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On the Evaluation of Photoreceptor Properties by Micro-Fluorimetric Measurements of Fluorochrome Diffusion

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Abstract. By use of the microfluorimetric technique it is possible to study the diffusion of the fluorochrome di-dansylcystine (DDC) within isolated frog rod outer segments (ros) which are immobilysed in agarose gel. For this purpose, by a short hypotonic shock a leak is applied to one end of the ros. By this open end the DDC enters the rod and migrates through the whole outer segment. Following the propagation of the fluorescence boundary with time the cytoplasmatic diffusion constant can be determined if a chromatographic model is used to allow for the considerable binding of DDC to the inner membrane surface. With a binding constant $K = 5 \cdot 10^{-4}$ cm the cytoplasmatic diffusion constant was found to be $D = 1.3 \cdot 10^{-6}$ cm^2/s whereas $D_g = 2 \cdot 10^{-6}$ cm^2/s and $D_r = 3.5 \cdot 10^{-6}$ cm^2/s were found in agarose gel or ringer solution, respectively. Using the mobility reduction factor given by $D/D_r \approx 0.4$ to calculate the cytoplasmatic conductivity an inner resistance per length of 1.7 M Ω/μ could be calculated for a frog rod which is in good agreement with corresponding data obtained from electrophysiological measurements.

Key words: Rod outer segment – Rhodopsin – Fluorochrome binding and diffusion – Cytoplasmatic space – Conductivity.

1. Introduction

A photoreceptor is highly specialized in its outer segment to carry out the transduction of light into electrical signals. In vertebrates the outer segment contains a stack of disc-like vesicles which in case of the rod cell are detached from the cell membrane (Cohen, 1968, 1970; see also Fig. 1). The cytoplasma is confined to the interdisc space and the narrow gap between the disk stack and the envelope membrane (Figs. 1a, b) and, in case of frog rods, also to several thin clefts in the discs which reach far down towards the center of the rod (Figs. 1a, c). Nevertheless, the cytoplasma is the medium for many chemical and electrical activities involved in either the primary phototransduction or the secondary regeneration processes.

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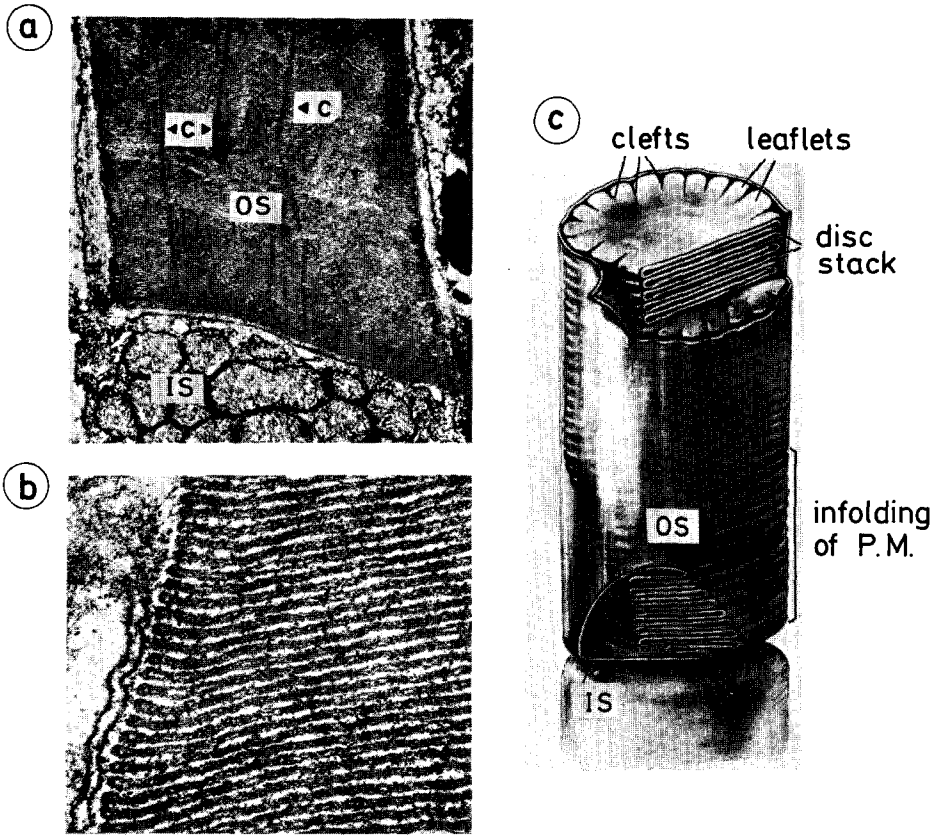

