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Chicken red-sensitive cone visual pigment retains a binding domain for transducin

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Iodopsin (a red-sensitive cone visual pigment) and rhodopsin (a rod pigment) were isolated from chicken retina. They were separately reconstituted into phosphatidylcholine liposomes and then mixed with rod transducin (Tα and T$\beta\gamma$) purified from bovine retina. Iodopsin enhanced, only when irradiated, the binding of GppNHp to Tα to a similar extent to irradiated rhodopsin. Furthermore, the binding of GppNHp to Tα in the presence of a photobleaching intermediate of iodopsin preferably required T$\beta\gamma$-2 rather than T$\beta\gamma$-1, which is very similar in profile to that in the presence of the intermediate of rhodopsin (J. Biol. Chem., in press). These results indicate that the binding domain for transducin in iodopsin should closely resemble that in rhodopsin.

Cone visual pigment; Iodopsin; GTP-binding protein; Transducin; Rhodopsin; (Chicken retina)

1. INTRODUCTION

In rod outer segments, a photobleaching intermediate of rhodopsin transiently binds to a GTP-binding protein, transducin (T) and catalyzes the formation of a T-GTP complex. T-GTP thus formed dissociates from the intermediate and in turn activates cGMP phosphodiesterase in the visual transduction process (for a review see [1]). Recently, T [2,3] and cGMP phosphodiesterase [4] have been identified in cone outer segments. Moreover, the amino acid sequence of visual pigments from human rods and cones showed significant homology [5]. Thus, it is now suggested that the transduction pathway in cones may be similar to that in rods. However, no direct evidence has been presented so far that a cone pigment might expose a binding domain for T when irradiated.

Although some cone pigments have been highly purified [6,7], the use of digitonin as a solubilizer has hampered biochemical investigations: since digitonin has a low critical micelle concentration and a high aggregation number, it is difficult to remove digitonin from purified proteins to prepare liposomes. We have recently succeeded in purifying one of the cone pigments, red-sensitive chicken iodopsin in Chaps (λ_{max}: 571 nm) and in reconstituting it into phosphatidylcholine (PC) liposomes. This preparation displayed a remarkable ability, only when irradiated, for enhancement of the binding of GppNHp (non-hydrolyzable analogue of GTP) to the α-subunit of bovine rod T (Tα) as well as chicken rhodopsin (λ_{max}: 503 nm).

We have previously shown that bovine T$\beta\gamma$ was composed of two species, T$\beta\gamma$-1 and T$\beta\gamma$-2 [8]. T$\beta\gamma$-1 exhibited a very low ability for enhancement of the GppNHp binding to Tα when compared with another component, T$\beta\gamma$-2, even when

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Abbreviations: GppNHp, guanosine-5'-(β,γ-imido)triphosphate; DTT, dithiothreitol; PC, fresh egg-yolk phosphatidylcholine; Chaps, (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate); $PhMeSO_2F$, phenylmethylsulfonyl fluoride

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rhodopsin was irradiated [8]. Here we demonstrate that substitution of rhodopsin for iodopsin gave no effect on the ability of both T$\beta\gamma$-1 and T$\beta\gamma$-2. All these observations indicate that the binding domain for T in iodopsin is very similar to that in rhodopsin beyond the difference in animal species.

