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THE BINDING OF G PROTEINS TO IMMOBILIZED DELIPIDATED RHODOPSIN

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We have shown that delipidated rhodopsin immobilized on Concanavalin A-Sepharose is capable of binding transducin from crude bovine rod outer segment proteins and GTP-binding proteins (G proteins) of Go/Gi-type from solubilized bovine brain membrane as well. The binding is reversible in the presence of a solution containing 1.2% octyl-β,D-glucopyranoside and 1 mM GTP. Also, alfa-subunits account for a large fraction of the G proteins which are bound to and then eluted from the immobilized rhodopsin. Concanavalin A-bound delipidated rhodopsin seems to be a useful model in isolating and purifying different G-proteins from crude cell lyzates and solubilized membranes as well as for studying G-protein - receptor interaction.

Photoexcited rhodopsin in retinal rod outer segments (ROS) is known to activate a light-dependent cGMP phosphodiesterase (PDE)in two steps: at the first step, a stable complex between rhodopsin and GDP-containing G-protein, transducin, is formed; then GDP is released from the active site of transducin and substituted for GTP; after the GDP/GTP exchange the rhodopsin - transducin complex dissociates, producing a GTP-bound transducin which, in its turn, activates PDE at the second step of the cascade /1,2/. Even though rhodopsin, transducin and PDE are membrane-associated proteins, the results of our previous investigations have shown that delipidated rhodopsin is capable of activating transducin and PDE in a solution of the non-ionic detergent, octyl glucoside (OG) /3,4/. At the same time, delipidated rhodopsin immobilized on Concanavalin A-Sepharose (Con A-Rh) has been shown to form a complex with purified transducin in a solution of lauryl sucrose in a light-dependent manner /5/. Since photoexcited rhodopsin incorporated in proteoliposomes can activate not only transducin, but other G-proteins (namely, Go and Gi) as well /6-8/, we have examined the possibility of a specific binding, by delipidated rhodopsin immobilized on Con A-Rh, of different G-proteins from crude cellular proteins mixtures - the ROS extract or solubilized brain membranes.

ABBREVIATIONS: Con A-Rh - rhodopsin, immobilized on Concanavalin A-Sepharose;G-proteins - regulatory GTP-binding proteins; OG - n-octyl-β, D-glucopyranoside;PDE - cGMP phosphodiesterase; ROS- rod outer segment.

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We have shown that immobilized delipidated rhodopsin is capable of binding transducin and G-proteins of Go/Gi-type, and that the binding is reversible in the presence of OG and GTP. We suppose that Con A-Rh can be used for "functional affinity chromatography" in isolating G-proteins from different sources and also as a model for G-proteins - receptor interaction.

EXPERIMENTAL PROCEDURES

ROS were isolated from dark-adapted bovine retinae at 0-4°C in dim red light /9/. Transducin and PDE were extracted from ROS by the method of Kuhn /1/ and purified to homogeneity as described earlier /3/. For the extraction of ROS proteins, pelleted ROS were suspended in a 10 mM Tris-HCl (pH 8.0), containing 2 mM EDTA-Na, 2 mM dithiotreitol, 0.1 mM phenylmetansulphonyl fluorid and 10 ug/ml of aprothinin, and disrupted in a Potter tephlon- to-glass homogenizer with 10-15 strokes in ice; then the homogenate was centrifuged at 50,000 x g for 20 min, the supernatant was collected and centrifuged again at 120,000 x g for 10 min to avoid membrane contamination. Then $MgCl_2$ was added to the clarified ROS proteins preparation to a final concentration of 5 mM. To obtain the extract of brain membrane proteins, the membranes isolated from the bovine brain tissue /10/ were dissolved in 30 mM Tris-HCl (pH 8.0) containing 6 mM $MgCl_2$,1 mM EDTA-Na, 4 mM 2-mercaptoethanol, 0,9% Na-cholate, 1 ug/ml of the soybean trypsin inhibitor, 0.1 mM $AlCl_3$ and 10 uM NaF; allowed for 120 min on ice and centrifuged at 50,000 x g for 20 min. The supernatant was collected, diluted 2-fold with the same buffer, but without the detergent and clarified using centrifugation at 100,000 x g for 30 min, 4°C . Partial purification of Go and Gi was performed according to the method of Neer et.al /10/ using steps of DEAE-Sepharose chromatography and high-performance gel-permeation chromatography on a Superose-12 column . The resulting preparation contained a mixture of Go and Gi subunits as the major polypeptides and a few contaminating proteins.