Fig. 1. Morphology of frog rod. *O.S./I.S.*: outer/inner segment, *c*: clefts. ⓐElectron micrograph of basal end of outer segment. ⓑSection out of disc stack [after Nilson, S. E. G.: J. Ultrastruct. Res. **12**, 209 (1965)]. The upper end of *I.S.* shows a dense package of mitochondria (a). ⓒChematic drawing of frog rod outer segment (after Young, R. W. In: Rodieck, R. W.: The vertebrate retina. San Francisco. Freeman 1973). *P.M.* plasma membrane

Although the significance of the cytoplasma is evident for the transport of ions like Na^+, Ca^{2+} (Yoshikami and Hagins, 1970, 1971) or metabolic compounds such as retinal isomers (Yoshikami and Nöll, 1978), ADP, ATP (Bownds et al., 1972) and GTP (Robinson and Hagins, 1979), very little is known yet about its chemical composition and transport properties.

Much more information is available on the properties of the plasma membrane which separates the cytoplasma from the outer medium. The intact rod outer segment (ros) has a strong osmotical activity (Korenbrot and Cone, 1972). The plasma membrane was found to be impermeable not only for large compounds as ATP, ADP etc., but also for such ions as H^+, Cl^-, Ca^{2+} and Mg^{2+} (Uhl, 1976; Schnetkamp et al., 1978).

Consequently the plasma membrane is readily disrupted by osmotic shocks. To try the integrity of the plasma membrane a specific staining test was developed by

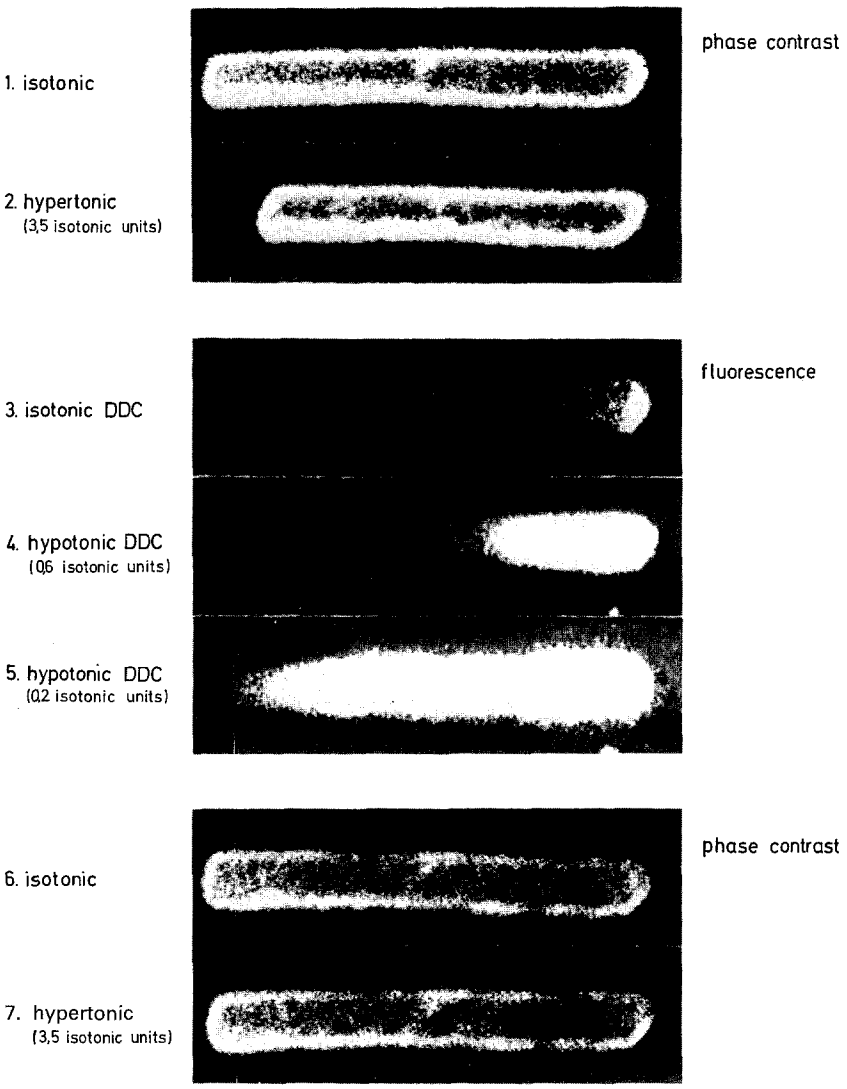

Fig. 2. Micrographs of gel fixed frog rod outer segment under different osmotic conditions applyed in subsequent steps 1.–7.

