Evidence that the photoelectric response of bacteriorhodopsin occurs in less than 5 picoseconds

R. Simmeth and G. W. Rayfield
Physics Department, University of Oregon, Eugene, Oregon 97403 USA

ABSTRACT The initial photoinduced charge separation in bacteriorhodopsin is shown to occur in <5 ps. This result is obtained by measuring the photovoltage rise time in an oriented film of bacteriorhodopsin (BR). A dye laser syncronously pumped by an Argon ion cw mode locked laser is used to produce 3-ps light pulses which, after passing through a dye amplifier chain, photoexcite the BR sample. The photovoltage transient is detected by an ultra-fast Josephson junction digital sampling oscilloscope with liquid-helium-cooled input circuitry.

INTRODUCTION

This paper describes an experiment in which very short (ps) laser pulses are used to produce a photovoltage in an ordered thin-film sample of bacteriorhodopsin (BR). The photovoltage is the result of light-induced charge separation within the BR sample (1). The rise time of the photovoltage is measured by using an ultrafast digital sampling oscilloscope based on the Josephson junction effect. To our knowledge this is the fastest photovoltage rise time ever observed in a biological sample.

BR is a membrane protein found in the cell membrane wall of *Halobacterium halobium* where it functions as a light-driven proton pump (2). The protein occurs naturally in an ordered two-dimensional array, the purple membrane (PM) sheet. Each PM sheet is ~1 μm in diameter and contains $\sim 10^5$ BR molecules. A BR molecule contains 248 amino acids and has a molecular weight of 26,000. Light is absorbed by a chromophore (retinal) buried within the interior of the protein (3). The retinal is connected through a protonated Schiff base to Lys-216 in the amino acid sequence of BR. A counter ion near the protonated Schiff base maintains charge neutrality within the hydrophobic interior of the protein. Upon absorption of a photon, a 13-*trans* to 13-*cis* isomerization of the all-*trans* retinal occurs and results in a red shift in the absorption spectrum of the protein. This photoreaction occurs in <1 ps (4, 5).

Numerous experiments have shown that illumination of an ordered sample of BR results in a photovoltage. The initial phase of the photovoltage is ultrafast and reflects the very early light-induced charge separation of the proton-pump cycle. The rise time of the initial phase of the photovoltage has not yet been resolved. Two recent papers report that it is faster than 20 ps (6, 7). However, the instrumentation used for these experiments had a bandwidth of <14 GHz, and the rise time quoted is based on a mathematical analysis of the experimental data. The instrumentation used for the experiments in the present work has a bandwidth in excess of 70 GHz, and the results show that the earliest charge separation in the proton-pump cycle is faster than 5 ps.

MATERIALS AND METHODS

BR in the form of PM sheets was isolated from strain JW-3 of *H. halobium* using a technique described by Becher and Cassium (8). An orientated sample of BR was prepared using the following procedure: PM is first washed in ultrapure distilled water and then resuspended at ~4 mg/ml in distilled water. A drop of this solution (30 μl) is placed on a transparent indium tin oxide (ITO) glass electrode and covered with a second electrode 1 mm away. The aqueous suspension of PM forms a bridge between the electrodes. A PM sheet at pH 7 has both a net charge (negative) and an electric dipole moment. Therefore, when a potential (2 V) is applied between the electrodes, the PM sheets migrate and orient within the aqueous solvent. The oriented PM sheets are deposited onto the anode (ITO glass electrode) with the cytoplasmic (negative) side toward the electrode. The PM sheets on the anode retain their orientation when the electrodes are separated and the sample is dried. Samples are exposed to room air and therefore retain bound water. The area of the dried BR film on the transparent glass electrode is ~4 mm^2. The degree of orientation of the BR molecule within the film is not known at this time. Fig. 1 shows the assembled BR sample cell with the ITO Glass electrode and BR film. A thin 6-μm Teflon film separates the BR film from the center electrode of the sample cell. Light-induced charge displacements within the BR molecule are capacitively coupled to the electrodes of the sample cell (1).

