INVESTIGATION BY FOCUSED LASER BEAM SCANNING OF THE PHOTOELECTRIC ACTIVITY OF BACTERIORHODOPSIN-CONTAINING LIPID BILAYERS

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ABSTRACT The photoelectric activity of different parts of lipid bilayer containing bacteriorhodopsin was investigated by moving a small actinic light spot across the Plateau-Gibbs border and the bimolecular part of this reconstituted model membrane. The results give direct evidence that bacteriorhodopsin incorporated into the bimolecular region of the lipid membrane is responsible for the photoelectric activity of this system. A technique for scanning the photoelectric activity of a modified bimolecular lipid membrane is described in detail.

It has been shown that bacteriorhodopsin-containing membrane sheets (BR) can generate electric fields and produce electric current upon illumination in different membrane model systems (1–5). Earlier we reported (4) oriented incorporation of BR into a bimolecular lipid membrane (BLM) having a positive surface charge. This model system (BR-BLM) exhibited photoelectric activity and represented one of the first cases of successful incorporation of a native pigment-protein complex into a BLM in a functionally active state. The BR-BLM proved to be a good model system for studying the connection of the photoelectric activity with the photochemical cycle of bacteriorhodopsin (6, 7). It remained to obtain direct proof as to whether the photoresponse in this system originates from the thin, bimolecular region or from the thick part (Plateau-Gibbs border) of the reconstituted membrane.

The isolation of BR, the incorporation, and the basic photoelectric measurement techniques used were as described earlier (4, 7). Scanning experiments on the photoelectric activity of BLMs modified with manganese porphyrins have been reported by Hong and Mauzerall (8). In our scanning experiments mapping of the photoelectric activity of BR-BLMs was provided by horizontal movement of the focused light beam of a He-Ne continuous laser (Spectra-Physics 155). The maximum intensity of the focused light spot was 10^{22} photons $m^{-2}s^{-1}$. The slow continuous movement of the scanning light spot was achieved by moving a glass lens horizontally in the light beam with a DC motor. For determination of the correlation between the definite position of the scanning light spot on the BLM and the photopotential generated, the intensity

of the light passing the BR-BLM was recorded simultaneously with the photopotential on a dual channel chart recorder.

The thicknesses of the BLMs were determined by capacitance measurements during the BLM formation and BR incorporation processes (9). As the planar thick membrane formed, it had a thickness of about 600–1,000 Å; this gradually decreased during thinning (usually lasting 5–10 min) to 70–90 Å and then stabilized. The initial resistance of the thick membrane was about $5 \cdot 10^8$ Ω cm^2, which also decreased by about one order of magnitude during the BLM formation.

After a BLM with constant resistance and thickness was obtained, the BR was added to one side of the bathing solution, while the scanning light beam reached the BLM from the opposite side. During incorporation (which usually lasted 30–60 min) of BR into the BLM, the resistance of the BLM dropped to approximately half its original value, and the thickness of the BR-BLM was slightly higher than that of the parent BLM.

The Teflon (E.I. DuPont de Nemours & Co., Inc., Wilmington, Del.) hole on which the BLM was prepared had a diameter of 1.65 mm, and the Plateau-Gibbs border usually formed a horizontally symmetric, 0.2 to 0.3-mm wide ring of thick lipid bilayer around the "black" bimolecular area. The diameter of the scanning light spot was 0.06 mm; this could be simply determined from the distance during which the intensity of the passing light reached its maximum value. Half of this distance determined the exact position of the edge of the Teflon hole on the scanning diagram.

