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# Photoactivation of rhodopsin and interaction with transducin in detergent micelles

## Effect of 'doping' with steroid molecules

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On detergent-solubilised bovine rhodopsin, we have studied the formation of the active photoproduct, metarhodopsin II (MII), and its interaction with the rod G-protein, transducin ($G_t$). The measured rate of flash-induced MII formation decreases by a factor of 300 from *n*-dodecyl-β-D-maltoside ($k = 4 \times 10^3$ at 18°C, pH 6.5), over (3-(lauroyloxy)propyl)-phosphorylcholine (deoxylysolecithin), *n*-octyl-β-D-glucopyranoside, sodium cholate to Chapso. For the two last agents, MII formation is similarly slow as in the native disc membrane; however, the micellar system does not display the very large decrease of the rate with lowering temperature, as is characteristic for the membrane. This points to entropic factors determining the rate in the micellar systems. An admixture of rigid steroid molecules (11-deoxycorticosterone) to lysolecithin micelles ('doped micelles') slows MII formation and shifts the MI/MII equilibrium to values typical for detergents of rigid structure. The observation gives further support to the surface free energy concept of MII formation outlined in previous studies. The free adjustment of the MI/MII equilibrium in these doped micelles allows $G_t$-induced formation of extra-MII to be measured, providing a convenient monitor of rhodopsin-$G_t$ interaction in solution.

Rhodopsin; Metarhodopsin II; Transducin; G-protein; (Detergent micelle)

## 1. INTRODUCTION

In retinal rods, early steps of signal transduction are mediated by a receptor/G-protein/effector system consisting of rhodopsin (R), transducin ($G_t$) and a cGMP phosphodiesterase (PDE). Binding of photo-activated rhodopsin (R*) to transducin catalyses the exchange of bound GDP for GTP, leading to stimulation of the PDE by the activated $G_t^{\alpha}$ subunit (see [1] for a recent review).

Upon absorption of light, rhodopsin runs over a series of photoproducts in a temperature- and pH-dependent equilibrium of the spectroscopically different intermediates, metarhodopsin I (MI) and metarhodopsin II (MII) [2]; the latter provides a conformation which is able to interact with other proteins, including transducin (reviewed in [3]) and 48 kDa protein [4]. Those interactions stabilize the MII state at the cost of MI and so-called extra-MII is formed, providing a spectroscopic monitor of the $R^*G_t$ complex in situ [3].

Specific interaction with MII, once formed, is characteristic of such proteins as $G_t$, 48 kDa protein and antibodies (König, B., Arendt, A., Hargrave, P.A. and Hofmann, K.P., unpublished). There is also an influence on MII of rhodopsin's hydrophobic environment; approximately 50% of the protein mass is localised in the hydrophobic portion of the lipid bilayer of the native disc membrane [5]. In contrast to protein interaction with MII, the environment affects the MI/MII transition itself and changes its activation parameters. Very different kinetics and equilibrium positions of the MI/MII transition are measured when rhodopsin is embedded in detergent micelles or lipid vesicles of different chemical composition ([6–8]; [9] and references therein). Numerous parameters, including polyunsaturation of lipid hydrocarbon chains [7,8], rigidity of detergent structure [9] or the nature of lipid polar head groups [10] have been investigated.

Here, we report on an investigation of the MI/MII transition in different detergent systems. The results confirm and generalize our previous result that flexible detergents tend to make the MI/MII transition faster while rigid structures cause slower kinetics and a shift of the equilibrium to MI.

We have also investigated the formation of MII in detergent-steroid micelles ('doped micelles') and find similar behaviour as in the biphenyl detergent micelle [9]. A doped micelle consisting of (3-(lauroyloxy)propyl)-phosphorylcholine (deoxylysolecithin) and 11-deoxycorticosterone also allows undisturbed measurement of MII-$G_t$ interaction, as monitored by the formation of $G_t$-induced extra-MII [3]. This provides a binding assay for the membrane-free receptor, comparable to the concanavalin A/rhodopsin system [11].

