Volume 249, number 1, 59–61 FEB 07160 May 1989

Enzymatic and immunological detection of a G-protein in *Halobacterium halobium*

Angelika Schimz, Klaus-Dieter Hinsch[+] and Eilo Hildebrand

Institut für Biologische Informationsverarbeitung der Kernforschungsanlage Jülich and [+]*Pharmakologisches Institut der Freien Universität Berlin, FRG*

Received 17 March 1989

In membrane preparations of *Halobacterium*, the hydrolysis of cGMP is accelerated by activators of G-proteins, namely GTP, GTP-γ-S, and fluoroaluminate. This suggests a type of G-protein which acts on a phosphodiesterase. Light stimuli which evoke behavioral responses in intact bacteria influence the rate of cGMP hydrolysis. Using an antiserum raised against a peptide identical with one of the sequences presumably involved in GTP binding of most G-proteins, a cross reactive protein with an apparent molecular mass of 59 kDa could be detected on immunoblots. The results support the idea that a G-protein may be part of the photosensory transduction chain of *Halobacterium* [(1987) Biochim. Biophys. Acta 923, 222–232].

G-protein; Peptide antiserum, α_{common}; Phosphodiesterase; cyclic GMP hydrolysis; Photobehavior; (*Halobacterium halobium*)

1. INTRODUCTION

Halobacteria spontaneously reverse their swimming direction with a rhythm of about 10 s [1]. This pattern is transiently changed by light or chemical stimuli [2–4]. We have reported previously that cGMP influences the reversal rhythm as well as the duration of refractoriness which occurs immediately after a reversal [5]. Addition of dibutyryl-cGMP to the medium slows down the reversal rhythm and shortens the refractory period. The phosphodiesterase inhibitor IBMX [6] has the same effect. The opposite effect, acceleration of the reversal rhythm and extension of the refractory period, is achieved by fluoroaluminate which is known to activate G-proteins [7]. We take this as an indication that in *Halobacterium* the level of cGMP may be controlled by a G-protein. Dibutyryl-cAMP had no effect on the behavior of halobacteria [5].

Correspondence address: A. Schimz, Institut für Biologische Informationsverarbeitung, Kernforschungsanlage Jülich, Postfach 1913, 5170 Jülich, FRG

Abbreviation: IBMX, 3-isobutyl-1-methylxanthine

We report here in vitro measurements of the influence of G-protein activators on the hydrolysis of cGMP and the immunological detection of a G-protein α-subunit by an antiserum against a highly conserved epitope on western blots.

2. MATERIALS AND METHODS

2.1. *Bacterial strain*

Halobacterium halobium, strain Flx 3 [8] was grown to the early stationary phase as described [9].

2.2. *Measurement of cGMP-hydrolysis in vitro*

Membranes and the soluble fraction were prepared by sonication and centrifugation as described [10]. Buffer: 5 mmol/l Hepes, 2 mmol/l $MgCl_2$, 4 mol/l NaCl, pH 7.9. Protein concentration was about 8 mg/ml in both fractions. The hydrolysis of cGMP was measured in 1 ml of suspension at 30°C under stirring by recording the pH changes [11]. GTP (final concentration 0.2 mmol/l), GTP-γ-S (Boehringer; 0.1 mmol/l), or fluoroaluminate (2.5 mmol/l) was added, and the reaction started with cGMP (2 mmol/l). The amount of protons released by the hydrolysis of cGMP was calculated after calibration with a known amount of HCl. Light for stimulation (150 W halogen lamp, cut-off filter OG 550, Schott, Mainz) was applied to the cuvette through fiber optics from above.

2.3. *Detection of a G-protein by antisera*

Samples were desalted to about 0.1 mol/l NaCl by Centricon centrifugal microconcentration (Amicon). About 120 μg of pro-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

tein per sample were separated by SDS-polyacrylamide gel electrophoresis [12]. Purified G-protein (transducin) from bovine retina (a gift from Dr U. Wilden, Jülich), 6 μg, was used as a control. Proteins were transferred to nitrocellulose (NitroScreen West, NEN) by electrophoresis (Transphor TE 42, Hoefer, CA) at 30–40 V, 250 mA for 4 h. Transfer buffer was 25 mmol/l Tris, 192 mmol/l glycine, pH 8.3, 20% methanol [13]. Part of the screen was stained for 5 min with 0.1% amido black, 45% methanol, 10% acetic acid, and destained for 3 min with 90% methanol, 2% acetic acid. The other part of the screen was incubated with block solution containing 0.4% gelatin in phosphate-buffered saline, PBS (8 g NaCl, 0.2 g KCl, 1.15 g $Na_2HPO_4 \cdot 2H_2O$, 0.2 g KH_2PO_4, ad 1 l), overnight and with the first antibody, diluted in PBS, for 2.5 h. The following antisera were used: (i) α_{common} peptide antiserum, AS 8, directed against a synthetic peptide identical with the GTP-binding site on the α-subunit of most vertebrate G-proteins [14], dilution 1:300; (ii) same antiserum, but blocked with the peptide (8 μg/ml) against which it had been generated; (iii) β_{common} peptide antiserum, AS 11, directed against a peptide from the β-subunit [14] (1:300); (iv) antiserum against purified bovine transducin, α, β, γ subunits [15] (1:250); (v) α_z peptide antiserum, AS 98, against a peptide which differs by 3 amino acid residues from the α_{common} peptide [16]. Second antibody: anti-rabbit Ig, biotinylated (Amersham), 1:400 in PBS and 0.1% gelatin, 1 h. Detection: Streptavidin-horseradish peroxidase complex (Amersham), 1:500 in PBS and 0.1% gelatin, 1 h. Substrate: 60 mg 4-chloro-1-naphthol in 20 ml methanol, immediately before use diluted with 80 ml PBS containing 100 μl H_2O_2. Between the incubations, membranes were washed 4 times for 5 min with PBS.

