TEMPERATURE DEPENDENCE OF G-PROTEIN ACTIVATION IN PHOTORECEPTOR MEMBRANES

Transient Extra Metarhodopsin II on Bovine Disk Membranes

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ABSTRACT The thermal activation barrier of guanosine triphosphate dependent dissociation of the light-induced rhodopsin–G-protein complex has been determined using a spectroscopic technique (enhanced formation of metarhodopsin II). The dissociation rate has been measured in the range $-2°C \leq t \leq 12°C$. The Arrhenius plot yields apparent activation energies: 166 ± 10 kJmol^{-1} with 5'-guanylylimidodiphosphate (GMPPNP) and 175 ± 15 kJmol^{-1} with GTP. The rhodopsin–G-protein dissociation rate is linearly related to the concentration of GMPPNP in the measurable range (≤ 200 μM). The data show that, at low temperature (1°C), the rate limiting step of G-protein activation is the bimolecular reaction between the protein and the nucleotide. This also seems to hold true for more physiological conditions as suggested by extrapolation and comparison with nucleotide exchange rates in the literature. The high activation barrier of the nucleotide exchange reaction is explained in terms of a rapid endothermic preequilibrium between an inactive and an exchanging state of the rhodopsin–G-protein complex.

INTRODUCTION

The light receptor protein rhodopsin (R)[1] activates a cyclic guanosine monophosphate (cGMP) phosphodiesterase (PDE) via a guanine nucleotide binding transmitter protein (G-protein or transducin, referred to as G in the following). This G-protein belongs to a family of transmitter proteins that mediate between the receptors for extracellular signals (such as light and hormones) and their effectors (such as PDE or adenylate cyclase). Signal transduction in these systems involves three subsequent steps: (*a*) activation of the receptor by an external stimulus, (*b*) activation of the G_α-subunit by interaction with the receptor and binding of GTP (or its analogues) (Godchaux and Zimmerman, 1979; Fung and Stryer, 1980), and (*c*) activation of the effector (Fung et al., 1981).

The R/G/PDE system seems to be a special case with regard to the photochemical activation of the receptor and the rapid sequential interaction of many peripherally bound G units per activated receptor unit.

Activation of G, however, follows a scheme which may apply to G-proteins in general: the receptor protein, in its activated conformation (see Liebman and Sitaramayya, 1984, for a review), catalyzes the transition of the G_α-subunit into the active form with guanosine 5'-triphosphate (GTP) as an effector (Fung and Nash, 1983) and the free energy gap between the guanosine 5'-diphosphate (GDP)- and the GTP-binding state as the driving force. This general scheme does not answer mechanistic questions regarding the succession of the reaction steps, the rate limiting step, and its thermal activation barrier.

Fast physical techniques are available for the photoreceptor which open a way to investigate these problems. There is a light-scattering signal linked to G-activation (so-called dissociation signal [Kühn et al. 1981]) whose rate saturation has been interpreted in terms of a rapid exchange of GTP and a subsequent rate-limiting conformation change with an intrinsic rate of 8 s^{-1} (Bennett and Dupont, 1985). This seems to be in conflict with the high speed of the R·G amplifier which requires lifetimes of the R·G complex shorter than 1 ms (Liebman and Pugh, 1982). Also spectroscopic data suggested the nucleotide uptake as the rate-limiting step and pointed in addition to a high activation energy of this reaction (Hofmann, 1985).

The present study reinvestigates the problem, using the same spectroscopic technique, which is based on the fact that the binding conformation of R (termed R_M in the following) coincides with the 380-nm intermediate metarhodopsin II (MII) (Emeis et al., 1982; Longstaff et al.,

[1] *Abbreviations used in this paper:* G, retinal GTP binding protein; G*, PDE-activating state of G; GDP, guanosine 5'-diphosphate; GMPPNP, 5'-guanylylimidodiphosphate; GTP, guanosine 5' triphosphate; GTPγS, guanosine 5'-0-(3-thiotriphosphate); M_{380}, total amount of 380-nm absorbing rhodopsin intermediates (sum of free and complexed MII); MI/MII, metarrhodopsin I/II; N, guanine nucleotide; PDE, retinal guanosine 3':5'-cyclic monophosphate phosphodiesterase; R, rhodopsin; RM, photo converted rhodopsin in its G-protein binding conformation; ROS, rod outer segments.

