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# Transducin GTPase provides for rapid quenching of the cGMP cascade in rod outer segments

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The role of transducin GTPase in rapid cGMP phosphodiesterase quenching was studied by simultaneous registration of GTP hydrolysis and phosphodiesterase activity in the same rod outer segments (ROS) preparation. The results thus obtained allow the conclusion that: (i) phosphodiesterase quenching coincides with transducin-bound GTP hydrolysis independently of ROS concentration; (ii) an increase in the ROS concentration results in the acceleration of cascade quenching due to the existence of a GTPase accelerating mechanism in ROS; (iii) approximation to physiological conditions (protein concentration, temperature) provides a transducin GTPase rate equal to 1–2 turnovers per second i.e., sufficiently high for satisfying the real rate of photoresponse reversion in dark-adapted rods.

Photoreception; Phosphodiesterase; Transducin GTPase; Signal quenching

## 1. INTRODUCTION

It is generally proposed that a photoresponse of retinal rods is caused by a decrease in the concentration of cytoplasmic cGMP, which is mediated by light-dependent activation of an enzymatic cascade including rhodopsin, GTP-binding protein T and PDE [1]. This cascade is capable of not only rapid activation in light, but also of sufficiently rapid quenching, responsible for rod photoresponse reversion. The mechanism of cascade quenching at the step of T activation by rhodopsin has been studied in detail; it includes rhodopsin phosphorylation and subsequent binding to phosphorylated rhodopsin of a rod 48 kDa protein [2–4]. However, the mechanism of cascade quenching at the step of PDE activation by T is a controversial point. Although it is known that PDE activation terminates after the hydrolysis of T-bound GTP [1], numerous measurements carried out in reconstituted systems indicate that the rate of GTPase reaction is too slow (tens of seconds) (review [5,6]) compared with the real cascade quenching time in an ROS suspension (seconds) [7]. To overcome the above contradiction two possibilities were postulated: (i) the rate of T GTPase under physiological conditions is higher than in reconstituted systems [6,8,9]; (ii) the rapid PDE quenching in ROS is caused by a mechanism, independent of T GTPase [10,11].

In the present work we studied the role of T GTPase in rapid PDE quenching by simultaneous registration of GTP hydrolysis and PDE activity in the same ROS preparation. The ROS used in this work remained sealed during their isolation, so all cytoplasm components which could possibly affect GTPase activity were present in the reaction mixture. Our experimental approach consisted of rapid cascade swithing-on by the addition of GTP substoichiometric amounts to T and of subsequent registration of cascade quenching under conditions excluding additional T and PDE activation during

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*Abbreviations:* ROS, rod outer segments; T, transducin; PDE, cyclic GMP phosphodiesterase; Mops, morpholinepropanesulfonic acid; AppNHp, adenylyl imidodiphosphate

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the experiment. The results obtained allow the conclusion that: (i) PDE quenching coincides with T-bound GTP hydrolysis independently of ROS concentration; (ii) an increase in the ROS concentration results in the acceleration of cascade quenching due to the existence in ROS of a GTPase accelerating mechanism; (iii) approximation to physiological conditions (protein concentration, temperature) provides a T GTPase rate equal to 1–2 turnovers per s, i.e. high enough to satisfy the real rate of photoresponse reversion in dark-adapted rods.

## 2. EXPERIMENTAL

Sealed bovine ROS were isolated in cold under infrared illumination acording to [12]. Rhodopsin concentration in the preparations was determined as in [5]. ROS were stored frozen in liquid nitrogen and disrupted by mild sonication before experiment. All the measurements were performed at 23°C in a buffer containing 20 mM Mops-NaOH (pH 8.0), 100 mM KCl, 5 mM $MgCl_2$, 1 mM dithiothreitol and 3 mM $NaH_2AsO_4$. 1 h preincubation of sonicated ROS in this arsenate-containing buffer at 0°C led to complete depletion of endogeneous nucleoside triphosphates. Dark experiments were carried out under infrared illumination; the bleaching of a calibrated amount of rhodopsin in ROS was performed with a photographic flash attenuated with neutral density filters.

GTPase activity was determined in 20 $\mu$l samples stirred on a vortex mixer. The reaction was started by adding 10 $\mu$l of [$\gamma$-$^{32}$P]GTP ($10^4$ cpm) to preliminary bleached ROS suspension and stopped by 50 $\mu$l of 0.5 M $HClO_4$. The amount of $^{32}P_i$ formed was determined as in [5].