In the first experiments, three peaks of photopotential generation were observed during the horizontal scanning of the BR-BLM (Fig. 1.). Two large peaks were located around the edge of the Teflon hole (but not at the border) and the third peak at the center of the BR-BLM, at light intensities under the saturation level. The highest photoresponse was observed when the actinic light spot was still far from the BLM

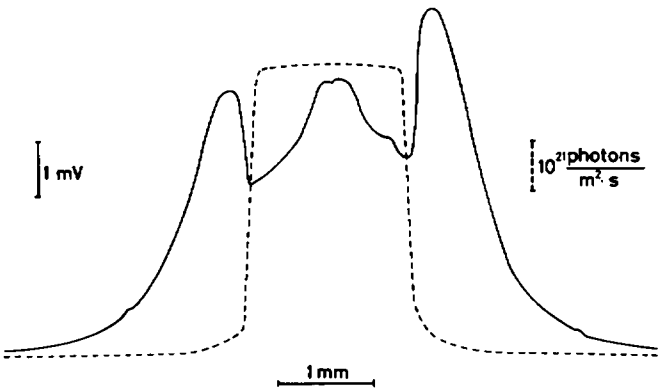

FIGURE 1 Scanning of the BR-BLM photopotential, the exciting light spot being moved in the horizontal direction. The exact position of the membrane is seen from the light intensity curve (----) measured behind the cup, after the beam has passed the membrane. The photopotential curve (——) is recorded simultaneously with the light intensity.

(1–2 mm); from this it was concluded that the white Teflon wall not only scatters the exciting light but acts as a light guide for a relatively long distance, disturbing the measurement. The light intensity dependence of this measurement also supports this explanation: when we reduced the light intensity, the two peaks on the sides decreased more rapidly than the one in the middle (originating from the direct excitation of the membrane). At the light intensity used, the photoelectric activity of the system is above the linear intensity-dependence region (7), i.e. the higher the light intensity, the smaller the increase in the photopotential caused by a certain fixed increase in the intensity of the exciting light. Because scattered light has a much lower effective intensity at the membrane than direct light (we are near the linear region of the photoresponse curve), the photopotential decreases faster with decreasing light intensity than the photopotential observed upon focused illumination at the same laser power. The absolute value of the photopotential is higher with scattered light, because in this case the whole surface of the membrane is illuminated. Thus, reduction of the exciting light intensity would seem to be a way to avoid the disturbing effect of light scattering; however, the fact that at sufficiently low light intensities the photopotential was also too small, made it impossible to carry out evaluation due to the electric noise level.

To avoid light scattering effects due to the Teflon wall, in the further experiments the edge or the Teflon wall was masked with a piece of black Teflon. The diameter of the hole in the mask was 1.55 mm, which proved sufficient to reduce the light scattering effect practically without shadowing even the border of the BR-BLM.

The result of a typical scanning experiment on the BR-BLM with the black mask is

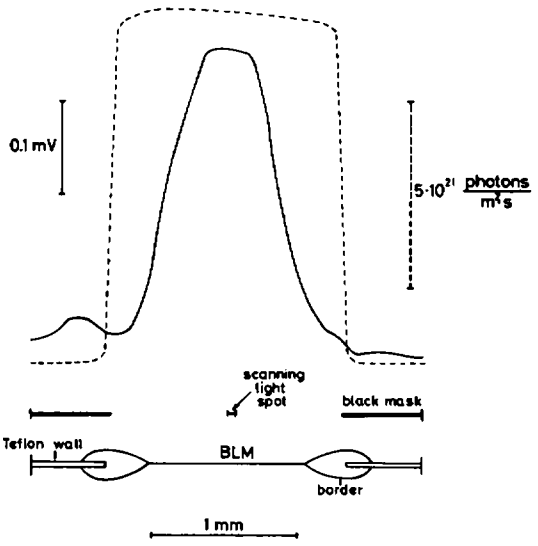

FIGURE 2 See Fig. 1. Scanning of the BR-BLM photopotential when the edge of the Teflon wall is masked. The scheme in the bottom shows the position and size of the mask, the edge of the hole on the white Teflon wall, the BLM with the Plateau-Gibbs border, and the scanning light spot. (Average of four measurements.)

