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Mirrorless all-optical bistability in bacteriorhodopsin

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Mirrorless all-optical bistability in bacteriorhodopsin

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We report direct observations of all-optical mirrorless bistability associated with saturable absorption in three kinds of bacteriorhodopsin (BR) samples: wild-type BR in water solution and dispersed in thin films of a polymer matrix as well as water solution of the genetically engineered mutant BR_{D96N}. The experiments are carried out with picosecond pulses at 532 nm. The values measured for the saturation intensity are explained in terms of the relaxation of the excited M state population to the B state of the BR photocycle for the three kinds of samples studied.

The biological photochrome bacteriorhodopsin (BR) is receiving much attention as an interesting alternative to conventional materials for a variety of applications¹⁻⁵ in optical recording and information processing. Recent advances in genetic engineering techniques⁶ for tailoring molecular properties (some of which were already optimized by evolution in nature) make it an outstanding photonic material. Models of "biochips" with BR as a molecular switch were already proposed.⁷ Second harmonic generation,⁸ saturable absorption,⁹ self-defocusing, and phase conjugation¹⁰ of thin films with low power visible continuous wave (cw) lasers are reported. Optical bistability in a Fabry-Perot resonator¹¹ and in a waveguide configuration¹² is investigated with varying degrees of success. Optical bistability and signal competition were studied in a resonant cavity¹³ containing a photochromic absorber on BR and an organic dye.

In this letter we report direct observations of all-optical mirrorless bistability which is due to optical absorption that decreases as the material becomes more excited, in wild-type BR in water solution and in a thin film dispersed in a polymer matrix, as well as for water solution of its genetic variant BR_{D96N} (Asp96 → Asn).

The purple membrane from *Halobacterium halobium* contains the photochromic protein BR in a lipid bilayer. In the halobacterial cell it acts as a light driven pump. The BR molecule consists of a single polypeptide chain of 248 amino acids. A retinal molecule linked via a protonated Schiff base to lysine 216 forms the chromophoric group. The proton pumping activity of light adapted BR is associated with conformational changes of the BR molecule and its chromophoric group during the photocycle shown in Fig. 1. The initial B state of BR has an absorption peak at 570 nm while the long-lived M state has an absorption peak at 412 nm. As the initial state has a broad absorption band it can be excited by means of red, yellow, or green light. The M state can revert to the initial state via a thermal process or by a photochemical process by excitation with blue light. The lifetime of the M state depends on the reprotonation process. It can be altered by different means: drying, supplying additives, reducing the temperature, and finally by genetic substitution of the aspartic acid in position 96 by asparagine (Asp96 → Asn).⁶ The asparagine sub-

stitution completely inhibits the reprotonation process as a result of which the lifetime of the M state is substantially increased by several orders of magnitude, milliseconds to seconds.

The BR thin films used in our experiments were purchased from Wacker Chemical (U.S.A.) Inc.. It is a product of Consortium fur Electrochemische Industrie GmbH. They are 35 μm thick and consist of wild-type BR dispersed in a polymer matrix. The BR and BR_{D96N} water solutions (at pH 7.0) were prepared at the Natick Laboratories as described by Oesterhelt *et al.*¹⁴

The experimental arrangement for studying intensity dependent absorption consists of a mode-locked, repetitively pulsed Quantel Nd:YAG laser, frequency doubled to give 532 nm. The duration of the laser pulse is 30 ps. The output energy per pulse is 25 mJ. A 10-cm focal-length lens is used for the high intensities. The intensity was varied using a standard combination of a half-wave plate retarder and two polarizers. A beam splitter was used to sample the input beam. Incident intensities are calculated from energy

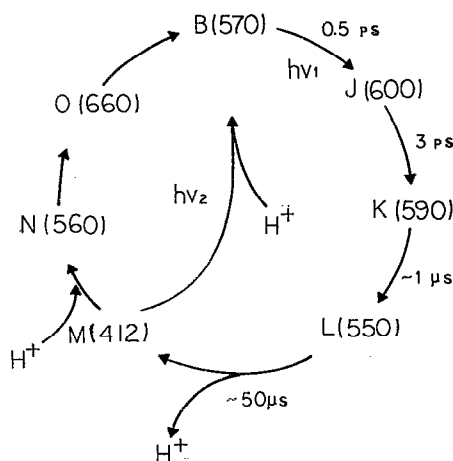


FIG. 1. Schematic of the BR photocycle. Upon excitation of the initial B state, the molecule passes through several intermediate states with short-lifetimes to the long-lived M state and relaxes to the initial state spontaneously with a lifetime of about 10 ms, or photochemically by irradiation with blue light. The numbers in parentheses indicate absorption peaks in nanometers.

