Biochimica et Biophysica Acta, 1101 (1992) 260–263

BBABIO 43666

Kinetics and energetics of the rhodopsin-transducin-cGMP phosphodiesterase cascade of visual transduction

Marc Chabre and T. Minh Vuong

Institut de Pharmacologie du CNRS, Sophia Antipolis, Valbonne (France)

(Received 14 April 1992)

Key words: Visual transduction; Retina; Rhodopsin; Transducin

In retinal rod cells, the absorption of a single photon by a retinal chromophore in a rhodopsin molecule elicits a hyperpolarisation of the whole cell, by about a millivolt, for a few hundred milliseconds. This results from the closure of ionic channels in the plasma membrane of the photorecepting outer segment, which induces a reduction by about a picoampere of the large Na^+ current that runs down a 35 mV potential difference across the cell membrane. The power gain of the equivalent amplifier, i.e., the ratio of the energy of the signal generated at the cellular level ($\sim 10^{-12}$ A $\times$ 3.5 $\cdot$ 10^{-2} V $\times$ 1 s = 3.5 $\cdot$ 10^{-14} J) over the energy of the absorbed photon (50 kcal/mol = 3.3 $\cdot$ 10^{-19} J), would thus be of the order of 10^5. This amplifier has its maximal energy consumption in the resting state in the dark, for the generation of the large 'dark' membrane current that flows through open channels in the outer segment membrane. The current generators are the ATP dependent Na^+/K^+ pumps located exclusively in the plasma membrane of the inner segment, in which all the ATP producing mitochondria are confined. The current modulators are cGMP dependent channels, located exclusively in the plasma membrane of the outer segment.

The key intracellular signal transmitter is thus cGMP, whose concentration in the outer segment is modulated by illumination. This is a negative control with a very high gain: one photoexcited rhodopsin (R*) induces the activation of a few hundred cGMP phosphodiesterases molecules (PDE). By hydrolysing 10^5 to 10^6 molecules of cGMP to 5'GMP (Fig. 1), enough to reduce its concentration locally by a few micromolar, the closure of a few hundred channels is induced [1,2]. The PDE activity results from a cascade of enzymatic activations: One R* catalyses the activation by GTP of a few hundred molecules of transducin, a heterotrimeric G-protein ($T\alpha_{GDP}T\beta\gamma$). The activated $T\alpha GTP$ subunits are released, diffuse away from R* and from $T\beta\gamma$, and in turn activate, non-catalytically (i.e., by stoichiometric persistent binding), an equivalent number of PDE molecules. Each PDE then starts hydrolysing cGMP at the rate of a few thousand per s per PDE. This PDE activity ceases when the GTP in PDE-bound $T\alpha GTP$ is itself hydrolyzed. The basal cGMP level was maintained in the dark and is regenerated after a flash by guanylate cyclases that consume GTP. All the energy required for this stage of signal amplification is thus provided by GTP. A high GTP level ($\sim$ 1 mM) is maintained in rod outer segments by a high NDP kinase activity which transphosphorylates GDP from ATP that flows in from the mitochondrion-rich inner segment.

We will focus here on the energetics of this light-induced rhodopsin-transducin-PDE cascade. It has become an archetype for a large variety of signal transduction processes, mediated by heterotrimeric G-proteins, between '7 helices' membrane receptors (to sensory, hormonal or neuronal signals), and effector proteins that modulate internal second messengers or intracellular ionic flows. The visual cascade differs quantitatively, however, from most other signal transduction processes by a larger amplification and faster activation and termination kinetics. We have developed a time-resolved microcalorimetry technique that enables us to monitor the yield and rate of heat released after a flash, and, thus, to analyse, under various metabolic conditions, the energetics of various stages of the reaction cascade, in vitro, in cattle retinal rod outer segment suspensions [3,4]. The heat sensor is a 40 μm thick poled poly(vinylidene difluoride) film, gold-plated on both faces, on the back side of a thin (0.4 mm) 36 $\times$ 36 mm sample chamber, which can be illuminated through a thin transparent Mylar front window. Dilation from heating causes charge dispacements in the

Correspondence to: M. Chabre, Institut de Pharmacologie du CNRS, Sophia Antipolis, 06560 Valbonne, France.

PVDF film, generating between its two metallic faces a displacement current proportional to the derivative of the film temperature, which is recorded by a high impedance preamplifier. The cell is glued on a heat insulating slab of balsa wood, which makes it adiabatic on the time scale of a minute, and is shielded from sonic and low frequency pressure waves. It is mounted, together with the preamplifier, in an air-tight cavity set between two 15 kg blocks of brass stabilized at 23°C inside a thick styrofoam box. The box has a double paned window through which the sample can be illuminated. The instrument impulse response time, limited mainly by the PVDF film heat capacity, is 140 ms (FWHM), as checked by flash-illumination of the cell filled with an absorbing dye. The sensitivity is of the order of 1 mcal/s.

