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Light scattering changes and protein distortion in the bacteriorhodopsin during the photocycle

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A change in light scattering was detected during the photocycle of the purple membrane. A systematic study of the pH, viscosity and photoselection dependence of scattering kinetics is given. From the experimental data it follows that purple membranes are bent, depending on the pH, and that the extent of bending changes during the photocycle is due to the functional protein. A theoretical treatment is also given. The model explains all the experimental data and, as an important consequence, calls attention to the fact that exact kinetic measurements cannot be performed without immobilizing the purple membranes.

Bacteriorhodopsin; Light scattering kinetics; Membrane shape; Protein deformation; (*Halobacterium halobium*)

1. INTRODUCTION

Bacteriorhodopsin is a light-driven proton pump contained in the purple membrane patches of the plasma membrane of *Halobacterium halobium*. There has been a number of trials to find out whether the parts of the bacteriorhodopsin molecules move or not during the proton pumping photocycle. It has been proved that the retinal, the chromophore of the protein, does not move [1-5] and no motion of the protein side chains has been found [6]. On the other hand, evidence exists for movement: by increasing the solvent viscosity with glycerol, some steps of the photocycle slow down [7] and then the proton movement can be directly demonstrated by the PERS method [8].

The results of the following light scattering kinetics measurements show that the proton pumping of the bacteriorhodopsin is accompanied by reversible mechanical deformations of the protein which are summed up and, as a result of this, is made detectable by the tight two-dimensional arrangement of the protein molecules in the purple membrane.

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2. MATERIALS AND METHODS

Purple membrane fragments were obtained according to standard procedures [9] from *H. halobium* strains JW3 and ET1001.

Light scattering kinetics were measured in a 0.5 mm flat cuvette by a home-made intelligent flash photolysis system [10]. The scattered light was collected over a wide range of the scattering angle (10°-80°) to get enough light to increase the signal-to-noise ratio. Other experimental details are described elsewhere [11].

In theoretical calculations, the Rayleigh-Debye approximation was used which was justified by the fact that the membranes were very thin (5 nm) as compared to the wavelength of the scattered light (320 nm). In this case the scattering intensity is determined by the form factor, P:

$$P(\theta) = \frac{1}{V^2} \left| \int_V e^{ihs} dV \right|^2$$

where V is the volume of the scattering particle, for the definition of the other parameters see below. P was derived for membranes of both spherical and cylindrical shape. (The spherical case is easier to handle, the cylindrical case is more realistic.)

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The corresponding form factors are

$$P(\theta) = \left| \frac{1}{l_0} \int_0^{l_0} J_0(h\varrho \sin\epsilon) e^{ihl\cos\epsilon} dl \right|^2$$

$$P(\theta) = \frac{\sin(hl\cos\psi)}{hl\cos\psi} \left| \frac{1}{2\psi_0} \int_{-\psi_0}^{\psi_0} e^{ihR\sin\psi\cos(\varphi - \Phi)} d\varphi \right|^2,$$

respectively, where θ is the scattering angle; J_0 denotes the zeroth order Bessel function; $h = 2k\sin(\theta/2)$; $k = 2\pi/\lambda$, where λ is the wavelength of the scattered light; $\varrho = \sqrt{R^2 - (R-1)^2}$; ψ is the angle between the axis of the cylinder and the line s; $\cos\Phi = \cos\epsilon/\sin\psi$, where ϵ is the angle between the central membrane normal and the line s; $2\psi_0$ and R are the angle and the radius of the membrane bending, respectively. For the remaining parameters see fig. 1.

3. RESULTS

The basic phenomenon can be seen in fig.2 where the meaning of the four characteristic kinetics is the following. All the kinetics are composed of two parts. The negative peaks with fast rise times are due to the change in reabsorption of the scattered light, while the remaining part makes the scattering change as follows from the concentration dependence of the measured scattering transients [11]. So, subtracting the ↔ and ↕ kinetics, we get a signal characteristic of only the scattering changes which do not contain the reabsorption-dependent component. (Because the flash intensity depended slightly on the polarization, a normalizing factor of near to 1 was needed during subtraction.) The resulting signal shows a strong pH dependence which is shown in fig.3.

