ENERGY TRANSFER TO CYTOCHROME *c* IN AN ARTIFICIAL LAMELLAR SYSTEM

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1. Introduction

During the last few years it was shown that it is possible to build up simple organized lamellar systems containing Cd arachidate and different dye molecules by means of a monolayer assembling technique [1]. This paper deals with similar systems containing Cd arachidate and cytochrome *c*. The cytochrome *c* layers are produced by adsorption of dissolved cytochrome *c* on an arachidic acid film at the air/water interface [2]. The lipid protein film can be transferred onto a solid support. By these means the cytochrome *c* layer is embedded between Cd arachidate layers in a multilayer assembly. In this system the cytochrome *c* molecules are partially oriented as indicated by absorbance measurements with polarized light. By embedding a cytochrome *c* layer at different distances from a fluorescent dye layer the trabsfer to excitation energy to cytochrome *c* is investigated. The range of the energy transfer corresponds to a Perrin Förster mechanism.

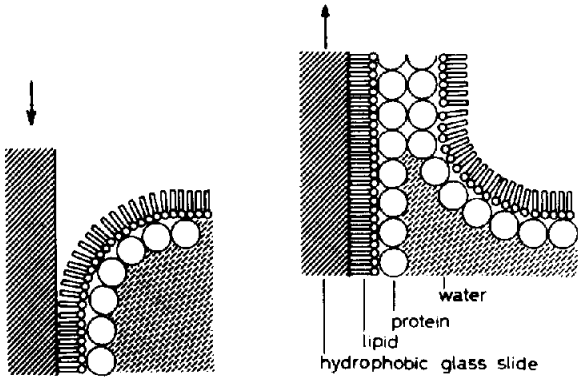

Fig. 1. By dipping a hydrophobic glass slide through a lipid protein film at the air/water interface the film is transferred onto the solid support (*left hand side*). By pulling the slide out through the same film a double protein layer is produced (*right hand side*). By replacing the lipid protein film at the air/water interface by a new lipid film before pulling out the slide, one protein layer can be embedded between two lipid layers.

2. Cytochrome *c* in an artificial lamellar system

In order to obtain a cytochrome *c* layer the protein (oxidized horse heart cytochrome *c*, Boehringer Mannheim) is adsorbed from the solution onto the hydrophilic side of an arachidic acid monolayer at the air/water interface (surface pressure of the monolayer: 35–40 dyn/cm; subphase: barbiturate buffer pH 7.8, ionic strength ca. 10^{-3} M; protein concentration: 0.24 g/l; time of adsorption 15 min). The lipid protein film is separated from the underlying protein solution by shifting it over a protein-free subphase in a multicompartment trough [2]. By dipping in and out a hydrophobic glass slide the lipid protein film is transferred onto the support (fig. 1).

By means of a special photometer [3] the absorption spectrum of one deposited cytochrome *c* layer can be measured. The absorbance of a dye layer in the proximity of an air/dielectricum interface depends on the phase of the light reflecting at the interface and interacting with the dye [4]. The measured absorbance *A* is a function of the distance of the dye layer from

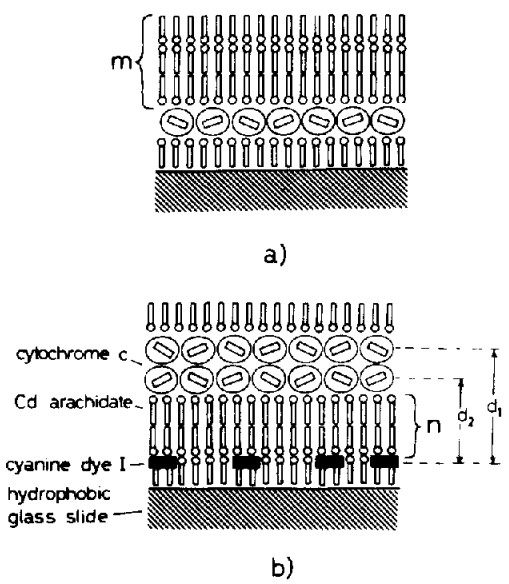

Fig. 2. Multilayer assemblies used for a) measuring the absorbance of a cytochrome *c* layer; b) investigating energy transfer from a cyanine dye to cytochrome *c*.