PDE activity was monitored through pH changes associated with cGMP hydrolysis [13] in a 0.8 ml cuvette equipped with a mechanical stirrer and a combined pH electrode (Radiometer). The reaction was started by 10 $\mu$l GTP. The half-time of the monitoring system response was 0.4 s.

## 3. RESULTS AND DISCUSSION

### 3.1. *Determination of T GTPase rate constant in ROS*

We conducted measurements of T-bound GTP hydrolysis during one turnover of the reaction in excess of T over GTP, which excludes additional T activation during the experiment (the amount of the GTP added never exceeded 1% of rhodopsin concentration in the probe; the fact that the T content in ROS is about 10% of rhodopsin guaranteed the excess of T over GTP). To suppress all non-T GTPase activities in the ROS, we added 1 mM AppNHp to the reaction mixture: this caused practically complete inhibition of dark GTPase activity without any effect on light-dependent T GTPase. The reaction course was described as an exponent from which GTPase rate constant could be calculated.

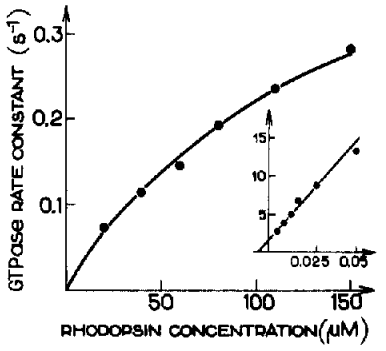


Fig.1. Dependence of the T GTPase rate constant on ROS concentration. Reaction mixture (see section 2) contained 1 mM AppNHp and completely bleached ROS in an appropriate concentration: the reaction was started by 200 nM GTP. (In the inset the same curve is presented as a double reciprocal plot).

Fig.1. shows the dependence of T GTPase rate constant on ROS concentration. It is seen that an increase in ROS concentration from 20 to 150 $\mu$M (by rhodopsin) results in the 4-fold acceleration of GTP hydrolysis. Assuming this is a hyperbolic dependence, one can calculate the the reaction rate at the saturating conditions, i.e. at an ROS concentration corresponding to physiological conditions (about 3 mM rhodopsin [6]); in 5 different ROS preparations this approximated rate constant varied from 0.35 to 0.7 $s^{-1}$ at 23°C. We could not determine this constant at physiological temperature, 37°C, because at high ROS concentrations the reaction was too fast to be monitored. But considering the fact that the temperature increase from 23°C to 37°C results in a 3-fold increase in the T GTPase rate [5,9], one can conclude that the rate of the reaction under physiological conditions is not less than 1 turnover per s.

A similar effect of GTPase acceleration upon ROS concentration increase was reported in [6] where a non-direct estimation of the GTPase reaction rate by light-scattering measurements was performed. But the authors described only the linear part of the plot and could not calculate the $V_{max}$ value. At the same time our results contradict the data [9] that the GTPase reaction rate is not dependent on ROS concentration and may attain 20–30 turnovers per min at 37°C only when the GTP con-

centration is about $10^2$-fold higher than in our experiments.

The data presented in fig.1 suggest that ROS contain a GTPase accelerating factor and that the increase obtained in the GTPase rate constant is due to the increase in the factor's concentration. Fig.2 provides the evidence for this proposition. It is seen (fig.2A) that the dilution of the ROS suspension with the buffer a few seconds after the onset of the GTPase reaction causes a considerable lowering of the GTP hydrolysis rate. This effect can be mediated by a decrease in the hypothetical factors's concentration. However, an alternative explanation is also possible: not all the GTP is bound to T before buffer addition – so the dilution decelerates T-GTP complex formation. To select between these possibilities, we diluted the sample with a suspension of unbleached ROS (which are practically unable to hydrolyse GTP) taken at the same concentration as in the starting mixture which allowed us to decrease the concentration of GTP and the rhodopsin-T complex without changing the concentration of other ROS components. Since this dilution did not cause any alterations in the course of the reaction, all of GTP was bound to T before the dilution. Therefore the effect of the buffer can be explainend only as a result of the decrease in the factor's concentration. (The changing rhodopsin-T complex concentration after the addition of dark ROS to the sample did not take place, because under our experimental conditions the bleached rhodopsin in the starting mixture was in a complex with T and GTP was added in an amount not higher than 10% of this complex). Another confirmation of this conclusion is shown in fig.2B: dilution of the reaction mixture with unbleached ROS taken at a higher concentration accelerated GTP hydrolysis.