The rhodopsin photoexcitation steps are too rapid to be resolved by our technique. The retinal photoisomerisation, completed within picoseconds, takes up about 30 kcal/mol from the photon energy, as was measured by classical slow microcalorimetry at liquid nitrogen temperature where the first intermediate 'bathorhodopsin' is stable. Most of this energy is invested in the separation, upon retinal isomerisation, of the positive charge of the protonated Schiff-base bond of the retinal with Lys-296 (in the middle of transmembrane helix VII) which moves away from the negative charge of Asp-113 (in helix III) to which it was tightly coupled in the dark 11-*cis* conformation [5]. At physiological temperature, the protein relaxes rapidly around the all-*trans* retinal which becomes deprotonated and decays in $\sim 10^{-4}$ s, with an enthalpy loss of about 15 kcal, toward the 'Meta II' state which remains stable for seconds. Meta II is the enzymatically active R* state. Its activity results from the uncovering of a catalytic binding site for transducin on the cytoplasmic pole of the transmembrane receptor protein. The lack of a high-resolution structure of rhodopsin limits our understanding of the propagation of a conformational change from the retinal-bound Lys-296, and from the charge-coupled Asp-113, both located in the transmembrane region, to this distant cytoplasmic surface of the protein.

Transducin binds to R* by collision coupling. A high collision rate is allowed by the fast lateral diffusion of rhodopsin in the fluid membrane, and the even faster lateral diffusion of transducin which, being only retained onto the membrane surface by a lipid modification (farnesyl) of its Tγ subunit, has its lateral mobility limited only by that of this protein-bound lipid in the membrane. The R*-T interaction induces the 'loosening' of the nucleotide site in Tα and allows the fast release of the bound GDP, which can be exchanged for GTP. If GTP has been suppressed from the medium, the exchange reaction cannot proceed and 'empty' transducin will remain stoichiometrically bound to R*.

