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12. The 293 cells were maintained in minimum essential medium (Earle's) containing fetal calf serum (10%). Wild-type and mutant α-subunit cDNAs were subcloned into the eukaryotic expression vector pcDNA1 (Invitrogen) and transfected into the 293 cells (10^6 cells on a 60-mm culture dish) in the presence of DEAE-dextran (400 μg/ml) and chloroquine (100 μM) for 2 hours. Cells were then shocked with phosphate-buffered saline (PBS) containing dimethyl sulfoxide (DMSO) (10%) for 2 min, washed once with PBS, and incubated in growth medium for 20 to 24 hours before reseeding in 12-well plates.
13. Expression of the exogenous α_{i1}, α_o, and α_z was verified by immunoblotting with antisera AS/7 (Du Pont Biotechnology Systems), GC/2 (Du Pont Biotechnology Systems), and P-961 (*8*), respectively. Antiserum to the α_{i3} peptide sequence AGSAQQGVMTSQLA (residues 111 to 124) was used to detect expression of α_{i3}. The procedure for protein immunoblotting was as described (*4*). All four proteins were expressed at readily detectable levels (~2-fold over endogenous α chains) (Y. H. Wong and H. R. Bourne, unpublished data). Single-letter abbreviations for the amino acid residues are as follows: A, Ala; G, Gly; L, Leu; M, Met; Q, Gln; S, Ser; T, Thr; V, Val.
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Quantum Conversion and Image Detection by a Bacteriorhodopsin-Based Artificial Photoreceptor

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A thin film (50 to 500 angstroms thick) comprising fragments of bacteriorhodopsin (bR)-containing purple membrane was formed on an SnO_2 conductive electrode by the Langmuir-Blodgett method. The film was put into contact with a thin, aqueous electrolyte gel to construct an electrochemical sandwich-type photocell with a junction structure SnO_2/bR/electrolyte/Au electrode. Under visible light irradiation, the bR-based photocell produced an efficient rectified photocurrent that proved to have unique differential responsivity to light intensity, which is characteristic of in vivo biological photoreceptors. An artificial photoreceptor comprising a pixel network of the bR photocell was fabricated in an attempt to study image-detecting and processing abilities of bR.

BACTERIORHODOPSIN (bR) IS A PROtein existing in the halobacterial purple membrane (PM), which is extremely stable as a visual pigment analog in vitro against light. Photochemistry of in vitro bR has recently stimulated extensive study of its potential application to the design of molecular electronic devices (*1*) and optical computers and memories (*2, 3*). The photovoltaic behavior of in vitro bR has been of particular interest in the field of bioelectronics and has been well elucidated with the use of dry PM films electrodeposited on conductive electrodes (*4–6*). In a manner similar to other photoconductive dyes in open-circuit voltaic cells (*7*), in vitro bR in dry films produces a steady-state photovoltage accompanied by an initial transient component. The photovoltage and concomitant photocurrent (normally very small) result from the displacement of charge in the bR molecule initiated by rapid photoisomerization of the chromophore retinal. Under direct contact between bR and electrode, the photoelectric response time of bR can thus reach the order of 10^{-11} s (*6*). However, the photoelectric behavior of dry PM films in contact with electrodes is inevitably affected significantly by the presence of water or ambient humidity (*4, 5*), reflecting the direct effect of water on the proton-pumping reaction cycle of bR.

In this study we attempted to construct a bR-incorporated electrochemical photocell with bR molecules in direct contact with both a conductive electrode as well as an aqueous medium of electrolyte. This photocell, having an ultrathin wet film of PM at the interface of electrode and electrolyte, can convert incident quanta to electric current at high efficiency with excellent linearity of output current against light intensity. As a light sensor, the bR photocell was found to have differential responsivity to light intensity, which is characteristic of in vivo biological photoreceptors. As an extended application, we fabricated an image-sensing photoreceptor based on this differential sensing type bR photocell. The bR photoreceptor, which is the first example of an artificial photoreceptor (image sensor) with immobilized biological materials, proved capable of detecting and processing an optical image in a manner similar to that of visual functions.

