EVIDENCE FOR STRUCTURAL CHANGES IN THE PHOTORECEPTOR DISK MEMBRANE, ENABLED BY MAGNESIUM ATPase ACTIVITY AND TRIGGERED BY LIGHT

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1. Introduction

To account for the immense amplification involved in visual transduction, Wald, in 1954, [1] proposed the existence of a diffusable transmitter, which was either released from a compartment (compartment theory) or produced by an enzyme (enzyme theory). Until very recently the Ca^{2+} theory of visual transduction [2], i.e., a specific compartment theory, was widely favoured. However, as more and more evidence accumulated against Ca^{2+} being the transmitter [3,4] the enzymatic hydrolysis of cGMP attracted increasing attention and is now considered the most likely candidate for the transmitter process [5,6].

An enzymatic transmitter model, however, would pose the question as to the functional significance of the special form of the photoreceptor disk membrane, i.e., the fact that the rhodopsin-containing membranes form topologically closed compartments with strikingly low ion-permeabilities [7]. The currently discussed mechanisms of light-regulation of cGMP levels in the photoreceptor do not make use of this particular feature of the cell.

Here we report the existence of a Mg-ATPase system in the rod outer segment (ROS) disk membrane, which, in many respects, resembles the ion translocating ATPases found in other systems. We correlate this enzyme activity with structural changes, which occur within the disk membrane in the dark, and also with subsequent changes which are triggered by light. Evidence is provided that structural integrity of the disk-compartment is a prerequisite for these structural events. The large size of these Mg-ATPase-enabled, light-triggered structural changes, as well as their rapid time course (20 ms at 37°C) suggests that they may be related to the photoreceptor function.

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2. Materials and methods

2.1. *ROS preparation*

ROS from bovine retinae were obtained employing a discontinuous sucrose density gradient procedure [8] and were frozen in small aliquots in liquid N_2. Preparation time was kept at a minimum (in some instances <75 min elapsed between the death of the animal and the freezing of the ROS) since it was found that the described effects were markedly diminished with increasing time of preparation.

The purity of the ROS was determined by their spectral ratio A_{280}/A_{500} (usually between 2.3 and 2.5 for unlysed ROS) and the levels of enzyme activity usually associated with inner segment (RIS) or mitochondrial contamination. No ouabain-sensitive Na/K-ATPase activity, nor significant succinate dehydrogenase or NADH dehydrogenase activity was found in our preparation.

2.2. *Kinetic light scattering apparatus*

The apparatus used was basically identical to that in [9], except that for angular measurements a 1 cm

square fluorescence cuvette, filled with ROS suspension, was placed in the centre of a larger, water-filled, self-focusing spherical cuvette. When measuring at angles other than 0° the photodetector (EG and G photodiode UV 444B) was moved on a circle around the centre of the cuvette combination.

A scattering angle of 10° was chosen for most measurements since at this angle interference from another light scattering transient 'P' [10] was minimal.

2.3. *ATPase activity determination*

ATPase activity was determined by measuring the amount of P_i released [11].

3. Results and discussion

3.1. *Mg-ATPase activity in ROS*

To determine ATPase activity in ROS accurately is difficult, since all of the possible contaminating cell material is known to exhibit high levels of ATPase activity. This is mostly why the numerous reports on this subject have been so conflicting (reviewed [12,13]).

When incubated with 3 mM ATP and 3 mM $MgCl_2$, our ROS preparations show relatively high ATPase activity (initial rate: 300–500 nmol ATP hydrolysed $.min^{-1}.(mg\ rhodopsin)^{-1}$ which rapidly decreases

