FINAL REPORT

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to the

DEPARTMENT OF ENERGY
OFFICE OF BASIC ENERGY SCIENCES

TIME RESOLVED LASER STUDIES OF THE PROTON PUMP
MECHANISM OF BACTERIORHODOPSIN

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I. Summary of Accomplishments

Our research group continued to be productive during the past year in spite of the fact that we were preparing to move by the end of the summer to Georgia Institute of Technology. A total of six publications have resulted from our research last year, appearing in reviewed journals such as Biophysical Journal, Biochemistry, Journal of Physical Chemistry, Advanced Materials, and Pure and Applied Chemistry. Details of the new research accomplished during the last year are set forth below.


Song, Li; Logunov, Stephan L.; Yang, Difei; El-Sayed, M. A.; Biophys. J. (1994), 67(5), 2008-12

The quantum yield of the retinal photoisomerization, the primary step in the Bacteriorhodopsin (bR) photosynthesis, is determined from the picosecond bleach recovery of the absorption at 560 nm in the pH range 6.89 - 11.3. From this and observed dependence of the observed retinal excited state decay on pH, the effect of the pH on both the photoisomerization and internal conversion rates are also determined. The results show that while the quantum yield of photoisomerization is independent of pH, each of the rate constants decreased by more than a factor of 2 in this pH range. This suggests that the perturbation resulting from the pH changes affects both rate constants similarly. The observation is discussed in term of the theoretically proposed potential energy surfaces of the ground and excited states of retinal in bR.

2. "pH Dependence of the Rate and Quantum Yield of the Retinal Photoisomerization in Bacteriorhodopsin"

Logunov, Stephan L.; Song, Li; El-Sayed, Mostafa A.; J. Phys. chem. (1994), 98(42), 10674-7

The pH dependence of the subpicosecond decay of the retinal photoexcited state in bacteriorhodopsin (bR) is determined in the pH range of 6.8 - 11.3. A rapid change in the decay rate of the retinal photoexcited state is observed in the pH range 9 - 10, the same pH range in which a rapid change in the M412 formation kinetics was observed. This observation supports the previously proposed heterogeneity model in which parallel photocycles contribute to the observed dependence of
the M₄₁₂ formation kinetics in bR.

3. "Recording of transient grating using the short lived bacteriorhodopsin photocycle intermediates."
Wu, Shuguang; Braeuchle, Christoph; El-Sayed, Mostafa; Adv. Mater (1993), 5(11), 838042

In this work, we reported the measurement of the diffraction efficiency of a transient grating formed in the bR film based on the earlier K intermediate on the ns time scale. The assessment of the intensity dependence of the grating efficiency was carried out in order to study the mechanism of grating formation. In addition, we also measured the time-resolved transient grating on three different time scales. By comparing these results with the kinetic results obtained from transient absorption measurements, the transient gratings formed on the different time scales were correlated with the formation and decay of the K and M intermediates. These results suggest cd be potentially used as a material for high-speed optical processing and transient storage.

4. "The C-terminus and the calcium low-affinity binding sites in Bacteriorhodopsin."
Zhang, Nancy Yi; El-Sayed, M. A.; Biochemistry (1993), 32(51), 141732-5

Bacteriorhodopsin (bR) is found previously to have two high-affinity and four to six low-affinity Ca²⁺ binding sites. Our previous studies with site-directed mutation suggested that the two high-affinity sites are located within the protein and close to the retinal pocket. In order to investigate the location of the four to six low-affinity binding sites, we studied Ca²⁺ binding to deionized bR as well as its variant in which its C-terminus is removed by papain treatment. Potentiometric titration with Ca²⁺ -selective electrodes were carried out and Scatchard plots were obtained from the titration data. We found that most of the low-affinity sites are eliminated upon removal of the C-terminus in bR. This suggests that the low-affinity sites in bR are on the surface. The involvement of the C-terminus in these sites is discussed.

Section II.1 describes our program on the study of the binding sites of metal cations in bR. In II.2, the problem of how protons are transported from the protonated Schiff base to the extra-cellular surface is discussed and our proposed work is described. In section II.3, the question is raised of how does the protein catalyzes the primary step in bR, i.e. the retinal photoisomerization. Our proposed effort in this direction is outlined.
5. "Binding of, and Energy-Transfer Studies from Retinal to Organic Cations in Regenerated Reduced Bacteriorhodopsin"


Regenerated reduced bacteriorhodopsin, RbR, from deionized RbR (dIRbR) and fluorescent 3,6-diaminoacridine cation (DAA+) is studied as a function of the cation to dIRbR ratio. Using the fluorescence intensity of DAA+, a Scatchard plot was drawn from which two DAA+ are found to be strongly bound while more than three are found to be weakly bound. The retinal in reduced bacteriorhodopsin (RbR) is fluorescent. The fluorescence intensity and decay of the retinal are examined under conditions were there are two strongly bound DAA+ per bR molecule. Assuming that energy transfer between the excited state of the retinal and the DAA+ is responsible for the observed shortening of the retinal excited-state lifetime, the Forster model was used to estimate a donor-acceptor distance of ≤14 Å. A discussion of the possible location of the DAA+ with respect to the retinal is given. A comparison between the organic and metal cation binding is made, and a discussion of the possible location of the metal cations in bR is presented.

6. "Binding Characteristics of an Organometallic Cation, Ru(bpy)32+, in Regenerated Bacteriorhodopsin."


Both metal cations and organic cations are known to regenerate the purple color and function of bR from its blue deionized nonfunctional form. To understand the role of cations in the function of bR, the difference between the binding properties of organic and metal cations needs to be determined. In this work, the binding constants and the number of Ru(bpy)32+ ions in each site in regenerated bR are determined from Scatchard plots by using fluorescence measurements in the 3.6-4.3 pH range. Three strongly binding sites are identified: the first two sites involve two Ru(bpy)32+ with the same high-affinity binding constant, and the third one has a medium affinity binding constant. An upper limit on the Ru(bpy)32+-retinal distance is estimated from Forster energy-transfer studies. The photocycle of fully regenerated bR is initiated by energy transfer from the excited state of bound Ru(bpy)32+ to the retinal. By monitoring the amount of M412 intermediate formed by this process relative to that formed
in native bR, an energy-transfer efficiency $E \geq 97\%$ is determined. From this low-limit value of $E$, the average distance between the retinal chromophore and the Ru(bpy)$_3^{2+}$ energy donor(s) in different binding sites is estimated to be $\geq 16$ Å.

II. Lectures and Meetings:

The principal investigator was invited to deliver the following special lectures at national and international meetings during the last year support period:


Tel Aviv University 30th Year Anniversary Symposium, Tel Aviv, Israel, "Molecular Mechanisms of Solar-to-Electric Energy Conversion of the Other Photo Synthetic System in Nature" - December 15, 1994.

III. Work Published During the Past Year of Funding


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6. "Binding Characteristics of an Organometallic Cation, Ru(bpy)$_3^{2+}$ in Regenerated Bacteriorhodopsin."

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