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A Theoretical Consideration how to Study Biochemical Interfacial Photoreactions

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A theoretical calculation for quantitative analysis of photoreactions in membrane interfaces is described. The method utilizes the photovoltage arising by the illumination of a pigmented membrane. Since the water-soluble substrate is only added to one side of the membrane an electrical double layer is produced. The corresponding photovoltage is quantified by the Gouy-Chapman theory. A possibility to investigate non-isotropic effects is discussed.

Some biochemical reactions depending on the absorption of light take place at the surface of biomembranes. For example the photosynthetic pigments chlorophyll and carotinoid are bound to the thylakoid membrane of the chloroplasts [1]. The visual process of the eye is initiated by the membrane protein rhodopsin which contains the chromophore retinal as cofactor. It is assumed that flavins act as light receptor in phototaxis and phototropy of plants [2, 3]. It is ultimately not clear, to what extent association of the pigments with the membrane proteins and direct lipid interactions contribute to the activity. In any case, the light induced reactions occur non-isotropically and are sterically selected.

Investigation of biological membranes is very complicated due to innumerable factors which influence an experiment. Therefore with the intention of bringing to light basic phenomena, studies of appropriate model systems have been performed. In this paper studies with bilayer membranes imitating non-isotropically and sterically influenced photoreactions will be suggested. The method utilizes photoelectric effects at artificial membranes [4, 5]. In the literature photovoltages and photocurrents with the pigments chlorophyll a [5], retinal [6, 7], flavin [8] and a cyanine dye [9] have been reported. A qualitative analysis of an interfacial photoreaction with lipid bilayer membranes containing magnesium porphyrin was done by Hong and Mauzerall [10]. They used the photocurrent measured by a voltage clamp method to determine chemical rate constants.

Consider a thin lecithin membrane which contains strongly bound pigment molecules with their reactive part in the membrane/water interface as shown in Fig. 1. Examples for suitable molecules are the above mentioned pigments chlorophylls, carotinoids and amphiphilic flavins [11]. A photochemical reaction of the pigment with a substrate dissolved in the water phase should be possible, which leads to a charge change of the pigment. The substrate as well as the reaction products (except the membrane bound pigment) should be water-soluble and should not influence the membrane permeability. If the substrate is only added to one side of the membrane the reaction occurs asymmetrically and a one-sided

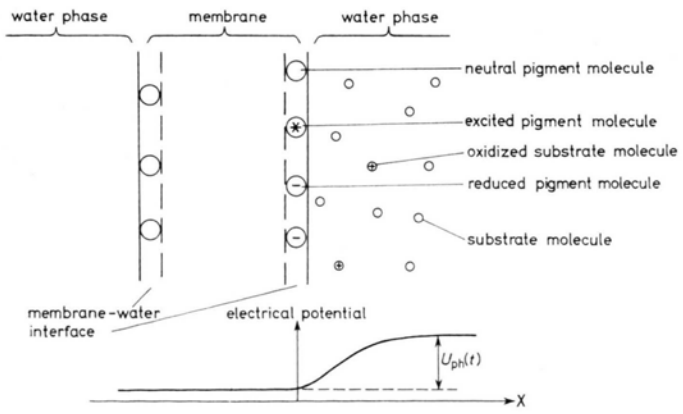

Fig. 1. Skeleton sketch of the charge distribution and the electric potential across the membrane. The membrane-bound pigment is assumed to be neutral in the initial state and is reduced in the photoreaction by a one electron step to a negative ion. The reducing agent is only added to the right side of the membrane.

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layer of charges fixed to the membrane developes. In this process the counter ions are statistically distributed in the water phase. The reaction leads to a change of one interface potential, which is detectable by electrodes protruding respectively into both aqueous phases. Protons which possibly participate in the photoreaction are negligible because their maximal concentration change is considerably smaller than their concentration in the aqueous solution. If the surface charge is not extremely high, the distribution of the counter ions and the developing potential can be described by the Gouy-Chapman theory [12]. When the membrane is kept in the dark and is then illuminated, a photovoltage arises from a stationary dark potential.

If the membrane is illuminated with monochromatic light of the quantum flux Q and the area concentration of the pigment in one interface is N_0, the temporal increase in surface charges $N(t)$ is proportional to the quantum flux, the quanta-capture cross-section q of one pigment molecule at the wavelength of the monochromatic light, the quantum yield γ of the photoreaction and the number of pigments which have not yet participated in the reaction. The surface charge decreases if a possible back-reaction (recombination) or another side-reaction occurs. This is taken into consideration by a term proportional to N and a corresponding time-constant τ_r:

$$\frac{dN(t)}{dt} = Q\,q\,\gamma(N_0 - N) - \frac{N}{\tau_r}\,. \tag{1}$$

The number of pigments in the excited state is neglected. The integration of (1) combined with the secondary condition $N(0) = 0$ leads to:

$$N(t) = \frac{N_0}{1 + \dfrac{1}{Q\,q\,\gamma\,\tau_r}}\left(1 - \exp\left[-t(Q\,q\,\gamma + \frac{1}{\tau_r}\right]\right). \tag{2}$$

