THE STUDY OF PHOTOCONDUCTION OF ARTIFICIAL LIPID MEMBRANES INCORPORATING RHODOPSIN. THE SIMULTANEOUS CHANGES OF MEMBRANE CONDUCTION AND RHODOPSIN FLUORESCENCE

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Abstract. The protein fluorescence changes of rod outer segment fragments during bleaching were studied. Flash caused a fluorescence intensity drop by about 6%. The time constant of this process was ~30 msec and coincided with the time constant of increasing the permeability of an artificial lipid membrane containing rhodopsin and of Metarhodopsin I decay. In the presence of hydroxylamine the fluorescence intensity increases after the initial drop. The second process time constant was about 300 msec and coincided with the conduction drop time constant of the artificial membrane containing rhodopsin. A new intermediate - Metarhodopsin II is proposed. It has the Metarhodopsin II absorption spectrum, lives for about 300 msec at room temperature, does not react with hydroxylamine, and increases the permeability of a disk membrane.

I. INTRODUCTION

Earlier we have developed a method for modification of the artificial lipid membranes (ALM) by rod outer segment (ROS) fragments obtained by ultrasonic treatment of ROS [1-4]. Such modified membranes responded to light by increasing the permeability [3, 4]. An essential feature of response was its reversibility: the conduction increase was followed by the spontaneous dark drop to the level identical or somewhat higher than the initial one. The rate of conduction increase coincided within the error limits of our experiments with the rate of Metarhodopsin I decay measured by means of the flash photolysis technique [5, 6]. The time constant of the conduction decrease at room temperature was about 0.3 sec while Metarhodopsin II lives at this temperature at least a few minutes [7]. This fact allows a conformational rearrangement to be suggested after Metarhodopsin II formation which does not lead to the absorption spectrum change. In order to obtain further evidence in favour of this suggestion we tried to detect this conformational transition by the fluorescence technique and to compare kinetic parameters of membrane response with those of luminescence changes.

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II. MATERIAL AND METHODS

ROS were prepared as follows: retinas excised from darkadapted cattle eyes were put in isoosmotic buffer solution (140 mM NaCl, 3.5 mM KCl, 1.8 mM CaCl₂, 0.5 mM MgCl₂ 10 mM Tris-HCl, pH 7.0), similar to that used by Korenbrot and Cone [8], 1 ml per 1 retina, and subjected to mild homogenization on the magnetic stirrer for 20 min. The homogenate was centrifuged for 10 min at 100 g. The supernatant was collected and centrifuged for 10 min at 5000 g. The sediment was suspended in 40% (w/v) sucrose solution prepared with the above mentioned isoosmotic buffer and subjected to flotation for 30 min at 30000 g. Flotation was repeated twice.

To determine the purity criterion, the ROS were solubilized with a 2% solution of cetyl trimethyl ammonium bromide (CTAB) (BDH, UK). The purity criterion measured according to Heller [9] was 2.1–2.5 for different preparations. Fragments of ROS (FROS) were obtained by sonic treatment of a ROS suspension in distilled water at 15 kHz. The membranes were formed by the method of Mueller et al. [10] on the aperture (1 mm in diameter) in a teflon cup, placed in a glass chamber. The lipid solution used consisted of egg lecithin (18 mg ml⁻¹) and cholesterol (12 mg ml⁻¹) in heptane. The modification was carried out by forming a membrane in the solution containing FROS (the optical density at 500 nm was 0.15). The solutions on both sides of the membrane were always identical. The current flowing through an ALM was measured with the electrometer amplifier (Keithley model 301) under the voltage clamp conditions. The 1 msec flashes from a xenon filled discharge tube were used to illuminate the samples.

The 289 nm line of the high pressure mercury lamp spectrum was used to excite fluorescence. The fluorescence was recorded with a photomultiplier after passing through a liquid filter transmitting the wavelength band from 320 to 340 nm. The photomultiplier was placed at the right angle to the exciting beam. The ‘dead time’ after the flash did not exceed 20 msec. The time constant of recording arrangement was about 5 msec.

III. RESULTS AND DISCUSSION

Figure 1 shows the registered change of FROS fluorescence caused by flash illumination. The fluorescence drops by about 6%, the time constant of this process being less than 30 msec. In our opinion this change can be attributed to the following three causes:

1. The Metarhodopsin II formation brings about the absorption increase in the band of fluorescence and, hence, the increase of fluorescence reabsorption.
2. The Metarhodopsin II formation causes the absorption increase at 365 nm and changes parasite light scattering originated due to intensive mercury lamp radiation at 365 nm, which passes to some extent through the whole optical system.
3. The cause of the registered effect is the change of fluorescence quantum yield.

The first two points were excluded by the results of the following experiments:

1. At the sample illumination by the light with the wavelength 334 nm the registered radiation (the scattered light with the wavelength 334 nm) did not change after rhodopsin bleaching. This points to sample absorption at 334 nm and, hence, fluorescence reabsorption did not change at bleaching.
2. The interference filter with the transmission maximum at 282 nm, placed in the exciting beam, changed significantly the relative amount of parasite light but did not change the relative effect amplitude.

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