The detection and characterization of G-proteins in the eyespot of *Chlamydomonas reinhardtii*

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The presence of G-proteins in the eyespot fraction of *Chlamydomonas reinhardtii* is shown. This fraction is capable of binding (GTPγ[^{35}S], possesses the GTPase activity and interacts with antibodies raised against a highly conserved peptide of most G-proteins' α-subunit. Cross-reaction with a 24-kDa protein is detected on immunoblots. Using an antiserum prepared from vertebrate β-subunit peptide, two additional proteins with apparent M_r 21 and 29 kDa could be revealed. The light-dependence of GTPase extraction from eyespot membranes is shown. The results make it possible to suggest the participation of G-proteins in the photosensory transduction chain of *Ch. reinhardtii*.

G-protein; GTPase; Rhodopsin; GTPγ[^{35}S]; *Chlamydomonas reinhardtii*

1. INTRODUCTION

The light-dependent motor reactions of unicellular green algae *Ch. reinhardtii* are realized with the help of a photosensory pigment which is localized in the special organelles, eyespots [1]. There is some evidence that the chromophore part of this pigment is similar to that of visual rhodopsin [2]. Moreover, we have previously shown that the protein parts of their molecules are also similar [3]. In particular, the rhodopsin-like pigment of *Ch. reinhardtii* has the domains which are capable of interacting with vertebrate photoreceptor G-protein (transducin) and participating in the visual transduction chain.

Taking into account these results, the question arises whether the unicellular algae possess their own G-protein(s) and what their possible functional role is.

The present work deals with the experimental investigation of this problem.

2. MATERIALS AND METHODS

2.1. *Algae strain*

Ch. reinhardtii wild type strain 494 c(−) was used. The cultivation of strain, isolation of cells and eyespot fractions have been described earlier [3].

2.2. *GTPγ[^{35}S]binding assay*

The GTPγ[^{35}S]-binding assay was performed as described [4] with some modifications: samples were incubated in buffer solution (200 mM NaCl, 30 mM Tris-HCl, pH 7.8, 1 mM EDTA, 1 mM DTT, 5 mM $MgCl_2$, 0.05% Lubrol PX) containing 0.1 μM GTPγ[^{35}S] for 40 min at 30°C. In the control probe 0.1 mM GTP was added.

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2.3. *Protein extraction*

The extraction of proteins from eyespot and other fractions was conducted in hypotonic buffer solution (5 mM Tris-HCl, pH 7.8, 2 mM DTT, 2 mM EDTA) for 40 min at 20°C. Afterwards the incubation samples were centrifuged (100000 × *g*, 1 h). In the case of extraction in dark 11-*cis* retinal (50 μM) was added before the extraction procedure.

2.4. *Immunological detection of G-proteins*

About 20 μg of protein per sample was separated by SDS-polyacrylamide gel electrophoresis [5]. The G-protein from bovine cerebellum was used as a control. Proteins were transferred to nitrocellulose by electrophoresis [6] and then part of the screen was incubated with block solution (3% BSA dissolved in phosphate-buffered saline, PBS) for 1 h and with first antibody for 10 h. Two types of antibodies were used: AS 8 (α_{common} peptide antiserum) and AS 11 (β_{common} peptide antiserum) [7]. The detection was performed with the [^{125}I]protein A. Between the incubations the membranes were washed 4 times for 5 min with PBS.

2.4. *ADP-ribosylation of G-proteins*

ADP-ribosylation in the presence of pertussis and cholera toxins was determined as described in [8].

2.5. *Measurement of GTPase activity*

The modified method [9] was used. Reaction mixture (100 μl) contained 30 mM Hepes, pH 7.5, 150 mM NaCl, 10 mM creatine phosphate, 50 μg creatine phosphokinase, 10^{-6} M GTP, 2×10^5 cpm γ-[^{33}P]GTP. Reaction was started by addition of 10–30 μg of protein.

3. RESULTS AND DISCUSSION

In our effort to determine and identify the G-protein(s) in *Ch. reinhardtii* the point of most interest seemed to be the eyespot, where, according to recent

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Table I

Identification of G-proteins in the eyespot fraction of *Ch. reinhardtii*

Fraction	Method		
	Binding of GTPγ[^{35}S] (pmol/mg protein)	GTPase activity (pmol/min/mg protein)	
		Gpp(NH)*p* absent	0.1 mM Gpp(NH)*p* present
Whole homogenate	13	n.d.	n.d.
Intermediate fraction	31	26.5 ± 4.6	25.1 ± 1.9
Eyespots	60	35.1 ± 0.6	20.3 ± 4.0

data [1,2], the photosensory rhodopsin-like pigment is localized. Fractions of eyespot were purified as described earlier [3] and used in all the experiments. In addition, the other fractions, which did not contain the photosensory pigment, and whole homogenates of *Ch. reinhardtii* cells were used in some experiments. The homogeneity of eyespot fraction was confirmed by electron microscopy.

