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Primary Photophysical and Photochemical Processes in Visual Excitation*

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Abstract. The color of visual pigments is experimentally shown to be controlled by excited state effects. These effects which define the primary absorption of light by rhodopsin are considered together with results obtained from emission and picosecond spectroscopy. In addition, the molecular changes induced in rhodopsin when a photon is absorbed are analyzed using resonance Raman spectroscopy. The molecular changes observed are compared in bacterial and photoreceptor rhodopsins. This comparison yields a unique explanation for the biological role of the cis-trans isomerization in visual transduction.

Key words: Visual pigment color – Energy transduction – M_{412} trans-biological role Cis-trans isomerization – Resonance Raman – Emission spectroscopy.

This short communication summarizes my discussion presentations at the EMBO-Workshop on transduction mechanisms in photoreceptors. It reviews results we have obtained on rhodopsin and bacteriorhodopsin using various experimental techniques which are based on the unique properties of laser radiation. The techniques we have used include resonance Raman spectroscopy, emission spectroscopy and subpicosecond and picosecond spectroscopy. The reader is cautioned that this summary is in no sense complete and includes some results that are in the process of being published. Reference should be made to these publications as they appear for further information.

The Energetics of Visual Excitation

When rhodopsin absorbs light it stores a significant fraction of the photon's energy in the primary photochemical product called batho-rhodopsin. As we have suggested

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[1, 2] experiments on iodopsin and bacteriorhodopsin (which have thermal transitions from batho-iodopsin and batho-bacteriorhodopsin back to iodopsin and bacteriorhodopsin, respectively) support this description of the energetics of visual excitation. Our applications of various forms of laser spectroscopy to investigate rhodopsin and bacteriorhodopsin are aimed at elucidating the molecular mechanism which is used to store this light energy and the use which is made of the stored energy in visual transduction.

Experiments on Rhodopsin and Bacteriorhodopsin

Resonance Raman experiments on all rhodopsins studied have indicated that the Schiff base of the retinylidene chromophore is initially protonated [3–12] and can be deuterated [6, 9, 12]. Furthermore, recent experiments have shown that the *ground state* resonance Raman spectrum of the retinylidene chromophore in opsin can be modelled with the resonance Raman spectrum of the free chromophore. These experiments indicate [12] that the color of visual pigments results from stabilization of the excited state of retinal by charges/dipoles on the protein.

Emission spectra [13–15] suggest that light absorbed by the retinylidene chromophore enters an allowed excited state and then evolves into the batho form and a forbidden region of the excited state surface. This is deduced from the observed emission life time of $\geqslant$ 15 psecs (depending on the extent of exciton annihilation phenomena) [13, 14] and the rise time of the photochemical product in $<$ 6 psecs [16, 19, 20].

Resonance Raman Studies of Batho Rhodopsin – The Primary Photochemical Product

Resonance Raman studies of the batho intermediate in several rhodopsins indicate that the $-\text{C}=\overset{\text{H}}{\underset{+}{\text{N}}}-$ stretching vibration does not vary in going from rhodopsin to bathorhodopsin [6, 17, 18] and this suggests that there is still a double bond between C_{15} and N, and a positive charge on the N after the photonic event. Furthermore, the C-CH_3 vibration indicates that in bathorhodopsin there are changes in the vicinity of the CH_3 groups [1, 17, 18], and it is interesting to note that these carbons are tertiary centers which can stabilize charge. In addition, even though the $-\text{C}=\overset{\text{H}}{\underset{+}{\text{N}}}-$ vibration does not appear to be affected by the light event the C=C vibrational mode is reduced in frequency [17]. This can be interpreted [17] in terms of a model for the structure of batho that considers this intermediate as a state in which charge is polarized [1, 17]. In the intermediates that follow the charge polarized, high energy batho form there appears to be a relaxation of the polarized state and relocalization of the electron density in the polyene. The concluding event of this electron polarization and relocalization process is the release of the Schiff

base proton which is detected by a reduction in the C=N vibrational frequency [1, 6, 21]. The deprotonation of the Schiff base nitrogen appears to be correlated with the generation of a proton gradient in bacteriorhodopsin and the generation of a neural response in vertebrates. Thus it appears that release of the Schiff base proton may be critically tied to the role rhodopsin plays in energy transduction.

The Biological Role of the 11-Cis-to-Trans Isomerization

The deprotonation of the protonated Schiff base occurs in all rhodopsins we have studied. In bacteriorhodopsin this deprotonation occurs apparently without a formal cis-trans isomerization as we have recently shown [22] by demonstrating that M_{412} is also in the all-trans conformation. However the biological roles of bacteriorhodopsin and photoreceptor rhodopsins are completely different. Bacteriorhodopsin is a light driven proton pump while the role of vertebrate and invertebrate rhodopsins is essentially that of a quantum detector. In order to have a good quantum detector there must be irreversibility and we believe this is the role of cis-trans isomerization in photoreceptor rhodopsins. On the other hand, in order to have a good energy converter, such as bacteriorhodopsin, endoenergetic conformational changes have to be minimized and reversibility has to be maximized [1]. Thus the bacteria have evolved a molecule which can accomplish the release of the Schiff base proton reversibly without major changes in the conformation of the retinylidene chromophore and this enhances the biological role of the bacteriorhodopsin to convert light energy into chemical energy. In so doing it sacrifices the high quantum efficiency ($\sim 65\%$) of photoreceptor rhodopsins by approximately a factor of two.

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