The relation between the surface charge N and the surface potential U_{ph} is stated in the Gouy-Chapman theory:

$$N = e_0\sqrt{I}\,\alpha \sinh\frac{U_{ph}}{\beta}\,, \tag{3}$$

where e_0 is the elementary charge, I the ionic strength and α and β are defined by

$$\alpha = \frac{1}{e_0}\sqrt{\frac{2\,D\,\varepsilon_0\,k\,T}{\pi}}$$
$$\beta = 2\,k\,T/e_0\,.$$

D is the dielectric constant between the separated charges, ε_0 the dielectric constant of the vacuum, k the Boltzmann constant and T the absolute temperature. Combining Eqn. (3) with (2) the photovoltage U_{ph} reads

$$U_{pg}(t) = \beta\cdot\operatorname{arsh}\left\{\frac{N_0(1 - \exp[-t(Q\,q\,\gamma + 1/\tau_r)])}{\alpha\sqrt{I}\left(1 + \dfrac{1}{Q\,q\,\tau_r\,\gamma}\right)}\right\}. \tag{4}$$

The timecourse of the photovoltage includes the information about the initial number of pigment molecules in the membrane and the quantum yield of the photoreaction. The other quantities of Eqn. (4) are either natural constants or can easily be derived by separate measurements, such as the quantum flux and the absorption cross-section. τ_r is derived by switching off the light. The time-constant of the decrease of the photovoltage leads directly to τ_r.

In order to find simple expressions for N_0 and γ an approximation is introduced. If the photovoltage does not exceed 10 mV the arsh can be substituted by its argument:

$$U_{ph}(t) = \left\{\frac{\beta N_0(1 - \exp[-t(Q\,q\,\gamma + 1/\tau_r)])}{\alpha\sqrt{I}\left(1 + \dfrac{1}{Q\,q\,\gamma\,\tau_r}\right)}\right\};$$
$$U_{ph} < 10\,\text{mV}. \tag{5}$$

The error made in this approximation is less than 1%. Since the photovoltage is proportional to N_0 the condition of small U_{ph} can easily be verified by using small pigment concentrations. Imagine for example the photoproduction of $1.3\cdot10^{12}$ charges/cm^2 causes a photovoltage of 10 mV.

The initial slope of the photovoltage of Eqn. (5) is

$$\left(\frac{dU_{ph}}{dt}\right)_{t=0} = \frac{\beta N_0(Q\,q\,\gamma + 1/\tau_r)}{\alpha\sqrt{I}\left(1 + \dfrac{1}{Q\,q\,\gamma\,\tau_r}\right)} \tag{6}$$

and the saturated value after the membrane has been subjected to light for a long time is

$$U_{ph}(t\to\infty) = U_{ph}^{\infty} = \frac{\beta N_0}{\alpha\sqrt{I}\left(1 + \dfrac{1}{Q\,q\,\gamma\,\tau_r}\right)}\,. \tag{7}$$

Eqns (6) and (7) can be solved for N_0 and γ:

$$N_0 = \frac{\alpha}{\beta}\sqrt{I}\,U_{ph}^{\infty}\left(1 + \frac{U_{ph}^{\infty}}{\left(\dfrac{dU_{ph}}{dt}\right)_{t=0}\cdot\tau_r - U_{ph}^{\infty}}\right) \tag{8}$$

$$\gamma = \frac{\left(\dfrac{dU_{ph}}{dt}\right)_{t=0}\cdot\tau_r - U_{ph}^{\infty}}{U_{ph}^{\infty}\,\tau_r\,Q\,q}\,. \tag{9}$$

Thus one single experiment enables the determination of the quantum yield $\gamma_{A,m}$ for the photoreaction with the substrate A. The subscript m indicates the sterically influenced (non-isotropic) membrane interface reaction. Another substrate B may react with the quantum yield $\gamma_{B,m}$. If analogous reactions were carried out in homogeneous solutions (isotropic case) other quantum yields are to be expected: $\gamma_{A,h}$ and $\gamma_{B,h}$ respectively. The subscript h indicates the homogeneously occuring reaction. In a general case one expects higher quantum yields for the isotropic reactions than for the non-isotropic, sterically influenced membranes reactions. Now one forms the quotient from the quantum yields of the isotropic to non-isotropic reaction for substrate A $\gamma_{A,h}/\gamma_{A,m}$ and the respective quotient for substrate B $\gamma_{B,h}/\gamma_{B,m}$. If these two ratios are the same no specifity is introduced by the membrane. If the ratios differ substrate specifity is introduced. The membrane reaction is hindered when the ratio is larger than 1 and is favoured when the ratio is smaller than 1.

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