2. EXPERIMENTAL

Both iodopsin and rhodopsin were purified from freshly dissected chicken retinas according to the method of Yen and Fager [6] with some modifications, the most important of which was the substitution of Chaps by digitonin, where 0.8–1.0 mg/ml PC was included to stabilize cone pigments. Detailed procedures will be described elsewhere. Briefly, all pigments were extracted from chicken rod and cone outer segment membranes with 0.75% Chaps supplemented with 1 mg/ml PC in buffer A (50 mM Hepes-NaOH, 140 mM NaCl, 1 mM DTT, 0.1 mM $PhMeSO_2F$, 4 μg/ml leupeptin, 50 kallikrein inhibitor units/ml aprotinin; pH 6.6). The extract was applied to a concanavalin A-Sepharose column, from which iodopsin was eluted with 1.5 mM methyl α-D-mannoside in buffer A supplemented with 0.6% Chaps, 0.8 mg/ml PC, 1 mM $MnCl_2$ and 1 mM $CaCl_2$. On the other hand, a mixture of rhodopsin and green-sensitive pigment was eluted from the column by increasing the concentration of methyl α-D-mannoside in the elution buffer to 100 mM. After desalting, the mixture was applied to a DEAE-Sepharose column. Rhodopsin adsorbed to the column was selectively eluted with buffer A containing 100 mM (instead of 140 mM) NaCl, 0.6% Chaps, 0.8 mg/ml PC and 20% (w/v) glycerol. Only a small amount of other pigments was detected spectrophotometrically in either purified iodopsin (iodopsin/rhodopsin/short wavelength-sensitive pigments [6] = 90:2:8) or purified rhodopsin (rhodopsin/green-sensitive pigment = 96:4). Iodopsin or rhodopsin thus obtained was incorporated into PC liposomes by dialysis against buffer B (10 mM Mops-NaOH, 2 mM $MgCl_2$, 140 mM NaCl, 1 mM DTT, 0.1 mM $PhMeSO_2F$; pH 7.5) after adjusting the molar ratio of PC/pigment to 100. Purified bovine rhodopsin was similarly reconstituted at the molar ratio of 150 as in a previous study [8] and stored at −80°C until use.

T was isolated from freshly dissected bovine retinas [3], and then Tα, T$\beta\gamma$-1 and T$\beta\gamma$-2 were purified as described previously [8].

3. RESULTS AND DISCUSSION

The purity of reconstituted iodopsin or rhodopsin was examined by SDS-polyacrylamide gel electrophoresis (fig.1: a, chicken iodopsin; b, chicken rhodopsin; c, bovine rhodopsin). Three bands corresponding to a monomer (about 38 kDa), a dimer (about 76 kDa) and probably a trimer (about 120 kDa) of each pigment were detected (lanes a, b and c). Only chicken iodopsin (lane a) had an additional band (16 kDa), which has not yet been

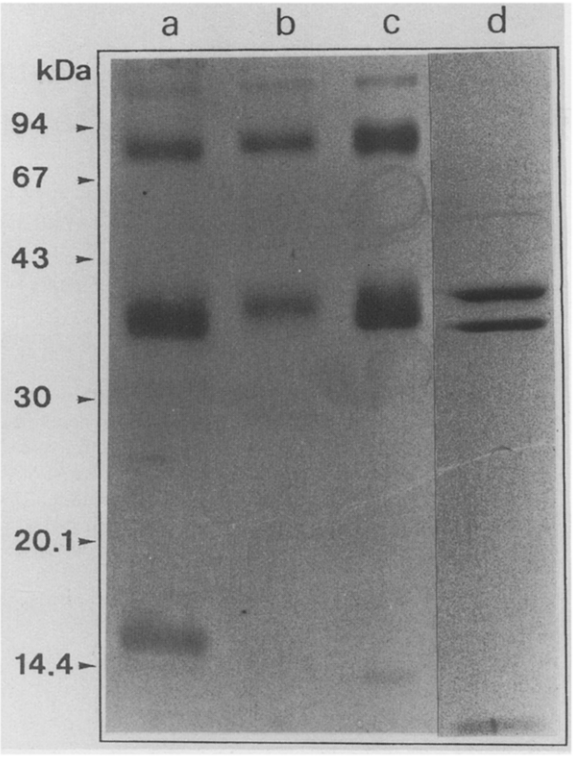

Fig.1. SDS-polyacrylamide gel electrophoresis patterns of purified proteins. Electrophoresis was performed by the method of Laemmli [11], and the gel was subjected to silver staining. Lanes: a, chicken iodopsin; b, chicken rhodopsin; c, bovine rhodopsin; d, bovine T.

characterized, while, T purified from bovine retina (lane d) exhibited only three bands corresponding to Tα (39 kDa), Tβ (36 kDa) and Tγ (6–8 kDa). Any trace of a band due to cone-specific Tα (41 kDa [2]) could not be detected. As reported by Lerea et al. [2], cone-specific T should have been lost during the course of isolation of outer segment membranes.

