Journal of Photoscience (2002), Vol. 9(2), pp. 47–50

A Possible Significance in Vertebrate Phototransduction of Multi-Protein Signaling Complexes on Raft-Like Membranes

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Raft is a distinctive membrane domain enriched in a certain class of lipids, cholesterol, and proteins observed on the plasma membrane. Growing evidence has revealed that such membrane domains play key roles in signal transduction, fertilization, development, transmitter release, and so on. Recently, we have isolated raft-like detergent-resistant membrane (DRM) fraction from bovine photoreceptor rod outer segments. Transducin and its effecter, cGMP-phosphodiesterase, elicited stimulus-dependent translocation between detergent-soluble membrane and DRM. This suggested potential importance of such distinct membrane domains in vertebrate phototransduction. Here, we will discuss physiological meaning of the translocation of major components of cGMP cascade to raft-like membrane in phototransduction. We would like to propose a hypothesis that raft-like membrane domains on the disk membrane are the place where cGMP cascade system could be quenched.

Key words: raft, rod outer segment, multi-protein complex

INTRODUCTION

The phototransduction system in vertebrate photoreceptor rod outer segments (ROS) has been extensively studied as a typical G protein signaling system. Photo-bleached rhodopsin interacts with GDP-form transducin, and stimulates nucleotide exchange on the α-subunit of transducin (Tα). Activated Tα is released from the complex, and approaches to its effector, PDE. The GTP-Tα binds with the inhibitory subunit of PDE, and releases its constraints to the PDE catalytic subuniti (Pαβ). Active Tα is quenched by the hydrolysis of GTP by its own GTPase, which is enhanced by a photoreceptor-specific regulator of G protein signaling protein (RGS9). The reduction of cytoplasmic cGMP-level causes cellular response of photoreceptors. It is interesting that the major phototransduction proteins are modified with lipids, by

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which they are anchored to the surface of the membrane, and they achieve their role by transient association with each other on the surface of the disk membrane. Although such an outline of vertebrate phototransduction has been authorized by many research works in these two decades, the inactivation process of phototransduction system, and the plastic adaptation mechanism of this system to background light has not been fully understood.

In order to characterize the phototransduction system from completely different point of view, we examined the presence of lateral organization of disk membrane in terms of detergent-insolubility of the membrane. Insolubility against neutral detergent has known to be a biochemical character of a distinct membrane domain so-called "raft" or "caveolae" observed on various cellular plasma membranes. It has been propsed that raft-like membrane domain exists in a separate phase from the rest of the bilayer, in a state similar to the liquid ordered (L_o) phase found in an artificial membrane. We have already reported the raft-like membrane fraction of bovine ROS, and stimulus-dependent accumulation of transducin and PDE to the detergent-resistant membrane (DRM)[1]. Here, we will discuss physiological meaning of the translocation of major components of cGMP cascade to raft-like membrane in phototransduction.

MATERIALS AND METHODS

Preparation of Triton X-100-insoluble Membrane Fraction from Bovine. ROS was prepared from dark-adapted bovine frozen retinas by using an image converter as described previously. ROS was suspended in Buffer A (10 mM MOPS (pH7.2), 60 mM KCl, 30 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 1 mM BAPTA, and protease inhibitor cocktail. ROS (5 mg protein in 400 μl of Buffer A) was incubated in the presence or absence of 400 μM of GTPγS, an unhydrolyzabel analogue of GTP for 30 min at 4°C under room light or in the darkness. Then, ROS was solubilized with 1% Triton X-100 in a volume of 750 μl at 4°C. The density of the solution was adjusted to 1.3 by adding 2.4 M sucrose in Buffer A, and the sample was placed in the bottom of centrifuge tubes. Low-density buoyant membrane fraction was obtained at the density ~1.10 after sucrose density gradient ultracentrifugation (200 k x g, 4°C for 20h). Fractions (500 μl) were collected from the top to the bottom of the cfg tubes. Proteins in each fraction were analyzed on SDS-polyacrylamide gel electrophoresis followed by western blotting.

RESULTS AND DISSCUSSION

Detergent-resistant membrane prepared from bovine ROS. Bovine ROS was solubilized with 1% of Triton X-100 in an isotonic buffer at 4°C and in a neutral pH condition. Slight opaque turbidity was observed in the solution. Following ultracentrifugation, we observed yellowish-white light-scattering diffuse band at a density ~1.10. Electron microscopic observation revealed that the low-density

opaque fraction contained uni- or multi-layered vesicles (100~300 nm in diameter) with electron dense particular substances on their surfaces. Neither light exposure of ROS nor the presence of GTPγS caused any obvious changes in electron microscopic images of these vesicles.