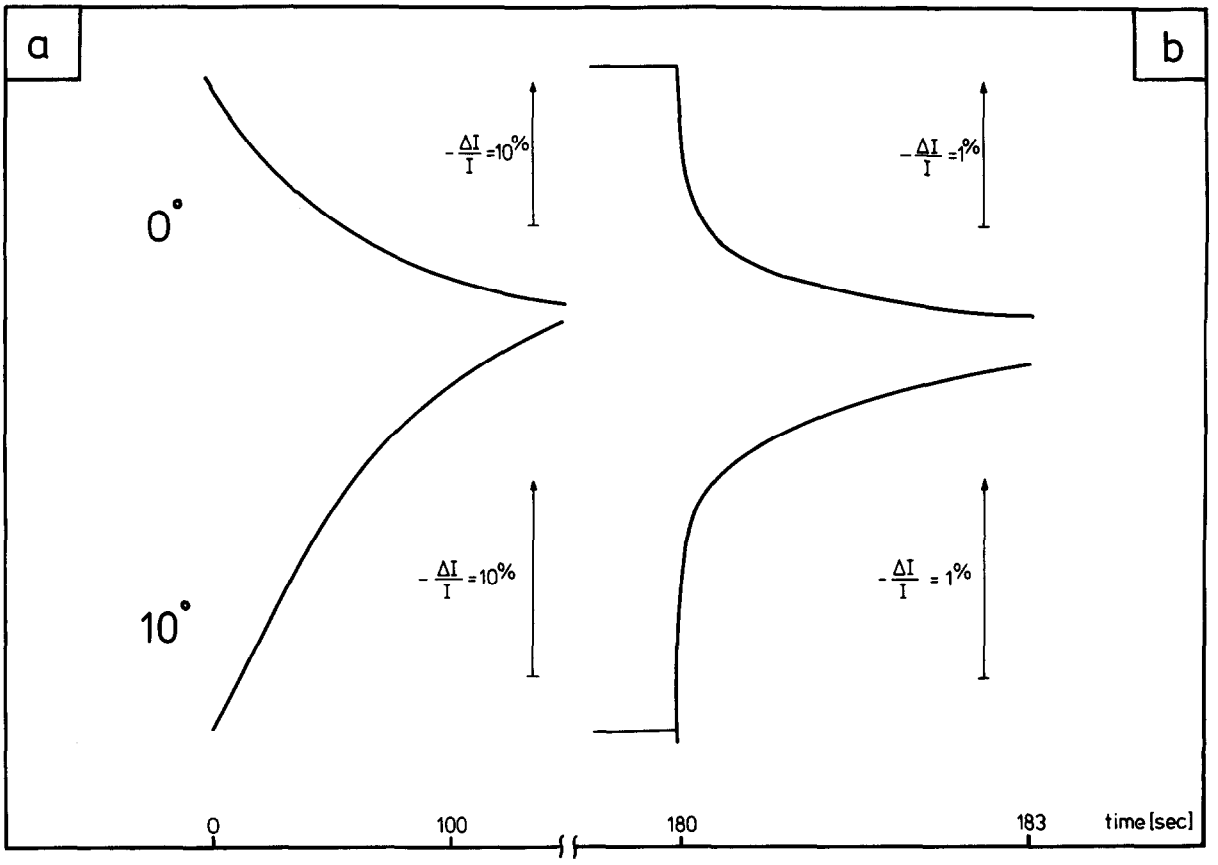

Fig.1. (a) 'A_D' signal at 0° and 10° scattering angle. Medium containing 60 mM Tris–HCl (pH 7.3), 3 mM ATP (sodium salt), 3 mM $MgCl_2$ and ROS suspension (1 μM rhodopsin). T = 20°C. (b) 'A_L' signal at 0° and 10° scattering angle, recorded immediately after 'A_D'. Underlying 'N' signals were electronically subtracted [15].

until it has reached a fairly constant level of ~50 nmol .min^{-1} .(mg rhodopsin)$^{-1}$ after 7–20 min. No ions other than Mg^{2+} were required for this ATP hydrolysis.

The initial rapid phase of ATP hydrolysis can be completely abolished by 100 μM DCCD (*N,N'*-dicyclohexylcarbodiimide) and shows ~50% inhibition with oligomycin at >10 μM. The more persistent slow phase of ATP hydrolysis is unaffected by these inhibitors. Since oligomycin-sensitivity is an exclusive feature of the mitochondrial Mg-ATPase and since DCCD is a specific inhibitor of proton translocating ATPase like the mitochondrial one, the observed inhibitory effects would suggest that the effects we are measuring are artefacts due to mitochondrial contamination. However, the findings below and their correlation with Mg-ATPase activity contradict this.

