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# Energy transfer in black lipid membranes

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SUMMARY

This paper reports on energy transfer between three pairs of pigments which were incorporated into black lipid membranes:

1. Chlorophyll *b* and chlorophyll *a*.

2. *p*-bis[2-(4-Methyl-5-phenyloxazolyl)] benzene (dimethyl-POPOP) and chlorophyll *a*.

3. 1,6-Diphenyl-1,3,5-hexatriene (DPH) and chlorophyll *b*.

From polarization measurements information on the orientation of the pigments was obtained.

Energy transfer processes between pigments of the thylakoid membrane play an important role in photosynthesis. This energy transfer depends on the pigment orientation in the membrane. To understand energy transfer and pigment orientation in biological membranes – the structure of which is still controversial – studies with well-defined model membranes seem necessary.

Alamuti and Läuger[1] reported on the fluorescence of artificial black lipid membranes. Their work and that of Steinemann *et al.*[2] suggested energy transfer between molecules of one species (chlorophyll *a*). The transfer of excitation energy between unlike molecules has been studied in micelles[3] and multilayer systems built up on a glass surface[4,5]. Black lipid membranes are more closely related to biological membranes than those model systems. They also have a well-defined geometry. Therefore, energy transfer has been studied with black lipid membranes.

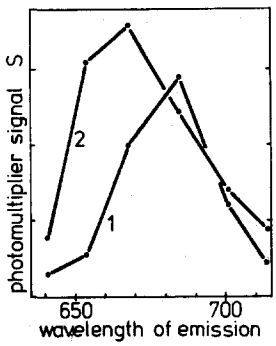
Abbreviations: dimethyl-POPOP, *p*-bis[2-(4-methyl-5-phenyloxazolyl)] benzene; DPH, 1,6-diphenyl-1,3,5-hexatriene.

The principle of the fluorescence measurements was the same as used earlier[1]. All lenses and cells were quartz instead of glass. Balzers B-40 narrow band interference filters (half-width about 15 nm) were used to select the excitation wavelength from a 250 W, 24 V halogen wolfram lamp. The emitted light was filtered with a combination of glass filters from Schott and Gen. Mainz and Balzers B-20 interference filters (half-width below 10 nm). The sensititity was increased by a chopped excitation beam and the photon counting method[6]. The detection system was an SSR 1551 B photomultiplier housing (Centronic Q 4283 R tube operated at 1500 V) and an SSR photon counting system (1120-1 amplifier/discriminator and 1110 digital synchronous computer). The excitation beam was chopped with a PAR variable speed chopper Model 222. The speed was controlled with a R-C oscillator (General Radio Comp. Type NO 1210-C) which also triggered the 1110 DSC chop mode. Signals from the light and dark periods were integrated separately, for 20 s (for small signals 200 s) and the difference formed. The measurements were made at 22 ± 2 °C. Membranes were formed on a black Teflon support, which was placed diagonally in a quartz cell and formed an angle of 45° with the excitation beam. The area of the membranes was 15 mm × 5 mm, the central illuminated spot had a diameter of about 1.5 mm. Emission was observed at an angle of 90° to the exciting beam. An alternative method for fluorescence measurements with black films has been described by Yguerabide and Stryer[7] who used spherical membranes of 1–4 mm diameter kept floating in a density gradient[8].

The membrane-forming solution contained 5 mg/ml synthetic dierucoyllecithin (lecithin with two $C_{22:1}$ fatty acid chains synthesized by K. Janko in our laboratory[9]) in *n*-decane (olefin-free, from Fluka). This lecithin was used because it forms extremely stable black lipid membranes even when the membrane area is large. Chlorophylls *a* and *b* (both puriss. Fluka), *p*-bis[2-(4-methyl-5-phenyloxazolyl)] benzene (dimethyl-POPOP) (p.a. Merck) and 1,6-diphenyl-1,3,5-hexatriene (DPH) (Schuchardt) were used without further purification. The aqueous phase was 0.1 M KCl, approx. 6.0. (In several experiments small amounts of *n*-butanol were added to the aqueous phase in order to facilitate the formation of the lamella.) The membranes turned black within about 30 to 90 min and were stable for many hours.

Concentrations of the pigments in the film-forming solutions were determined by measuring the absorption spectra with a Beckmann DB-G spectrophotometer. Fluorescence and excitation spectra of all solutions were measured both in the fluorimeter described above and for comparison in a Perkin–Elmer MPF-3 fluorimeter.

