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Signaling Protein Complex Formation in Detergent Resistant Membrane of Bovine Photoreceptor Rod Outer Segments

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We have recently found that a detergent-resistant raft like membrane (DRM) can be prepared from bovine rod outer segment membranes as a low-density buoyant fraction in sucrose density gradient ultracentrifugation. G protein (transducin) and its effector enzyme (phosphodiesterase: PDE) drastically change their affinities to DRM in the process of phototransduction. We report here that the recruitment of transducin and/or PDE to DRM has close relationship with their states in signal transduction. Active Tα/PDE-complex has a high affinity to DRM, whereas inactive transducin, or inactive PDE are excluded from DRM. Active Tα/PDE-complex seems to bind to a GTPase activating protein (GRS9) in multi-protein complexes localized on DRM. Physiological significance of the multi-protein complex on the raft-like membrane in vertebrate phototransduction would be discussed.

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

INTRODUCTION

Rafts are a collection of membranes characterized by insolubility in nonionic detergents. Insolubility of such micro membrane domain is thought to be due to compact packing of highly saturated fatty acid of sphingo- or glycerol-lipids with cholesterols.

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The disk membranes of rod photoreceptor outer segments (ROS) has been know to be enriched in highly unsaturated phospholipids. Thus, one can speculate that proteins involved in vertebrate phototransduction can move freely on the disk membrane. Although membrane domain enriched in highly unsaturated phospholipids is susceptible to detergent, we observed some membrane protein components in ROS were insoluble in Triton X-100, e.g. guanylate cyclase. Thus, we tried to prepare detergent-resistant membrane (DRM) fraction from bovine ROS preparations. A low-density buoyant fraction could be

obtained in sucrose density gradient ultracentrifugation following solubilization of ROS membranes by Triton X-100 [1]. It is important that transducin and its effector cGMP-phosphodiesterase showed massive translocation between DRM and Triton X-100-soluble fraction. Outline of our study was presented in a symposium (evolution of phototransduction) of this conference. Here, we report on the stability of the DRM against detergent, and putative multi-signaling protein complex on the raft-like region of disk membrane.

MATERIALS AND METHODS

Intact bovine ROS was prepared from dark-adapted bovine frozen retinas accordingly to Molday [2]. DRM was prepared by the method described before [1] with a little modification. One mg protein of ROS was solubilized by Triton X-100 in final concentration of 0.5, 1, and 2% (w/v) Triton X-100, then the solution was subjected to sucrose-density gradient ultracentri-fugation (200,000 x g, at 4° C for 20h). Fractions were collected from the top, and proteins in each fraction were analyzed on SDS-PAGE, and western blotting. In order to examine the complex formation of signaling proteins in DRM, DRM was solubilized again with 0.5% lauryl sucrose and subjected to ultracentrifugation on a second sucrose-density gradient ultracentrifugation from upper position. The supernatant and pellet fractions were analyzed by SDS-PAGE and immunoblotting. For the activation of transducin in the dark, $AlCl_3$ and NaF were added to ROS.

RESULTS AND DISSCUSSION

Detergent/lipid-ratio is a decisive factor to prepare DRM from photoreceptor rod outer segments

Raft is defined by detergent insolubility, and low density (n_{d20} ~ 1.10). Unlike other rafts, DRM of photoreceptor rod outer segments appeared more sensitive to detergent. Thus, we examined the detergent-sensitivity of DRM of bovine ROS. ROS was solubilized with various concentration of Triton X-100, and then subjected to sucrose-density gradient centrifugation. Figure 1 shows protein concentration of every fraction from the sucrose gradient from the top to the bottom. It shows that 0.5% Triton X-100 is not enough to solubilize ROS, whereas 2% Triton X-100 is too much to observe DRM. Proper detergent/ rhodopsin weight ratio for obtaining DRM was about 2.

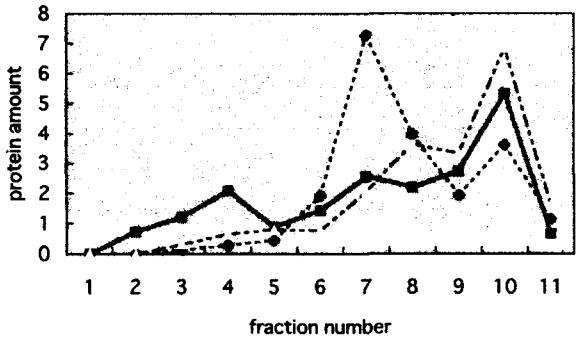

Figure 1. Raft-like membrane was observed at a proper lipid/detergent ratio. ROS was solubilized with 0.5% (diamond), 1% (squre), 2% (triangle) Triton X-100, and subjected to density gradient centrifugation.

Phototransduction may be accomplished by multi-protein complex localized on the raft-like membrane domain. As shown in our previous paper [1], the accumulation of transducin to DRM was completely light-requiring process. Since bleached rhodopsin has a high affinity to GDP-Tαβγ, rhodopsin on DRM is most likely candidate for the recruitment. Recently, it has been suggested that this type of transducin pool has a slower kinetic character in nucleotide exchange than those on more fluid membrane.

PDE accumulation to DRM occurred in the presence of an unhydrolyzable analog of GTP under light condition [1]. It was found that aluminum fluoride also causes PDE accumulation with a minor fraction of Tα in the dark. This result indicates that active Tα/PDE complex has a high affinity to DRM. There should be target protein(s) for the active Tα/PDE on the raft-like membranes.

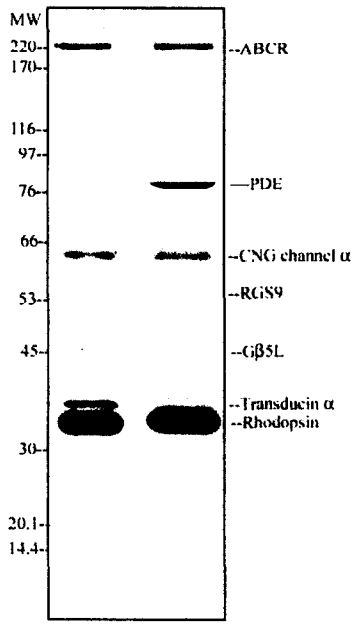

Figure 2. Protein components in DRM prepared in the absence (left) or presence (right) of GTPγS.

In order to identify the target protein for Tα/PDE, we have tried to examin the other components in DRM. Figure 2 indicates CBB stained pattern of proteins in DRM. Major protein bands could be identified with amino acid sequencing and/or western blotting. The regulator of G protein signaling (RGS9), and its cofactor Gβ5L were constantly distributed on DRM.

Raft-like membrane fraction could be solubilized with a mild neutral detergent such as lauryl sucrose, and multi-protein complex was released from DRM vesicles. The complex consisted of proteins that we have already identified as above. Multi-protein complexes containing RGS9 may be the target of active Tα/PDE, and they seems to have important function in phototransduction.

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