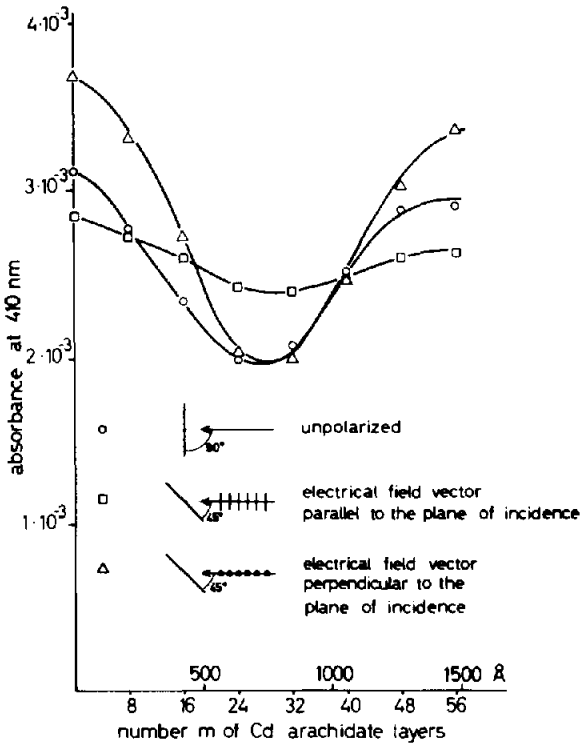

Fig. 3. Absorbance of a cytochrome *c* layer at 410 nm as a function of the number *m* of Cd arachidate monolayers deposited upon it (fig. 2a). Different polarizations and angles of incidence of the measuring light are applied as indicated in the figure.

the interface with a highest value A_{max} and a lowest value A_{min}. The absorbance in a dielectric continuum is calculated from these two values [5, 6]:

$$\bar{A} = 1/2\,(A_{max} + A_{min})$$

To determine the absorbance of a cytochrome *c* layer embedded in Cd arachidate this layer is covered by Cd arachidate films (fig. 2a). Fig. 3 shows the measured absorbance as a function of the number *m* of superimposed Cd arachidate layers. Different polarisations and angles of incidence of the measuring light are applied as indicated in the figure. Taking the values of A_{max} and A_{min} from fig. 3 the following values of $\bar{A}$ are obtained:

$\bar{A}_1$ (unpolarized) $= 2.6 \times 10^{-3}$
$\bar{A}_2$ (el. field parallel to the plane of incidence) $= 2.7 \times 10^{-3}$
$\bar{A}_3$ (el. field perpendicular to the plane of incidence) $= 2.9 \times 10^{-3}$

The ratio $R = \bar{A}_3/\bar{A}_2$ depends on the orientation of the chromophore considered. By comparing the experimental value of R with values calculated for different orientations of the porphyrin chromophore [5, 7], information is obtained on the orientation of the cytochrome *c* molecules embedded in the multilayer system. Taking into account that cytochrome *c* has approximately the shape of an ellipsoid, (main axes 35 Å, 25 Å, 25 Å) the porphyrin ring being embedded in a cleft nearly parallel to the long axis [8], and using the refractive index of Cd arachidate 1,52 [5, 6] the calculations provide the following values of the ratio R:

$R = 1$ in the case of a statistical orientation of the cytochrome *c* molecules;
$R = 1.27$ in the case of an orientation with all porphyrin rings parallel to the layer plane;
$R = 1.08$ assuming all cytochrome *c* molecules to be adsorbed with their long axes parallel to the layer plane and their short axes statistically oriented.

The experimental value $\bar{A}_3/\bar{A}_2 = 1.08$ agrees with the last mentioned partially ordered arrangement.

If the cytochrome *c* molecules are assumed to be

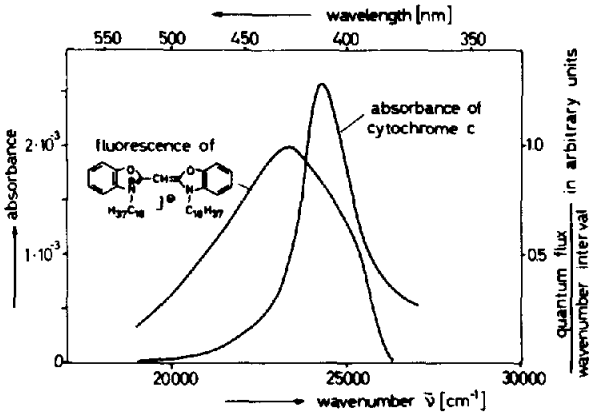

Fig. 4. Quantum spectrum of the fluorescence of a monolayer consisting of cyanine dye I and Cd arachidate in the molar ratio 1:20 and absorption spectrum of a cytochrome *c* layer.