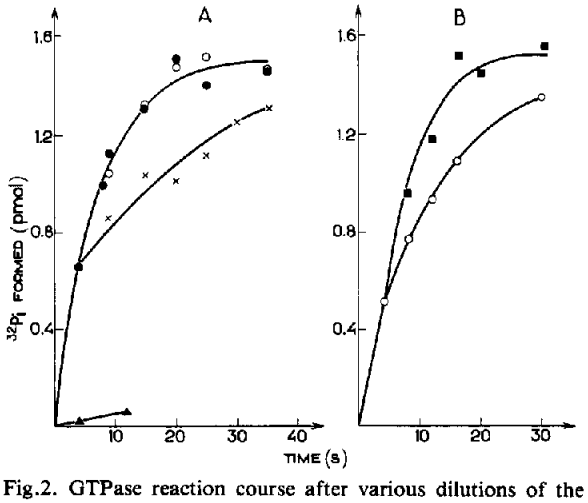


Fig.2. GTPase reaction course after various dilutions of the reaction mixture. All samples (see fig.1 for details) contained 30 $\mu$M rhodopsin bleached by 2.5%: the reaction was started by 75 nM GTP and diluted 4 s after the start. (A) ○, without dilution; ●, 15-fold dilution with 30 $\mu$M unbleached ROS; ×, 15-fold dilution with sample buffer; ▲, the activity of 30 $\mu$M unbleached ROS. (B) ○, without dilution; ■, 2-fold dilution with 170 $\mu$M unbleached ROS. (The data presented in parts A and B were obtained with two different ROS preparations.)

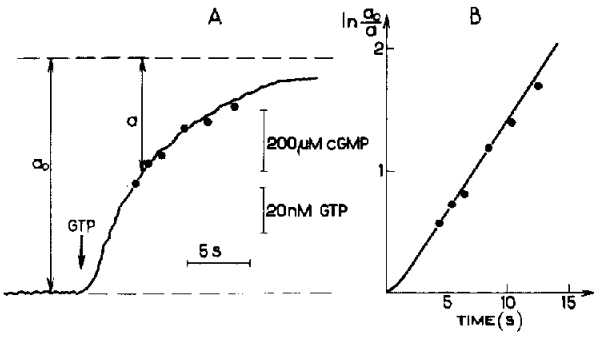


Fig.3. Determination of the T GTPase and PDE quenching rate constants in the same ROS preparation. The mixtures (see section 2) contained 100 $\mu$M completely bleached rhodopsin, the reaction was started by 200 nM GTP. (A) Solid line, PDE monitoring; ●, the monitoring of $P_i$ formation; (B) the same data are presented as semilogarithmic plots.

Nothing could be said about the nature of GTPase accelerating factor from ROS. Apparently, it is possible to exclude rhodopsin, PDE, 48 kDa-protein and $\beta\gamma$-subunits of T because these proteins have no effect on T GTPase in a

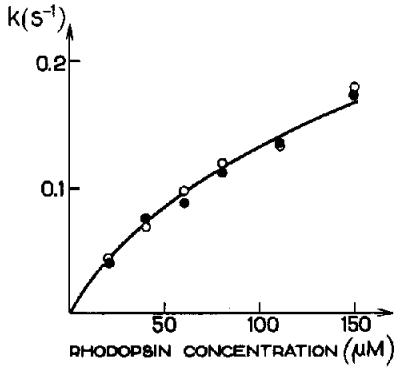


Fig.4. Dependence of PDE quenching (○) and T GTPase (●) rate constants on ROS concentration.

reconstituted system [5]. The recent finding of a special protein, accelerating the GTPase of $p21^{ras}$ oncoprotein [14], allows the proposition that the acceleration of GTPase activity can be a general principle of GTP-binding proteins regulation.

### 3.2. *The coincidence of PDE and T quenching in ROS*

By using the one-turnover approach we have measured both the hydrolysis of the T-bound GTP and the PDE reaction in the same ROS preparation. Fig.3 shows shows that the courses of both processes coincide and can be approximated by an exponent. This coincidence occurs at all the ROS concentrations used (fig.4). So, in our ROS the rate of T-bound GTP hydrolysis determines the rate of PDE quenching.

Is the rate of cascade quenching obtained in our experiments sufficient to explain rapid signal reversion in retinal rods? The above approximation of the GTPase reactio rate to physiological conditions gives a value of more than 1 turnover per s. This is high enough to provide the real reversion of a photoresponse in dark-adapted rods. Thus, it is possible to conclude that GTPase can really serve as a mechanism of rapid cascade quenching at the step of T-PDE interaction.

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