An ultrafast digital sampling oscilloscope (model PSP-1000; Hypres Inc., Elmsford, NY) was used to measure the transient photovoltage from the oriented BR sample. The sample cell (Fig. 1) was attached directly to the "K" connector on the front of the oscilloscope. This instrument has a 70-GHz bandwidth (5 ps rise time) limited by the "K" connector on the input. It uses a Josephson junction and superconduct-

0006-3495/90/05/1099/03 $2.00

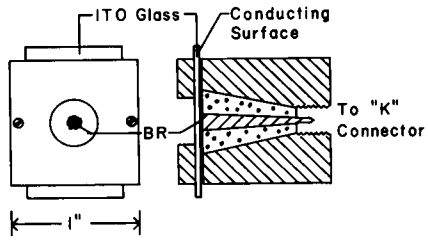

FIGURE 1 A drawing of the BR sample cell is shown. An oriented film is deposited on the ITO glass electrode and a thin 6-m Teflon film separates the BR film from the center electrode of the sample cell. The sample cell is threaded to screw directly on the "K" input connector of the Hypres oscilloscope.

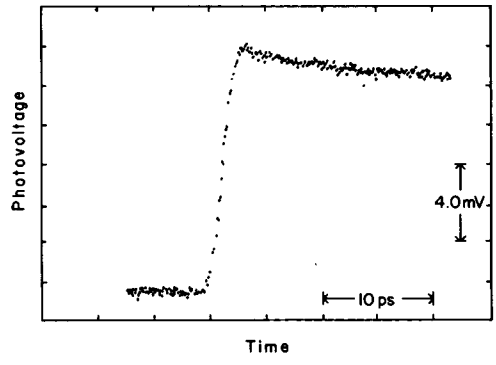

FIGURE 2 The photovoltage rise time of BR sample cell is shown. It was generated by a 300-μJ, 3-ps laser light pulse at 595 nm. The photovoltage was recorded using a Hypres ultrafast sampling oscilloscope. The rise time of the photovoltage is <5 ps.

ing input circuitry to achieve ultrafast response. It is necessary to internally trigger the oscilloscope to achieve the highest time resolution, and this requires at least a 10-mV signal. The present response of our BR sample (<1 mV per μJ) requires that we have picosecond laser pulses with an energy in excess of 10 μJ per laser light pulse.

A dye laser (model 375B; Spectra Physics, Inc., Mountain View, CA) synchronously pumped by an Argon ion mode locked cw laser (Spectra Physics) was used as a source of the picosecond light pulse. The light pulses from the dye laser have an energy of 5 nJ, a repetition rate of 82 MHz, a pulse width of 3.0 ps, and a wavelength of 595 nm. A longitudinal pumped three-stage dye amplifier chain (9) was built to amplify the pulses from the dye laser. The amplifier was pumped by a Q-switched Nd:YAG laser (model GCR-11-3; Spectra Physics, Inc.) operating at 10 Hz. The energy of the output light pulse from the dye amplifier was ~300 μJ with a pulse width of 3.0% 0.5 ps, a repetition rate of 10 Hz, and a beam diameter of 6 mm. The last stage of the amplifier is run at saturation to produce stable (constant-energy) light pulses.

RESULTS AND DISCUSSION

Fig. 2 shows the transient photovoltage from the oriented BR sample when it is illuminated with 300-μJ, 3-ps laser light pulses at a wavelength of 595 nm. Data were taken using the Hypres ultrafast digital sampling oscilloscope and was sampled at 10 Hz for ~25 min. The rise time of the transient photovoltage shown in Fig. 2 is slightly faster than 5 ps and is consistent with the fastest rise time that can be resolved using the Hypres oscilloscope. The photovoltage rise time of the BR sample is therefore <5 ps. The rise time of the photovoltage signal from the BR sample cell is determined by the time required for charge separation to occur in the BR molecule after absorption of a photon.

Charge separation within the BR molecules causes a change in the surface charge on the electrodes (1). This launches the electromagnetic wave in the sample cell. The limited bandwidth of the "K" connector, which separates the sample cell from the superconducting circuitry of the Josephson junction, causes a rolloff of the high-frequency response.

The measured decay time of the photovoltage after illumination by the laser light pulse was ~250 ps. This decay time is in agreement with the system's RC time constant, which is determined by the input impedance of the oscilloscope (50 Ω) and the capacitance of the BR sample cell (4–5 pF).