3.2. *Structural consequences of the Mg-ATPase in the dark: 'A_D'*

The ATPase activity is accompanied by a very dramatic light scattering change from the ROS as is seen in fig.1a. At 0° scattering angle the transmission increases by ~20% in 3 min, whereas the amount of light scattered at 10° decreases by 20%. This ATP-dependent light scattering increment in the dark, whose only ionic requirement is the presence of Mg^{2+}, we have designated 'A_D'. A preliminary study of the

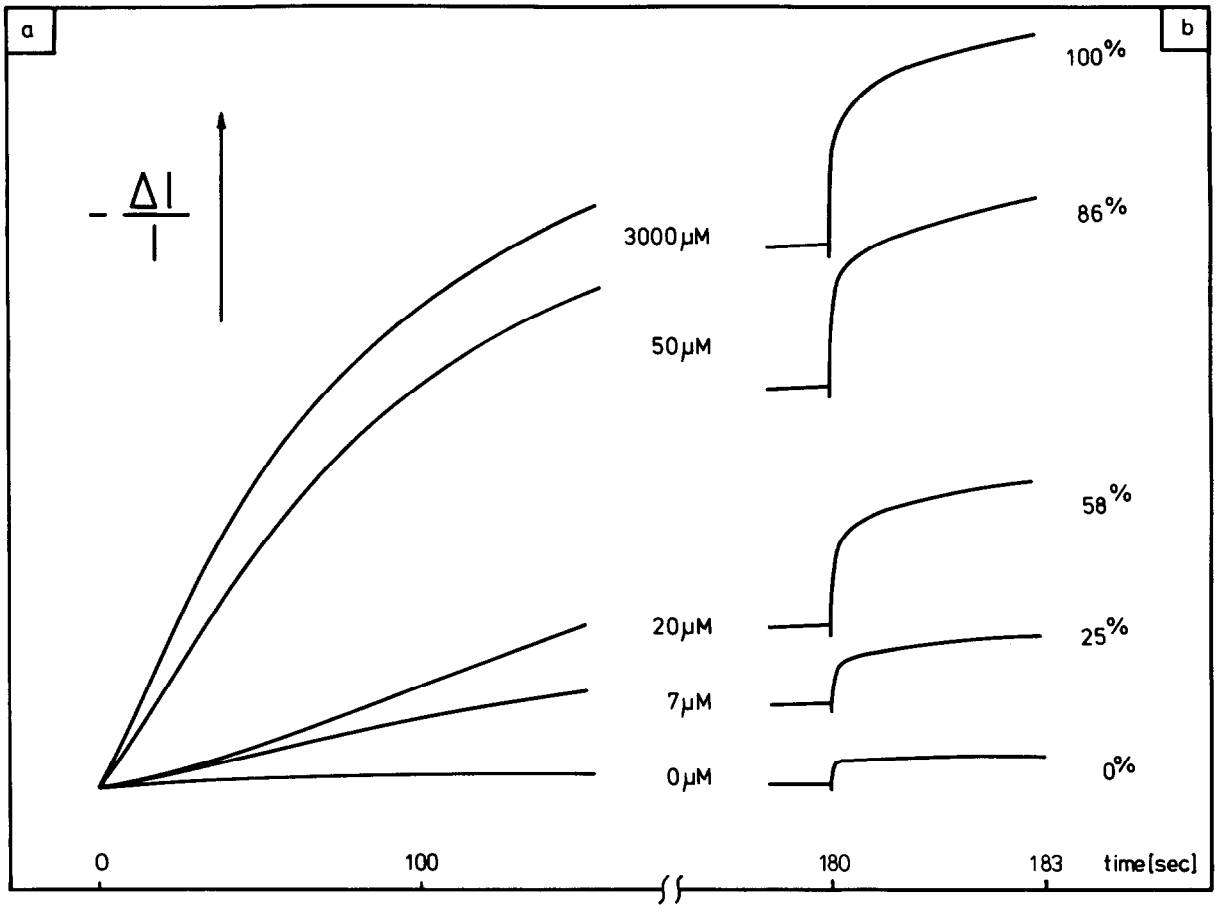

Fig.2. (a) 'A_D' and (b) corresponding 'A_L' signals at 10° scattering angle, obtained with 0–3000 μM ATP. 'N' signals (the lower trace of (b), 0 μM ATP, shows a pure 'N' signal) were not subtracted. The percentages given indicate the amplitude of 'A_L' relative to the maximal 'A_L' amplitude at 3000 μM ATP. Medium and temperature as in fig.1.

angular and wavelength dependence of 'A_D' suggests that the underlying structural event is a change in refractive index rather than a change in shape and/or volume of the ROS. The large amplitude of 'A_D' absolutely precludes the possibility that this effect originates in contaminating membrane material. Neither the ATP-analogues AMP-PNP (adenylyl-imidodiphosphate), which cannot be hydrolysed, nor GTP can replace ATP in starting 'A_D', therefore suggesting that an adenosine specific nucleotide triphosphatase is responsible for it. Figure 2a shows that the rate of 'A_D' decreases with decreasing concentrations of ATP and fig.3a indicates the inhibitory effect of increasing concentrations of the ATP analogue AMP-PNP at a fixed ATP concentration. DCCD and aligomycin also show strong inhibition (fig.3a), the difference between the two being that with DCCD complete inhibition can be achieved, whereas even the highest concentrations of oligomycin yielded <50% inhibition.