The measurement of GTPγ[^{35}S] binding by all the fractions studied revealed that the degree of specific binding increased in the course of eyespot purification and was maximal in the fraction of eyespot (Table I). These data allowed us to suppose the presence of GTP-binding proteins in *Ch. reinhardtii* and gave some reasons to assume their possible localization in one eyespot.

The determination of high-affinity GTPase activity gave support to this supposition. Gpp(NH)*p*-inhibited GTPase, inherent in G-proteins, was also revealed in eyespot fractions (Table I).

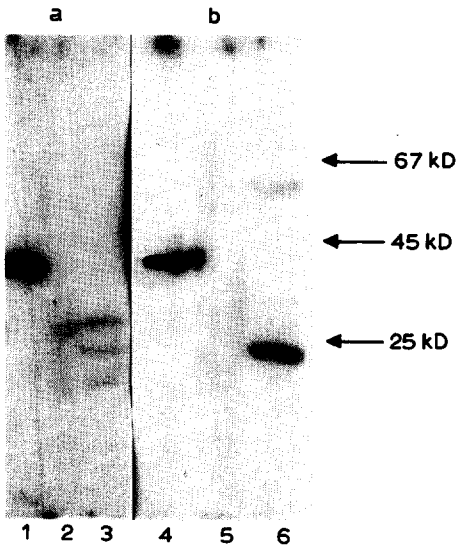

Fig. 1. Immunoblot of *Ch. reinhardtii* proteins with β_{common} (a) and α_{common} (b) antiserum after SDS electrophoresis. Lanes: 1, 4, control; 2, 5, whole homogenate; 3, 6, eyespot fraction.

For the immunological detection of G-proteins, two types of antibodies raised against highly conserved domains of vertebrate G-protein subunits were applied. An immunoblot of electrophoretically separated eyespot proteins with α_{common} antiserum, AS 8, showed a major band with a molecular mass of about 24 kDa (Fig. 1). A weak band with M_r 57 kDa may be explained as a result of negligible tubulin contamination. In the case of β_{common} peptide antibodies, AS 11, two additional bands with molecular mass of about 21 and 29 kDa were revealed (Fig. 1). It should be mentioned that both types of antibodies interacted on immunoblots with corresponding proteins only in the case of eyespot fraction and gave no reaction with electrophoretically separated proteins of whole cell homogenate (Fig. 1) and of intermediate fractions (not shown).

The data obtained strongly suggest that eyespot fractions of *Ch. reinhardtii* contain at least 3 types of G-proteins with M_r of about 21, 24 and 29 kDa, which are homologous to vertebrate G-proteins α- and β-subunits. The possibility of 24-kDa protein to interact with both AS 8 and AS 11 antisera should be mentioned. Obviously, this fact needs further experimental study.

We did not succeed in our attempt to perform the ADP-ribosylation of revealed G-proteins. The ADP-ribosylation in the presence of pertussis and cholera toxins did not take place. Incapability of some low molecular weight G-proteins to be ADP-ribosylated is known [10].

Taking into account an essential role of visual G-protein, transducin, in the photoexcitation process, possible functional significance of *Ch. reinhardtii* G-proteins is of considerable interest.

The important property of transducin, which allows it to be involved in cGMP cascade of reactions, is the light-dependence of its interaction with photoreceptor membranes [11].

It was shown that the extraction of Gpp(NH)*p*-inhibited high affinity GTPase from *Ch. reinhardtii*

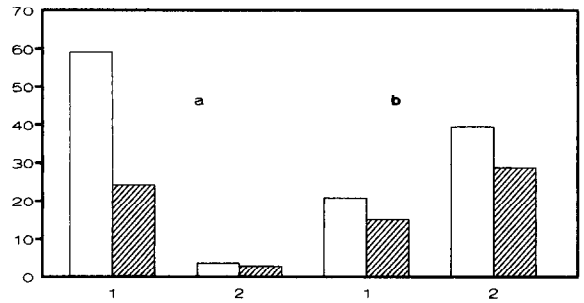

Fig. 2. GTPase activity in the eyespot fraction of *Ch. reinhardtii*. The ordinate axes: activity pmol GTP/mg protein/min. (a) Extraction was performed under room light. (b) Extraction was performed in dark; 1, extract; 2, pellet; □, control with Gpp(NH)p; ▨, −0.1 mM Gpp(NH)*p* was added in incubation mixture for GTPase activity measurement.

eyespot is also dependent on illumination (Fig. 2). In contrast to extractability of transducin increasing in darkness, the *Ch. reinhardtii* eyespot GTPase extracted much better under illumination.

If the activity of this enzyme really reflects some properties of G-proteins, these results give a reason for the directed investigation of *Ch. reinhardtii* G-proteins' role in photodependent reactions.

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