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## 2. MATERIALS AND METHODS

### 2.1. *Preparation of washed disc membranes*

Isolated washed disc membranes were prepared under dim red light by a combination of Papermaster and Dreyer [12] and De Grip et al. [13]. About 40 retinas were collected per tube in 15 ml 50% sucrose in Ringers (10 mM Pipes (pH 7.2), 130 mM KCl, 0.5 mM $MgCl_2$, 2 mM $CaCl_2$, 0.1 mM EDTA, 1 mM DTT and 1 mM phenylmethylsulfonyl fluoride (PMSF)) and filtered through a 100 $\mu$m nylon mesh. The suspension was layered upon a 50% sucrose cushion and centrifuged (46000 × $g$, 30 min, 4°C). The floating rod outer segments (ROS) were washed with 40 ml isotonic saline to lower the sucrose concentration and centrifuged again (15000 × $g$, 20 min, 4°C). The pellet (ROS) was resuspended in 2 ml isotonic saline, layered upon a continuous (42–25%) sucrose gradient and centrifuged for 1 h (100000 × $g$, 4°C). The band containing the ROS was collected with a syringe, washed in 80 ml isotonic saline and centrifuged (15000 × $g$, 20 min, 4°C). The pellet was twice suspended in 40 ml hypotonic buffer (5 mM Pipes (pH 7.0), 1 mM DTT and 1 mM PMSF) and centrifuged 30 min (46000 × $g$, 4°C). The resulting hypotonic extract was again centrifuged (100000 × $g$, 30 min, 4°C), the supernatant concentrated by ultrafiltration (Amicon, YM-10 membrane), and stored at −80°C. The washed disc membranes were resuspended in 8 ml isotonic saline and also stored in aliquots at −80°C. Routinely, the $A_{280/500}$ ratio was 1.9:2.0. Transducin was purified according to Fung et al. [14].

### 2.2. *Rhodopsin solubilisation and purification*

Purification of rhodopsin was performed according to de Grip [15]. The following detergents were used for solubilizing washed disc membranes: (i) *n*-dodecyl-$\beta$-D-maltoside (Boehringer, Mannheim), 10 mM; (ii) *n*-octyl-$\beta$-D-glucopyranoside (Bachem, Bubendorf, Switzerland), 100 mM; (iii) deoxylysolecithin (Berchtold, Bern, Switzerland), 10 mM; (iv) Chapso (Sigma), 40 mM; (v) sodium cholate (Sigma), 100 mM.

The various detergents were found to behave quite differently during the purification procedure.

40 mM Chapso (5 × cmc) solubilized disc membranes completely within 30 min. A disadvantage of this detergent is that approximately 30% of the rhodopsin bound to the concanavalin A column does not elute.

*n*-Dodecyl-$\beta$-D-maltoside in a concentration of 10 mM (cmc = 0.2 mM) is necessary to solubilise disc membranes within 1 h to 80%. This detergent eluted rhodopsin quantitatively. With deoxylysolecithin (cmc = 0.2 mM), the results are similar to those of *n*-dodecyl-$\beta$-D-maltoside.

*n*-Octyl-$\beta$-D-glucopyranoside is distinguished by the fact that only a 4-fold cmc (100 mM) is required for complete solubilisation of disc membranes within 10 min. Phosphate analysis [16] showed that maximally 0.5 mol phospholipids/mol rhodopsin are present after purification, which was the best result of all the detergents tested so far.

Purified rhodopsin had a 280:500 ratio between 1.61 and 1.67 and no other proteins were found in SDS-PAGE.

For all the detergents, rhodopsin eluted to more than 98% from the column, with the exception of Chapso (<70%). This is probably due to the negative charge of the terminal sulfate group of the sulfobetain chain which cannot be completely neutralized by the positively charged nitrogen. This might cause interaction with the positive charges of concanavalin A.

In spite of its zwitterionic headgroup, deoxylysolecithin behaves like a non-ionic detergent. This might be due to the arrangement of the phosphatidylcholine headgroup in the micelle which tends to arrange opposite charge in close neighbourhood to one another [17].