Lipid compositions of DRMs prepared in various conditions were compared to that of ROS. Among DRM prepared in different light and nucleotide conditions, no difference in lipid compositions was observed. The recovery of sphingolipids, especially ganglioside GD3 and sphingomyelin, to DRM was considerably high. About 50% of GD3, sphingomyelin, and cholesterol in ROS were condensed in DRM fraction. In contrast, the contents of glycerol-phospholipids such as PC, PE, PI, and PS were reduced considerably, whereas they were still major lipid components of the DRM.

Protein composition of raft-like membrane.

In our experimental conditions, approximately 10 % of rhodopsin in ROS was constantly localized on the DRM. No obvious change in the content of rhodopsin on DRM was observed in any conditions except for the concentration of detergent. In higher concentrations of Triton X-100, lesser rhodopsin was observed in DRM. Rhodopsin on the raft-like membrane has been suspected to have reduced binding strength to transducin, and kinetics [2].

On the basis of western blotting data and partial amino acid sequencing data, we have identified some components of the DRM prepared from bovine ROS; ATP-binding cassette transporter (ABCR, 220 kDa), guanylate cyclase (110 kDa), photoreceptor-specific regulator of G protein signaling (RGS9, 55 kDa) and its cofactor Gβ5L (44 kDa), Rh (37 kDa) were constantly observed in DRM; PDEαβ (90 and 88 kDa), Tα (40 kDa), Tβ (37 kD), Pγ (10 kDa), Tγ (9 kDa) were observed dependently on their states in phototransduction process.

Light-dependent recruitment of transducin to DRM.

Upon light exposure of ROS, a significant change in protein composition of DRM was observed only on the transducin. At most 50 % of transducin was recruited to the DRM prepared from light-exposed ROS. Since all the subunits of transducin showed the same behaviors, transducin seems to translocate in the form of trimeric holo-enzyme. This transducin pool is reported to have slow nucleotide exchange rate [2]. In this context, the accumulation of transducin to DRM might cause reduction of effectiveness of transducin to activate PDE. This massive translocation of transducin to raft-like region before nucleotide exchange may be a process for the

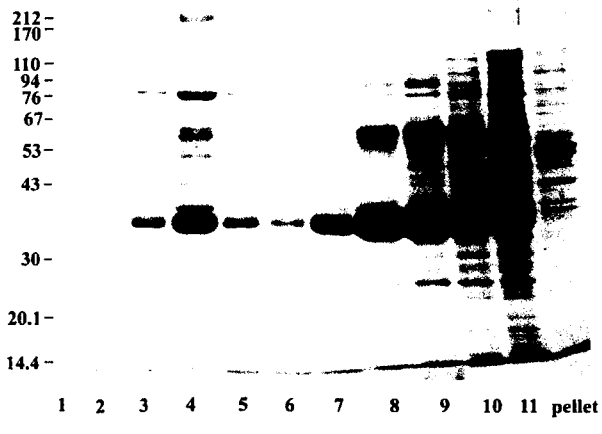

Figure 1. Detergent-resistant membrane fraction from bovine rod photoreceptor outer segments (Fraction # 4)

adaptation of vertebrate photoreceptors.

Active PDE/Tα complex is recruited to raft-like membrane. We have examined the form of PDE that was recruited to DRM under light in the presence of GTPγS. Since phototransduction system on the disk membrane was stimulated by light and GTPγS, PDE could be activated by GTPγS-Tα. In fact, PDE assay data suggested that PDE in the DRM fraction was in its full activation state. However, western blotting analysis indicated that the all subunits of PDE comigrated to DRM. Therefore, it was strongly suggested that PDE holoenzyme comigrates with active Tα to the DRM [1].

Comigration of PDE with active Tα was supported by an experiment in which we used AlF_4^- instead of GTPγS as a cofactor for Tα. The addition of AlF_4^- to the dark-adapted ROS elicited massive accumulation of PDE with approximately equimolar amount of Tα. Thus, it was confirmed that bleached rhodopsin is not required to this phenomenon. Furthermore, addition of excess amount of Pγ to AlF_4^- -activated ROS resulted in inhibition of the recruitment of Pαβ to DRM, while Tα/Pγ-complex comigrated to DRM.

Conclusively, our data strongly suggested that active GTP-Tα binds with PDE to activate it, and then recruits it to raft-like region on the disk membrane. The recruitment is thought to be due to an affinity of Tα to something in the raft-like region. From our experi-mental results, RGS9 is the most likely candidate.

Together with the massive translocation of transducin before nucleotide exchange, the trans-location of signaling molecules to DRM is highly likely to be indispensable process to quench or reduce the activity of the cGMP cascade.

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