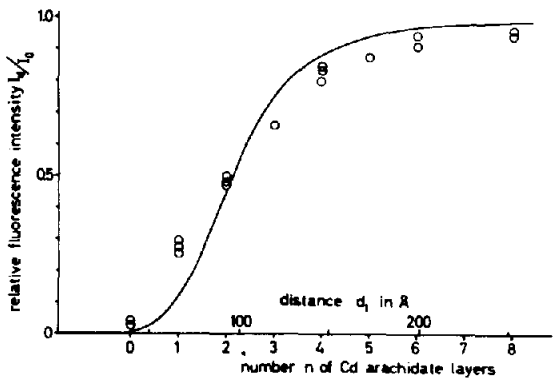

Fig. 5. Relative fluorescence intensity I_d/I_0 of a monolayer of cyanine dye I (fig. 4) at 430 nm as a function of the number n of Cd arachidate between the dye layer and a double layer of cytochrome *c* (fig. 2b). In addition the function:

$I_d/I_0 = (1 + (\frac{d_0}{d_1})^4 + (\frac{d_0}{d_2})^4)^{-1}$ is plotted with $d_1 = d_2 + 25$ Å,

$d_2 = n \times 26.5 + 12.5$ Å and $d_0 = 65$ Å.

embedded in a two-dimensional closely packed array with their long axes parallel to the layer plane they must occupy an area of 750–800 Å^2/molecule. The experimental value of the area per adsorbed molecule is obtained from the absorbance measurements. Using the value of the absorbance at 410 nm with perpendicular angle of incidence $\overline{A_1} = 2.6 \times 10^{-3}$, the extinction coefficient of oxidized cytochrome *c* at 410 nm $\epsilon = 1.065 \times 10^8$ cm^2/mole [9] Avogadro's number $N_A = 6 \times 10^{23}$ and taking into account that for the observed orientation of the chromophores the absorbance is 9/8 times the absorbance for statistical orientation, the number N of cytochrome *c* molecules per cm^2 is obtained:

$$N = \frac{8 \times N_A \times \overline{A}}{9 \times \epsilon} = \frac{8 \times 6 \times 10^{23} \times 2.6 \times 10^{-3}}{9 \times 1.065 \times 10^8} = 1.3 \times 10^{13}$$

molecules/cm^2

This value correspons to an area of 770 Å^2 per molecule indicating a closely packed layer of cytochrome *c* molecules being incorporated in the lamellar assembly.

3. Energy transfer from a fluorescent cyanine dye to cytochrome *c*

The excitation energy of a fluorescent dye molecule can be transferred to another molecule in the proximity through the electromagnetic field if the fluorescence spectrum of the energy donor overlaps with the absorption spectrum of the energy acceptor [10–12]. By separating monolayers of two suited dyes by Cd arachidate monolayers in a multilayer assembly, information is obtained on the distance dependence of the energy transfer and on possibly occurring rearrangements of the multilayer system [1].

Fig. 4 shows that the fluorescence spectrum of the cyanine dye I overlaps with the Soret band of cytochrome *c* indicating that cytochrome *c* is suited as acceptor of the excitation energy of this dye molecule. Multilayer systems are built up according to fig. 2b containing a cyanine dye monolayer (mixed with Cd arachidate at the molar ratio 1:20) and a cytochrome *c* double layer separated by different numbers n of Cd arachidate monolayers. The dye is excited by light of 366 nm and the fluorescence intensity is measured at 430 nm in a special fluorometer [3]. Fig. 5 shows the values of the ratio I_d/I_0 of the fluorescence intensity I_d for different distances of the acceptor from the fluorescent dye divided by the fluorescence intensity I_0 of similar systems without a cytochrome *c* layer.