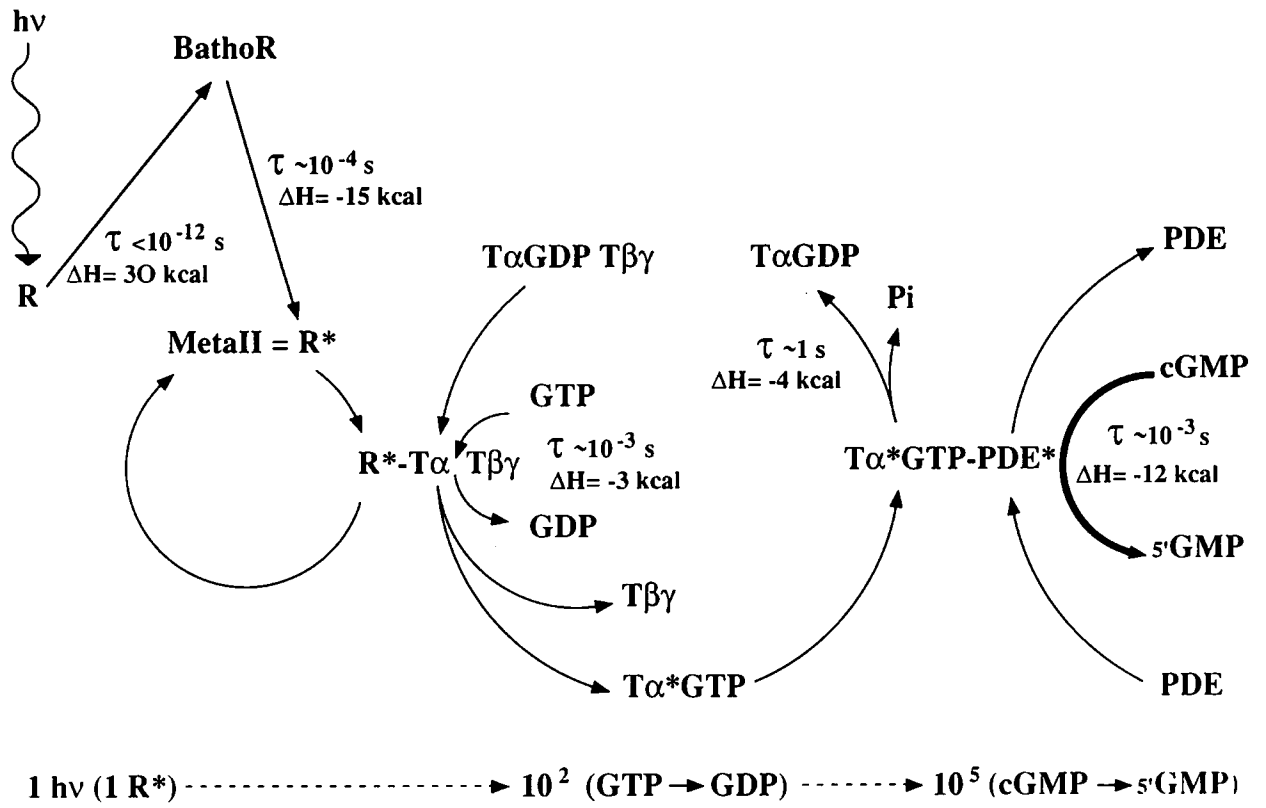

Fig. 1. Scheme of the rhodopsin-transducin cGMP-phosphodiesterase reaction cascade

The R^*-T_{empty} interaction in this blocked transition state is extremely tight and can stabilise the conformation of R^* for 1 h, which would otherwise decay and release its retinal in a few seconds. The binding of GTP in the open and empty nucleotide site induces its closure and a conformational change of $T\alpha$GTP, which dissociates from $T\beta\gamma$ and from R^*. As a catalyst, R^* is released in the same state as before this first collision coupling, and is, therefore, able to activate other transducin molecules catalytically. The time taken by R^* to perform the chemistry of catalysis on transducin, as measured by near-infrared light scattering techniques, is 1.2 ± 0.2 ms at the saturating level of GTP encountered in the cell, and is not limited by lateral diffusion of the proteins. The exchange reaction is rapidly driven forward by the binding energy of GTP, which is larger than that of GDP in $T\alpha$. We have measured the corresponding enthalpy release by time-resolved microcalorimetry, using non-hydrolyzable GTPγS instead of GTP to avoid interferences with the GTPase step. The time-course of the microcalorimeter signal corresponded to the fast exchange kinetics, and its integrated amplitude amounted to 2–3 kcal/mol per bound GTPγS, i.e., about 40% of the ΔH that would be released upon the hydrolysis of GTP to GDP + P.

With GTP, but with no cGMP added, the PDE activation should not give rise to any significant microcalorimetric signal. Only the inactivation step, correlated with the hydrolysis of GTP in the PDE activator $T\alpha$GTP, should be observable. There remained a major puzzle of the visual transduction process: The k_{cat} for GTP hydrolysis in transducin seems low, as usually measured biochemically in vitro (below 0.1 s^{-1}), whereas the termination of the physiological response requires the inactivation of the PDE to proceed with a k_{off} higher than 1 s^{-1}. Our time-resolved microcalorimetric technique suggested a solution to the puzzle by demonstrating the occurrence after a relatively strong flash of a heat pulse decaying within less than a second, and followed by a lower but persistent heat flow plateau. We interpreted the heat pulse as due to the enthalpy released, within a second at 23°C, by the fast hydrolysis of GTP in $T\alpha$GTP that interacted with the available pool of PDE. The low heat flow plateau would then correspond to the recycling, as long as some R^* persists, of the 'fast' transducin pool after its first row of GTP hydrolysis, at a rate governed by slow post-hydrolytic processes. The plateau was indeed rapidly suppressed when the same experiment was conducted in the presence of enough hydroxylamine to hydrolyse specifically the Schiff-base bond of all-*trans* retinal and to inactivate R^* within seconds of its photoactivation. The hydrolysis of GTP in $T\alpha$ in situ, where it activates the PDE, seems, therefore, to proceed in less than a second, making it kinetically consistent with the physiological response. However, a sepa-