### 2.3. *Preparation of steroid-detergent micelles*

For the preparation of the doped micelle, washed disc membranes were solubilized in a solution containing 20 mM Pipes (pH 6.5), 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1 mM EDTA, 150 mM NaCl, 10 mM deoxylysolecithin and 2 mM 11-deoxycorticosterone (Serva). After 30 min, the solution was centrifuged (100000 × $g$, 30 min, 4°C), and the supernatant was loaded onto a 2 ml concanavalin A-Sepharose column (Pharmacia) equilibrated with the same buffer. Bound rhodopsin was washed with the same buffer as above, except that the concentration of deoxylysolecithin was lowered to 2 mM. Purified rhodopsin was eluted and stored at −80°C. Under these conditions the doped micelle was stable for at least 6 months.

### 2.4. *Flash photolysis experiments*

All experiments were performed by using the two-wavelength spectrometer described by Hofmann and Emeis [18]. For flash excitation, a neodymium-YAG Laser YG 580 (Quantel, Les Ulis, Orsay, France; 530 nm) or a photo-flash (maximal intensity at 520 nm) were used, both bleaching 5% of the rhodopsin. The buffer for the MII experiments consisted of 40 mM Pipes (pH 6.5), 100 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 0.1 mM EDTA and 1 mM DTT. The buffer for the extra-MII experiments was the same, except that 40 mM Hepes (pH 8.0) was used instead of 40 mM Pipes. The detergent, rhodopsin and transducin concentrations are stated in the legends to figs 1 and 2. MII formation was measured as the absorbance difference between 380 nm ($A_{max}$ of MII) and 417 nm (isosbestic point of MI/MII transition).

## 3. RESULTS

### 3.1. *MII formation in different detergents*

*n*-Dodecyl-$\beta$-D-maltoside, deoxylysolecithin and *n*-octyl-$\beta$-D-glucopyranoside proved to be good detergents for purification of rhodopsin. MII formation was very different in its extent and kinetics in the various detergents (fig.1 and table 1). MII is formed

Table 1

Amplitudes and rate constants of metarhodopsin II formation in different chemical environments

| Detergent | pH 6.5; $T$ = 18°C | | pH 8.0; $T$ = 4°C | | Ratio |
|---|---|---|---|---|---|
| | $A$ (rel. units) | $k_1$ ($s^{-1}$) | $A$ (rel. units) | $k_2$ ($s^{-1}$) | $k_1/k_2$ |
| *n*-Dodecyl-$\beta$-D-maltoside | 0.95 | $4.2 \times 10^3$ | 0.90 | $5.0 \times 10^2$ | 8.4 |
| Deoxylysolecithin | 0.90 | $4.6 \times 10^2$ | 0.90 | $1.1 \times 10^2$ | 4.2 |
| *n*-Octyl-$\beta$-D-glucopyranoside | 0.90 | $3.1 \times 10^2$ | 0.85 | $9.4 \times 10^1$ | 3.3 |
| Sodium cholate | 0.60 | $2.1 \times 10^1$ | 0.15 | 2.5 | 8.4 |
| Chapso | 0.50 | $1.5 \times 10^1$ | 0.10 | 2.2 | 6.8 |
| Doped micelle | 0.45 | $1.3 \times 10^1$ | 0.10 | 1.7 | 7.6 |
| Washed membranes | 0.85 | $1.1 \times 10^2$ | 0.25 | 4.0 | 27.5 |

$A$ is the relative signal amplitude, normalized to the amplitude measured with disc membranes at pH 6.5, 35°C. $k_1$ and $k_2$ are the rate constants $k$ of the apparent first-order signal time course $S(t)$ in: $S(t) = A(1 - e^{-kt})$.

The measured quantities $A$ and $k$ are related to properties of the MI/MII equilibrium:

$$\mathrm{MI} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} \mathrm{MII}.$$

Flash-induced MI equilibrates with the sum of the on and off rates: $k = k_+ + k_-$, with $A = [\mathrm{MII}]/([\mathrm{MII}] + [\mathrm{MI}]) = K/(1 + K)$, and $K = k_+/k_-$

very fast in *n*-dodecyl-β-D-maltoside, with a biphasic time course. The kinetic distortion might be due to a transformation of the micelle during MII formation. In *n*-octyl-β-D-glucopyranoside and deoxylysolecithin, the kinetics are similar to the one in disc membranes, whereas sodium cholate and Chapso cause even slower MII formation.