The results of fluorescence measurements with membranes containing chlorophyll *a* or *b* are shown in Figs 1 and 2. Fig. 1 shows the fluorescence spectra with excitation at the maximum of the Soret band. The maxima of both fluorescence spectra are red-shifted compared to a solution in *n*-decane (*plus* 4% v/v *n*-butanol) by about 15 nm. Figs 2A and 2B show the excitation spectra with emission measured at 654 and 685 nm, respectively. Due to the overlap of the fluorescence spectra of chlorophylls *a* and *b* it was not possible to find two wavelengths where only one of the two substances fluoresces. Fig. 3 shows the excitation spectra of membranes containing chlorophyll *a* and



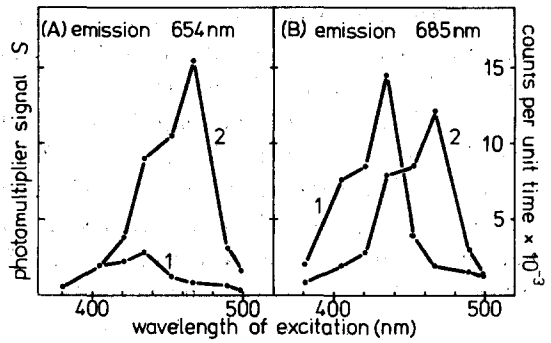




Fig. 1. Fluorescence spectra of black lipid membranes containing either chlorophyll *a* (Curve 1) or *b* (Curve 2). Concentration of the film-forming solutions: (1) chlorophyll *a*, 0.5 mg/ml; (2) chlorophyll *b*, 1.0 mg/ml. Both with 5 mg/ml dierucoyllecithin in *n*-decane. The excitation wavelength was 435 nm for Curve 1 and 467 nm for Curve 2.

Fig. 2. Excitation spectra of black lipid membranes containing either chlorophyll *a* (1) or chlorophyll *b* (2). Concentrations as indicated in Fig. 1. (A) Emission measured at 654 nm. (B) Emission measured at 685 nm.

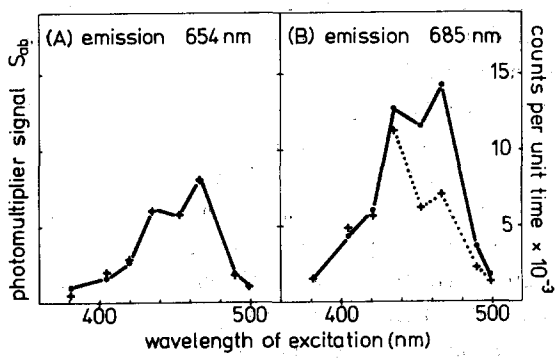



Fig. 3. Excitation spectra of black lipid membranes containing chlorophyll *a* and *b* together (measured within 20 min after the membrane was completely black). Concentrations: chlorophyll *a*, 0.5 mg/ml; chlorophyll *b*, 1.0 mg/ml; dierucoyllecithin, 5 mg/ml in *n*-decane. (A) Emission measured at 654 nm. (B) Emission measured at 685 nm. •, measured with two pigments in the membrane. +, calculated from measurements of Fig. 2 with $S_{ab} = 0.5\,(S_a + S_b)$.

*b* together. For the following calculation the assumption was made that both chlorophylls are incorporated into the membrane independent from each other and in the same proportion as present in the film-forming solution. This assumption seems justified by the following experimental results. The fluorescence of chlorophyll *b*-containing membranes depended on the concentration of chlorophyll *b* in the bulk phase in the same way as found for chlorophyll *a* (ref. 1). First the increase was fairly linear but reached a constant value for bulk phase concentrations of chlorophyll *b* between $1.0 \cdot 10^{-3}$ and $1.5 \cdot 10^{-3}$ M. Comparing the fluorescence of the membrane with the fluorescence of a 0.001-cm-thick layer of a dilute chlorophyll *b* solution of known concentration showed again (as with

chlorophyll $a$[1,2]) that chlorophyll $b$ is concentrated in the membrane with respect to the bulk phase by a factor of about ten. The fact that chlorophyll $b$ behaves so similar to chlorophyll $a$ shows that the assumption made above is very reasonable, as one would expect from the chemical similarity of the two pigments.