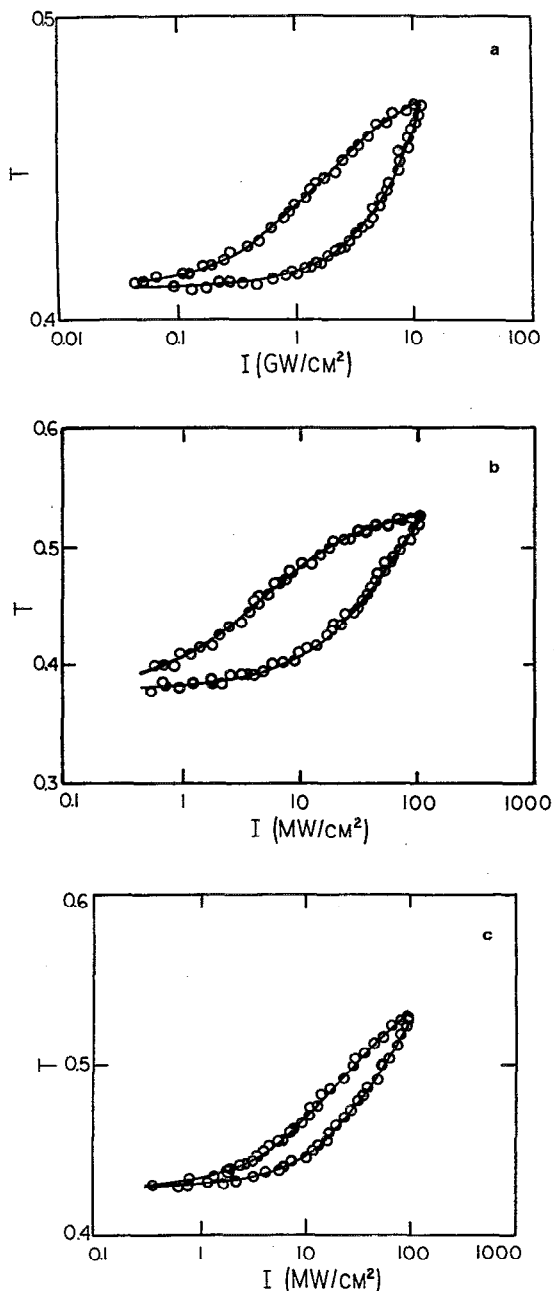


FIG. 2. Total energy transmission of the BR samples measured as a function of the incident intensity: (a) wild-type BR in water solution, (b) wild-type BR thin film in a polymer matrix, (c) mutant BR_{D96N} in water solution. The circles represent average values of observed results with a spread of about 3%.

measurements. The uncertainty in the absolute values of the intensity may be $\pm 5\%$ but relative values are more accurate. Total energy transmission is obtained directly from a pair of Molelectron pyroelectric energy meters. The meters were interfaced to a personal computer for data acquisition and analysis. The nonlinear sample was placed in the path of the laser beam. 2-mm glass cuvettes are used for the water solutions. Thin films obtained from Wacker are sandwiched between glass plates.

All the samples studied displayed the bistable behavior. The experimental results are shown in Fig. 2 illustrat-

ing hysteresis in the transmission response. The bistability is induced at low incident intensities for dry samples of wild-type BR (thin film) and solutions of the mutant, for both of which the reprotonation process is somewhat inhibited and the lifetime of the M state is long. For water solutions of wild-type BR, on the other hand, the lifetime of the M state is relatively short due to reprotonation and the optical bistability occurs only at two to three orders higher intensities. The hysteresis loops are nonsquare, making the actual switching transitions diffuse. This can be attributed to different extinction coefficients for each state causing a differential absorption at the two absorption peaks so that the local absorption is a function of position along the optical path, thus smearing out the sample's response. A similar behavior was observed by Kirkby *et al.*¹⁵ in an organic material. The experiments were repeated several times with the same set of samples and consistent results are obtained demonstrating the remarkable regenerative properties of bacteriorhodopsin based materials. In the case of the film we observe some damage at intensity levels of order 1 GW/cm². For the solution samples we do not see any damage even at these levels.