1986). MII is in equilibrium with its tautomeric form metarhodopsin I (MI) (Matthews et al., 1963). Binding of G-protein to R_M leads to an enhanced formation of 380-nm photoproducts (free MII and MII·G). The resulting extra MII (Emeis and Hofmann, 1981) is easily measurable because of the large spectral difference between MI (λ_{max} = 480 nm) and MII (λ_{max} = 380 nm).

Within the limits imposed by the properties of the metarhodopsin I/II equilibrium (Parkes and Liebman, 1984) ($T \leq 12°C$, pH $\geq$ 7.5), extra MII is a real time monitor of R_M-G complex formation and dissociation.

MATERIALS AND METHODS

Chemicals

GTP, GTPγS, GMPPNP, and Ficoll 400 were obtained from Sigma Chemical GmbH München, Federal Republic of Germany.

Preparation

Bovine rod outer segments were prepared according to a standard procedure (Emeis and Hofmann, 1981). Retinas from freshly dissected bovine eyes were shaken in isotone saline (buffer A: 130 mM KCl, 0.5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 1 mM dithiothreitol [DTT], 0.5 mM EDTA, 10 mM PIPES, pH 7.0), and filtered through a nylon mesh (100 μm mesh width). The resulting crude suspension was layered on a discontinuous sucrose gradient (37.7% wt/vol sucrose solved) and washed in buffer A.

The extraction of the peripheral proteins was done according to Kühn's method (Kühn, 1980). ROS were osmotically shocked in a low ionic strength buffer (1 mM DTT, 1 mM EDTA, 5 mM PIPES, pH 7.0), gently homogenized and sedimented in a Sorvall SS34 rotor (E. I. du Pont de Nemours & Co., Inc., Wilmington, DE) at 4°C and 18,000 rpm. The supernatant was centrifuged again and yielded the extracted peripheral proteins. This protein extract was stored at −80°C.

Isolated osmotically intact disk membranes (Smith Disks) were prepared from ROS. The procedure used was similar to the standard procedure described by Smith et al. (1975) except that (*a*) according to Bauer and Mavromatti (1980), 2.5% wt/vol Ficoll 400 instead of 5% was used in order to reduce interaction of the swollen disk membrane vesicles with Ficoll, and (*b*) after flotation of the disk membranes Ficoll was not removed by a separate washing step. The resulting Ficoll concentration after dilution to the final measuring concentration was ~0.5% wt/vol. Isolated disks were used within 48 h after preparation without freezing.

All measurements were done in isotonic saline (124 mM KCl, 0.5 $MgCl_2$, 1 mM $CaCl_2$, 0.5 mM EDTA, and 1 mM DTT). After adding protein extract and adjusting the pH with 200 mM HEPES, pH 8.0, the sample was diluted to give a final rhodopsin concentration of 5 μM. Then the sample was kept in a dark box for 2 h at room temperature to allow the decay of photoexcited rhodopsin.

Measuring Technique

MII formation was measured according to the method described by Hofmann and Emeis (1981) which minimizes scattering artifacts by comparing the flash-induced changes in the absorbances at 380 and 417 nm. In such measurements, the absorbance change at 417 nm (MI isosbestic to MII) serves as a reference for determining the level of MII (λ_{max} = 380 nm).

All measurements were done on a UV300 two-wavelength spectrophotometer (Shimadzu Scientific Instruments, Inc., Japan) (2 nm slit, 1 cm path). The sample was placed in a thermostated cuvette holder next to the photomultiplier. The temperature was measured with a thermodiode (1N4148). A green photoflash (1 ms) was delivered via a light guide onto the cuvette. Flash artifacts were avoided using the differentiated "S-Pulse"-output of the spectrophotometer for triggering the flash at the beginning of a voltage-free period of the photomultiplier, which was additionally protected with a blue-green glass filter (BG 24, 2 mm, Schott, Mainz, FRG).

GTP or analogues were added after the sample had reached the scheduled measuring temperature. The measuring light shutter was opened just at the start of the measurement. The sample was handled in complete darkness; some steps were performed using an infrared image converter.