Purple membrane was purified from the S-9 strain of *Halobacterium halobium* by the method of Oesterhelt and Stoeckenius (*8*). PM was suspended in pure water and emulsified by mixing with *N,N*-dimethylformamide (DMF) and *n*-hexane to yield an opaque dispersion. The dispersion was carefully applied on a surface of pure water in a Langmuir film balance to form a film consisting of a two-dimensional array of PM

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fragments, that is, a monolayer of bR. Under a controlled surface pressure of 30 mN/m, the monolayer was deposited and built up on the surface of a SnO_2 transparent conductive electrode (1.5 cm by 2.5 cm, with a 4500 Å SnO_2 layer on a glass plate) by horizontally attaching the surface to the monolayer. This yielded a multilayer film of PM with an optical density of approximately 0.0017 per monolayer (at 560 nm) and a thickness of ≤50 Å per monolayer (*9*). A small amount of an aqueous electrolyte, which was typically an aqueous gel comprising 6% carboxymethylchitin (degree of carboxymethylation, 78%) and 1 M KCl (pH 7 to 8), was then applied on the PM film, and the electrolyte was sandwiched with a counter electrode (an Au-sublimated glass plate) by use of a Teflon ring spacer (200 μm thick). A thin-layer photocell thus prepared (Fig. 1A) immobilizes an ultrathin layer of bR (estimated thickness ~40 nm for ten bR monolayers) that is directly attached to the electrode (SnO_2) and simultaneously impregnated with an aqueous electrolyte.

Irradiation of green light (10 mW/cm²) onto the PM film through the SnO_2 electrode by a 150-W xenon arc lamp caused a photocurrent response with the time profile shown in Fig. 2. Positive and negative transient photocurrents are generated when light is switched on and off, respectively; these pulses are followed by rapid relaxation to the background level. This characteristic response is attributed to a charge displacement occurring in the photoexcited bR molecule. Excitation of a light-adapted bR causes a rapid trans-to-cis isomerization of the chromophore retinal accompanied by a change of dipole moment, which is followed, via thermal intermediates named **K** and **L**, by the vectorial transfer of protons (proton pumping) within the bR molecule (*10*). Excitation of bR ultimately brings about charge displacement that induces an electric current on an electrode adjacent to the molecule. The action spectrum of the transient photocurrent (Fig. 3) coincides with the broad absorption spectrum of bR (at 450 to 650 nm) and indicates that the origin of photoresponse is bR. Oscilloscopic measurement revealed the response time to be less than 300 μs, which may correspond to the time range of the proton displacement associated with the retinal Schiff base of **K** to **M** intermediates (less than 1 ms).

In this photocell, the photocurrent induced by bR is strongly rectified in the cathodic direction (from the electrolyte to the electrode side). Rectification of the photocurrent may result from an electric field established spontaneously at the interface of an oxide electrode (negatively charged) and an aqueous electrolyte phase including the thin PM film, which is a phenomenon characteristic of electrochemical interfaces (*11*).

An important aspect that distinguishes the nature of this photocell from previous voltaic cells (*1, 4–6, 12*) is that an efficient photocurrent can be obtained with a single layer of PM (a monolayer of bR). In desirable light sensors, including in vivo biological photoreceptors (the retina), sensing is performed in the quantum conversion mode, which guarantees linear sensitivity of a sensor against the incident photon flux. In our cell, excellent linearity of photocurrent is confirmed over the light intensity region of more than three orders of magnitude (Fig. 4).

On the basis of incident flux determination by chemical actinometry under irradiation of monochromatic light (550 nm), we obtained the quantum efficiency of this system to be on the order of 10^{-2} (*13*), which is considered to be larger by a factor of 10^1

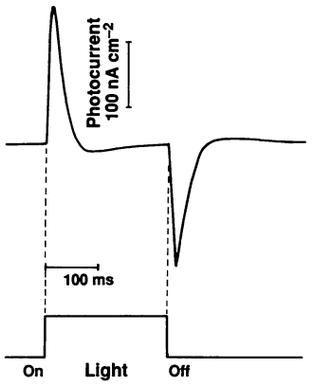

Fig. 2. Typical profile of the time response of bR-induced photocurrent obtained with a sandwich-type photocell consisting of junctions of SnO_2/PM LB film (six layers)/aqueous electrolyte gel (6% carboxymethylchitin and 1 M KCl)/Au. The photocurrent responds positively (in the cathodic direction) and negatively to an increase and decrease of light intensity, respectively, on switching of light irradiation with the pattern shown. The photocell thus responds differentially to the light intensity. Here, PM film was irradiated with green light supplied by a 150-W xenon arc lamp in combination with a filter system (Toshiba G-55S and IRA-05, transmittance peak 540 nm, half-width 130 nm) that gave an incident intensity of about 10 mW/cm². The SnO_2 electrode was biased by −0.6 V against the Au counterelectrode. Such negative-bias application improves the photocurrent peak value some without changing the differential response profile. A pulse-excitation study with a flash lamp revealed the response time of the initial transient photocurrent to be less than 300 μs.

