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Efficiency of Phototransduction Cascade in Carp Cones[§]

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In the vertebrate retina, rods mediate twilight vision and cones daylight vision. Rods have been purified easily from the retina, and thus the phototransduction mechanism in rods is now well documented. However, it has not been possible to purify cones in large quantities, and therefore, the knowledge on the mechanism in cones is limited. Here we report purification of carp (*Cyprinus carpio*) cones with a stepwise Percoll gradient. Using purified cells, we compared the phototransduction mechanism between rods and cones. The results showed that both transducin activation and phosphodiesterase activation are less effective, and visual pigment phosphorylation is faster in cones. These differences explain lower light-sensitivity and briefer photoresponse time course in cones.

Key words : cone photoreceptors, visual pigment, transducin, PDE, phototransduction, phosphorylation

INTRODUCTION

In the vertebrate retina, there are two types of photoreceptor cells, rods and cones. Rods mediate twilight vision and cones daylight vision. The photoresponse characteristics differ in rods and cones [for review, see refs. 1-3]. The light-sensitivity of a cone is ~100 times lower than that of a rod, and the response is much briefer in cones than in rods [4-6].

It has been known that the phototransduction cascades in rods and cones are basically similar, and there are rod- and cone-version of phototransduction enzymes (for example, visual pigment, transducin (photoreceptor-specific trimeric G-protein), cGMP phosphodiesterase (PDE) and cGMP-gated channel). Therefore, it is likely that the difference in the photoresponses between rods and cones are due to differences in the efficiencies of phototransduction reactions in rods and cones. Thus, to know the molecular mechanisms that characterize the rod and cone photoresponses, it is inevitable to compare biochemically the efficiency of a reaction in rods with the corresponding reaction in cones. However, it has not been possible to isolate cone cells in quantities large enough to do biochemistry yet.

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Recently, we have succeeded in obtaining rods and cones simultaneously from the retina of carp (*Cyprinus carpio*), and measured some of the phototransduction reactions in both types of the cells [7].

MATERIALS AND METHODS

Isolation of rod and cone photoreceptor cells, and preparation of rod and cone membranes. Carp (*Cyprinus carpio*), 25-30 cm in length, was dark-adapted for >3 hr before use, and the retina was dissected after pithing the animal. The photoreceptors were brushed off in a Ringer's solution (4 mM HEPES, 119.9 mM NaCl, 2.6 mM KCl, 0.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.5 mM $MgSO_4$, 1 mM $NaHCO_3$, 16 mM glucose, 0.5 mM NaH_2PO_4, pH 7.5), and the resultant suspension of the photoreceptors was filtered through a nylon mesh. The filtrate containing isolated photoreceptors was layered on the top of a stepwise Percoll gradient (see Fig. 1), and centrifuged for 20 min at 10,000 g. The purified cells at the interfaces were collected and resuspended in the Ringer's solution, and sedimented by centrifugation firstly at 600 g for 12 s and then at 3,000 g for 4 s.

The cells were disrupted by freeze-thaw, and the resultant membranes were washed with a potassium-gluconate buffer (K-gluc buffer; 115 mM K-gluconate, 2.5 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 0.1 mM $CaCl_2$, 0.2 mM EGTA, 10 mM HEPES, pH 7.5). The membranes were then resuspended in the same buffer. All manipulations were carried out in complete darkness with the aid of an

infrared image converter.

Spectroscopic measurement. To quantify visual pigments in the rod and the cone membranes, absorption spectrum of each type of the pigment was measured as described previously [8].

Transducin activation assay. Radionucleotide filter binding assay was carried out as described [9] with some modifications [7].

Phosphorylation assay. Rod or cone membranes (15 μl) were mixed with 10 μl of the K-gluc buffer containing [γ-^{32}P]ATP, GTP, and EGTA (final concentrations: 0.3 μM visual pigment, 0.1 mM [γ-^{32}P]ATP, 0.5 mM GTP, 0.8 mM EGTA). After pre-incubation for 30 s, the sample was irradiated with a light flash bleaching 1.9 % rhodopsin or 4.0 % cone visual pigments. The reaction was terminated by adding 150 μl of 10% TCA. After centrifugation (14,000 g, 10 min), the precipitate was washed with the K-gluc buffer and subjected to SDS-PAGE. The amount of ^{32}P incorporated into the visual pigment band was quantified by an image analyzer (BAS 2000, Fuji Film). All manipulations were carried out at room temperature.

PDE assay. PDE activity was measured as described previously [10] with the pH assay method that relies upon the pH drop accompanied by hydrolysis of cGMP.

RESULTS AND DISCUSSION

Purification of Rods and Cones.

