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Fluorescence of thin chlorophyll membranes in aqueous phase

The primary processes of photosynthesis are still unknown, but there are some hints that the first chemical reaction after the absorption of the light quantum is a redox process in which the excited chlorophyll molecule exchanges an electron with its environnement. With respect to this hypothesis model studies in which photosynthetic pigments are incorporated into artificial lipid bilayer membranes are of great value[1–3]. For the interpretation of these experiments, especially for the calculation of the quantum efficiency of the observed photoelectric effects, the composition of the membrane must be known. This is not a trivial problem, because the bilayer membrane must be considered as a distinct phase which is in thermodynamic equilibrium with the solution from which the membrane has been formed. Therefore, the chlorophyll to lipid ratio in the membrane and in the film-forming solution may be quite different. In this communication we describe a sensitive fluorescence method by which the chlorophyll concentration in the bilayer membrane can be determined.

Membranes were formed in the usual way[4] on a Teflon frame with a circular hole of 8 mm in diameter. A solution of 0.5 % (w/v) synthetic dioleoyl lecithin in *n*-decane with various proportions of chlorophyll *a* (molar ratios chlorophyll to lecithin between 1:100 and 2:1) was used. The aqueous phase was a 0.1 M KCl solution (pH approx. 6). Membranes containing chlorophyll *a* were considerably stabler than membranes formed from a pure lecithin solution.

The experimental arrangement for the fluorescence measurements is shown schematically in Fig. 1. The central part of the membrane was illuminated with blue light of about 500 ergs/cm^2 per sec (Osram HBO 200-W high-pressure mercury source combined with Schott BG 12 filter). The light beam had a diameter of 2 mm (compared with a membrane diameter of about 8 mm). The red fluorescence of the film was detected with a photomultiplier (Philips 56TVP with S20 cathode) arranged at a right angle to the light beam. A red filter (Schott GG10 + RG 630) served to protect the phototube from stray blue light. Special care was taken to prevent the red emission of the thick membrane border which is excited by stray light to reach the phototube.

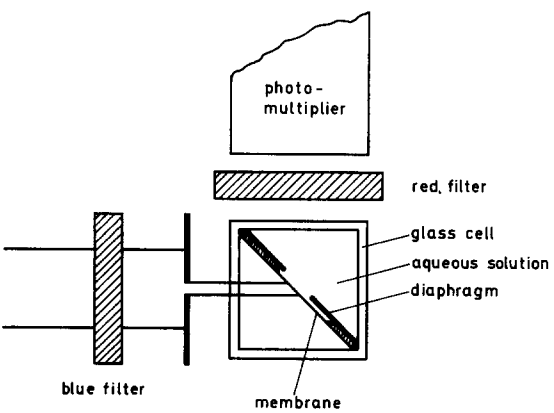


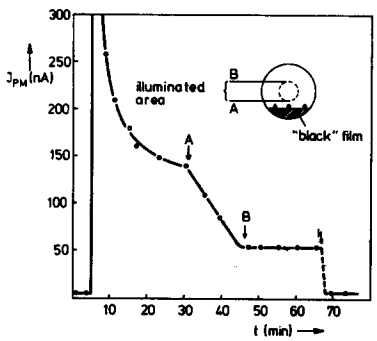
Fig. 1. Experimental arrangement for fluorescence studies with thin lipid membranes.

This was achieved by a diaphragm with a hole of 4 mm in diameter placed behind the membrane at a distance of about 0.5 mm.

In order to obtain the thickness of the black film, the film capacitance was determined with an a.c. bridge at 2 kC/sec. This gave a value of 0.41 ± 0.04 μF/cm^2 which was independent of the chlorophyll to lecithin ratio within the limits of error. With a dielectric constant of 2.1 (ref. 4) the film thickness is calculated to be about 50 Å.