Yoshikami et al. (1974). In this method a fluorescent dye di-dansylcystin (DDC) is employed which does not penetrate the receptor membranes but binds to them. In binding to the lipid membrane phase the DDC fluorescence efficiency is enhanced up to a factor of 20 if compared with its fluorescence in the water phase and shifts the fluorescence maximum from 550 to 505 nm. In staining rod outer segments with DDC the disc stack which contains about 97% of all the rhodopsin is not accessible to DDC as long as the envelope membrane is intact and thus the ros do not fluoresce at all (Yoshikami et al., 1974; see Fig. 2).

However, if the plasma membrane is damaged the disc stack is stained and hence the ros gives a strong fluorescence, i.e., they are DDC-positive. The fluorochromic DDC-method was used to test isolated ros of frogs (Yoshikami et al., 1974; Hochstrate and Rüppel, 1976). The DDC-test was applied also to investigate the aging process of isolated frog ros and their stability against influences of light and pH-changes (Hochstrate and Rüppel, 1980). The DDC-test failed, however, with cattle rods. Applying several methods which definitely destroy the plasma membrane a fluorescence enhancement could never be detected in cattle ros. The same finding was made by Schnetkamp et al. (1978).

In the course of these investigations the conditions of the osmotic shock treatment had been carefully studied which leads to a disruption of isolated ros when immobilized in agarose gel. Applying a relatively small hypotonic shock to an intact frog ros it was found that only one end of the ros was opened up whereas the rest of the membrane stayed intact. This phenomenon might be explained by the assumption that the ros was opened up at the end where it was broken off from the remaining receptor and had been sealed off again.

Once the ros is opened up DDC is able to flow into the ros thus making the end leak detectable in the fluorescence microscope. As the migration of DDC appears to be a slow process (10–30 min all over the ros) it could be easily followed by observing the fluorescence boundary in the ros (see Fig. 5).

In this study the DDC-injection and measuring technique is used to obtain information on unknown properties of the cytoplasma in frog rod outer segments. From DDC-migration measurements inside the cytoplasma and a separate determination of the binding constant of DDC to the membrane the DDC diffusion constant was evaluated. Furthermore some evidence is given on the dimensions of the cytoplasmic space in the ros and also on the transport properties of the cytoplasma.

2. Methods and Materials

Incubation Media

The experiments were performed at room temperature in the following solutions:

Frog Ringer: 119 mM NaCl, 2,5 mM $CaCl_2$, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 24 mM Na_2HCO_3, pH = 7.0.
"Hypotonic" Ringer: like frog ringer only NaCl concentration changed to 50 mM.
DDC-Ringer: 0.2–0.3 mM N, N′-di-dansylcystine (Sigma Chemical) added to frog ringer.

Flow System

The system used for microfluorimetric experiments is shown in Fig. 3. The preparation (see below) is kept between two cover glasses fixed at a distance of 300 μm. The

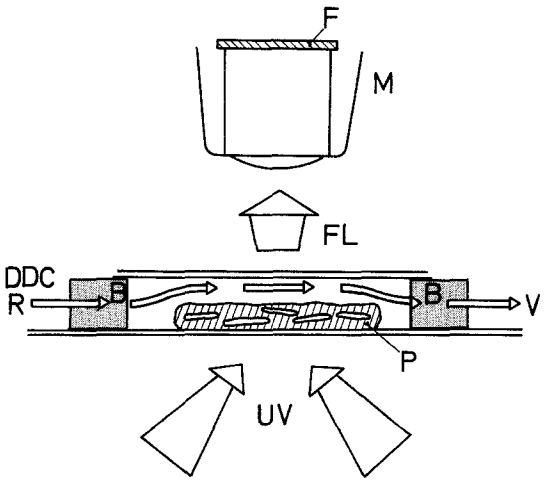

Fig. 3. Schematic drawing of the flow system mounted in a fluorescence microscope. *R*: reservoir of incubation solution; *V*: water jet vacuum; *B*: blotting paper; *P*: ros preparation; *uv*: ultraviolet light focussed with high aperture by a dark field Heine condensor to excite the preparation; *Fl*: fluorescence light from preparation; *M*: objective 10 x 0.25; *F*: cut off filter for u.v., Wratten No. 8

incubation solution is sucked through the interspace in between these slides with aid of slices from blotting paper. In this way the incubation medium could be exchanged within less than 2 s.

Microscopic Observation

The preparation (see below) was observed by a microscope (Zeiss-Ortholux with automatic camera attachment Zeiss-Orthomat). The fluorescence was excited by the 365 nm band of a high pressure mercury lamp (HBO 200 – Osram with UG5 cut-off filter Schott). The u.v.-light was focussed into the preparation by a dark field Heine condensor. The irradiance of about $3 \cdot 10^{15}$ photons/cm^2·s at 365 nm did not produce DDC-positive receptor cells if applied for less than 4 min. After prolonged u.v.-illumination the plasma membrane of the receptor is destroyed with a half life time of 20–30 min. In DDC-ringer suspension this membrane destruction is accompanied by a remarkable prolongation of the receptor cell and a formation of a curled, snake like structure. In the microfluorimetric experiments described in this paper the outer segments were never illuminated longer than 2 min.