We use a simple approximate two level model similar to Werner *et al.*⁹ to describe the saturation behavior of the system. The rate equation for the level population may be written as

$$\frac{dM}{dt} = \sigma_1 F B - \frac{M}{\tau} - \sigma_2 F M, \quad (1)$$

where F is the photon density flux of the incident beam, M and B are the populations per unit volume in the M and B levels, respectively, σ_1 , σ_2 are the absorption cross sections for the nonradiative transitions B \rightarrow M and M \rightarrow B, respectively, τ is the relaxation time for the transition M \rightarrow B. The populations in M and B levels are obtained by the steady-state solution of Eq. (1).

$$B = N \left(\frac{1 + \sigma_2 F \tau}{1 + (\sigma_1 + \sigma_2) F \tau} \right), \quad (2)$$

$$M = N - B, \quad (3)$$

where N is the density of BR molecules in the sample.

The intensity dependent nonlinear absorption is described by the expressions

$$\alpha = N \sigma_1 \left(\frac{1 + 2\sigma_2 F \tau}{1 + (\sigma_1 + \sigma_2) F \tau} \right) = \alpha_0 - \frac{gI}{1 + (I/I_S)}, \quad (4)$$

$$\frac{dI}{dz} = -\alpha(I)I,$$

where $\alpha_0 = N\sigma_1$, $g = N\sigma_1(\sigma_1 - \sigma_2)\tau/h\nu$ and the saturation intensity $I_S = h\nu/(\sigma_1 + \sigma_2)\tau$. For light of wavelengths close to or greater than 570 nm, $\sigma_1 \gg \sigma_2$ and $\alpha(I)$ exhibits saturable absorption. The term α_0 includes linear absorption as well as any loss due to scattering.

The experimental points of transmissivity of the samples versus incident intensity are displayed in Fig. 2. The solid lines obtained by the theoretical fit of Eq. (4) closely follow the experimental points and the parameters ob-

TABLE I. Values of best fit parameters for the samples studied.

Sample	Concentration (mg/ml)	α_0 (cm^{-1})	Trace	I_S (MW/cm^2)	g (cm/GW)
Wild-type BR water solution	0.80	4.45	Upper	1500	0.105
			Lower	70 000	0.014
Wild-type BR thin film	50.0	277	Upper	4	85.0
			Lower	70	7.7
BR _{D96N} water solution	0.76	4.25	Upper	18	14.0
			Lower	70	5.0

tained by best fit are shown in Table I for the three samples studied, for both the upper and lower curves. It may be seen from the table that the saturation intensity I_S is about three orders higher for the wild-type BR in water solution compared to the value for thin film and BR mutant water solution. The ratio of time constants in any two samples can be obtained from the theoretical relations for the parameters at the same wavelength, as the absorption cross sections are the same for all the samples

$$\frac{I_S^i}{I_S^j} = \frac{\tau_j}{\tau_i}, \quad \frac{g_i}{g_j} = \frac{N_i \tau_i}{N_j \tau_j}. \quad (5)$$

The results indicate that thin film sample of wild-type BR and water solution of the mutant are characterized by relaxation times for the M state about three orders longer relative to wild-type BR water solution. The increase is attributed to relative values of humidity in the first case and mutagenesis in the second case.^{3,6} This correlates well with the observed behavior of switching at low light intensities (two to three orders) for wild-type BR thin film and water solution of mutant compared to wild-type BR in water solution. It will be interesting to look at the film of the mutant which may exhibit a relaxation time for the M state of a few hours.

In conclusion we observed all-optical mirrorless bistability in three different samples of BR: (a) wild-type BR in water solution, (b) thin film of wild-type BR dispersed in a polymer matrix, and (c) genetically engineered mutant BR_{D96N} in water solution. For samples (b) and (c) the bistability is obtained at intensity levels approximately two to three orders lower compared to sample (a), which can be explained in terms of the relaxation time of the excited M state to the initial B state of the BR photocycle. The relaxation times for samples (b) and (c) are about three

orders long compared to sample (a). Detailed studies of the dynamics involved in the transient process are in progress and will be reported elsewhere.

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