The rhodopsin immobilization and delipidation procedure was carried out in dim red light at 0-4°C by using the method /11/ in 2% OG. The rhodopsin content in the resulting preparation of Con A-Rh was about 1 mg per ml of gel media.

The reaction mixture for ADP-ribosylation, containing 50 mM Tris-HCl(pH 8.0), 2 mM $MgCl_2$, 10 mM dithiotreitol, 1 mM ATP, 1 mM NADP, 0.1 mM GTP, 10 uM NAD^+, 10 ug pertussis toxin and 3 uCi [^{32}P] NAD^+ and aliquote of fraction eluted from the Con A-Rh column was incubated for 1 hour at 37°C. After electrophoresis /12/, the labeled proteins were detected by radioautography.

RESULTS AND DISCUSSION

The results of chromatography of purified transducin on the Con A-Rh column are shown in Fig.1. Trancducin, either when alone or when mixed with bovine serum albumin and myoglobin (Fig.1.b), binds to Con A-Rh and can be eluted with a buffer containing 1.5% OG and 1 mM GTP. The concentration range for the most effective binding of transducin is found to be 0-0.4% OG, and that for the effective elution of this protein - 0.7-1.5%. It is photo-excited rhodopsin that is needed for the transducin binding in this case, because as a result of bleached Con A-Rh washing with 1 mM hydroxylamine prior to transducin application, no rhodopsin-transducin complex formation is obtained (data not shown).

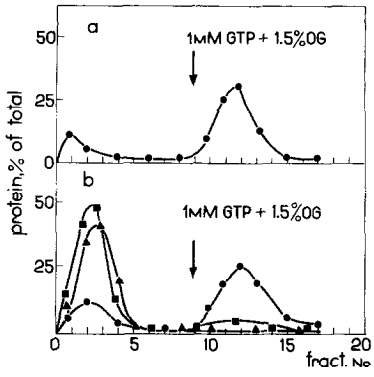

FIG.1. Transducin chromatography on a Con A-Rh column. A Con A-Rh column (1 ml of the gel) was washed in the dark with 5-10 volumes of 10 mM Tris-HCl (pH 8.0),containing 100 mM NaCl, 5 mM $MgCl_2$ and 1 mM dithiotreitol without OG (buffer A). All the subsequent procedures were carried out in light at 0-4°C . Purified transducin, dissolved in the same buffer, was applied to the illuminated gel media. The column was washed with buffer A to withdraw the unbound protein; the bound proteins were eluted with the same buffer containing 1.2% OG and 1 mM GTP at flow rate of 0.3 ml/min. The proteins eluted were separated by polyacrylamide gel electrophoresis using the Laemmli discontineous buffer system /12/, followed by densitometry of the Coomassi-stained electrophoregrams. Transducin (●) was applied onto the column (a)- alone ; or (b)- mixed with bovine serum albumin (■) and myoglobin (▲), 200 μg of each protein. The quantity of the protein in the fractions was plotted as percentage of the total amount of each protein, respectively. Elution - from left to right.The arrow indicates OG and GTP addition to the elution buffer.

We have carried out a chromatography of the total extractable ROS proteins on the Con A-Rh column. As shown in fig.2, most of the polypeptides presented in the crude protein extract did not bind to the affinity media except for transducin and a few other proteins. After the removal of the unbound proteins and subsequent elution of the retained proteins with OG and GTP, mainly transducin (alfa and beta subunits) was found in the peak fractions with a purity of about 70%. About 30% of the contaminating proteins consisted of polypeptides with a molecular weight about 90 kDa (presumably PDE subunits) and 26 kDa. The nature of the latter is not yet clear.

It is known that the other members of the G-proteins family - Gi, Gs and Go - are very similar to transducin in structure and in the mechanism of activation by their specific receptors /6-8/. Moreover, in model membrane systems it has been shown that photoexcited rhodopsin can activate not only transducin but both Go and Gi proteins as well /6-8/. Taking into account the close similarity between these proteins, we have verified whether Go/Gi-type proteins can bind to immobilized delipidated rhodopsin. After the chromatography of the partially purified G-proteins on Con A-rhodopsin (see Fig.3), G alfa polypeptides were found in the fractions eluted with a GTP-containing OG solutuion, with a slight admixture of the beta subunit(s). All of the contaminating proteins and most of G-beta subunits unbound to the