Kinetic Analysis

To obtain the effective rate constant k_d of the complex decay the curves were digitized, differentiated with respect to time, and logarithmically plotted against time. To smooth the data points before differentiation the curves were fitted with a sum of three exponentials:

$$\sum_{n=1}^{3} a_n [1 - \exp(-b_n \cdot t)] + c. \quad (1)$$

This evaluation procedure reduces the problems with small irregularities of the signal time course. In the time domain where the reaction follows a first order time law, the analysis yields straight lines with slopes equal to k_d. Data processing is illustrated in Fig. 1.

RESULTS

A typical measurement is shown in Fig. 1. The transient MII-formation is described by a reaction scheme (Hof-

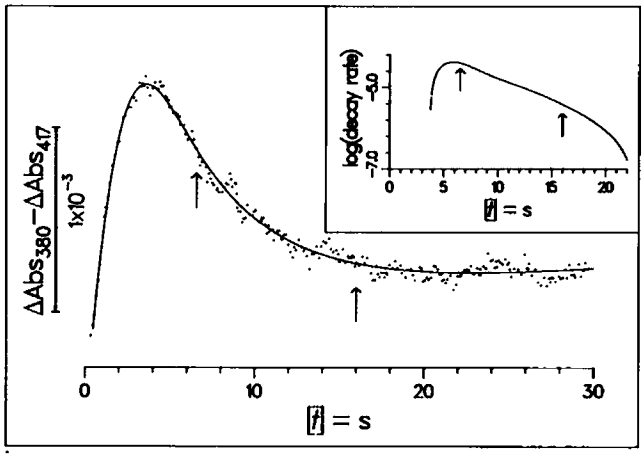

FIGURE 1 Kinetic analysis of extra MII decay. The figure shows a typical recording (100 μM GMPPNP, pH 7.5, 1°C, first flash). After adding the nucleotide to the disc membranes (recombined with protein extract; rhodopsin concentration 5 μM, concentration of G-protein ~0.2 μM) the reaction was started at t = 0 by bleaching 3% rhodopsin with a green flash. Formation of 380-nm absorbing photoproducts (M_{380}, i.e., MII + MII · G) is seen as an absorbance increase, decay of M_{380} as a downward deflection. The final absorbance level is determined by the value of the MI/II equilibrium. The decay of M_{380} is a direct measure of the MII · G dissociation rate as derived in Results. Smoothing was achieved by fitting a sum of three exponential functions (*solid line*) to the original curve. The fitted waveform was further processed as demonstrated in the inset. After the inflexion point, seen as a maximum in the inset figure, the reaction follows a first-order time law, which results in a straight line with its slope equal to the dissociation rate k_d. The arrows indicate the margins of the pseudo-first-order behavior.

mann, 1985):

$$R + G \xrightarrow{k_i} MI + G \underset{k_{-e}}{\overset{k_e}{\rightleftharpoons}} MII + G \underset{k_{-b}}{\overset{k_b}{\rightleftharpoons}} MII \cdot G \xrightarrow{N \searrow k_d} MII + G'_N.$$

Scheme I
N-Guanosine Triphosphate, G–activated G-Protein with N Bound

Virtually all photoconverted rhodopsin is present as MI after milliseconds (rate k_i, initial downward deflection, as seen in Figs. 2–4). The system then relaxes (with $k_e + k_{-e}$, ~0.8 s^{-1} under the conditions of Fig. 1) into an equilibrium between MI and MII (absorbance increase).

Free MII does not only take part in the equilibrium with MI but also in that with MII · G (Emeis et al., 1982). The total amount of 380-nm photoproducts (termed M_{380} in the following) is the sum of complexed and free MII.

MII · G complex formation leads to an enhanced level of M_{380} (extra MII). Under the measuring conditions, the actual binding step (index *b* in Scheme I) is fast and the formation of MII is rate limiting for the total amount of M_{380} (Emeis et al., 1982).

Binding of GTP or its analogues leads to dissociation of the complex into PDE-activating G* and free MII; after dissociation, the levels of MI and MII are determined by the conditions of the metarhodopsin equilibrium (final absorbance level).

Because released MII is quickly redistributed over the species MI, MII, and MII · G the measured decay of M_{380} (absorbance decrease) is, in a certain time domain, where it follows a first-order time law, a measure of the R_M · G complex dissociation rate k_d. With the amount of flash-activated rhodopsin applied in this study (its molarity being approximately equal to that of the G-protein), most of the MII · G is formed in a rapid 1:1 association reaction. The subsequent dissociation is kept rate-limiting for the overall sequence by the relatively low nucleotide concentrations. On the other hand, the concentration of the nucleotide is always much higher than that of its reaction partner, the R_M · G complex. Thus the dissociation reaction and its measure, the M_{380} decay, are well described by a pseudo-first-order behavior.