Preparation of Rod Outer Segments

Rod outer segments were prepared according to Yoshikami et al. (1974). A frog retina (*Rana pipiens* or *temporaria*) is isolated in frog ringer solution at room temperature. By gently touching the retina onto a slide the rod outer segments are detached from the rest of the receptor cells. The isolated ros remain on the slide in a drop of rather pure

suspension in ringer solution. In order to observe one and the same cell over a long period of time inspite of the perfusing procedure the ros had to be immobilized in agarose gel in the following way: A drop of warm 2% agarose solution in ringer (40° C) is mixed with the drop of ros suspension and rapidly spread out by pressing a second cover glass onto the drop to form a thin film of 30–50 μm thickness. After gelatinization of the agarose the cover glass is cautiously removed and the preparation mounted into the flow chamber. For opening one end of the outer segments the preparation is incubated in "hypotonic" ringer for 0.5–1 min. It has to be realized that the velocity of changing the osmotic conditions decides whether a ros remains undamaged, is opened one side or completely disrupted. Therefore, as the gel diffusion barrier flattens the osmolarity jump the thickness of the gel film determines the optimum osmolarity change to open as many cells as possible at only one end. At a film thickness of 40 μm the "hypotonic" ringer of 0.6 isotonic units opens about 30% of the inbedded cells at one end. After the osmotic shock the cells are incubated in DDC-ringer. The diffusion of the DDC into the ros was observed by taking photographs at different times.

Spectrophotometric Measurements

The determination of rhodopsin and DDC-concentrations were performed in the scattering light attachment of the spectrophotometer Beckman model 5260. DDC-diffusion in ringer solution was measured in a special diffusion cell using initial boundary sharpening (stop flow) and Schlieren optics (Institut für Technische Chemie, TU Berlin). To follow the DDC-diffusion in a test gel the gel scanning accessory of a Beckman spectrophotometer model 25 was used (Robert-Koch-Institut, Berlin). All preparations and experiments were performed at room temperature.

3. Analysis of DDC-Diffusion in a Receptor Cell

The propagation of DDC inside a receptor cell is not a simple solute-solvent diffusion. There are two strong arguments that the observation of the fluorescence boundary might yield merely an apparent diffusion constant:

1. Having entered the receptor cell DDC can only diffuse in the space between the disc stack and the plasma membrane.

2. To explain the enormous increase of fluorescence yield and the shift of the emission maximum one has to assume that a considerable amount of DDC is bound to the receptor membranes.

By the following model (Fig. 4) these two facts are taken into consideration:

The free diffusion space inside the ros is regarded as the interspace between two long coaxial cylinders with radii r and r-b. In the model, the total surface S of the inner disc membranes is projected onto the surface A of the inner cylinder which is by a factor $\alpha = S/A$ smaller than the real inner membrane surface S. This simplification can be introduced to the model because a concave fluorescence boundary was never observed so that a slow dye diffusion parallel to the disc surface (should be excluded see Fig. 2). The DDC-distribution within the disc stack may be enhanced by the clefts in the discs as