The ratio between the formation rates at pH 6.5, $T$ = 18°C and pH 8.0, $T$ = 4°C remains relatively small (3–7) for all detergents, in line with the finding of others [6,19] of a much smaller activation enthalpy in detergent micelles, as compared to the disc membrane. It is interesting to note that this ratio does not approach the high value (~25) found in disc membranes, even if the rate becomes similarly slow.

The data corroborate our previous finding [9] that MII formation depends on the chemical structure of the detergent. In Chapso or sodium cholate, both of which contain rigid steroid structures, the MII kinetics is slow and the MI/MII equilibrium shifted towards MI; the same was found with the rigid biphenyl detergent [9].

### 3.2. *Rhodopsin in the doped micelle*

A doped micelle consisting of deoxylysolecithin and 11-deoxycorticosterone was prepared as described in section 2. As can be seen from fig.2, kinetics and yield of MII formation are different from those of the pure detergent micelle. The time constant for the formation of MetaII increased by a factor of 16 from 1.6 ms (pH 6.5, $T$ = 18°C) in the deoxylysolecithin micelle to 26 ms in the doped micelle. The MII/MI equilibrium constant was decreased by a factor of 2.1.

### 3.3. *Binding of transducin*

Because of its well-suited MI/MII equilibrium, we tested rhodopsin in Chapso micelles for the interaction with transducin, using the extra-MII assay (see section 2). Extra-MII was not seen either in Chapso or sodium cholate. The doped micelle system, however, exhibited a strong extra-MII signal, as shown in fig.2b and c.

## 4. DISCUSSION

### 4.1. *Detergent structure determines MII formation*

The results suggest a simple classification of the detergents: flexible detergents cause fast and complete MII formation, while rigid detergents tend to make MII slow and to shift the MI/MII equilibrium to MI. This agrees with our previous results on deoxylysolecithin and a biphenyl detergent of rigid structure [9].

An interesting new finding is that the observed rates, while being even slower than in the native disc membrane, do not display the large temperature coefficient known from the membrane system. This could mean that entropic terms rather than a large activation enthalpy limit the reaction rate in the micellar system (dominance of the term $S/R$ over $H/RT$ in the Arrhenius equation); however, there is also a difference between the doped and normal lysolecithin micelle. This point will be investigated in more detail elsewhere.

