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INCORPORATION INTO LIPID BILAYER MEMBRANES OF A PHOTOSENSITIVE PIGMENT FROM THE HONEYBEE COMPOUND EYE

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Summary

An increase of electrical conductance up to a factor 10^2—$5 \cdot 10^2$ was obtained by adding, in the dark, the honeybee photopigment to a positively charged lipid bilayer. The increase in conductance was made slower by illuminating the system during the incorporation of the protein into the membrane and it was negligible when the photopigment was bleached before the incorporation. The interaction of the photopigment with the membrane is tentatively interpreted in terms of formation of channels.

Incorporation of rhodopsin into bilayer lipid membranes in order to clarify the still unknown mechanisms underlying the electrical signals triggered by the illuminated rhodopsin in the visual cell membrane, has been in recent years [1—3] an exciting task. The rhodopsin incorporated into planar lipid bilayer as reported by Montal and Korenbrot [3] resulted in the formation of very unstable membranes, making difficult the measurements of the effects of illumination. The hindrance of building such rhodopsin-containing membranes seems to us to lie in the difficulty of obtaining rhodopsin free from detergents and in the number of manipulations needed to extract the water soluble rhodopsin lipoprotein into organic solvents.

We used a light sensitive pigment from the honeybee compound eye, similar to that described by Goldsmith [4], which is the only example of a rhodopsin-like protein soluble in aqueous buffer and easily extractable by preparative electrophoresis on polyacrylamide gel [5,6]. As shown by measurements on gel electrophoresis [5] and on thin-layer chromatography [6], the pigment binds retinene, releases the chromophore when illuminated in solution and it is negatively charged at pH 7.

The photopigment, added to the bath containing the planar lipid

bilayer, strongly interacted in the dark with lipids bearing positively charged groups, obtained by mixing at various percentages egg lecithin in decane (50 mg/ml) and oleylamine in decane (18 mg/ml). This interaction induced an increase of the electrical conductance of the membrane up to a factor of 10^2—$5 \cdot 10^2$ as shown in Fig. 1. With negatively charged lipids such as soybean lecithin or egg lecithin, smaller or negligible interactions were observed.

In order to examine the possibility of a mere interaction of retinene with the membrane, a control experiment was performed adding to the bath, in the dark, an amount of retinene in ethanol up to 100-times the concentration of retinene bound to protein in the experiments of Fig. 1. Even at such a high concentration no change in conductance was found.

The transport properties of the membrane change by illuminating the system with white light, as shown in Fig. 2. In the initial stage, the changes in conductance coincide, within the experimental error, with those obtained in the dark (data of Fig. 1 are plotted in broken lines for the comparison). The increase in conductance is much slower after about 15 min, which corresponds to the lapse of time necessary to have a sufficient number of bleached molecules [5].

In agreement with this behaviour a little or negligible increase in conductance is obtained if the photopigment is completely bleached in the presence of hydroxylamine, before the incorporation into the membrane

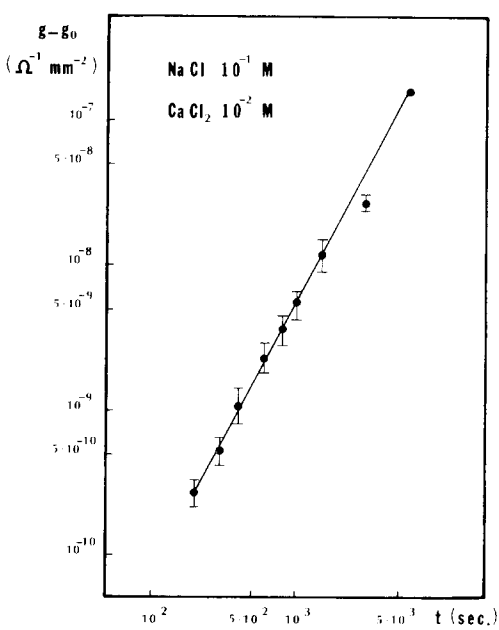

Fig. 1. The figure shows, in a log-log scale, the specific conductance variation, $g-g_0$, as a function of time, t, after the addition (at $t = 0$) of 10^{-3} mg/ml (final concentration in the bath) of the photopigment in the dark, to both sides of the membrane. The membrane was made by mixing 20% of the oleylamine and 80% of the egg lecithin solutions. Ionic solutions were NaCl 10^{-1}M + $CaCl_2$ 10^{-2}M buffered at pH 7 with Tris-HCl 10^{-2}M. Each point on the graph represents the mean value from seven experiments. All the measurements were performed in the dark or in dim red light, with an experimental apparatus described elsewhere [7].