3. RESULTS AND DISCUSSION

3.1. *Effect of G-protein activators on the hydrolysis of cGMP*

Addition of cGMP to a crude preparation of *H. halobium* membranes resulted in a slow acidification of the suspension, which indicates a slow hydrolysis of cGMP (fig.1a). Preincubation of the suspension with GTP-γ-S, which is not hydrolyzed by GTPases and thus leads to a permanent activation of G-proteins caused an about 3-fold increase in the rate of proton release upon addition of cGMP. This rate was reduced in the presence of the phosphodiesterase inhibitor IBMX. Preincubation with GTP stimulated cGMP hydrolysis by a factor of about 32 (calculated after subtraction of the proton release caused by the hydrolysis of GTP itself). Fluoroaluminate which is known to activate bovine transducin-GDP by mimicking the γ-phosphate of GTP in its binding site [7] stimulated the hydrolysis of cGMP by about the same amount as GTP-γ-S. No G-protein dependent hydrolysis of cGMP was observed in the soluble fraction (not shown). Step-like increase and decrease of yellow light which are stimuli known to cause attractant and repellent behavioral responses, respectively [2,3], changed the rate of cGMP hydrolysis (fig.1b).

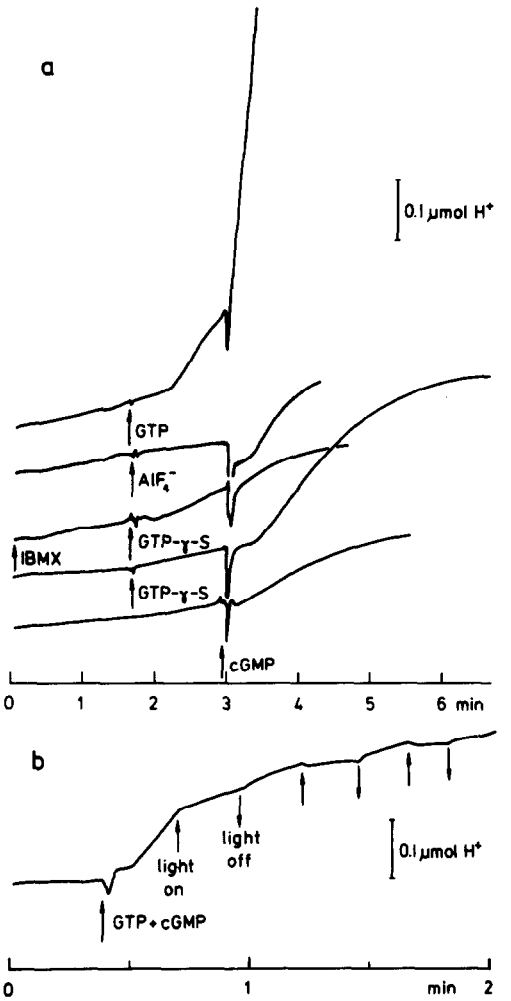

Fig.1. Measurement of the hydrolysis of cGMP in crude membrane preparations of *H. halobium* by recording the time course of proton release. (a) Effects of G-protein activators added at the time indicated by arrows. The reaction was started with cGMP always at about 3 min. (b) Effect of light stimuli. Increase (↑) or decrease (↓) of light of $\lambda > 550$ nm.

These results show that in *H. halobium* the rate of cGMP hydrolysis is enhanced by substances which are known to activate G-proteins. We therefore conclude that the bacteria contain a G-protein, the target of which is most likely a cGMP-phosphodiesterase. The light-induced changes of the rate of cGMP hydrolysis support our

hypothesis that a G-protein is involved in the signalling pathway of *H. halobium* [5].

3.2. *Immunological detection of a G-protein α-subunit*

An immunoblot of the electrophoretically separated proteins of *H. halobium* with the α_{common} antiserum, AS 8, showed a predominant band with a molecular mass of about 59 kDa, which was equally strong in both the membrane and soluble fraction (fig.2). Additional faint bands showed up at 87 kDa and at 44 kDa, in some experiments also at 34 kDa, and 22 kDa. The main band at 59 kDa, together with the faint bands at 44, 34 and 22 kDa disappeared after the antiserum had been blocked by the peptide that had been used to generate the antibody and can therefore be regarded to be specifically recognized by antibodies against the α_{common} peptide. Only the 87 kDa band remained detectable under these conditions. Its staining, therefore, is non-specific. The 34 kDa and 22 kDa bands did not occur in all experiments and may be degradation products.