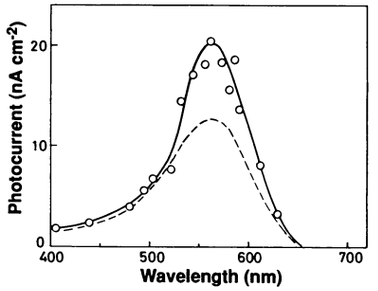

Fig. 3. Action spectrum of bR-induced photocurrent (solid line) measured with a film of two PM layers (two bR monolayers), and optical absorption of bR (dashed line in arbitrary units). For a precise analysis that excluded light reflection, the result shown was obtained in a conventional electrochemical cell with a three-electrode system including a reference electrode (saturated calomel electrode, SCE) and a Pt wire counterelectrode (*15*); the electrode potential of SnO_2 was regulated at −0.35 V versus SCE. The electrolyte was 0.1 M aqueous KCl solution. Thicker PM films with an increased number of layers gave similar action spectra after the absorption of bR, confirming that the origin of photocurrent was bR.

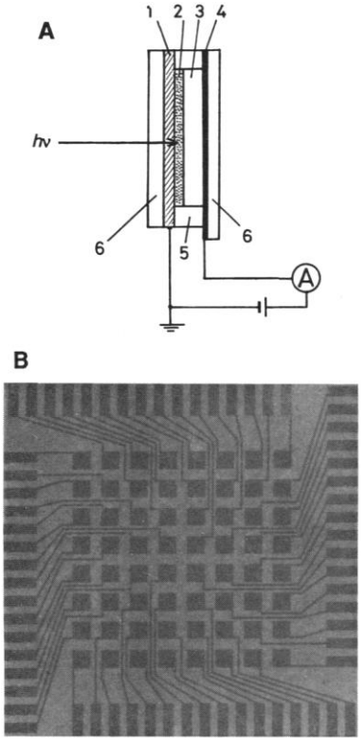

Fig. 1. (**A**) Structure (cross section) of the bR-immobilizing, thin sandwich-type photocell; 1, SnO_2 transparent conductive layer (4500 Å thick); 2, PM LB film (typically six to ten layers); 3, aqueous electrolyte gel layer comprising 6% carboxymethylchitin and 1 M KCl (200 μm thick); 4, Au layer (<1000 Å thick) as counterelectrode; 5, Teflon ring spacer; 6, glass substrate. We normally applied bias voltage (0 to −0.7 V) to the SnO_2/PM electrode to improve the magnitude as well as the signal-to-noise ratio of photocurrent output by controlling the background dark current. (**B**) ITO electrode patterned with 64 pixels used in the image-sensing study (see text). Pixels of ITO (2.5 mm by 2.5 mm square, 1000 Å thick transparent layer, electric resistance 30 ohm/cm²) are two dimensionally arrayed on a glass plate; each pixel has an independent wire (width 300 μm) lead to the four edge terminals along the sides, where parallel circuits for signal amplification and display are to be connected. This patterned electrode is positioned in the place of the SnO_2 single electrode (1 with 6) in the junction structure of (A).