First, a fixed amount (1.2 μM) of T$\alpha\beta\gamma$ without separation into the subunits (Tα and T$\beta\gamma$) was mixed in the dark with various amounts of iodopsin (chicken) or rhodopsin (chicken or bovine) in liposomes. Just after adding [^{3}H]GppNHp (10 μM in final concentration) to the mixtures, they were incubated for 30 min in the dark or in the light (red light above 660 nm for iodopsin and orange light above 590 nm for rhodopsin). Then, upon irradiation chicken rhodopsin (fig.2, —△—) or even iodopsin (—○—) enhanced the binding of GppNHp

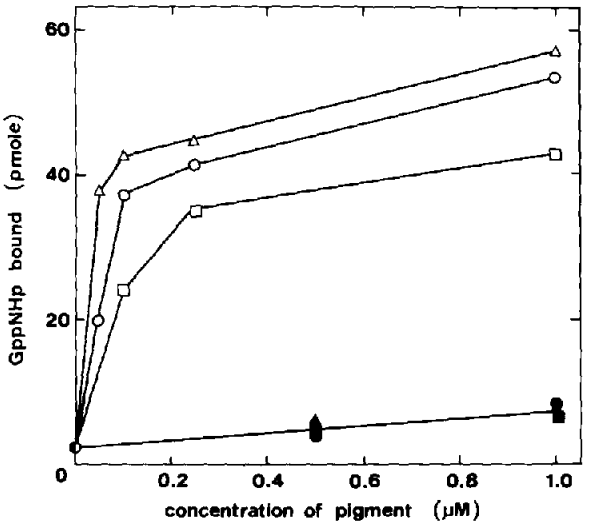

Fig.2. Binding of GppNHp to bovine T in the presence of rhodopsin, iodopsin or the photobleaching intermediate. The reaction mixtures (0.15 ml of buffer B) included 1.2 μM bovine T ($T\alpha\beta\gamma$), 10 μM [^{3}H]GppNHp (0.27 mCi/mmol; Amersham) and various amounts of either chicken iodopsin (circles), chicken rhodopsin (triangles) or bovine rhodopsin (squares) in PC liposomes. After initiating the reaction by the addition of [^{3}H]GppNHp, the mixtures were incubated at 4°C for 30 min in the dark (closed symbols) or under continuous irradiation with 1 kW tungsten lamp fitted with a cut-off filter (Toshiba), VR-61 (>590 nm) for irradiation of rhodopsin or VR-68 (>660 nm) for iodopsin. After the incubation, [^{3}H]GppNHp bound to Tα was isolated according to a conventional filtration method [12]. The molar extinction coefficient of chicken rhodopsin or iodopsin was postulated to be the same as that of bovine rhodopsin.

to bovine T to almost the same or a larger extent than bovine rhodopsin (—□—). Since iodopsin was irradiated with the red light, which cannot be absorbed by pigments other than iodopsin, the enhancement of the binding by iodopsin (—○—) cannot be ascribed to the bleaching of other contaminated pigments (see section 2). Furthermore, addition of chicken pigments at a concentration (<0.1 μM) far lower than that of T (1.2 μM) greatly enhanced the binding in light, excluding a possibility that a small fraction of contaminating pigment is responsible for the enhancement of the binding. This result clearly indicates that not only rhodopsin but also iodopsin act like a catalyst in the formation of Tα-GppNHp. On the other hand, GppNHp binding was suppressed completely without irradiation (fig.2, closed symbols), demonstrating that the binding domain for T in each pigment is well masked in the dark.

