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TECHNICAL PROGRESS REPORT

MASTER

for

Photo-initiated Processes in Vision¹

For the Period November 14, 1969 to November 15, 1970

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The Principal Investigator(A.Guzzo) has spent approximately 50% of his time on the project and will continue to do so for the remainder of the contract year.

I. DYNAMICS OF THE PROTEIN REGION AUTOFLUORESCENCE OF RETINAL COMPONENTS

In the past years renewal proposal we planned experiments aimed at finding the time course of the protein fluorescence change reported previously. The preprint COO-1627-12 documents our latest findings and was submitted to Photochem. Photobiol.. Several good points were brought up by the referees and work is now in progress to clarify these points.

Their criticisms were

- 1) The emission maximum at 365nm seems to red shifted to be characteristic of protein alone.
- 2) The noise level in Figure 4. of the preprint is too high to allow the time constant to be measured to the accuracy we claim.
- 3). The correlation of the fluorescence change to the meta I - meta II change should be supported by absorption kinetics.

Polarization Studies

In investigating the protein fluorescence further we have measured the polarization of the emission. In numerous studies we have found that the polarization increases about 30% upon flash illuminating the pigment side of the retina, and not at all if the neural side is illuminated.

These observations can be interpreted in two ways;

- 1) Since we are very likely measuring the emission from tryptophyl residues associated with the opsin part of the pigment we may conclude that the small polarization initially measured is due to rapid rotation of the emitting groups.

Upon illumination, a structural change takes place that imposes restrictions on the movement of the emitting groups. The higher polarization observed would result.

2) The same experimental observations can be rationalized by assuming extensive energy transfer between tryptophyl groups before bleaching and little transfer after. This would also be the case if a structural change altered the proximity of the emitting groups.

We may distinguish between these alternative explanations by carrying out the polarization - bleaching experiment at some low temperature. We may be able to find conditions that will prevent gross molecular structural changes but still restrict rotational motion. We may thus be able to identify the kind of motion that results in the polarization increase.

II. TRIPLET STATE STUDIES

In COO-1627-13 it was reported that the triplet level of all-trans retinal appeared to be located 38 kcal. above the ground state. This measurement was rather indirect in that energy transfer techniques were used to detect the level. We have now measured this quantity directly using the oxygen perturbation technique in absorption spectroscopy. The triplet level was very clearly observed and vibrational fine structure can be discerned. This is discussed somewhat further in the attached preprint(COO-1627-14). If our conclusions are correct then the two values obtained for the triplet level in all-trans retinal are in good agreement.

Model Compounds

In order to pursue our studies on the effect of methyl group hyperconjugation on triplet levels, especially in

compounds related to the vitamin A structure we have synthesized or obtained the following series

Some preliminary spectroscopic work has been carried out on these systems. In the renewal proposal some of the impetus behind these studies is discussed.

III. COPPER CONTENT AND BINDING CAPACITY OF RHODOPSIN

Several years back we discussed our preliminary work which indicated the possibility of rhodopsin being a metal protein complex. Mrs. Nui-Oi Cheng has completed a Master's degree on this topic and the evidence gathered is much more convincing that rhodopsin is a metal-protein complex.

In the first part of her work she analyzed a number of rhodopsin solutions (in digitonin) for bound copper. This was accomplished by passing the solution through an ionexchange column first and then analyzing for the metal using the Perkin Elmer Atomic Absorption spectrometer. A ratio was computed giving moles of copper to moles of

rhodopsin. Clearly we expected this ratio to be near unity. It was not and in fact was quite small indicating that rhodopsin in the state of purity that we had achieved had only a small contamination of copper. Some further work then indicated that the copper binding site was such that dissociation was occurring in the washing stages of the rhodopsin preparation. We found that after seven(7) washings the ratio R was $.08 \pm .05$. After four(4) washings the ratio measured was $.25 \pm .05$. (We unfortunately do not have similar ratios for samples with still fewer washings).

In a second series of experiments we have taken this relatively pure rhodopsin solution and re-introduced copper. There appear to be several different kinds of binding sites, as would be expected, however, one site is unique. This site appears to have a dissociation constant of approximately 5×10^{-6} which would account for the ease with which copper is lost in the purification procedure.

If we begin with 100% of the rhodopsin complex then after the first washing only about 40% remains as complex. For our conditions this would produce a copper concentration in the wash of 0.2 ppm. A value of .16 ppm was observed. Upon the addition of 1 ppm Cu back to the rhodopsin then nearly 70% should be tied up as the complex.

We must carry this work further and also duplicate the previous experimental findings if this is to reach the publication stage.

IV. ESR STUDY OF MODEL COMPOUNDS RELATED TO VISUAL PIGMENTS

The compounds of greatest interest in this study are the Schiff base analogs of the visual pigments. However, these are complicated molecules and radicals of these are certain to be difficult to discuss because of the large number of interacting centers. Some profit may then be made in the study of less complicated systems.

Of some interest in any of the retinyl compounds is the possible twist about the 6-7 bond. The conjugated double bond system is certainly not planar because of steric hindrances which arise between the ring and the polyene chain. Pullman, Langlet, and Berthod(1969) have shown, via calculations, that two minima exist for twist about the 6-7 bond. In each case the S-cis conformation is preferred.

In the interpretation of the ESR spectrum from such a system, care would have to be taken in considering interactions from protons in the ring. In the case of one of the simplest analogs - β -ionone- we found that the spectrum was impossible to reconcile with the spin densities calculated through simple Huckel theory. A series of calculations were carried out considering various angles of twist about this bond. It was found that for an angle of $50 \pm 5^\circ$ the best fit was obtained.

There are other points concerning the magnitude of the couplings which we will discuss at a later time.

Other systems of interest are anion radicals of α -ionone and 5,6-dihydro- β -ionone. The report COO-1627- 15 describes this work in further detail.

Further we have measured the ESR spectra of the retinal radical anion as well as that of the N-retinylidene - butylamine anion. These spectra have been interpreted and will be described in a subsequent report. Attached is the abstract sheet from C.M. Lang's doctoral thesis which summarizes this recent work.

B.Pullman, J.Langlet, and H.Berthod, J.Theoret.Biol. 23, 492(1969)