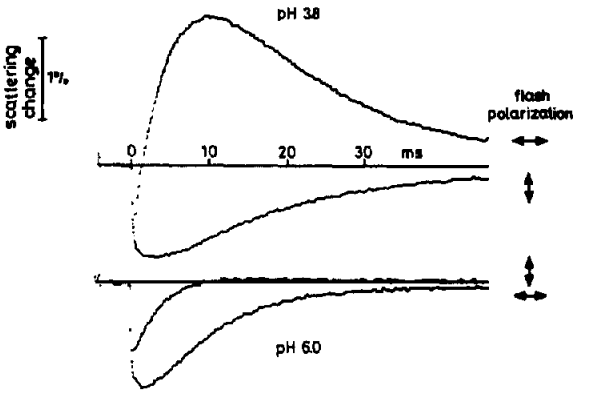

Fig.2. Change of the scattered light during the photocycle. Note the effect of pH and the flash polarization. ↔ and ↕ denote the case when the flash polarization is parallel with and perpendicular to the scattering plane, respectively. Temperature is 25°C, $\lambda = 320$ nm.

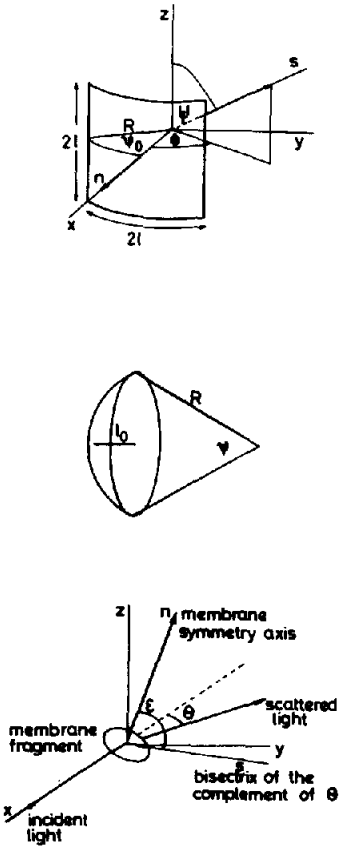

Fig.1. Geometry of the light scattering and the meaning of the parameters used in the formula of the form factors.

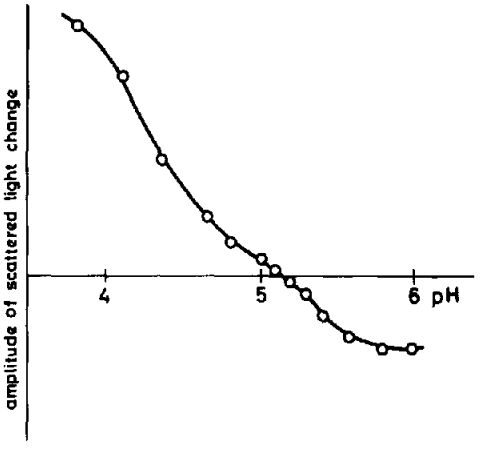

Fig.3. The pH dependence of the scattered light kinetics. The curves are corrected (see text). $T = 25$°C, $\lambda = 320$ nm.

It is worth noting that the 'true' scattering kinetics just changes its sign when the flash polarization is changed from ↔ to ↕ position.

Another important observation can be seen in figs 4 and 5: increasing the solvent viscosity by adding more and more sucrose to the buffer, the scattering kinetics becomes slower and smaller in amplitude. (In contrast to glycerol [7], sucrose practically does not affect the speed of the photocycle.) In the case of a sample, with an extremely high viscosity, which contains purple membranes embedded in polyacrylamide gel, only the reabsorption signal can be observed (fig.5).