This justifies the first-order analysis of the data (Fig. 1). It was confirmed by calculating waveforms for a realistic choice of the rate constants, using a computer program designed for the simulation of coupled chemical reactions (KISS, Gottwald, 1981).

When the nucleotide concentration is raised, the initial rate of M_{380} formation remains unaffected (Fig. 2) while the amount of M_{380} transiently formed, as well as the time to peak, decreases (Fig. 2) until, at very high nucleotide levels, only undetectable amounts of extra MII are formed. The latter behavior has been described previously (Hofmann, 1985).

The rise of the dissociation rate k_d with increasing GMPPNP concentration is demonstrated in the inset of Fig. 2. In the measurable range (≤200 μM), k_d increases linearly and no saturation is observed.

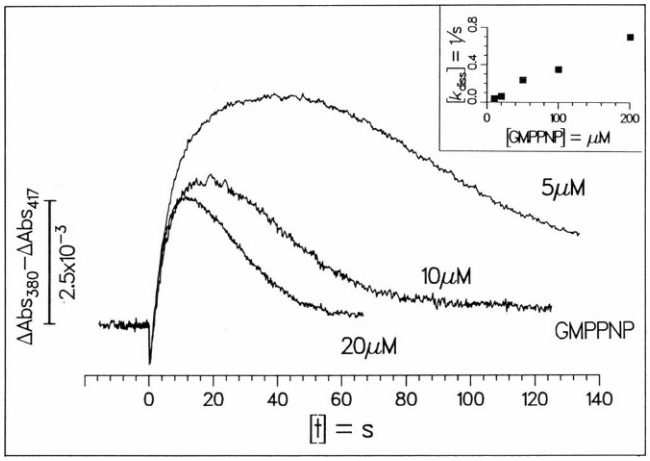

FIGURE 2 Rise of the rhodopsin–G-protein complex dissociation rate k_d (k_{diss} in the inset) with increasing GMPPNP concentration. The inset shows the resulting rate vs. concentration plot. All points were taken from the first flash bleaching 3% rhodopsin; conditions are 1°C, pH 7.5.

Fig. 3 shows that the dissociation rate k_d is determined by the type of nucleotide. Both nucleotides, applied in this experiment, yield the same waveform; however, with GMPPNP, a ~20 times higher concentration is required to achieve the same k_d as with GTP. The GTP-analogue GTPγS has been found to be as effective as GTP (data not shown).

The transient formation of M_{380} highly depends on temperature: Fig. 4 *a* reflects, apart from the well-known acceleration of MII-formation with increasing temperature, a similar high acceleration of the decay reaction. Fig. 4 *b* shows an Arrhenius representation of the k_d values. Linear regression of the data yields the following information: (*a*) In the measured range of k_d (from 1.5×10^{-2} to 1 s^{-1}), only one reaction step of the overall dissociation reaction is rate limiting and is seen as a straight line in the Arrhenius plot. (*b*) The apparent activation energy E_a of this reaction step is: 166 ± 10 kJmol^{-1} with GMPPNP and 175 ± 15 kJmol^{-1} with GTP.

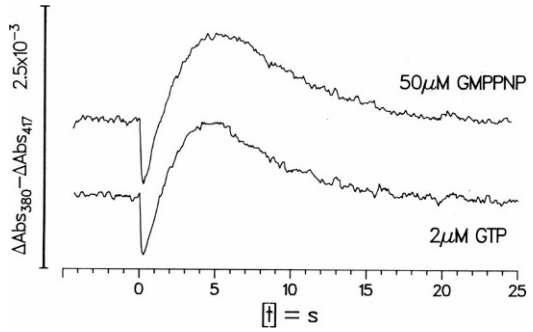

FIGURE 3 Comparison of the effects of GTP and GMPPNP on the rate of rhodopsin-G-protein complex decay. Temperature 1°C, pH 7.5, recordings are from the first flash bleaching 3% rhodopsin.