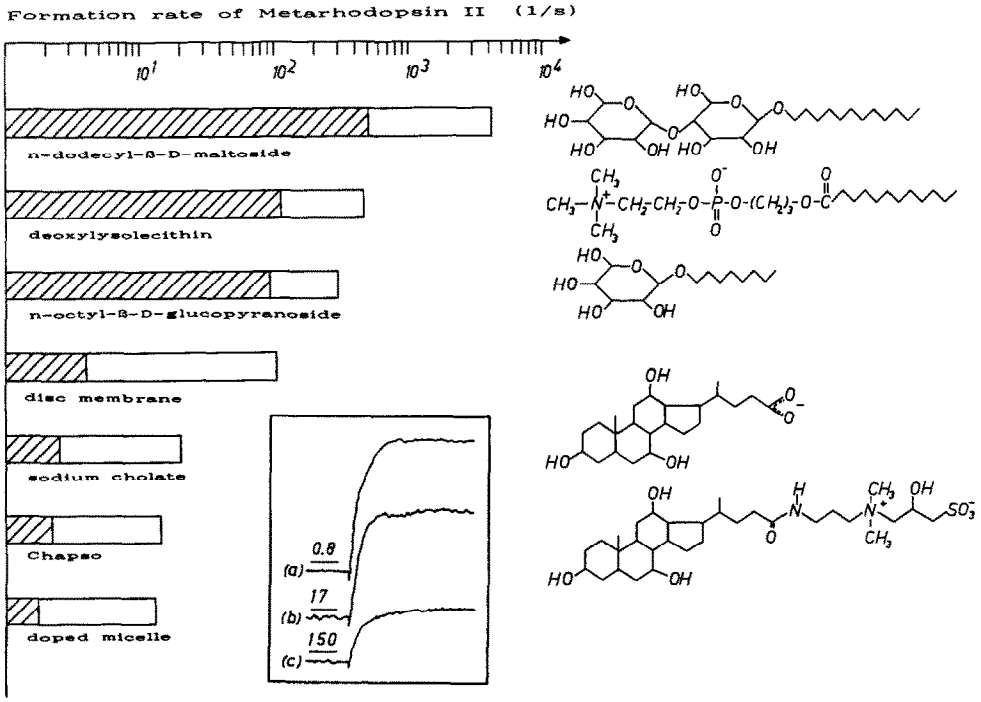


Fig.1. Formation rate of metarhodopsin II (MII) in different detergents. Empty bars indicate values for pH 6.5, $T$ = 18°C, hatched bars for pH 8, $T$ = 4°C (compare table 1). MII formation is the faster and more flexible structure of the detergent (shown at the right). The inset shows measurement examples for *n*-dodecyl-β-D-maltoside (a), *n*-octyl-β-D-glucopyranoside (b), and Chapso (c). Rhodopsin concentration was 5 μM, concentrations of detergent were as described in section 2, and mol fraction of photolysed rhodopsin was 3.7%.

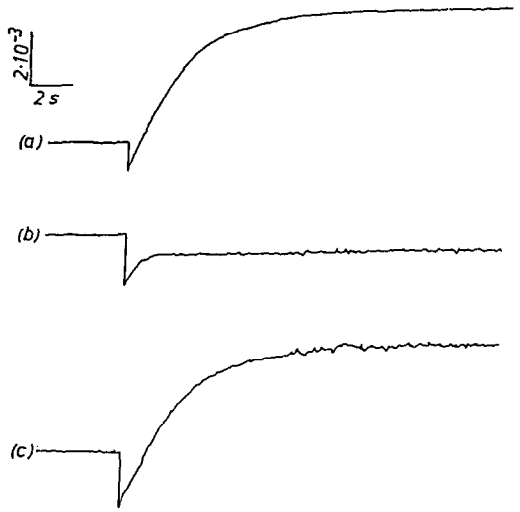


Fig.2. Formation of metarhodopsin II (MII) and $G_t$-induced extra-MII in washed disc membranes and in doped micelles: (a) washed membranes in the presence of $G_t$ (extra-MII); (b) rhodopsin solubilised in equimolar 11-deoxycorticosterone and deoxylysolecithin ('doped micelle'); (c) solubilised in the doped micelle in the presence of $G_t$ (extra-MII). pH 8.0, $T$ = 4°C. Concentrations of rhodopsin and $G_t$ were 0.5 $\mu$M and 1.5 $\mu$M, respectively. Mol fraction of photolysed rhodopsin was 5%. Each trace represents the average of two recordings. Signals are the absorbance change at 380 nm (absorption maximum of MII) minus the absorbance change at 417 nm (isosbestic point of MI/MII transition). After the negative deflection, which is the absorption change due to early photoproducts including MI, the production of MII follows on the s-time scale under the conditions.

### 4.2. *Insertion of steroids mimics rigid detergent*

The tendency of rigid components in the micelle core to reduce the formation of MII was already found in our previous study [9]. In the two component system (equimolar deoxylysolecithin and 11-deoxycorticosterone) kinetics and extent of MII formation was the same as in the rigid biphenyl detergent investigated previously [9]. The fact that small molecules, which have no solubilising property in themselves, can replace the bulky biphenyl detergent argues against a side-effect due to the specific structure of the detergent.

We interpret these results within the framework of the surface free energy concept [7–9]: insertion of small molecules of rigid structure into the micelle enhances the packing of the detergent, by analogy to the effect of cholesterol in lipid membranes [20]. The interfacial tension at the surface of the solubilised protein is enhanced and acts in favour of the less extended MI conformation.

### 4.3. *Rhodopsin interacts with transducin in the doped micelle*

The shift of the MI/MII equilibrium to MI in this system provided the basis for measuring transducin-induced extra-MII in deoxylysolecithin. Apparently, the doped micelle provides a good reaction milieu for the R–$G_t$ interaction and the $G_t$-enhanced formation of MII ('extra-MII') is functioning as in the native membrane.

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