(hydroxylamine prevents the regeneration of the photopigment by reacting with free retinene and forming the retinene oxime). As shown by the lower curve of Fig. 2 the bleached photopigment, which has the same isoelectric point, did not induce relevant changes in conductance.

No changes in conductance were observed upon adding to the bath amounts of bovine serum albumin (negatively charged at pH 7) up to 10-times the concentration of the pigment.

While these facts exclude the possibility that the observed increase in conductance has to be ascribed to an aspecific screening effect of the photopigment on the charges of the membrane [8,9], further experiments confirm the importance, at least in the first stage of incorporation, of the electrostatic interaction. In fact the increase in conductance is hindered or even nullified by either increasing the ionic strength of the solution to 1 M NaCl, or decreasing the positive charge on the membrane surface, resulting from a decrease in the percentage of oleylamine. Since, it does not seem plausible to imagine specific electrostatic interactions between photopigment and membrane, it seems more logical to suppose that, while the initial force determining the interaction is mainly electrostatic, the overall incorporation involves important hydrophobic bonds.

The same conclusion can be reached independently if one considers the kinetics of such an interaction, which both in the time scale and dependency greatly differs from that observed for the simple adsorption of a protein at an oil-water interface [10].

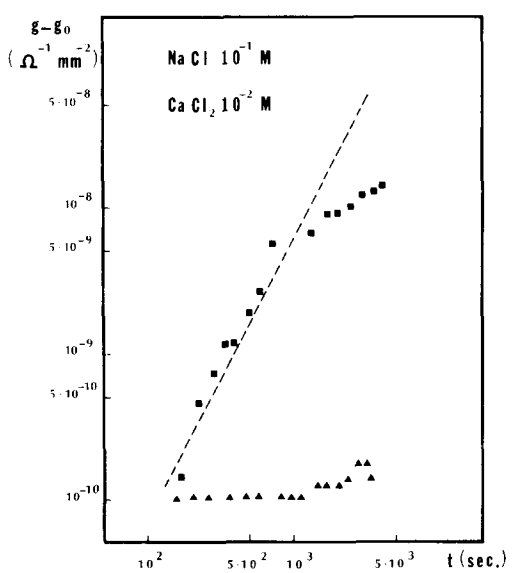

Fig. 2. The upper curve shows, in a log-log scale, the specific conductance variation, $g—g_0$, as a function of time, t, after the addition to both sides of the membrane (at $t=0$) of 10^{-3} mg/ml (final concentration in the bath) of the photopigment in a cold and diffuse white light. The lower curve refers to the addition of the same amount of photopigment bleached in the light in the presence of hydroxylamine 0.1 M, after removal of retinene oxime and hydroxylamine by dialysis. Other conditions are the same as in Fig. 1.

On the basis of these findings the most challenging question remains the role played by the retinene in the incorporation and in the transport process. In answering this question, further complication lies in the difficulty to separate, in the overall conductance changes, the contribution due to the formation of new permeation pattern, from a possible gating role played by retinene, upon illumination, in the ionophoric properties of the photopigment already incorporated. While this question remains still open, some further informations can be drawn from the analysis of the data of Fig. 1. The points of Fig. 1 can be fitted by the equation:

$$g - g_0 = kt^y \tag{1}$$

where g is the specific conductance at time t, g_0 is the specific conductance of the membrane at $t=0$ and k is a parameter which should depend on the photopigment concentration and on the composition of the lipid membrane. It is of interest to note that the t^y dependency of the conductance variation, $g-g_0$, with $1<y<2$ is a general feature observed in differently charged membranes.