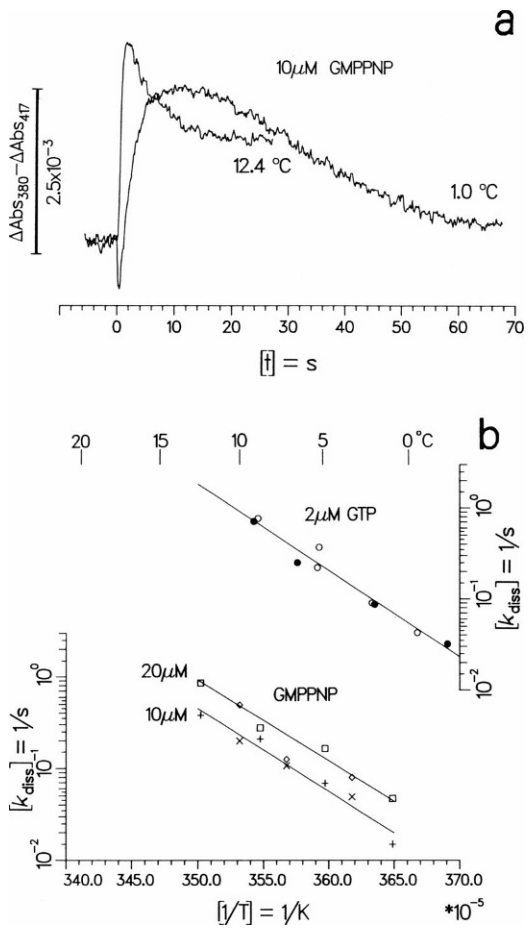

FIGURE 4 Acceleration of the rhodopsin-G-protein association/dissociation cycle with temperature. (*a*) Comparison of two measurements with 10 μM GMPPNP at 1° and 12.4°C, respectively. Since metarhodopsin I (MI) formation is accelerated with temperature, the initial downward deflection seen at 1°C is no longer time resolved at 12.4°C. The rate of M_{380} formation and decay is greatly increased. The higher final absorbance level at 12.4°C reflects the shift of the MI/MII ratio with temperature. (*b*) Arrhenius analysis of the rhodopsin-G-protein dissociation rate k_d (k_{diss} in the figure): +, ×, 10 μM GMPPNP; □, ◇, 20 μM GMPPNP; ○, ●, 2 μM GTP. All measurements are from the first flash bleaching 3% rhodopsin; pH 7.5. Different markers indicate samples from different batches.

DISCUSSION

For the discussion of the data we go back to Scheme I which describes the reaction sequence leading to activation of G-protein (G) for the interaction with the phosphodiesterase (PDE) inhibitor. An entire recycling of photoactivated rhodopsin (termed R_M in the following) includes $R_M \cdot G$ binding, GTP/GDP exchange, $R_M \cdot G$ dissociation, and the diffusion to the next successful $R_M \cdot G$ interaction. As described in Results the time required for diffusion and interaction of R_M and G is comparably short under the conditions of our measurements and the kinetics of the dissociation step (index *d* in Scheme I are directly observed in the decay of extra-metarhodopsin II.

This dissociation step shall now be discussed in detail.

$$R_M \cdot G \xrightarrow{N\ k_d} R_M + G^*.$$

Scheme II

The overall process leading to the observed dissociation involves, besides the nucleotide uptake, a conformation change of G_α which has been shown, for example, by a GTP dependent difference in trypsination (Fung and Nash, 1983).

The data presented above have shown that the lifetime of the $R_M \cdot G$ complex depends both on the concentration as well as on the specific nucleotide presented. This agrees with the result of previous studies (Fung and Stryer, 1980; Kühn et al., 1981; Bennett and Dupont, 1985) that nucleotide binding is the event triggering the transition of the $R_M \cdot G$ complex into the PDE-activating state G*. Moreover, the observed linear rise of k_d with increasing concentration of nucleotide over a large range shows that the rate of dissociation is limited by the uptake of the nucleotide (at 1°C).