4. DISCUSSION

The viscosity dependence and the immobilization effect immediately give the idea that the origin of the scattering kinetics outlined above is in the motion of purple membranes which change their shape in correlation with the photocycle. (Assuming the other possibility, the change in size, we would be unable to explain the opposite signs of the ↔ and ↕ cases.) As for the shape, the bending of purple membrane fragments is the only possibility for change.

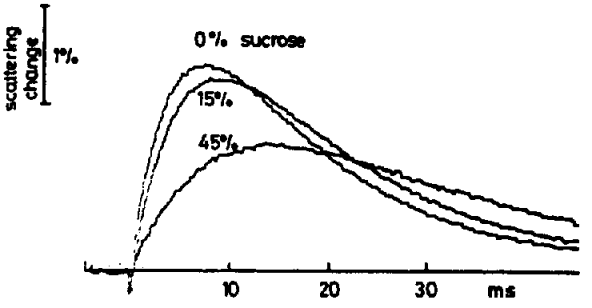

Fig.4. Viscosity dependence of the scattering kinetics. The slow down occurs only in the scattering signal, the absorbance kinetics do not change. The curves are corrected. $T=26°C$, pH = 3.9.

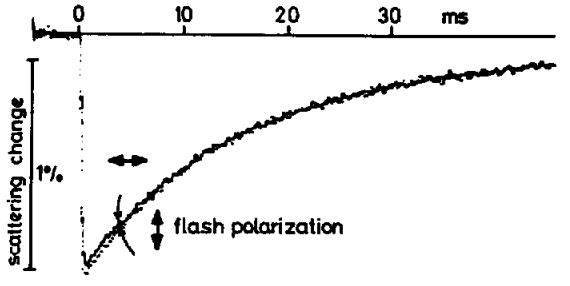

Fig.5. Light scattering kinetics in a sample embedded in polyacrylamide gel. Only the reabsorption signal remains, the scattering changes disappear. $T=28°C$, pH = 3.8.

This concept can easily explain the pH dependence of the sign of the scattering transients if we assume basic bending of the fragments. The basic bending hypothesis becomes quite natural if we realize that the surface charge distribution of the purple membranes is asymmetric [12,13] and this involves the energetically more stable bent shape of the membranes. (The outer side has more space for charges and counterions.) Moreover, it is well-known that charge distribution asymmetry depends on pH and changes its sign around pH 5 [14]. Taking into account that the direction of proton pumping does not change with pH [14–16], the resulting asymmetry easily explains the pH dependence of the scattering kinetics.

It is far more complicated to understand the flash polarization effect but the following qualitative picture will provide some help. In figs 6 and 7 the form factor curves of a single bent membrane can be seen. (In the cylindrical case an average for the rotation around the central normal of the membrane is shown.) Considering the two bundles of curves, we found the following photoselection phenomenon.

Since the retinal chromophore is near perpendicular (~70°) to the membrane normal [5,12,17], by exciting the sample using a ↔ polarized laser

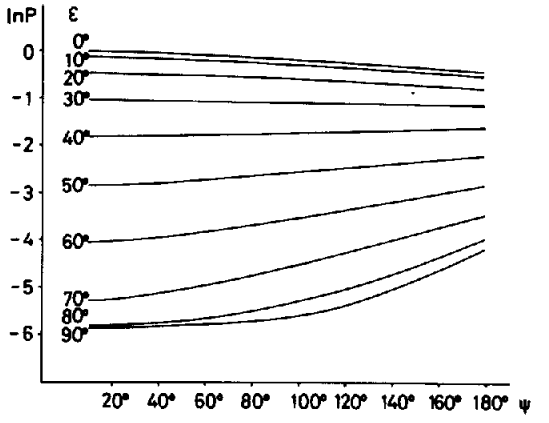

Fig.6. The light scattering of a spherically bent membrane as a function of the bending and the direction of its symmetry axis. The size is fixed, 0.25 μm^2, $\lambda = 320$ nm, $\theta = 45°$. The intensity of the scattered light is proportional to the form factor P. Note the log scale.

