MASTER

Technical Progress Report

for

Photo-Initiated Processes in Vision

for the period November 15, 1971 to November 14, 1972

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The substitute director of this program Dr. Robert White spent approximately 50% of his time on this project and aided in the direction of Mr. Rodger Raubach's work. From June 1, 1972 to the time of submission of this report A. Guzzo has spent 100% of his time on the project. He will spend approximately 50% of his time on the project until November 14, 1972.

I. Completed Projects

Photoisomerization Pathways in the Visually Important Polyenes

In our study of the isomeric retinals (all-trans, 11-cis, and 13-cis) and of the protonated Schiff bases of the retinals we have, within the limits of our assumptions, determined the main pathways for isomerization. The studies are reported on in detail in COO-1627-27 and COO-1627-28. Briefly we found, through sensitization techniques, that the all-trans and 13-cis isomers of retinal isomerize with only a low quantum efficiency through the triplet state. This observation combined with the measured intersystem crossing yield would assert that the main pathway for the direct isomerization of these isomers is through the singlet state. On the other hand, 11-cis retinal has a high isomerization efficiency (~80%) through the triplet. Therefore we conclude that the singlet and triplet states participate almost equally in the direct photoisomerization process for this isomer.

In the protonated Schiff bases the situation is somewhat different. For this class of compounds no singlet ($n-\pi^*$) state exists beneath the first excited singlet ($\pi-\pi^*$) state, therefore intersystem crossing is reduced. Nevertheless, we find for the 11-cis N-retinylidene protonated Schiff base a yield of direct photoisomerization of approximately 0.3. This low yield would appear to be insufficient to amount for the high yield of transformation of rhodopsin to prelumirhodopsin (0.5 to 0.6). However, as we argue below it is of the correct magnitude if we consider the variable extinction coefficient of the 11-cis isomer.

It is well known that the extinction coefficient of 11-cis retinal and the Schiff base prepared from 11-cis retinal is temperature dependent. At liquid nitrogen temperatures the value is almost double that at room temperatures, at least for the aldehyde. Further it is known that once incorporated into rhodopsin, the 11-cis chromophore no longer exhibits this temperature dependence and the extinction is at its maximum value. Thus the protein must constrain the polyene chain and remove certain degrees of freedom in much the same way that a lower temperature does.

We may postulate then that the extinction coefficient for the 11-cis-N-retinylidene protonated Schiff base would rise once it is incorporated into the protein. If this occurred the quantum yield of the isomerization would rise proportionately and become consistent with the measured yield in rhodopsin. We conclude then that the 11-cis protonated Schiff base is an excellent spectroscopic model for the rhodopsin chromophore.

II. Continuation Studies.

1. Metal Binding Studies in Rhodopsin

In a previous report we stated that our experiments had shown that copper was present in high concentration in the retina and further that upon only minimum washing, copper was found bound to rhodopsin in amounts that seemed to approach a 1:1 ratio. Experiments aimed at adding copper back to highly purified rhodopsin seemed to confirm the existence of this unique binding site.

Some further work by R. Moore has shown much the same situation. However, upon close examination of the first study (a Master's degree

project of Mrs. Nui Cheng) I discovered that the standards prepared for this work did not agree with each other. Therefore all of this work is cost in doubt. Clearly copper is bound but whether a unique binding site exists or not can not be verified. We again must report this study.

2. Light Induced Changes in the Rhodopsin Protein Fluorescence.

Our work attempting to measure the rapid protein fluorescence change induced by a flash of light was successful. It is described in detail in COO-1627-26. We have proposed to extend this work now to quantitative measurements on the frog retina. Further we will attempt to measure the absorption change in the same visual system in order to see if the effect is identical to the appearance of meta II rhodopsin or to the disappearance of meta I rhodopsin. Further work will of course, be determined by our findings on these points.