The photomultiplier signal as a function of time is shown in Fig. 2. Prior to the formation of the lamella, only the dark current of the phototube (approx. 6 nA at 2.1 kV) is observed. As long as the lamella shows interference fringes, corresponding to a thickness of the order of 1 μ, the signal is very high. The gradual thinning of the film which may be followed visually is accompanied by a steep decrease of the photocurrent. The expansion of the black film always starts at the lower rim of the hole. When the black film reaches the illuminated area, an inflection point in the signal–time curve is observed (point A). As soon as the whole illuminated area is in the "black" state (point B), the photomultiplier signal remains constant. When the film is destroyed by applying a small d.c. voltage between the external solutions, the signal falls to nearly the dark current.

In order to obtain the number of chlorophyll molecules per cm^2 of the black film, the method was calibrated in the following way. After each experiment the membrane was replaced by a 0.25-mm-thick layer of a dilute chlorophyll *a* solution in ethanol of known concentration. An optical microcell was used for this purpose. In this case the measured photomultiplier signal could be correlated to the fluorescence of a known number of chlorophyll molecules. As the fluorescence quantum yield, Φ, of hydrated chlorophyll *a* is practically independent of the polarity of the solvent[5], we may assume that Φ is the same whether the chlorophyll molecule is dissolved in



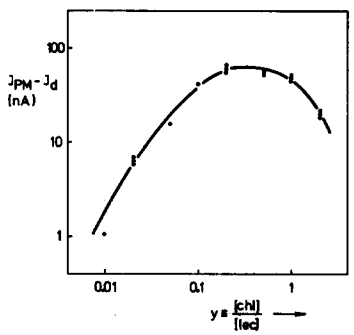


Fig. 2. Photomultiplier signal as a function of time. The lamella is formed at $t = 5$ min from a solution of chlorophyll *a* and dioleoyl lecithin (molar ratio 1:5) in *n*-decane. At point A the "black" film reaches the illuminated area, at point B the whole illuminated area is in the "black" state. The film is destroyed by an electric impulse at the end of the experiment. In order to minimize photochemical reactions of the chlorophyll, the film is illuminated only during short periods needed to record the signal.

Fig. 3. Fluorescence of the black film at different compositions of the solution used for film formation. The ordinate is the difference between the photomultiplier current J_{PM} and the dark current J_d. The single points represent different membranes (values corrected for small variations in the intensity of the exciting light). A photomultiplier signal of 10 nA corresponds to $1 \cdot 10^{12}$ chlorophyll molecules per cm^2 in the absence of concentration quenching.

ethanol or incorporated into the lipid membrane. This assumption, however, is justified only as long as the distance between neighbouring chlorophyll molecules in the membrane is sufficiently large; at higher packing densities concentration quenching of the fluorescence is expected to occur[6–8].

In Fig. 3 the fluorescence intensity of the black film is plotted as a function of the molar ratio, y, of chlorophyll to lecithin in the bulk solution. The fluorescence intensity increases with y at low chlorophyll concentration and goes through a maximum at $y \simeq 0.3$. The decrease of the fluorescence at $y > 0.3$ is presumably due to concentration quenching. This conclusion is supported by recent measurements of the optical absorption of black chlorophyll films[9]. If concentration quenching is assumed to be absent for $y \leqslant 0.1$, then the calibration method described above leads to the following values for the number, ν, of chlorophyll molecules per cm^2 of the membrane: $6.5 \cdot 10^{11}$ ($y = 0.02$), $1.5 \cdot 10^{12}$ ($y = 0.05$), $4 \cdot 10^{12}$ ($y = 0.1$). The same order of magnitude of ν is found by optical absorption measurements.

The geometrical arrangement of the chlorophyll molecules in the film is not known with certainty. One may tentatively assume that the strongly hydrophobic phytol chain is inserted between the hydrocarbon chains of the lecithin molecules, whereas the plane of the slightly hydrophilic porphyrin ring lies in the polar surface of the membrane. If this picture is correct, the mean distance between the porphyrin rings in the membrane surface is calculated to be 71 Å at $y = 0.1$. It is interesting to note that the critical distance, R_0, at which intermolecular energy transfer and emission have the same probability, is equal to 80 Å for chlorophyll *a* in solution[10]. This is in agreement with the observation that fluorescence quenching becomes noticeable in the bilayer membrane at chlorophyll to lecithin ratios greater than 0.1.

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