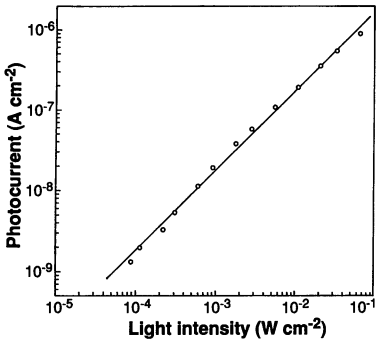

Fig. 4. Relation between the bR-induced photocurrent (transient peak value) and the light intensity incident to the PM film. Light intensity represents the quantity of a transient change that occurred in the intensity from an ambient intensity level, to which the photocell can respond. The lowest intensity of the linear correlation may correspond to a sensitivity limit for light detection. The upper sensing limit of intensity, on the other hand, lies beyond the maximum intensity shown here; its detection may require a laser-irradiation experiment. Experimental conditions were as in Fig. 2.

to 10^3 than those of previous bR-mounted cells, as determined from a comparison of photocurrents and irradiation conditions or as estimated from reported power conversion efficiencies (*5*). Such essential improvement in efficiency should be contributed by the cell structure established here. In our cell, bR molecules are accommodated in a salt-containing aqueous medium favorable to their proton pumping activity, and, simultaneously, they have direct contact with the electrode so that the charge displacements in bR immediately induce current on the electrode. Molecules of bR thus accommodated in the aqueous medium are, needless to say, free from any effect of ambient humidity, which was a serious problem in the case of dry PM films.

The most significant function of this bR photocell is its differential responsivity to light. This responsivity is demonstrated in Fig. 2, in which bR responds to the change of light intensity, not to the intensity itself. Under a constant light intensity, the transient photocurrent undergoes rapid and perfect offset down to the background level without leaving any steady-state response. This function makes possible various types of optical image processing, such as mobile image extraction and edge line enhancement, that are essential for visual information processing.

We have extended this study by fabricating an in vitro photoreceptor bearing a pixel network of small bR photocells for the purpose of examining the image-detecting function of bR. An indium–tin oxide (ITO) electrode that bears a lithographically printed, 64-pixel ITO pattern on glass was prepared for this purpose (Fig. 1B). On this pixel-patterned electrode, ten monolayers of PM were uniformly deposited by the above-described manner. An artificial photoreceptor comprising 64 pixels of the bR-electrolyte junction cell (2.5 mm by 2.5 mm) was thus constructed by superposing thereon a carboxymethylchitin electrolyte gel (500 μm in thickness) and an Au common counter-electrode. A small photocurrent from the pixel electrodes (3 to 30 nA) was electrically amplified in the voltage mode up to the order of 1 to 10 V by means of simple operational amplifier circuitry. The amplified photoresponse (differential response) was output to a light-emitting diode (LED) array composed of 64 picture units in order to simultaneously display the image detected by the bR photoreceptor.

We first examined image detection for simple alphabetical letter images with this system. An optical image incident to the bR photoreceptor was provided by a commercial slide projector (150 W) and was chopped at a frequency of 20 to 50 Hz (with a light to dark ratio of 1/3) in order to give an oscillation in the light intensity. This method of light modulation gave rise to a series of transient photocurrents that was effective for monitoring a still image. The letter image could be detected in this manner and simultaneously displayed on the LED panel. When no such modulation was applied, the photoreceptor was insensitive to still images; nevertheless, it proved to be highly sensitive in response to mobile images. It is noteworthy that the bR-based artificial photoreceptor is capable of selectively detecting a mobile optical object in still vision. This sensing ability, intrinsic to the bR photoreceptor, is an important function of visual reception.

A light-modulating application further demonstrates that geometrically oscillating the optical axis incident to the photoreceptor, mechanically or with a spatial light modulator, enables the detection (and even vectorial extraction) of the outline component from both still as well as mobile images. Thus, under suitable light modulations, the bR photoreceptor is capable of processing input information in real time.

A self-processing ability in image reception is peculiar to biological visual perception (*14*). This study emphasizes that the simulation of visual functions by molecular assemblies of bR is highly important for understanding the mechanism of image processing in vivo as well as for constructing intelligent image sensors in the future.

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9. In the Langmuir-Blodgett (LB) film used, the orientation of the two sides (intracellular and extracellular) of PM is regarded as random because no specific treatment for orientation (for instance, electric-field application) was applied during the film-preparation processes. We have confirmed the existence of a random orientation for a PM monolayer deposited from an aqueous surface onto a carbon microgrid for electron microscopy by visualizing the orientation morphology of the PM fragments using the immuno-gold labeling technique, in which a specific side of bR molecules in PM had been selectively modified with colloidal Au particles with the use of an anti-bR monoclonal antibody Au conjugate (N. Yamaguchi, T. Miyasaka, K. Koyama, in preparation).
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