Kononenko et al. (10) suggest that the initial phase of the photovoltage transient is associated with the 3-ps rise time of the K photointermediate. This is consistent with the experiments of Trissl et al. (7), which indicate that irradiation of K to produce BR leads to a light-induced charge displacement that is opposite in direction to the BR to K light-induced charge displacement. K is a 13-*cis* conformation of the retinal. The 13-*trans* to 13-*cis* isomerization of the retinal causes a movement of the protonated Schiff base away from its negative counter ion on the opsin and may be the molecular event associated with the initial photovoltage transient. This molecular event takes place in ~450 fs (4, 5).

The techniques used for the photovoltage–rise-time measurements on BR are directly applicable to light-induced charge-separation measurements in other inorganic, organic, and biological systems and, in particular, early events associated with charge separation in photosynthesis.

The techniques described here could be extended to give even better time resolution. The bandwidth of the Hypres oscilloscope can be extended to 100 GHz by changing only the input connector of the oscilloscope. A Josephson junction oscilloscope is capable, in principle of resolving 1-ps rise times (11). However, the geometry of the superconducting input circuit of the Hypres oscillo-

scope would have to be changed to accomplish this. The longitudinal dye-amplifier chain can amplify laser-light pulses with a pulse width of 1 ps (9), and pulse compression can be used to shorten the pulse width of the dye laser.

We would like to acknowledge the important contributions of H. Palm and P. Schmidt to this work. Part of the work was performed in the shared laser facilities at the University of Oregon.

Contract support was provided by the United States Army Armament Research Development and Engineering Center.

Received for publication 4 December 1989 and in final form 26 January 1990.

REFERENCES

1. Rayfield, G. W. 1983. Events in proton pumping by bacteriorhodopsin. *Biophys. J.* 41:109–117.
2. Stoeckenius, W. R., and R. A. Bogomolni. 1982. Bacteriorhodopsin and related pigments of Halobacteria. *Annu. Rev. Biochem.* 52:587–616.
3. Heyn, M. P., J. Westerhausen, I. Wallat, and F. Seif. 1988. High-sensitivity neutron diffraction of membranes. Location of the Schiff base end of the chromophore of bacteriorhodopsin. *Proc. Natl. Acad. Sci. USA.* 85:2146–2150.
4. Dobler, B. J., W. Zinth, W. Kaiser, and D. Oesterhelt. 1988. Excited-state reaction dynamics of bacteriorhodopsin studied by femtosecond spectroscopy. *Chem. Phys. Lett.* 144:215–220.
5. Pollard, W. T., C. H. Cruz, C. V. Shank, and R. A. Mathies. 1989. Direct observation of the excited-state *cis-trans* photoisomerization of bacteriorhodopsin: multilevel line shape theory for femtosecond dynamic hole burning and its application. *J. Chem. Phys.* 90:199–208.
6. Groma, G. J., F. Raksi, G. Szabo, and G. Varo. 1988. Picosecond and nanosecond components in bacteriorhodopsin light-induced electric response signal. *Biophys. J.:* 54:77–80.
7. Trissl, H. W., W. Gaertner, and W. Leibl. 1989. Reversed picosecond charge displacement from the photoproduct K of bacteriorhodopsin demonstrated photoelectrically. *Chem. Phys. Lett.* 158:515–518.
8. Becker, B. M., and J. Y. Cassium. 1975. Improved isolation procedures for the purple membranes of *Halobacterium halobium. Prep. Biochem.* 5:161–178.
9. Koch, T. L., L. C. Chiu, and A. Yariv. 1982. Analysis and performance of a picosecond dye laser amplifier chain. *J. Appl. Physiol.* 53:6047–6059.
10. Kononenko, A. A., E. P. Lukashev, S. K. Chamorovsky, A. V. Maximychev, S. F. Timashev, L. N. Chekulaeva, A. B. Rubin, and V. Z. Paschenko. 1987. Oriented purple-membrane films as a probe for studies of the mechanism of bacteriorhodopsin functioning. II Photoelectric processes. *Biochim. Biophys. Acta.* 892:56–67.
11. Tuckerman, D. B. 1980. A Josephson ultrahigh-resolution sampling system. *Appl. Phys. Lett.* 36:1008–1010.