The question arises whether k_d remains proportional to the nucleotide concentration also under more physiological conditions of nucleotide (GTP $\geq$ 100 μM) and temperature ($\geq$20°C). This is not directly testable by use of extra MII which becomes undetectable at high dissociation rates. However, this question can be discussed on the basis of the data listed in Table I which includes the observations of other studies. In line 2 of the table we first extrapolate the data (according to Fig. 4 *b*) to the case prevailing at 22°C. In lines 3–5 data contained in other studies are summarized. Entries for GTP level are those used in the studies. Entries for the dissociation rate were calculated from data contained in these studies as described in the legend of Table I. It is evident that the rates in lines 3 and 4 highly exceed the rate value of 20 s^{-1} predicted to prevail at 2 μM GTP. We assume these high values to result from the use of the higher GTP concentrations. For example, from the linear relationship of our Fig. 2 (where GMPPNP was used), one would predict a 62.5-fold increase in the GTP (2 → 125) level to yield a 62.5-fold increase in the R_M-cycling rate (20 → 1,250). The result of Liebman and Pugh (1982), i.e., a recycling rate of 1,700 s^{-1}, appears consistent with such a prediction of linear extrapolation.

Accordingly, we propose that the nucleotide uptake is rate limiting for the dissociation step (and for the entire R_M-cycle of G-activation) also at more physiological temperatures and nucleotide concentrations.

The Arrhenius plot of the dissociation data (Fig. 4 *b*) shows that the temperature dependence of the $R_M \cdot G$ dissociation is, in the range of the measurements (−2°C–12°C), described by only one exponential function. This means, in terms of kinetic theory, that the R_M catalyzed bimolecular reaction between G-protein and the nucleotide has an apparent Arrhenius energy E_a given by the equation:

$$k_d = A \cdot \exp(-E_a/R \cdot T). \quad (2)$$

TABLE I

RATE CONSTANTS OF THE $R_M \cdot G$ DISSOCIATION AND $R_M \cdot G$ ACTIVATION CYCLE

Authors	T	[GTP]	Rate	Material	Method
	°C	μM	s^{-1}		
Dissociation step only					
This study	4	2	0.2	Bovine	Extra MII
(Temp. extrapol.)	22	2	20		
Entire R_M-cycle of G-activation					
Robinson et al. (1986), Fig. 1	Room temp.	10	300	Frog	GTP-binding
Liebman and Pugh (1982), Fig. 2	Room temp.	125	1,700	Toad	GTP-binding
Fung and Stryer (1980), Fig. 2	4	1.5	0.16	Bovine	GDP-release

The rate constants of the studies of the other authors were derived by a procedure described in Liebman and Pugh (1982): From the figure given in the table the initial rate of G-activation was calculated. This value was extrapolated to $R_M/G|R_M \rightarrow 0$ by multiplying it with the ratio of the gain in the experiment to the gain of the single R_M case. This ratio was obtained from light titration curves of G-activation presented in the same study.

In this representation, the high temperature dependence of the reaction expresses itself in a high value for E_a determining the slope of the straight lines in Fig. 4 *b*. This observed value for E_a supports the idea that a conformation change accompanies the transition of $R_M \cdot G$ to G*. This is consistent with the observation of a GTP-dependent conformation change of the G_α-subunit (Fung and Nash, 1983), mentioned above.

We propose a reaction sequence that incorporates thermal activation and nucleotide exchange:

$$R_M \cdot G \underset{\text{fast}}{\overset{T}{\rightleftharpoons}} R_M \cdot G' \overset{\text{GTP GDP}}{\rightleftharpoons} R_M \cdot G' \underset{\text{fast}}{\longrightarrow} R_M + G^*.$$

Scheme III

A key feature of reaction Scheme III is that a thermally activated conformation change precedes the nucleotide binding. In the presence of bound catalyst R_M, the G-protein exists in a rapid equilibrium between two forms, $R_M \cdot G$ and $R_M \cdot G'$. Higher temperature favors the latter state in which nucleotide can be exchanged at its binding site. Uptake of a guanosine triphosphate triggers the rapid relaxation of G_α into the biochemically activated and dissociated state G*. That is, the nucleotide acts as a trapping agent. The final product G* forms faster the higher temperature and nucleotide concentration.

Note that the $R_M \cdot G$ complex remains in the forms $R_M \cdot G$ or $R_M \cdot G'$ until the lifetime of $R_M \cdot G'$ is terminated by the GTP-induced relaxation to G*. Thus temperature does determine the level of $R_M \cdot G'$ and thereby the rate of formation (but not the level) of G*. The apparently slow uptake of GTP is explained by the "bottle neck" of the low $R_M \cdot G'$ concentration. The intrinsic rate of nucleotide uptake k' (time required for GTP to be bound to a given G-protein molecule in the state $R_M \cdot G'$) may be quite fast. This cannot be determined from these data since the effective dissociation rate k_d depends both on the nucleotide level and on the unknown amount of $R_M \cdot G'$ ($k_d = k' \cdot [R_M \cdot G'] \cdot [N]$).