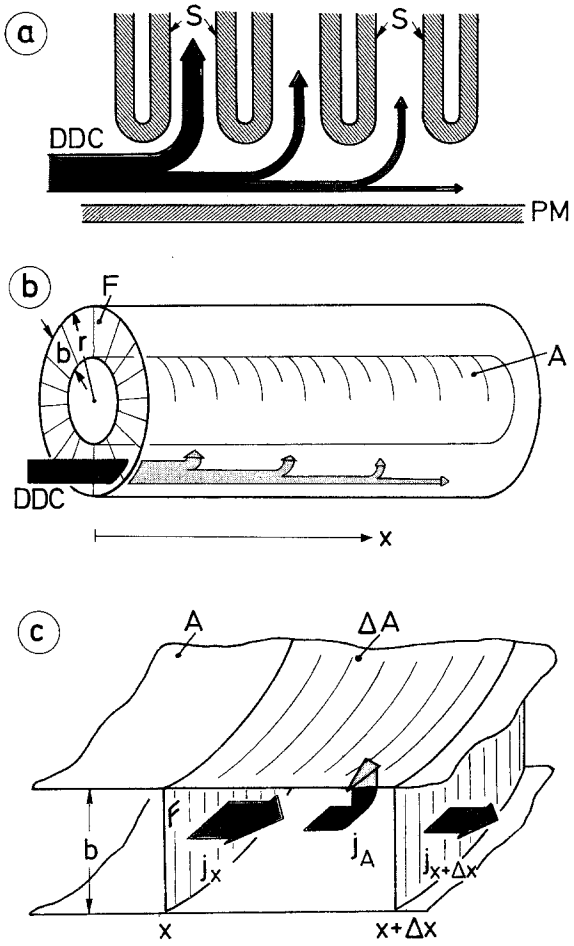

Fig. 4. Schematic drawings to illustrate the process of DDC migration and binding inside the rod outer segment (ros). ⓐ Cross-section through ros (compare Fig.1a). *S*: total disc surface; *PM*: plasma membrane. ⓑ Coaxial cylinder model for rod outer segment. *r*: radius of ros; *b*: mean gap width between disc stack and plasma membrane; *F*: free diffusion cross section; *A*: surface of inner cylinder (mean envelope of disc stack); *x*: direction of DDC flow. ⓒ Volume element of free diffusion gap shown in (b). $\Delta V = F \cdot \Delta x = b \cdot \Delta A$, $j_x/j_{x+\Delta x}$: DDC- in/out-flux in volume element ΔV, j_A: DDC-flux to surface element ΔA

shown in Fig. 1. An equilibrium is assumed between the DDC bound to the cylinder surface A and the DDC solved in the cytoplasma according to the equation

$$C_A/C_V = K_A\,, \tag{1}$$

where C_A is the apparent surface concentration (moles/area) and C_V the real volume concentration (moles/volume) of DDC. Since the local adjustment of the equilibrium is much faster than the DDC propagation within the receptor cell this equilibrium can be taken as stationary as far as the diffusion process is concerned. Thus for any place x in the ros it is

$$\left(\frac{\partial C_A}{\partial t}\right)_x = K_A \cdot \left(\frac{\partial C_V}{\partial t}\right)_x . \tag{2}$$

The flux j_A of DDC molecules onto the adsorbing surface (see Fig. 4c) is given by

$$j_A = + \left(\frac{\partial C_A}{\partial t}\right)_x . \tag{3}$$

The number of molecules δn which are transfered within the time interval δt into the volume element $\Delta V = F \cdot \Delta x = b \cdot \Delta A$ ($F \approx 2\,\pi rb$) between x and $x + \Delta x$ thereby raising the DDC concentration by δC_V is

$$\delta n = \Delta V \delta C_V = (-F\Delta j_x - \Delta A j_A) \cdot \delta t \tag{4}$$

j_x is the DDC-fluc in x-direction in the interspace and $\Delta j_x = j_{x+\Delta x} - j_x$ (see Fig. 4c).
Dividing by ΔV and δt yields

$$\frac{\delta C_V}{\delta t} = -\frac{\Delta j_x}{\Delta x} - \frac{j_A}{b} \text{ or in the limit } \Delta x \to 0$$

$$\left(\frac{\partial C_A}{\partial t}\right)_x = -\left(\frac{\partial j_x}{\partial x}\right)_t - \frac{j_A}{b}. \tag{5}$$

Using Eq. (2) and Fick's law I: $j_x = -D \cdot \left(\frac{\partial c}{\partial x}\right)_t$, one obtains:

$$\left(\frac{\partial C_V}{\partial t}\right)_x = D \frac{1}{1 + K_{A/b}} \left(\frac{\partial^2 c}{\partial^2 x}\right)_t. \tag{6}$$

This differential equation corresponds to Fick's law II with an apparent diffusion constant

$$D_a = \frac{1}{1 + K_{A/b}} \cdot D. \tag{7}$$

For the diffusion of DDC injected into the receptor cell at one end ($x = 0$) one has the following initial and boundary conditions:

① $t = 0$: $C_V = 0$ for $x > 0$
② $t > 0$: $C_V = C_0$ at $x = 0$

C_0 = DDC concentration in the incubating solution;
C_V = DDC concentration in the cytoplasma.

Under these conditions the solution of Eq. (6) is

$$C_V(x, t) = C_0 [1 - \text{erf}\,(x/2\sqrt{D_A t}]\ ^{1)}. \tag{8}$$

Using the migrating fluorescence boundary x_f as measure for the DDC-propagation (see Fig. 5) and introducing the corresponding threshold concentration C_f for fluores-

1 Eq. (8) can easily be derived from the common solution for a two phase system given by Jost (1937) assuming that the DDC partition coefficient is one and the diffusion constant in the outer medium ($x < 0$) is much larger than in the cytoplasmic space ($x \geqslant 0$)

cence observation Eq. 8 delivers a relation between x_f and the time t:

$$1 - \frac{C_f}{C_0} = \operatorname{erf}\left(\frac{x_f}{2\sqrt{D_a t}}\right). \tag{9}$$

4. Results

In Figure 5 an example is given for the experiments conducted to measure the DDC-propagation inside an outer segment of a frog receptor cell fixed in agarose gel.