Carp rods and cones were brushed off the retina (Fig. 1). Based on the number of the cells, the ratio of rods and cones at this stage was ~50 : 1. The cells were then separated by using a stepwise Percoll gradient: rods were obtained at the 45 / 60 % (w/v) interface and cones at the 75 / 90 % (w/v) interface (Fig. 1). Contamination of the other type of the photoreceptors was negligible in both the rod and the cone fraction. The cone fraction, however, contained erythrocytes (Fig. 1) so that in the following studies we always did control experiments to confirm that erythrocytes does not show the effect.

Purified rods had outer and inner segments, but lacked nuclear region (Fig. 2A). Most of purified cones also had outer and inner segments, but it also lacked nuclear region (Fig. 2B). Some of the cone cells lacked outer segment, but they are purified together with cones that retained outer segments. This fact indicated that the inner segment of a cone is heavier than that of a rod. This is probably

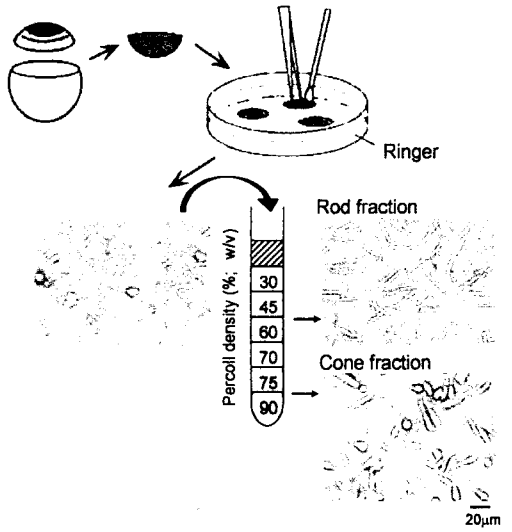

Fig 1. Purification of rod and cone photoreceptor cells from carp retina. Photoreceptor cells were brushed off the retina, and then separated by using Percoll stepwise gradient.

the reason why we were able to separate cones from rods by using the density gradient method.

With the partial bleach method, we detected only a single visual pigment, rhodopsin, in the rod fraction, and three types of visual pigments in the cone fraction. The ratio of the three types of the pigments, and therefore possibly that of other phototransduction proteins, was 3 (red):~1 (green):~1 (blue).

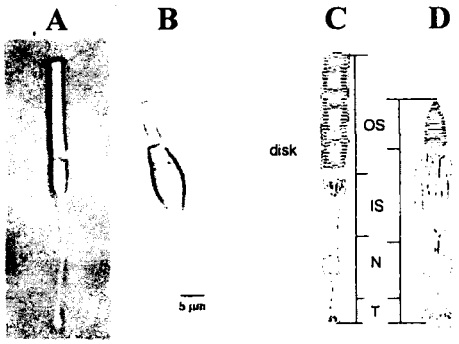

Fig. 2. Purified rod and cone. A, purified rod. B, purified cone. C and D, sketches of a rod and a cone. OS, outer segment. IS, inner segment. N, nuclear region. T, terminal region.

Comparison of Phototransduction Efficiencies in Rods and Cones.

By using purified rods and cones, we compared three reactions in the phototrasduction: (1) transducin activation by visual pigment, (2) PDE activation by visual pigment (through transducin activation by visual pigment), and (3) inactivation of visual pigment by phosphorylation.

The result showed that transducin is activated by an activated visual pigment at the rate of ~50 molecules/sec in rods. In the case of cones, the rate was less than 2 molecules/sec. The result showed that the activation of transducin is about 25 times less effective in cones.

The PDE activation was found to be about 250 times less efficient in cones, which is partly because the transducin activation is about 25 times less effective in cones (see above). These results, therefore, indicated that PDE activation by an activated transducin molecule is about 10 times less effective in cones. The phosphorylation of light-activated visual pigment was too fast to measure in cones, and was more than 20 times faster in cones than in rods.

Molecular Mechanism Characterizing the Cone Photoresponses.

The transducin activation is about 25 times , and PDE activation is 10 times less efficient in cones. These differences should be the underlying molecular mechanisms that characterize the rod and cone photoresponses. The lower efficiencies of the reactions in cones should be responsible for the slower rise of a photoresponse in cones when compared with the photoresponse elicited in rods by the light flash of the same intensity. This should result in lower light sensitivity in cones. In addition, the visual pigment phosphorylation is faster in cones, and therefore, the lifetime of light-activated visual pigment is much shorter in cones. As a result, photoresponse starts to recover at a much shorter period of time after a light flash in cones. This fast onset of the recovery explains the briefer photoresponse and in addition, the lower light-sensitivity in cones.

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