$S_a$ and $S_b$ are the photomultiplier signals obtained from a chlorophyll $a$ or chlorophyll $b$ membrane, respectively, and $S_{ab}$ is the signal from a membrane containing both pigments. $S_a$ and $S_b$ stayed quite constant for 2 h after the membrane was completely black. $S_{ab}$ decreased by about 20% during this time. The maximal values of $S_a$, $S_b$ and $S_{ab}$ were reproducible within ± 10% with different membranes.

If the excited chlorophyll $a$ and chlorophyll $b$ molecules would fluoresce independently from each other, $S_{ab}$ should be the sum of $S_a$ and $S_b$, because the concentration of $a$ and $b$ in the two-pigment film was the same as in the one-pigment films (see concentrations indicated in Figs 2 and 3). It was found that $S_{ab}$ is smaller than the sum of $S_a$ and $S_b$. This can be formulated by $S_{ab} = f_a \cdot S_a + f_b \cdot S_b$ where $f_a$ and $f_b$ are factors which indicate that $S_a$ and $S_b$ can be changed in the mixed film by quenching and energy transfer. At the emission wavelength 654 nm (Fig. 3A) the best fit for $S_{ab}$ was obtained with $f_a \cong f_b = 0.5$. The points calculated with this factor from Fig. 2 agree close to the measured $S_{ab}$. The factor $f_a$ could also be between 0.5 and 1.0 and still give a reasonable fit between measured and calculated $S_{ab}$. It was found that the form of the excitation spectra of chlorophyll $a$ and chlorophyll $b$, respectively, are the same when measured at 654 and 685 nm (see Fig. 2). Therefore, in the absence of energy transfer $S_{ab} = f_a \cdot S_a + f_b \cdot S_b$ should also hold at 685 nm with the same values of $f_a$ and $f_b$ found at 654 nm. With $f_b = 0.5$ and $f_a$ between 0.5 and 1.0 the calculated $S_{ab}$ is always below that measured between 453 and 490 nm excitation (see Fig. 3B). This indicates that efficient energy transfer is possible in the membrane from chlorophyll $b$ to chlorophyll $a$. This energy transfer is the reason for the strong quenching of chlorophyll $b$ fluorescence at 654 nm by chlorophyll $a$. From the concentration dependance of fluorescence observed with membranes containing only chlorophyll $b$ (mentioned above) the conclusion is drawn that self-quenching of chlorophyll $b$ is less efficient. The fact that $S_{ab}$ still decreased when the black membrane was observed for longer time may be explained by a decreasing decane content of the membrane leading to a closer proximity of pigment molecules and more efficient quenching and energy transfer. This is supported by the well-known result that the capacitance of black lipid membranes increases with time, which indicates decreasing membrane thickness (I found for dierucoyllecithin–decane membranes an increase from 0.32 to 0.36 $\mu F/cm^2$ within the first 2 h).

In further experiments the concentration dependance of the energy transfer was studied. In a macroscopic phase of the film-forming solution energy transfer was efficient above $2 \cdot 10^{-3}$ M and was absent at pigment concentrations below $5 \cdot 10^{-4}$ M. At the lower concentration the mean distance between two molecules in a cubic lattice would be about 150 Å. Membranes were formed from solutions with a total chlorophyll concentration (molar ratio chlorophyll $b$ : chlorophyll $a$ = 2:1) of $1.3 \cdot 10^{-4}$, $0.8 \cdot 10^{-4}$ and $0.3 \cdot 10^{-4}$ M. According to the results of Steinemann *et al.*[2], the mean distance between two chlorophyll

*a* molecules in the membrane at these bulk concentrations would be about 80, 110 and 125 Å. It is assumed that chlorophylls *a* and *b* are present in the membrane in the same molar ratio as in the bulk phase, which seems justified by the similarity of the pigments as discussed above. The mean distance between a chlorophyll *b* molecule and its nearest chlorophyll *a* neighbour would then be the same as the chlorophyll *a* – chlorophyll *a* distance just calculated. Whereas at the highest concentration energy transfer in the membrane was still efficient, it was greatly reduced at $0.8 \cdot 10^{-4}$ M chlorophyll bulk concentration (that means $S_{ab}$ was similar to the calculated, dashed curve of Fig. 3B). At the lowest concentration the exact shape of the excitation spectrum could not be measured due to the low signal-to-noise ratio. These results show that energy transfer is found when the mean distance of the chlorophyll *b* to *a* molecules is below 100 Å. In general, the results agree with the findings of Watson and Livingston[12] on solutions of both chlorophylls, who investigated different possibilities of self-quenching, quenching and energy transfer.