Before applying an osmotic shock no DDC-fluorescence is detectable in the ros (Fig. 5a) indicating that the plasma membrane is intact. Such cells are osmotically active (Yoshikami et al., 1974) and show a shrinkage of about 10% if they are embedded in 2% agarose and exposed to a hypertonic shock of threefold osmolarity in the incubating medium. The extent of the shrinkage is reduced in the agarose fixation. In contrast, free suspended ros (Korenbrot and Cone, 1972) as well as receptor cells still attached to the retina shrink as much as 30% under the same osmotic conditions. Agarose fixation also preserves the shape of the ros even under such hypotonic conditions where the plasma membrane is destroyed. In hypotonic suspensions without agarose embedding the ros are largely prolonged and show a curling similar as after excessive u.v.-illumination in isotonic solution (Hochstrate and Rüppel, 1980). Therefore it is nearly impossible to perform diffusion measurements in suspended but unfixed receptor cells.

After opening the gel fixed ros at one end by a short time hypotonic shock (Fig. 5a) the DDC instantly starts to penetrate into the ros to propagate inside the cytoplasma. The position x of the fluorescence boundary at different times t after the hypotonic shock was analysed under the following assumptions:

1. The penetration of the dye into the ros is independent of the random shape of the leakage produced by the osmotic shock.

2. The threshold DDC concentration C_f at the fluorescence boundary is approximately 10^{-6} M/l.

Assumption 1 seems to be realysed within the experimental error of these microfluorimetric experiments. The analysis of DDC propagation in eight cells yields an apparent diffusion coefficient within a maximum error of 35% (see Fig. 5).

As for assumption 2 the DDC concentration C_f at the fluorescence boundary could not be determined exactly. Titrating C_f by varying the DDC concentration of the incubating medium it was found to be difficult to reproduce a) the illumination conditions of the single ros in the microscope and b) the developement procedure of the film material. The value $C_f = 10^{-6}$ M/l was chosen because $C_0 = 10^{-5}$ M/l gives a well detectable staining of the cells whereas no staining is observed at $C_0 = 10^{-7}$ M/l under the experimental conditions. On the other hand using $C_0 = 2.5 \cdot 10^{-4}$ M/l in all the experiments the erf. value $1 - C_f/C_0$ of Eq. (9) changed from 0.999 to 0.980 in varying C_f from $2 \cdot 10^{-7}$ to $5 \cdot 10^{6}$ M/l, which changed the apparent diffusion constant by less than 50%.

Under these two assumptions an apparent DDC-diffusion coefficient in frog rod outer segment was deduced from the experiments as

$D_a = 2.3 \cdot 10^{-10 \pm 0.3}$ cm²/s (see Fig. 5c)