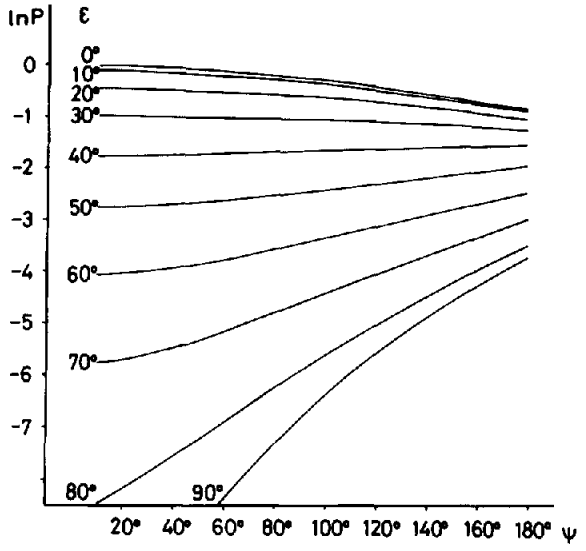

Fig.7. The form factor curves for cylindrically bent membranes (see also fig.6).

flash, the membranes with a big ϵ (near to 90°) will be excited more. Hence, the form factor increases with increasing ψ.

In the case of a flash with ↕ polarization, among the properly oriented membranes (having retinal direction near to that of the polarization of the exciting flash) every ϵ occurs. However, the scattering of the membranes with small ϵ values is much bigger than that of purple membranes with near 90° ϵ. Thus the curves with small ϵ values determine the scattering which implies that P decreases when ψ increases.

From the above arguments and the experimental data in fig.2, it follows that the curvature of purple membranes increases during the photocycle at a pH below 5 and decreases at a pH above it.

At the same time this implies that the transient pH changes cannot cause the bending change for the following reasons. Since the cytoplasmic side of the purple membranes is less negative at pH values below 5 [12,13], the basic bending is the same as that of the purple membranes in the living cells. Due to their positive charges, the protons released by the bacteriorhodopsin molecules can only decrease the negative charge on the convex side. At the same time, because of the protons picked up on the concave side, the negative charge on this side of the membrane cannot decrease. Both of these effects result in the decrease of the curvature which is

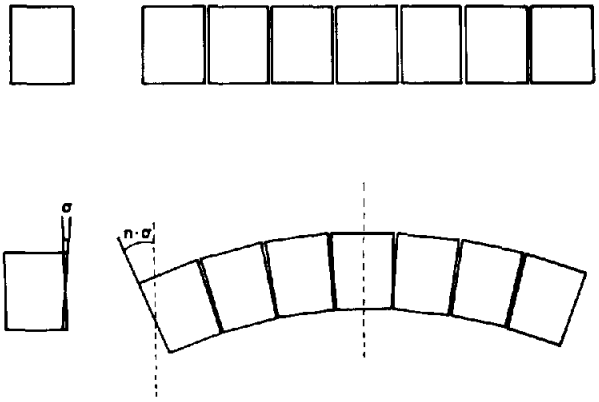

Fig.8. The amplification of the slight distortions of purple membrane building blocks in the lattice.

opposite in direction to the curvature change deduced from the scattering data. The discussion of the case where pH is above 5 also leads to a result contradicting to the experimental data.

Therefore the local pH changes due to the proton pump would result in an opposite change of the membrane bending, so one has to conclude that a tiny deformation of the excited protein is amplified into a membrane shape change as can be seen in fig.8.

As a methodical conclusion it is important to note that, depending on the particle size, the wavelength and the experimental circumstances (geometry, refraction index etc.), scattered light always distorts the absorption kinetics measurements. As the best solution to avoiding this disturbing effect, this paper suggests the immobilization of purple membranes, at least, when the kinetics to be measured are in the millisecond range.

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