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Interaction of liposomes with black lipid membranes

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SUMMARY

Liposomes labelled with fluorescent pigments were allowed to interact with black lipid films. The transfer of label from the liposomes to the film was studied by fluorescence and photoelectric measurements. With the neutral lipid lecithin, in contrast to negatively charged phosphatidylinositol, a rapid transfer was observed. The results are discussed with respect to fusion of liposomes with black lipid films.

Cell fusion is believed to play an important role in many cellular processes such as exocytosis, synaptic transmission or membrane transformation. The molecular mechanism responsible for the fusion process has been rather obscure until now. It is not even known which component of the cell membrane is essential for fusion. We therefore decided to study the problem on the basis of simple artificial lipid membranes. The basic idea is to add labelled liposomes to the aqueous phase in contact with planar lipid films and to look for an incorporation of the label into the film.

As a label we used the hydrophobic fluorescent pigments magnesium octaethylporphyrin or chlorophyll *a* (Fluka, puriss.). The liposomes were made by sonication of egg lecithin, prepared in our laboratory, or phosphatidylinositol *ex* bovine brain (Koch-Light Laboratories Ltd). Lipids and pigment (molar ratios between 10:1 and 100:1) were dissolved in ethanol or ethanol *plus* chloroform. After evaporation of the solvent, 0.1 M KCl was added to obtain 10 mg lipid/ml solution. The emulsion was sonified for 30 min with an MSE 150 W ultrasonic disintegrator with a microprobe at maximum output. The vessel was flashed with argon and cooled to about 20 °C. The solution was centrifuged at 4 °C and 40 000 × *g* for 1 h. Pigment concentrations were measured spectrophotometrically. Lipid concentrations were determined by phosphate analysis. In a few cases

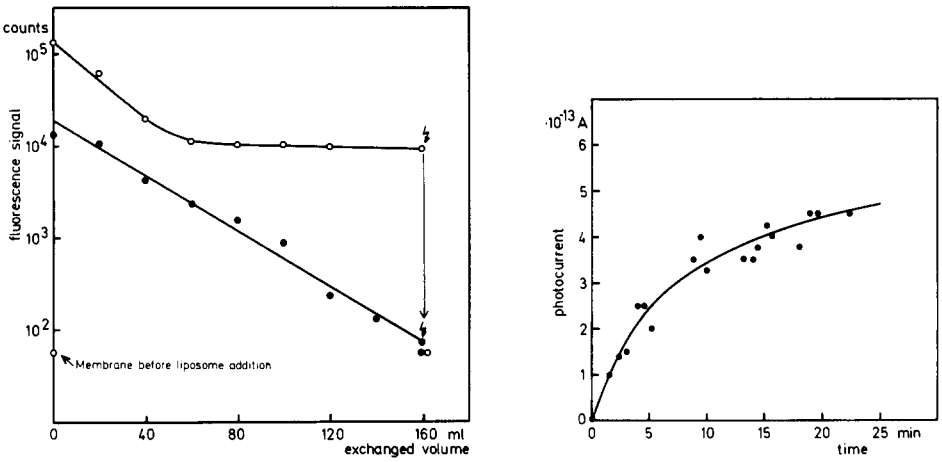

Fig. 1. Fluorescence signal as a function of exchanged volume of liposome-containing solution. Liposomes (with magnesium octaethylporphyrin) were allowed to interact for 10 min with the black lipid film under stirring. The volume of one compartment of the cuvette was 10 ml. Subsequently liposomes were removed by exchange. At the point indicated by the arrows the membrane was destroyed. o——o, lecithin liposomes against lecithin membrane; ●——●, inositol liposomes against inositol membrane.

Fig. 2. Photocurrent as a function of time at a fixed voltage of 40 mV. At t=0, egg-lecithin liposomes with magnesium octaethylporphyrin were added to one side of a dierucoyllecithin membrane to give a final concentration of 30 μg lipid/ml solution.

liposomes were formed according to the method of Batzri and Korn[1]. For the formation of the black lipid films the same kind of lipids were used as for the liposomes. Instead of egg lecithin, however, we used synthetic dierucoyllecithin (lecithin with two $C_{22:1}$ fatty acid chains, synthesized by K. Janko in our laboratory) to improve membrane stability. The concentration of the lipids was 0.5–1% (w/v) in n-decane with up to 10% (v/v) of n-butanol. Membranes were formed on a teflon support in 0.1 M KCl, pH about 6.