shown in Fig. 2. With the choice of scanning speeds (30–40 s/mm) slow enough to allow the steady-state potential changes to follow the changes in light intensity without delay, several scans were made horizontally in both left and right directions. When the average of the photopotential changes during four scans was plotted (Fig. 2.), only one large maximum with a small plateau situated at the center of the BR-BLM was observed. As a small photopotential could also be detected outside the membrane, due to scattered light, this experiment too proves that (due to the divergence of the focused laser beam) the mask used did not shadow the membrane significantly. The border of the BR-BLM had no or very small photoelectric activity compared to the central bilayer structure. Every BR-BLM examined by the scanning technique described (eight independently reconstituted BR-BLMs with 10–20 scans on each) had the same properties. It is seen, however, from Fig. 2 that the plateau in the curve of photopotential is less than the width of the black (but not necessarily bimolecular) part of the membrane (this is typical for the measurements). Because the size of the Plateau-Gibbs border has been determined visually, it may be, that the thickness of the remaining part of the membrane is not uniform. Namely, it has a bimolecular structure only in the central region (where photoelectric activity is the highest), whereas near to the edges its thickness grows (causing the photopotential to fall), however still appearing as black. (Up to a thickness of about 1,000 Å—the range of the wavelength of visible light—the membrane can be seen as black membrane [9].)

The distance resolution of this photoelectric scanning technique has been determined on a thick planar lipid membrane (not a BLM) containing unoriented BR. This model membrane was prepared from membrane-forming solution with BR simply mixed in (1, 2), and was investigated under the same conditions as the BR-BLMs. In this thick planar lipid membrane, the BR patches were aggregated and distributed unevenly on

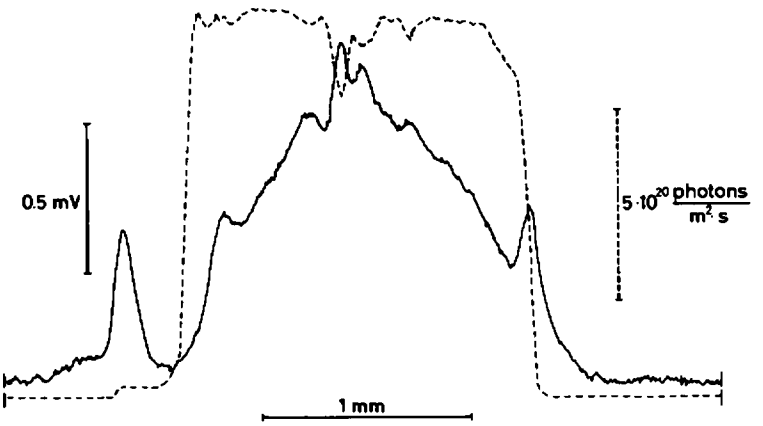

FIGURE 3 See Fig. 1. Scanning of the photopotential of a planar lipid membrane containing nonoriented BR, to investigate the distance resolution of the technique used.

the membrane surface, and consequently they gave sharp, irregularly located peaks of photopotential generation during photoelectric scanning (Fig. 3). The positions of the photopotential maxima coincide with the positions of the light transmission minima, showing the direct correlation between the membrane absorption and photopotential generation. Inasmuch as the photopotential peaks are distributed randomly (as are the BR), the distance of two nearest but well-distinguishable peaks gave us the upper limit of the scanning resolution. As expected, a value (0.1 mm) close to the determined diameter of the light spot was obtained for the distance resolution.

According to these data, the distance resolution of the system was two to five times higher than the thickness of the border ring of the BLM, and thus, in the case of the BR-BLM, this sensitivity should have resolved the photoelectric activity of the border if it had any.

With regard to the experimental data obtained on the thickness and resistance, and to the visual observations on the BR-BLM, no doubt remains that we were dealing with a real bilayer structure. The scanning experiments show that the photoelectric activity of the BR-BLM is connected with the BR incorporated into the bimolecular part of the membrane. It can not be ruled out, however, that during attachment of BR to the bilayer structure the latter might form microlenses (10, 11) of smaller size than the distance resolution of the scanning technique used.

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