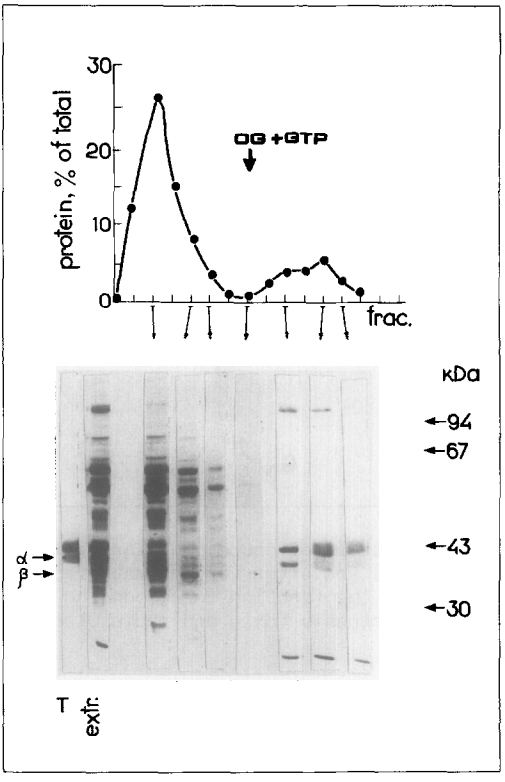

FIG.2. Chromatography on Con A-Rh column of proteins, extracted from ROS. Fractions, obtained after chromatography, were analyzed by SDS-PAG electrophoresis. `T` - purified transducin as a standard, `extr` - original sample before chromatography.

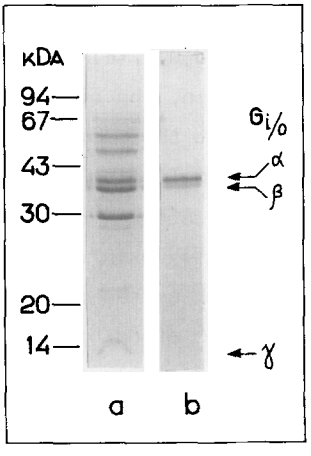

FIG.3. Electrophoretic pattern of partially purified Gi/Go proteins fraction from bovine brain membranes before (a) and after (b) chromatography on a Con A-Rh column.

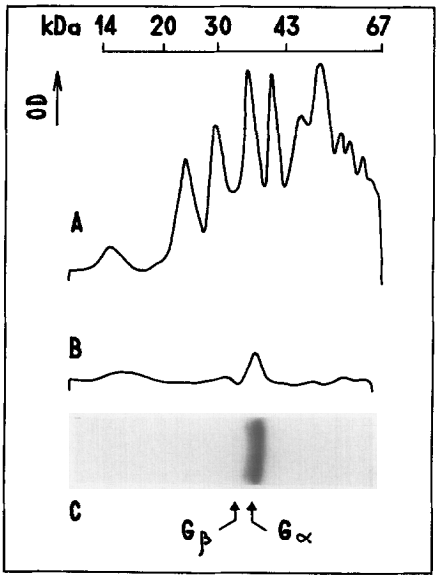

FIG.4. Electrophoretic pattern of crude proteins from solubilized bovine brain membranes before (A) and after (B) chromatography on a Con A-Rh column. The proteins from the fraction corresponding to "B" were then [^{32}P] ADP-ribosylated by pertussis toxin and radioautographed (C).

column were separated from alfa subunits during washing without OG and GTP.

Thus, not only transducin, but Go/Gi-type proteins too, can be attached to the bleached delipidated rhodopsin and the resulting complexes dissociate in the presence of a high OG and GTP concentration. This fact enabled us to investigate whether or not any of the G proteins could be isolated from the solubilized membranes directly, using its reversable binding to immobilized rhodopsin. Fig.4 shows an electrophoretic pattern of the detergent-solubilized brain membrane proteins before the application onto a column (line A) and after the elution with OG and GTP (line B). One can see that after the removal of the contaminating protein, the addition of OG and GTP causes the elution of a number of proteins, but the most intensive band on the electrophoregramm corresponds to those of molecular weight about 40 kDa comigrating with alfa-subunits of Gi/Go marker. After [^{32}P] ADP-ribosylation of the eluted proteins in the presence of pertussis toxin radioactivity comigrates with the 40 kDa - polypeptide (Fig 4.C). Because the pertussis toxin-dependent ADP-ribosylation is a characteristic of Go/Gi-type proteins /13/, the 40 kDa band ought to correspond either to a single alfa- subunit, or to several ones with very close molecular weights.

Thus, the results presented in this paper indicate that both transducin and different Go/Gi type proteins` alfa-subunits can interact specifically with immobilized delipidated rhodopsin. It appears from the data presented

here that receptor molecules can form complexes with corresponding G proteins via its alfa-subunits.

Also, the chromatography on Con A-Rh seems to be a useful approach in isolation and purification of different G-proteins from crude cell lysates and solubilized membranes.

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