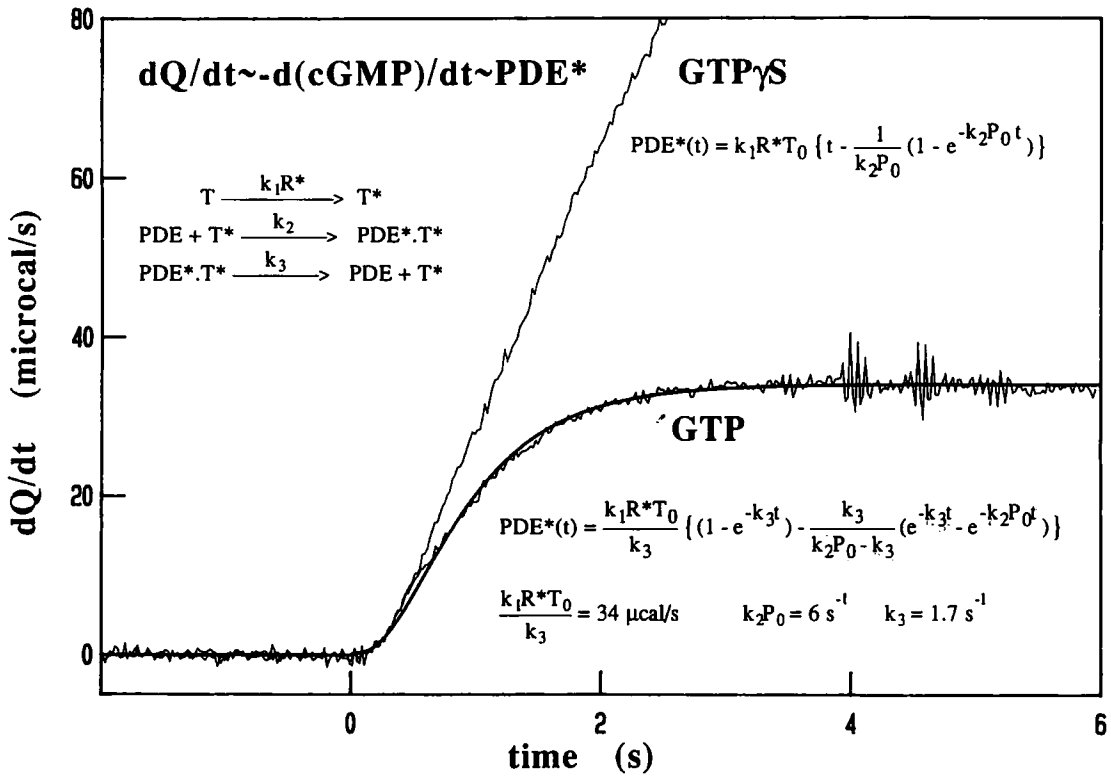

Fig. 2. Kinetic analysis of the cascade from time-resolved microcalorimetric measurements of the cGMP hydrolysis rate after flashing in the presence of GTP or GTPγS.

rate type of experiment by a stopped flow technique combined with fast filtration (Antonny et al., unpublished data), reasserted that the hydrolysis of GTP is much slower in isolated TαGTP that has been eluted from the membrane immediately after its activation ($k_{cat} = 0.05\ s^{-1}$. This led us to suggest that the GTPase step is accelerated by the interaction of TαGTP with a membrane-bound GAP (GTPase Activating Protein) that is here possibly a component of the effector, the PDE.

The large ΔH of cGMP hydrolysis ($\sim$ 12 kcal/mol) and high turn-over rate of an activated PDE ($\sim 10^3$ cGMP molecules hydrolyzed per s) make time-resolved microcalorimetry very convenient to monitor PDE activity: in the presence of both GTP and cGMP, weak flashes elicit large heat signals which are almost entirely due to cGMP hydrolysis (Fig. 2). The rate of cGMP hydrolysis measured by its associated heat release is directly proportional to the population of activated PDE (PDE*). With GTP this population, builds up to a plateau after a slight delay, i.e., to a steady state level where production of PDE* is balanced by its destruction. The rising phase reflects the production of the PDE* and the bend to the steady state plateau reflects its deactivation. If deactivation depends on GTP hydrolysis, then with GTPγS there should be no steady state but a continuously rising phase, which is indeed observed. After very low intensity flashes ($\sim$ 30 photons/rod) only a very small proportion of the total transducin and PDE pools are activated, and the rate of PDE activation and deactivation can be deduced from a simple analysis of the slope and curvature of the heat response. The deactivation rate of PDE is $1.7\ s^{-1}$. This deactivation requires GTP hydrolysis, which itself takes less than one second for the pool of TαGTP that activates the PDE. This implies that the PDE is reinhibited very quickly once the Tα is deactivated. The simple reset mechanism where endogenous GTP hydrolysis turns off transducin, which then swiftly loses its activating power on PDE, as originally proposed 10 years ago, seems valid, but with the supplementary postulate that the interaction between TαGTP and PDE, which increases the hydrolysis rate of cGMP in PDE*, also increases the hydrolysis rate of GTP in the bound Tα.

References

1 Stryer, L. (1991) J. Biol. Chem. 266, 10711–10714.
2 Chabre, M. and Deterre, Ph. (1989) Eur. J. Biochem. 179, 255–266.
3 Vuong, T.M. and Chabre, M. (1990) Nature 346, 71–74.
4 Vuong, T.M. and Chabre, M. (1991) Proc. Natl. Acad. Sci. USA 88, 9813–9817.
5 Khorana, H.G. (1992) J. Biol. Chem. 267, 1–4.