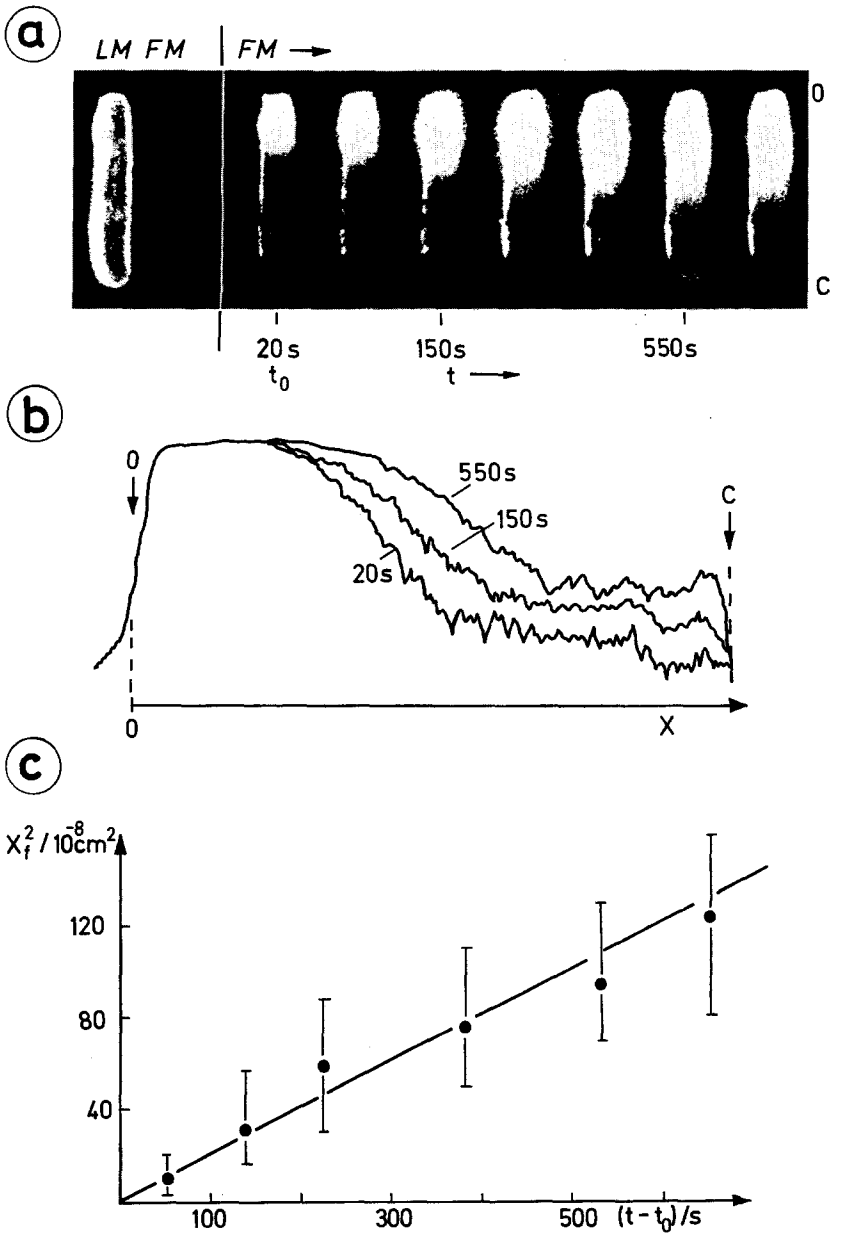

Fig. 5. Example for observation ⓐ, plotting ⓑ, and evaluation ⓒ of a DDC-diffusion experiment in a gel fixed frog rod outer segment (ros). ⓐ Observation. Left hand: light (*LM*) and fluorescence (*FM*) micrographs of ros before osmotic shock. Right hand: fluorescence micrographs (*FM* →) of ros showing DDC-migration at $t = 20, 70, 150, 250, 400, 550$, and 670 s after osmotic shock. ⓑ Plotting. Densitometer curves of fluorescence micrographs given in right hand. Arrows indicate open (o) and closed (c) end of ros, x: direction of DDC-flux. ⓒ Evaluation ⓐ of the apparent diffusion constant for DDC. Square of fluorescence boundary x_f versus time $t - t_o$, $t_o = 20$ s. Error marks give variation for 6 ros. The apparent diffusion constant was determined according to Eq. 9 by $x_f/2\sqrt{D_A \cdot t} = 2.1$ for $1 - c_f/c_0 = 0.996$. (Two further ros give a slope above or below the error marks. The mean value between both, however, is the same as that of the first 6 ros)

with an inaccuracy factor of 2 ($10^{\pm 0.3}$). To calculate the real diffusion constant from Eq. (7) the equilibrium constant K_A has to be determined first by independent measurements.

The equilibrium constant K for DDC-adsorption at the disc membrane surface S was determined by titrating a certain volume of DDC-ringer solution with a suspension of frog ros of known rhodopsin content. The palsma membranes of the ros were disrupted osmotically so that the DDC could reach the whole surface area of the disc membrane S. The concentration change of DDC in the solution after adding a certain amount of ros yields the total amount of DDC bound to the disc membranes. For this purpose the ros fragments were spun down in the DDC-solution by a laboratory centrifuge so that the remaining DDC concentration in the supernatant could be measured in the spectrophotometer. The total disc surface S was calculated by measuring the rhodopsin content of the added ros-suspension using the known surface concentration of frog rhodopsin with $2.5 \cdot 10^{+4}$ Rh/(μm)^2 (Liebman et al., 1974). The titration experiments gave the following results: The equilibrium relation between surface concentration C_S of DDC and the volume concentration C_V

$$C_S/C_V = K \tag{10}$$

holds at least for the concentration range between $2 \cdot 10^{-5}$ and $3 \cdot 10^{-4}$ M/l studied in these experiments. The equilibrium constant in this range was determined as $K = 5 \cdot 10^{-4}$ cm.

Since in the diffusion model for ros the total inner surface S of the disc stack is projected onto the cylinder area A the constant K_A in the model is higher according to the ratio $\alpha = S/A$. For frog rods this factor is $\alpha \approx 100$ so that $K_A = \alpha \cdot K \approx 5 \cdot 10^{-2}$ cm [2]. The model distance b between the plasma membrane and the disc stack was determined from electron micrographs of frog ros (Cohen, 1968). Under the assumption that the intact plasma membrane is tightly streched all over the irregulary shaped leaflets of the discs the average value of b is about 90 nm. Using the values of b, α and K the real diffusion constant of DDC in the ros cytoplasma is calculated from Eq. (7) to

$$D = 1.3 \cdot 10^{-6 \pm 0.3} \text{ cm}^2/\text{s} .$$

The real diffusion constant of DDC in Ringer solution was determined to be $D_r = (3.5 \pm 0.5) \cdot 10^{-6}$ cm^2/s.