In order to get some deeper insight into the meaning of Eqn. 1, we shall consider an heuristic model in which the interaction of the photopigment with the membrane results in the formation of a permeation pathway or a channel of constant conductance λ. Moreover, we shall assume that a cooperative process is involved so that the rate of channel formation, dn/dt, is increasing with time, t, according to the empirical law

$$\frac{dn}{dt} = \alpha t^{\beta} \tag{2}$$

hence

$$n(t) = n(o) + \frac{\alpha t^{\beta+1}}{\beta+1} \tag{3}$$

where $n(0) = 0$ is the number of channels at $t=0$. The total conductance variation, $G-Go$, will be given by

$$G - Go = \frac{\alpha\lambda t^{\beta+1}}{\beta+1} \tag{4}$$

which, upon dividing by the area A, is identical to Eqn. 1 where

$$k = \frac{\alpha\lambda}{(\beta+1)A} \tag{5}$$

and

$$y = \beta+1. \tag{6}$$

The best fit of data from Fig. 1 yields: $k = 1.4 \cdot 10^{-14}$ ($\Omega^{-1} \cdot s^{-y} \cdot mm^{-2}$) and

$y = 1.8$. These values of k and y allow one to evaluate, by use of relations 3, 4, 5 and 6, the number of pores formed in the membrane at a certain time t, provided the single channel conductance, λ, is known. We may try heuristically to put $\lambda = 2 \cdot 10^{-12}\ \Omega^{-1}$, which is the same value as that estimated for the Na^+ pore in frog and rat rod [11] and is of the same order of the lower limit of Na^+ channel conductance in various preparations of nerve membrane [12]. Thus, recalling that the membrane area is $A \simeq 1\ mm^2$, the number of channels formed in the membrane after 30 min, is of the order of 10^4, with a specific area of 100 μm^2/pore. Such a value of the area is many orders of magnitude greater than that estimated for the mean cross sectional area of a photopigment molecule. This consideration suggests that it is highly improbable for a single photopigment molecule to form a channel, since in this case the mean distance between molecules would be too large to account for a cooperative process. A more convincing model might be one in which the channels are formed by the statistical aggregation of different molecules. In this case the number of molecules inside the membrane would be much greater than the mean number of open pores. Further support to this model will be given by direct measurements of single channel conductance.

Although the net negative charge of the protein without chromophore does not change (as shown by electrophoresis experiments [5]), the incorporation into the membrane produces changes in conductance only in case the chromophore is not detached.

We may therefore conclude that the first electrostatic interaction cannot be considered responsible for the changes in conductance, but there might be some important hydrophobic bonds in the protein, which are masked by a conformational change following the illumination. A different explanation is that retinene might confer to the protein the ability, by associating with others, to form a channel. In both cases it will be interesting to examine the responses to illumination of the membrane when the incorporation of the photopigment has already taken place in the dark.

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References

1 Hong, K. and Hubbell, W.L. (1972) Proc. Natl. Acad. Sci. U.S.A. 69, 2617
2 Drachev, L.A., Kaulen, A.D., Ostroumov, S.A. and Skulachev, V.P. (1974) Febs Lett. 39, 43–45
3 Montal, M. and Korenbrot, J.I. (1973) Nature 246, 219–224
4 Goldsmith, T.H. (1958) Proc. Natl. Acad. Sci. U.S.A. 44, 123
5 Pepe, I.M., Perrelet, A. and Baumann, F. (1976) Vision Res. 16, 905–908
6 Pepe, I.M., Perrelet, A. and Baumann, F. (1976) in VIIth International Congress on Photobiology, Rome, Abstract No. P283
7 Ciani, S., Gambale, F., Gliozzi, A. and Rolandi R. (1975) J. Membrane Biol. 24, 1–34
8 McLaughlin, S.G.A., Szabo, G., Eisenmann, G. and Ciani S.M. (1970) Proc. Natl. Acad. Sci. U.S.A 67, 1268–1275
9 Montal, M. (1972) J. Membrane Biol. 7, 245–266
10 Davies, J.T. and Rideal, E.K. (1961) in Interfacial Phenomena, pp. 154–216, Academic Press, New York
11 Cone, R.A. (1973) in Biochemistry and Physiology of Visual Pigments (Langer, H., ed.), pp. 275–282, Springer-Verlag, Berlin
12 Armstrong, C.M. (1975) Q. Rev. Biophys. 7, 179–210