The results strongly suggest that the 59 kDa band is at least in part highly homologous to α-subunits of G-proteins, and may have a similar GTP-binding site as most of the vertebrate G-proteins. Possibly the faint 44 kDa band may also represent a halobacterial G-protein. The enzymatic experiments suggest that the halobacterial G-protein has a close functional relationship to bovine transducin which is known to regulate a phosphodiesterase [17]. The total structure of the G-proteins in halobacteria and vertebrate photoreceptors, however, must be different, since there is no cross-reaction with an antiserum against purified transducin (not shown). Neither could a β-subunit of the halobacterial G-protein be detected with the β_{common} peptide antiserum. As expected, the antiserum against the α_z peptide (AS 98), which differs by 3 amino acids from the α_{common} peptide [16] did not recognize the α-subunit from halobacteria.

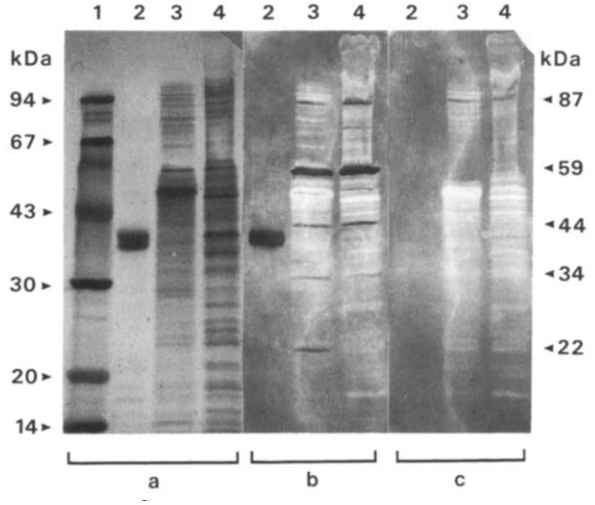

Fig.2. Immunoblot of *H. halobium* proteins with α_{common} antiserum after SDS electrophoresis and protein transfer to nitrocellulose. Lanes: 1, molecular mass standard; 2, bovine transducin; 3, soluble fraction of *H. halobium*; 4, membrane fraction of *H. halobium*. (a) Nitrocellulose stained with amido black; (b) incubated with α_{common} antiserum; (c) incubated with α_{common} antiserum blocked with the peptide that was used for the generation of the antiserum.

Acknowledgements: We thank Dr Ursula Wilden for the purified transducin, and Dr Ingo Weyand for his advice and help with the immunoblots. This work was supported by grants of the Deutsche Forschungsgemeinschaft.

REFERENCES

[1] Schimz, A. and Hildebrand, E. (1985) Nature 317, 641–643.
[2] Hildebrand, E. and Dencher, N. (1975) Nature 257, 46–48.
[3] Spudich, J.L. and Stoeckenius, W. (1979) Photobiochem. Photobiophys. 1, 43–53.
[4] Hildebrand, E. and Schimz, A. (1983) Photochem. Photobiol. 37, 581–584.
[5] Schimz, A. and Hildebrand, E. (1987) Biochim. Biophys. Acta 923, 222–232.
[6] Amer, M.S. and Kreighbaum, W.E. (1975) J. Pharmacol. Sci. 64, 1–37.
[7] Bigay, J., Deterre, P., Pfister, C. and Chabre, M. (1985) FEBS Lett. 191, 181–185.
[8] Spudich, E.N. and Spudich, J.L. (1982) Proc. Natl. Acad. Sci. USA 79, 4308–4312.
[9] Hildebrand, E. and Schimz, A. (1983) Photochem. Photobiol. 38, 593–597.
[10] Schimz, A. (1982) FEBS Lett. 139, 283–286.
[11] Yee, R. and Liebman, P.A. (1978) J. Biol. Chem. 253, 8902–8909.
[12] Laemmli, U.K. (1970) Nature 227, 680–685.
[13] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[14] Mumby, S.M., Kahn, R.A., Manning, D.R. and Gilman, A.G. (1986) Proc. Natl. Acad. Sci. USA 83, 265–269.
[15] Rosenthal, W., Koesling, D., Rudolph, U., Kleuss, C., Pallast, M., Yajima, M. and Schulz, G. (1986) Eur. J. Biochem. 158, 255–263.
[16] Spicher, K., Hinsch, K.-D., Gausepohl, H., Franke, R., Rosenthal, W. and Schultz, G. (1988) Biochem. Biophys. Res. Commun. 157, 883–890.
[17] Fung, B.K., Hurley, J.B. and Stryer, L. (1981) Proc. Natl. Acad. Sci. USA 78, 152–156.