Two methodes were used to detect an incorporation of the label into the black film. One was a sensitive device for fluorescence measurements of black films. The principle of the set-up has been described previously[2]. The photomultiplier tube was cooled to 0 °C to reduce the dark current. The fluorescence signal was integrated for 10^3 chopper cycles with a chopper sampling time of $2 \cdot 10^{-3}$ s. The membrane was formed without liposomes in the aqueous phases. When the film was completely black, a small amount of liposomes was added to one side, under stirring up to a final concentration of 20 μg lipid/ml solution. The liposomes were allowed to interact with the film for 10 min. Afterwards, the solution containing the liposomes were exchanged against a pure 0.1 M KCl solution. This was done to allow the measurement of membrane fluorescence without interference of liposomes in the aqueous phase. Fig. 1 shows the results. The large fluorescence signal, 10 min after addition of the liposomes (exchanged volume 0 ml), is mainly due to the liposomes in the aqueous phase and only in part

derived from the black film. This may be concluded from the strong decrease in fluorescence on the dilution of the liposome-containing solution. In the lecithin system a stationary signal remains after complete exchange. It goes back to the dark signal of the detection system when the membrane is destroyed by an electrical pulse. With phosphatidylinositol no dye could be detected inside the black films as the signal reached the dark value when the exchange was complete.

Similar results were obtained with the second method. Hong and Mauzerall[3,4] found that magnesium octaethylporphyrin-containing black films show an increased conductance during illumination of the membranes. While they used ferricyanide in the aqueous phase, one of us(Trissl, H.-W., unpublished result) observed similar effects without this component. Although the mechanisms by which the charge carrier concentration in the membrane increases upon illumination is not completely understood, it seems that molecular oxygen acts as a redox component with the excited porphyrin. Despite the uncertainty with respect to the mechanism, photoconductivity can serve to detect incorporated magnesium octaethylporphyrin. The experimental set-up was similar to that used earlier[5]. The current across the membrane at a fixed voltage of 40 mV was plotted on a strip chart recorder. Within certain time intervals the membrane was illuminated with white light for 1 s. When the membrane had reached the black state liposomes were added, under stirring, to one compartment to give final concentrations between 30 and 120 μg lipid/ml solution. As shown in Fig. 2, a photoconductance appeared which increased with time. It again indicates incorporation of magnesium octaethylporphyrin into the black film for the lecithin system. In agreement with the fluorescence measurements, no photoconductance was observed with the inositol system within the experimental error. A small effect appeared with lecithin liposomes (containing magnesium octaethylporphyrin) and inositol membranes. No photoconductance could be observed without magnesium octaethylporphyrin in the liposomes.

Our results clearly show that pigment molecules are transferred from the liposomes into the black lipid membrane. The possibilities that the observed effects are only due to the special pigment magnesium octaethylporphyrin or due to the kind of liposome preparation are made unlikely by qualitatively similar results with chlorophyll *a* (only fluorescence measurements) instead of magnesium octaethylporphyrin and another liposome preparation according to the method of Batzri and Korn[1]. The absence of fluorescence and photoelectric effects with the inositol system may be easily understood considering the electrostatic repulsion between negatively charged liposomes and a negatively charged black membrane. Control experiments exclude a trivial exchange of pigment through the aqueous phase. Form the experiments of Hong and Mauzerall[3,4], a transfer of magnesium octaethylporphyrin from the membrane into water seems improbable. In addition, the following experiments completely exclude this possibility. First, magnesium octaethylporphyrin without lipids was sonified and treated according to the above-mentioned procedure of liposome preparation. Neither fluorescence of the aqueous phase nor photoelectric effects could be observed. Second, liposomes containing magnesium octaethylporphyrin were separated from their aqueous solution by ultrafil-

tration (pore size of the filter, 50–100 Å). The filtrate showed no fluorescence. Two other possibilities of pigment transfer remain. One is that pigment molecules are exchanged when liposomes and black membrane come into close contact and the second is that complete liposomes are incorporated into the black membrane by fusion. The second possibility would certainly be of more biological significance. Both possibilities, however, would show a new way of transferring hydrophobic molecules (such as pigments or even proteins) from artificial or natural membrane vesicles into black lipid membranes. At the moment we cannot decide definitively which of the two transfer mechanisms occurs. The size of the effect and the short time it needs to appear seem to make a fusion of complete vesicles with black membrane more likely. A preliminary quantitative treatment of the observed effects supports this suggestion. Experiments are under way which should give a clear-cut answer to this question. Fusion of lipid vesicles with one another and with biological membranes were reported recently[6].

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