3.3. *Light-triggered structural consequences of the Mg-ATPase activity: 'A_L'*

We have reported the existence of rapid, light-induced ATP-dependent structural changes in ROS [14]. We have subsequently demonstrated that these structural changes, which can be monitored as a light scattering transient 'A' (here we shall call it 'A_L', in

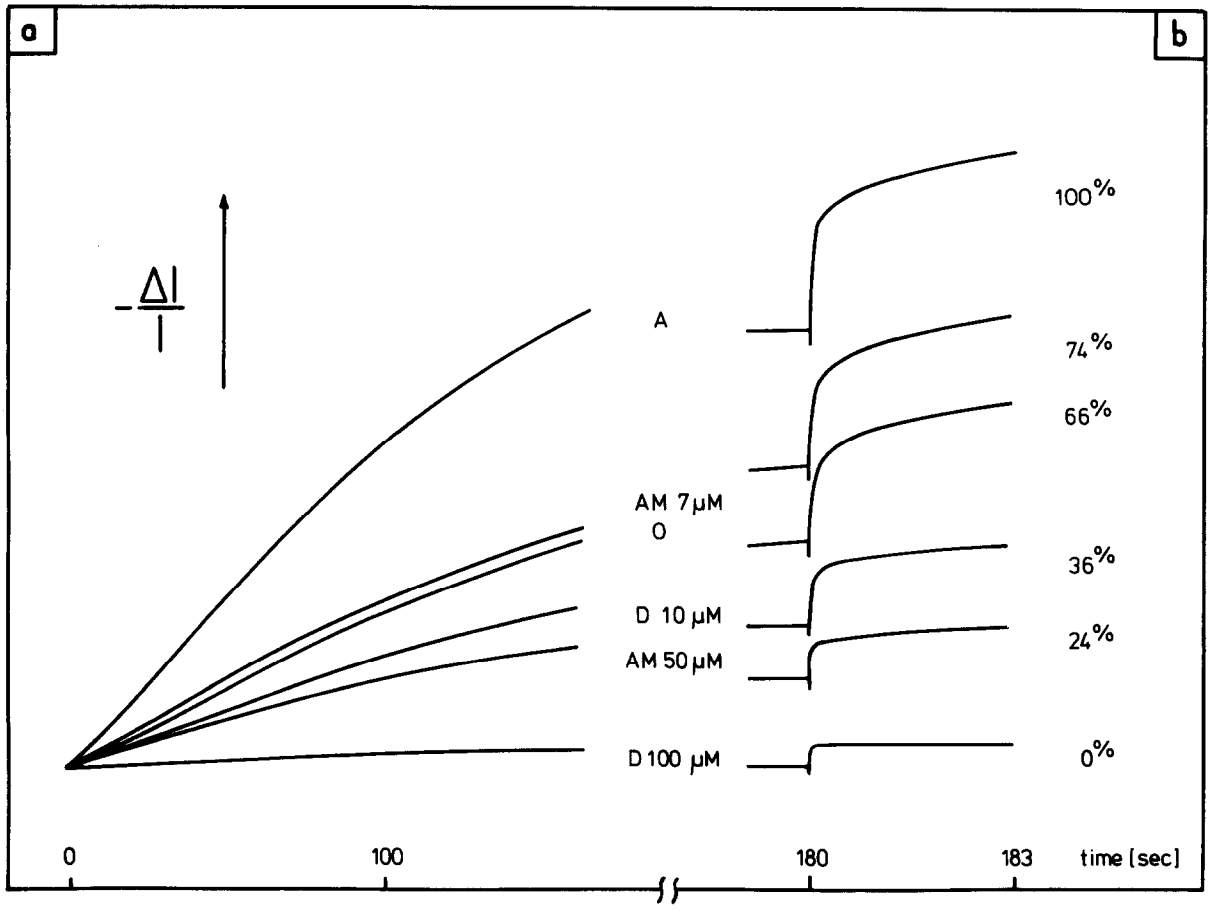

Fig.3. (a) 'A_D' and (b) 'A_L' signals at 10° scattering angle, obtained with 50 μM ATP and various inhibitors present: (A) 50 μM ATP; (AM) 50 μM ATP + AMP-PNP; (O) 50 μM ATP + Oligomycin; (D) 50 μM ATP + DCCD.

contrast to 'A_D'), require ATP hydrolysis in the presence of Mg^{2+} to precede illumination [15]. Now we report possible connections between 'A_L', 'A_D' and Mg-ATPase activity.