Two other pairs of pigments were studied in black lipid membranes: dimethyl-POPOP and chlorophyll *a*; diphenylhexatriene and chlorophyll *b*. Both dimethyl-POPOP and DPH showed a fluorescence maximum at 418 nm. With both of the pigment pairs mentioned it was found that the fluorescence at 418 nm decreased to about one third of the value found in the absence of the chlorophylls. At the same time the fluorescence at 685 nm of membranes containing both dimethyl-POPOP and chlorophyll *a* and the fluorescence at 654 nm of membranes containing both DPH and chlorophyll *b* increased by a factor of about two compared to those containing either chlorophyll *a* or *b* alone, when the fluorescence was excited at the absorption maximum of dimethyl-POPOP or DPH. Although these effects were large on a relative scale, their absolute size was small. The reason is that the chlorophylls themselves absorb quite strongly between 340 and 380 nm and their concentration must be kept so low that their own contribution to the fluorescence is about the same size as that from the other pigments. (The concentrations of the film-forming solutions were: (1) chlorophyll *a* 0.05 mg/ml, dimethyl-POPOP 0.1 mg/ml, dierucoyllecithin 5 mg/ml in *n*-decane; (2) chlorophyll *b* 0.25 mg/ml, DPH 0.6 mg/ml, dierucoyllecithin 5 mg/ml in *n*-decane.)

When the fluorescence of the membranes was excited with polarized light, the observed signal was dependant on the relative orientation of the polarization plane. In the geometry described earlier, horizontally polarized light (wavelength 380 nm) gave a four times stronger emission signal (at 418 nm) with both dimethyl-POPOP and DPH membranes, than vertically polarized light. Yguerabide *et al.*[7] have analyzed the orientation of dimethyl-POPOP in membranes from oxidized cholesterol by studying the polarization of fluorescence. They found that the transition moment of this pigment has a preferential orientation in the membrane. It is nearly perpendicular to the plane of the membrane. My result with dimethyl-POPOP is consistent with that more detailed analysis. It also indicates that DPH may have a similarly oriented transition moment when incorporated in black lipid membranes. The orientation of the porphyrin rings of chlorophyll *a* and *b* was determined independantly by Steinemann *et al.*[10] and Cherry *et al.*[11]. The transition

moments form an angle of about 30° with the plane of the membrane.

The orientation of the transition moments of all three pigment pairs investigated in this study are such that optimal energy transfer cannot be expected. Greater effects will be observed with pigments having such an orientation in the membrane that their transition moments are parallel to each other.

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REFERENCES

1 N. Alamuti and P. Läuger, *Biochim. Biophys. Acta*, 211 (1970) 362.
2 A. Steinemann, N. Alamuti, W. Brodmann, O. Marschall and P. Läuger, *J. Membrane Biol.*, 4 (1971) 284.
3 F.W.J. Teale, *Nature*, 181 (1958) 415.
4 D. Möbius, *Z. Naturforsch.*, 24a (1969) 251.
5 V.A. Sineshchekov, F.F. Litvin and M. Das, *Photochem. Photobiol.*, 15 (1972) 187.
6 F.T. Arecchi, E. Gatti and A. Sona, *Rev. Sci. Instr.*, 37 (1966) 942.
7 J. Yguerabide and L. Stryer, *Proc. Natl. Acad. Sci. U.S.*, 68 (1971) 1217.
8 R. Pagano and T.E. Thompson, *Biochim. Biophys. Acta*, 144 (1967) 666.
9 G. Stark, R. Benz, G.W. Pohl and K. Janko, *Biochim. Biophys. Acta*, 266 (1972) 603.
10 A. Steinemann, G. Stark and P. Läuger, *J. Membrane Biol.*, (1972) in the press.
11 R.J. Cherry, Kwan Hsu and D. Chapman, *Biochim. Biophys. Acta*, 267 (1972) 512.
12 W.F. Watson and R. Livingston, *J. Chem. Phys.*, 18 (1950) 802.