Photo-Initiated Processes in Vision

Final Report

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The project "Photo Initiated Processes in Vision" began in 1966 with the intent of investigating the early event in visual excitation. We proposed an examination of rhodopsin, retinal, and retinal Schiff bases under illumination by various spectroscopic techniques including electron spin resonance. The object of this study was to gather enough information to allow us to create a model for the generation of the early receptor potential (1) and hence the subsequent electrical action of the visual cell.

Much of the proposed work was based on the early observations of ESR signals from retinal materials (2,3,4). Our repeated effort, however, to observe an ESR signal failed to produce anything that could unequivocally be linked to rhodopsin. We observed from rod outer segments a signal due to Cu^{++} , but were unable to show that the copper was a definite part of the rod itself and not simply an artifact of the extraction procedure. Copper is present in other layers of cells in the retina and upon disruption of the retina it may become free to bind to other sites particularly free amino groups. In this situation rods would always be extracted with bound copper. If the extractions were carried out with a metal scavenger such as EDTA we could not be sure that if the copper was bound to the rods it would not be exchanged away. Some effort was made to show that rhodopsin did have at least one strong copper binding site (5) and that extensive washing with distilled water would leach out any bound copper. Thus the more care taken in the extraction of the rhosopsin the greater the chance of obtaining copper free material.

At times another light dependent ESR signal was detected from illuminated whole retinas and at times in rod preparations. We felt this clearly was due to remnants of the pigment epitheleum which contains quinones and was not due to any structure containing only rhodopsin.

We then turned our attention to the possibility of observing an ESR signal from a retinal based chromophore. Thus we attempted to produce free radicals from vitamin A aldehyde itself, the alcohol, the N-retinylidene Schiff bases, and a series of Schiff base model compounds.

From our studies of radical formation in retinal and the N-retinylidene Schiff bases, by electrolysis, we showed that well resolved ESR signals could be elicited from anions of these materials. We were not able to observe signals from cations, however. Thus the failure to observe an ESR signal from the biological sample either in the dark or in light could not be due to a broadening phenomenon. Rather we could assume that if electron transfer takes place in responses to light in rhodopsin an ESR signal is not observed for one of the following reasons:

- 1) The separated electrons remain coupled as a singlet state.
- or 2) the lifetime of transfer, even at liquid N₂ temperatures is short and no measurable population of radicals is maintained,
- or 3) the separated electrons couple as a triplet and this is not easy to observe at the half field region.

With regard to the latter point, it has been reported that half field signals have been observed from illuminated rhodopsin (4) although our efforts were negative.

One of the reasons for postulating radical participation or some kind of electron pair separation in the initial event of vision is that the quantum efficiency of isomerization of the 11-cis chromophore in rhodopsin is quite high i.e. ~.64 (6,7) while that for retinal is rather low (7). However, our work with the protonated 11-cis retinylidene Schiff base shows that at room temperature the quantum efficiency of isomerization is between .3 and .4.

It is also well known that the extinction coefficient of the 11-cis isomer is temperature dependent i.e. at room temperatures $\epsilon = 27,000$ and increases at low T (8). This does not happen with the native visual pigment since its extinction is high at all temperatures and we know it has the 11-cis isomeric form. Thus if we simply postulate that the quantum efficiency of isomerization is proportional to the extinction coefficient then in rhodopsin we should have a $Q \approx .5$ simply due to the absorption of more light by rhodopsin than does the retinal Schiff base. Thus nothing more need be postulated and it becomes clear that the protonated Schiff base (11-cis) at low temperatures is a very good model for the visual pigment at least from the spectroscopic viewpoint.

In work centered on the possible use of fluorescence as an indication of structural changes near the visual pigment chromophore we surveyed the emission properties of rhodopsin. Such work (9,10) produced the emission spectrum of most of the intermediates in the bleaching sequence of rhodopsin (11). Further the triplet level of retinal itself was postulated by indirect evidence to be near 38 kcal/mole and this was verified later by a direct measurement (12). Thus basic spectroscopic data collection was a part of this work.

While investigating the fluorescence of visual materials, numerous studies were carried out using intact frog retinas. It was found that a distinct emission change in a lifetime of several msec. was observed after flashing of an intact retina. We concluded after extensive analysis that we were probably not watching a direct process i.e. of the photo induced decrease in the substance that was fluorescing, rather the increase in the meta II concentration caused by illumination was effectively blocking by absorption the underlying, background fluorescence (in the 350-360 nm, region) of various protein material. Thus our kinetics were possibly related to the generation

of meta II rhodopsin not the decay of any other material. This work was not pursued.

While investigating model Schiff base systems we began to analyze the E.S.R. spectra of a number of dieneones. In particular the E.S.R. spectra of α -ionone, β -ionone and 5,6-dihydro- β -ionone were analyzed. The spin densities in radicals of these compounds were predicted using Hückel theory and the non-planarity of β -ionone (useful as a model for the vitamin A-aldehyde non-planar system) was satisfactorily described by allowing a resonance integral in the theory to vary as a function of twist angle. The experimental data then combined with theory allowed us to calculate the angle of twist to be nearly 40° in the radical state. Presumably this would also apply to the vitamin A aldehyde radical also. The angle had been calculated for the neutral vitamin A aldehyde by several workers and their values were near $47-48^\circ$ (14,15). Thus features of the structure of vitamin A were found in this research.

Finally our latest work centered on the possibility of using spin labels to indicate changes in environment in illuminated visual materials. We could not find clear indications of environmental changes subsequent to a flash of light yet there were distinct changes in the label signals. We found that once oxygen was excluded from our systems, the photoreduction of the nitroxide label could be sensitized by a number of carbonyl containing compounds. The mechanisms involved energy transfer from the excited carbonyl to the spin label via a singlet-doublet transfer. This was followed by self reduction of the excited label into a hydroxylamine like ground state molecule. Our results with a series of donor molecules did indicate such transfer was taking place and the efficiency of transfer (as expected) was a function of the properties of the label $^2(n-\pi^*)$ state involved and the $^1,3(n-\pi^*)$ or $^1,3(\pi-\pi^*)$ nature of the donor. This work is being prepared for publication at this time.

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