Figure 1b shows 'A_L' signals at 0° and 10° scattering angles. The corresponding 'A_D' signals, obtained from the same ROS suspensions just prior to 'A_L' are shown in fig.1a. It was found that both the angular and wavelength dependence of 'A_D' and 'A_L' were identical, suggesting that they both arise from a decrease in refractive index of the ROS.

At lower ATP concentrations (which were accompanied by lower amplitudes of 'A_D' after 3 min), 'A_L' was reduced accordingly as depicted in fig.2a,b. It is interesting to note, however, that in a number of instances it was observed that maximal light response was obtained after 'A_D' had reached ≤50% of its maximum amplitude.

From fig.3a,b it is obvious that not only low ATP concentrations are responsible for reduced 'A_L' amplitudes, but also higher ATP concentrations in the presence of inhibitors which block ATPase activity and hence reduce 'A_D'. Thus it strongly appears that it is the extent to which 'A_D' is completed which defines the amplitude of the subsequent light response.

The fact that this ATP- and Mg^{2+}-requiring light response not only has the action spectrum of rhodopsin bleaching, but also decreases linearly with the amount of bleached rhodopsin present in the ROS and can be regenerated with 11-*cis* retinal [15], proves that the responsible enzyme resides in the outer segment. Furthermore, the fact that the response is as fast as 20 ms at 37°C strongly suggests that the rhodopsin-containing disk, and not the plasma membrane, is the site of action.

3.4. *Connections between ATPase activity: 'A_D' and 'A_L'*

Given the evidence here it appears very likely that the oligomycin- and DCCD-sensitive Mg-ATPase presented in section 3.1 and the ATP hydrolysing systems responsible for 'A_D' and 'A_L' are identical and reside in the photoreceptor disk membrane. A plausible scheme for the action of the Mg-ATPase which enables the rapid light-triggered structural changes in the disk is proposed in fig.4.

It is surprising to note that both the enabling process 'A_D' and the enabled and light-triggered

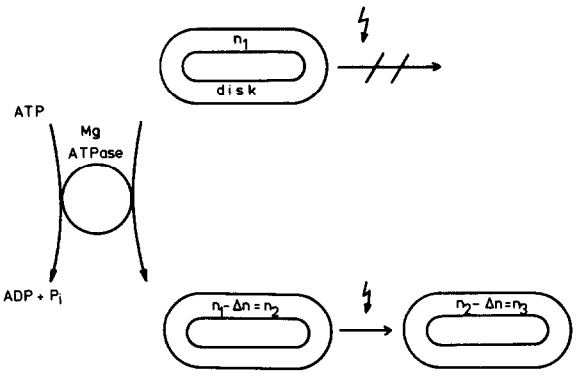

Fig.4. Proposed scheme for the action of the Mg-ATPase and light in bovine photoreceptor disk membranes.

phenomenon 'A_L' are both reflected by a decrease in light scattering. One would expect the enabling process to be reversed and not further enhanced by light. This is even more surprising considering the following observation:

Small amounts of Triton X-100 (0.001%), when added to ROS suspensions, make little pits in the disk membrane, and this is accompanied by a small decrease in light scattering. After formation of these little pits both 'A_D' and 'A_L' are completely suppressed. Moreover, when Triton is added after 'A_D' has already taken place, the light scattering increases again, i.e., 'A_D' is reversed and 'A_L' is consequently disenabled. As yet we have no explanation for this behaviour. However, it strongly suggests that the structural intactness of the disk compartment is a prerequisite for the observed structural events.

Currently conducted physico–chemical studies are directed towards determining the exact structural origin of 'A_D' and 'A_L'. Other studies, in which chemical parameters, particularly the concentrations of various inhibitors, uncouplers and ionophores are varied, are being undertaken in order to determine:

(1) Whether or not the disk Mg-ATPase, like similar enzymes in other membranes, is involved in ion translocation;
(2) Which ions may be involved.

These experiments are intended to further substantiate the functional significance of the described phenomena.

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