbone vibrations
- Labeling just the arrestin with ^{15}N total isotope results in a shift of difference bands indicating that the arrestin is undergoing a conformational change during the rhodopsin two photon photocycle.
- The frequencies of the arrestin bands that change indicate it reflects alpha-helical structure undergoing changes, consistent with the alpha-helix anchor model.

References:
1. Murakami, et al., Nature, 453 (7193) 2008
2. Sutton, et al., Journal of Molecular biology, 354 (5) 2005
3. Gurevich, Handbook Exp Pharmacology, (186) 2008

This work was supported by National Institutes of Health grants R01GM069969 (to K. J. R.).