These results suggest that the active domain in iodopsin is very similar to that of rhodopsin. This suggestion seems reasonable because the amino acid sequence of the carboxy-terminus of rod Tα, which may be an interaction site with rhodopsin [9], is highly homologous to that of cone Tα [2]. In contrast, the sequence of rod Tα diverges somewhat from that of cone Tα at the amino-terminus which is supposed to interact with T$\beta\gamma$ [9], suggesting that a cone outer segment might also contain a specific T$\beta\gamma$ which has not yet been identified. Recently, we have found that bovine Tγ is composed of two components, tentatively named Tγ-1 and Tγ-2 [8]. Interestingly, T$\beta\gamma$-1 exhibited a far lower ability to enhance the binding of GppNHp to Tα than T$\beta\gamma$-2, even when rhodopsin was irradiated. One of the possible interpretations was that T$\beta\gamma$-1 might be a cone-specific subunit and active only in the presence of a cone pigment [8]. Success in reconstituting iodopsin to a functionally active form has now enabled us to test the possibility.

After separation of T$\alpha\beta\gamma$ into Tα, T$\beta\gamma$-1 and

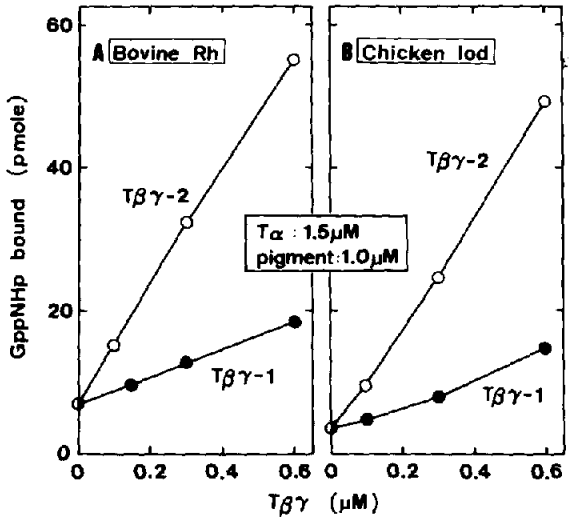

Fig.3. Effect of the photobleaching intermediate of rhodopsin or iodopsin on the abilities of T$\beta\gamma$-1 and T$\beta\gamma$-2 to enhance the binding of GppNHp to Tα. The concentrations of [^{3}H]GppNHp (10 μM), Tα (1.2 μM) and bovine rhodopsin (1.0 μM, A) or chicken iodopsin (1.0 μM, B) were held constant in the reaction mixtures, to which T$\beta\gamma$-1 (closed symbols) or T$\beta\gamma$-2 (open symbols) was added at various concentrations. All mixtures were continuously irradiated (see the legend of fig.2) during the incubation. Other experimental conditions are the same as in fig.2.

T$\beta\gamma$-2, various amounts of T$\beta\gamma$-1 or T$\beta\gamma$-2 were added to the reaction mixture which included fixed amounts of both Tα and a purified pigment in liposomes (bovine rhodopsin or chicken iodopsin). Just after the addition of [^{3}H]GppNHp to the mixture, it was continuously irradiated under the same condition as that in fig.2. In the presence of bovine rhodopsin (fig.3A), the amount of GppNHp bound to Tα increased in proportion to the amount of T$\beta\gamma$-2 added (—○—), while T$\beta\gamma$-1 enhanced the binding only slightly (—●—) as reported previously [8]. Chicken iodopsin again showed an activation profile very similar to bovine rhodopsin (cf. fig.3A and B). This observation would not necessarily exclude the possibility that T$\beta\gamma$-1 might be a cone-specific subunit, because the Tα used was derived from rods. It should be tested in the presence of cone Tα, which has been identified [2] but has not yet been isolated. More importantly, this result together with that of fig.2 indicates that the active domain of iodopsin, which is most probably exposed to the cytosolic surface to interact with T only when irradiated, is very similar to that of rhodopsin. We reported previously that photobleaching of iodopsin activated cGMP phosphodiesterase in a mixture of chicken rod and cone outer segments in the presence of GTP [10]. The present study demonstrates more convincingly and directly that the first step in the visual transduction process in cones is most probably similar to that in rods. This conclusion will be confirmed when cone T is isolated in the future.

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