The state $R_M \cdot G'$ is probably correlated with the R_M-induced opening of the otherwise poorly accessible nucleotide binding site of G-protein inferred from nucleotide exchange experiments (Fung and Stryer, 1980; Bennett and Dupont, 1985). In Scheme III the "open" state is not stable but exists only in a rapid equilibrium with $R_M \cdot G$. However, we do not know whether the apparently open form of the $R_M \cdot G$ complex is actually realized in the G-protein. It could also be determined by the structure of R_M. By this, the GTP binding site of G-protein would always be open while interacting with R_M but its accessibility would be sterically blocked. The steric effect would be relieved in a second state R'_M, in endothermic equilibrium with R_M, allowing the nucleotide to enter into the binding site. The two forms of R_M could well have the same absorption spectrum because spectrally silent (and G-protein dependent) transitions of R_M are documented in the literature (for a review see Hofmann, 1986). The advantage of this interpretation would be that the shut-off of R_M could simply consist in a block of the R'_M conformation.

The basic evidence of our study supporting Scheme III is: (*a*) a rapid, endothermic preequilibrium of the two forms $R_M \cdot G$ and $R_M \cdot G'$ is consistent with the observation of only one exponential function describing the temperature dependence of $R_M \cdot G$ complex dissociation. (*b*) The apparent overall activation energies of the dissociation are essentially equal with GMPPNP and GTP (Fig. 4 *b*). This is easily understood in terms of the above reaction sequence (III) since the thermal equilibrium between $R_M \cdot G$ and $R_M \cdot G'$ precedes nucleotide binding and is therefore independent of the specific guanosine triphosphate. (*c*) The k_d increases for $R_M \cdot G$ dissociation rate concentrations of GMPPNP (inset of Fig. 2) much higher than its apparent dissociation constant (K_D = 20–100 μM; Bennett and Dupont, 1985).

There is evidence from other studies that is consistent with Scheme III: The total amount of complexed G-protein ($R_M \cdot G + R_M \cdot G'$) is essentially temperature independent. This is best measured in the absence of nucleotide and with excess G-protein when a stable $R_M \cdot G$ complex is formed. The measured amount of M_{380} and the amount of MII · G calculated from it is at 0°C only 10–20% lower than at 15°C (Emeis and Hofmann, 1981; Schleicher et al., to be published). On the other hand, the light- and GTP-induced GDP release from the G-protein binding site exhibits a temperature dependence similar to that $R_M \cdot G$ dissociation (line 5 of Table I).

The scheme appears to be consistent with the available evidence but it is certainly not unique. For example, we cannot exclude a different effectiveness of the different nucleotides, once bound, to induce the complex dissociation. This is discussed by Yamanaka et al. (1986) who have recently observed that the ability of certain GTP analogues to compete with [α-^{32}P]GTP for the binding site of G is not parallel to their effectiveness in activating PDE.

On the other hand, our scheme relates by itself the effectiveness of a nucleotide to induce G-activation with the on-rate of its binding reaction (reflecting the speed of its fitting into the binding site). Its (equilibrium) affinity does not seem to be very relevant. This can occur if the rate of the subsequent transition to G* is comparable to or faster than the back-reaction accompanied by the release of bound nucleotide from its binding site.

It would be desirable to demonstrate the existence of the hypothetical intermediate $R_M \cdot G'$ more directly. An investigation of the temperature dependence of nucleotide exchange is currently in progress. Monoclonal antibodies blocking the G'-G* transition without impeding nucleotide binding could help to determine which reaction steps are accompanied by a detectable conformation change.

Finally, we note the discrepancy between this study and the light-scattering data by Bennett and Dupont (1985) who derived a rate limiting reaction step ($k = 8\ s^{-1}$) in the final transition to the activated G (G* in our Scheme III). The reason is the experimental fact that the initial rate of their dissociation signal saturates with increasing nucleotide concentrations while the decay of extra MII does not. We have also found a much higher effectiveness of GTP relative to GMPPNP (~20-fold) than these authors (threefold). The basis of these differences is still unknown. However, it seems possible that the kinetics of the dissociation signal are co-determined by scattering changes other than G-protein activation (Schleicher and Hofmann, 1987).

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