The quenching of the fluorescence of a dye molecule due to Perrin Förster energy transfer to a thin acceptor layer decreases with increasing distance d of the donor molecule from the acceptor layer according to [12]:

$$I_d/I_0 = (1 + (\frac{d_0}{d})^4)^{-1}.$$

The characteristic distance d_0 is given by:

$$d_0 = \alpha \times 1/r \times \lambda_f \times q_f^{1/4} \times A_f^{1/4}$$

with

$$A_f = \int_0^\infty \bar{A}(\bar{\nu}) \times f(\bar{\nu}) \times (\frac{\bar{\nu}_f}{\bar{\nu}})^4 \times d\bar{\nu}.$$

$\bar{A}(\bar{\nu})$ is the absorbance of the acceptor layer as a function of the wavenumber $\bar{\nu}$; $\bar{\nu}_f$ is the wavenumber of the maximum of the fluorescence band and $f(\bar{\nu})$ is the distribution function of the quantum spectrum of the fluorescence normalized according to $\int_0^\infty f(\bar{\nu})\, d\bar{\nu} = 1$. q_f is the quantum efficiency of the fluorescence of the energy donor, λ_f is the wavelength of the maximum of the fluorescence band and r is the refractive index of the surrouding dielectricum. α is a numerical factor.

For one cytochrome c layer, $A_f = 0.84 \times 10^{-3}$ is obtained from fig. 4. For the cyanine dye I, $q_f = 0.43$ [13] and $\lambda_f = 430$ nm. For Cd arachidate $r = 1.52$ [5, 6]. If the transition moments of the energy donor lie parallel to the layer plane [1] and the moments of the acceptor are statistically oriented, $\alpha = 0.14$ [12]. The transition moments of the acceptor being oriented as observed with cytochrome c this value of α is changed only little. Thus the theoretical value of d_0 is calculated to be d_0 (theor.) = 56 Å.

Taking into account that the measured fluorescence intensities (fig. 5) are obtained with two acceptor layers at the distances d_1 and d_2 (fig. 2b), an experimental value of d_0 is found by fitting the function

$$I_d/I_0 = (1 + (\frac{d_0}{d_1})^4 + (\frac{d_0}{d_2})^4)^{-1},$$

varying d_0 by trial and error. d_1 and d_2 depend on the number n of Cd arachidate layers, on the thickness of one Cd arachidate layer (26.5 Å [6]) and on the thickness of one cytochrome c layer (ca. 25 Å) according to (fig. 2b):

$$d_1 = d_2 + 25 \text{ Å},$$

$$d_2 = n \times 26.5 \text{ Å} + \tfrac{1}{2} \times 25 \text{ Å}$$

The function plotted in fig. 5 corresponds to d_0 (exp.)= 65 Å. Thus by interpreting the measured fluorescence quenching being caused by Perrin Förster energy transfer, a characteristic distance d_0 is obtained very similar to that predicted by the theory. The difference may arise from the not very accurate value of q_f. The deviation of the measured fluorescence intensity from the theoretical curve (fig. 5) is not yet well understood.

The possibility of measuring a reproducible distance dependence of fluoresence quenching indicates that the arrangement of the lipid protein multilayer assembly is as to be expected from the depositing procedure of the monolayers. Little disturbances cannot be excluded by these macroscopic measurements. Electron microscopic studies are in progress to get information on this problem.

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References

[1] H.Bücher, K.H.Drexhage, M.Fleck, H.Kuhn, D.Möbius, F.P.Schäfer, J.Sondermann, W.Sperling, P.Tillmann and J.Wiegand, Mol. Crystals 2 (1967) 199.
[2] P.Fromherz, Thesis, Science Faculty University Marburg (1969).
[3] D.Möbius and H.Bücher, in: Physical Methods in Organic Chemistry, Vol. IV, eds. A.Weissberger and B.W.Rossites (Wiley, New York) in press.
[4] H.Mayer, Physik dünner Schichten, Stuttgart (1950).
[5] K.H.Drexhage, unpublished.
[6] H.Bücher, Thesis, Science Faculty University Marburg (1970).
[7] K.H.Beck, Thesis, Science Faculty University Marburg (1970).
[8] R.E.Dickerson, M.L.Kopka, J.Weinzierl, J.Varnum, D.Eisenberg and E.Margoliash, J. Biol. Chem. 242 (1967) 3015.
[9] E.Margoliash and N.Frohwirt, Biochem. J. 71 (1959) 570.
[10] F.Perrin, Annales de Physique 10e série, t.XVII (1932) 283.
[11] Th.Förster, Annal. Phys. Leipzig 6. Folge 2 (1948) 55.
[12] H.Kuhn, J. Chem. Phys. 53 (1970) 101.
[13] P.Tillmann, Thesis, Science Faculty University Marburg (1966).