ABSTRACT

Bacteriorhodopsin, a retinal protein, is a light-activated proton pump that converts directly electromagnetic energy into electrochemical energy. In its native form, it exists as a hexagonal array of protein trimers known as the purple membrane. Anesthetics induce a pH-dependent equilibrium between two spectral forms of bacteriorhodopsin. In the presence of the halogenated general anesthetic enflurane, the maximal absorption of bacteriorhodopsin reversibly changes from 570 nm to 480 nm. This is accompanied by a structural and functional transition. But, structural and functional changes of the purple membrane do not recover completely. Irreversible effects are found, both in the structure of purple membranes and the photochemistry of proton pumping.

The absorption changes as a function of anesthetic concentration show a very strong cooperativity among the bacteriorhodopsin molecules. However the concentration that induces absorption transition depends on the history of the sample. Samples that have been treated with anesthetics may retain part of previously added anesthetics. Besides anesthetics, high temperatures (~70 °C) induce the same reversible pH-dependent absorption transition of bacteriorhodopsin. This suggests that anesthetics induce, at room temperature, a transition that otherwise, would only occur above 70 °C. Analysis of this phenomenon by calorimetry indicates that this transition is strongly related to the crystalline array.

Anesthetics inhibit the bR proton pumping activity and change the kinetics of the bR photocycle. The photocycle of bR_{480} is characterized by a fast deprotonation and a slow reprotonation of its chromophore, corresponding to a fast rise and a slow decay of its intermediate M. The decay of intermediate M is split into two components, a fast one and a slow one, which can be detected even in the presence of a very small amount of anesthetic. Comparing the decay of intermediate M in the presence of different concentrations of anesthetics to that of the native bR, we find that bR photochemistry does not

completely recover after anesthetic-treatment and that the remaining effect of anesthetic is not due to an excess amount added to the sample.

The structure of purple membranes was also examined by several physical techniques. Circular dichroism spectroscopy of the purple membrane in the presence of anesthetics shows that bR trimers change to monomers. After removal of anesthetics, most of bR monomers recover their trimeric structure. Differential scanning calorimetry measurement indicates lost of organization of the crystalline lattice, while x-ray diffraction and atomic force microscopy reveal that the crystalline array is seriously damaged after anesthetic removal from the sample.

On the other hand, bR secondary and tertiary structures were examined by Fourier transform infrared spectroscopy. The changes of bR secondary structure are very small and reversible but the change in the lipid seems not to be completely reversible. The asymmetric bands corresponding to CH_3 groups prove that. In addition, H/D exchange measurements show that the change of bR tertiary structure occurs at the surface region of the membrane.

Experiments with simple DPPC lipid membranes provide additional evidence that the effect of anesthetics at the surface of the membrane is not as easily removed as that in the core of the membrane. We thus conclude that the irreversible effect of anesthetics, namely the irreversible modification of the crystal array may be due to strong interactions of anesthetics and polar groups of the membrane surface.

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