In 2% agarose gel the diffusion constant seems to be slightly reduced. A first determination using the gel scanning technique yielded a diffusion constant of $(2 \pm 1) \cdot 10^{-6}$ cm^2/s.

5. Discussion

A comparison of the DDC-diffusion data obtained in the cytoplasma of frog rod outer segments, in 2% agarose gel and in normal ringer solution suggests that the diffusion is slowed down by a mobility factor $\beta = D/D_r = 0.4$ with an error range between 0.2 and

2 In a preliminary report on these measurements given by J. Brown (1979) (Dahlem Konferenz 1978) the diffusion constant was derived not considering the surface reduction factor. Therefore the numerical value given in the report of the Dahlem Konferenz was smaller by a factor of 100

0.8. As the current flow through the outer segment is mediated through the same free cross-section $F = \pi \cdot d \cdot b$ as that used by the DDC diffusion the inner resistance per unit length

$$r_i = \frac{1}{F \cdot \gamma_i} = \frac{1}{\beta \gamma_r \pi d b} \tag{11}$$

can readily be calculated by estimating the conductivity γ_i of the cytoplasma (γ_r = conductivity in ringer solution). Using the available concentration data for the main movable ions in cell cytoplasma (140 mM K^+, 10 mM Na^+, 3.5 mM Cl^-) and the equivalent conductivities at an ionic strength of $I = 0.14$ one obtains $r_i = 1.7$ M Ω/μ. This value is in reasonable agreement with data used by Ehrhardt and Baumann (1977) in describing the current flow in frog rods and also by Hagins and Rüppel (1971) following the time course of the fast photovoltage of rat rods. However, it should be emphasized that according to Eq. (7) the diffusion constant D of the cytoplasma and consequently the mobility factor is related to the free space parameter b:

$$\beta = \frac{D}{D_r} = \left(1 + \frac{K_A}{b}\right) \frac{D_a}{D_r} \approx \frac{K_A}{b} \cdot \frac{D_a}{D_r} \quad \text{as} \quad \frac{K_A}{b} \gg 1 \,. \tag{12}$$

For a given value of the apparent diffusion constant D_a the product $\beta \cdot b \approx K_A \cdot D_a/D_r$ is constant. The model parameter b is derived only by electron micrographs which do not necessarily reflect the in vivo morphology of the photoreceptor. Therefore b might be subject not only to statistical but also to a considerable systematical error. On the other hand according to Eq. (11) the inner resistance r_i depends only on the product $\beta \cdot b$. That means, assuming that the cytoplasma has the same diffusion properties as the outer medium ($\beta = 1$), the resistance is unchanged if b is reduced from 90 to 35 nm. As a matter of fact, in calculating $r_i = 1.9$ M Ω/μ Ehrhardt and Baumann (1977) used the cytoplasmic conductivity given by Stämpfli (1952), which is only slightly smaller than that of the outer receptor medium.

The F value they used according to Falk and Fatt (1973) is 2% of the whole ros cross section which corresponds to a b value of 30 nm.

In any case, the main result of this investigation on DDC diffusion of freshly isolated intact ros is that a free space between disc stack and envelope membrane exists with a mean gap width of at least 20 nm according to the lower limit of D_a. This conclusion agrees also with the following considerations:

It had been suggested that the muco-polysaccharides bound to the receptor membranes (Cohen, 1973) might be the source for a higher viscosity of the cytoplasma. However, if the free space parameter is larger than 20 nm the influence of the polysaccharides can be neglected. The saccharide side chains should stick out of the membrane – if at all – at maximum only 1–2 nm into the cytoplasma. Furthermore, electron micrographs using normal and high voltage techniques[3] do not show any hint for microtubuli structures or inner compartimentations in the ros which might impede DDC diffusion or ion migration inside the cytoplasma. Such microtubuli structures are found in many cells (Burnside, 1975). Similar protein structures have been postulated

3 Electron micrographs of critical point dried frog ros were taken by H. R. at the 1.2 MV-electron microscope at the Max-Planck-Institut für Metallforschung, Stuttgart

in the invertebrate photoreceptor to mediate the propagation of light excitation in the microvilli (Hamdorf, 1979). Thus, if a slightly lower mobility exists in the cytoplasma it should be due to macromolecular compounds solved or suspended in the cytoplasma.

Finally it might be mentioned that the fluorochrome DDC is mostly bound to the receptor membranes in the rod outer segment and only 0.1‰ of the total amount of DDC is solved in the cytoplasma. At a DDC concentration in the cytoplasma $C_0 = 2.5 \cdot 10^{-4}$ M/l, with the binding constant $K = 5 \cdot 10^{-4}$ cm it follows that about 50 DDC molecules are reversibly bound to the mean membrane area per rhodopsin.

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