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# Relationship between the fraction of closed photosynthetic reaction centers and the amplitude of the photovoltage from light-gradient experiments

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**The light-gradient technique is a method to measure electrically the primary photosynthetic charge separation under in vivo and in vitro conditions. The importance of the technique lies in the high time resolution and the good signal-to-noise ratio that can be obtained in the presence of antenna pigments. Although the effect has been known for almost 20 years, there is little theoretical basis for it. The analysis of recent studies on trapping times and trapping yields in various photosynthetic organisms, however, requires a quantitative treatment. Here we derive a theory that describes the basic experimental observations of the light-gradient photovoltage and that correlates the photovoltage with the fraction of reaction centers closed by nanosecond flashes, picosecond flashes, and trains of picosecond flashes. Furthermore, the effect of singlet-singlet annihilation in the case of picosecond flashes, the effect of energy transfer between photosynthetic units, and the time-course of the photovoltage in the case of nanosecond flashes measured with high time resolution are analyzed with respect to molecular parameters that govern the excitation dynamics in photosynthetic membranes. Some experimental examples for the predictions of the theory are given.**

## Introduction

The early historical proof for the now well-established generation of a transmembrane potential by the primary photochemistry in reaction centers had been achieved by measurements of the electrochromic shift of absorption bands [1,2]. In search of independent evidence for the electric field, Fowler and Kok reported in 1972 an elegant experiment which demonstrates the generation of a transmembrane photopotential by electrical detection with two macroscopic electrodes [3]. The experiment was termed light-gradient experiment because it rests on the asymmetry of excitation of two membrane sides in a vesicle with respect to the direction of the incoming light.

According to the original explanation of Fowler and Kok, the photovoltage transient evoked by non-saturating flashes exciting photosynthetically active vesicle preparations (e.g., chloroplasts) originates from the higher dipole density in the vesicle half-spheres that

*List of terms used*

| | |
|---|---|
| $\alpha$ | coefficient relating the competition between annihilation and trapping ($\alpha = \gamma/2\Gamma k_0$) |
| $E$ | energy of excitation flash (photons·cm$^{-2}$ or $\mu$J·cm$^{-2}$) |
| $f$ | proportionality factor in the light-gradient theory |
| $\gamma$ | bimolecular annihilation rate constant |
| $\Gamma$ | quantum yield of primary photosynthetic charge separation |
| $I_0$ | intensity of dc-light (photon·cm$^{-2}$·s$^{-1}$) |
| $I(t)$ | intensity of excitation flash (photons·cm$^{-2}$·s$^{-1}$) |
| $J$ | number of vesicles in series |
| $k_i$ | rate constants |
| $N$ | antenna size/size of photosynthetic unit |
| $\Delta q_c$ | fraction of RCs closed by the flash |
| $q_c(t)$ | fraction of closed RCs |
| $Q_0$ | fraction of open RCs before the flash |
| $\sigma$ | absorption cross section of one mean antenna pigment |
| $T_0$ | transmission of one membrane |
| $T_{eff}$ | transmission of one membrane of an effective vesicle |
| $V$ | photovoltage |
| $V_0$ | photovoltage created by closure of all RCs in a single membrane |
| $V_{eff}$ | photovoltage from one effective vesicle |
| $z$, $z_i$ | normalized excitation energies in hits per trap ($z = \sigma NE$) |

Abbreviations: PS I, Photosystem I of green plants; PS II, photosystem II of green plants; PSU, photosynthetic unit; RC, reaction center.

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face the light source as compared to the shaded other half-spheres. Consequently the polarity depends decisively on the position of the light source with respect to the electrodes. The basic properties of the light-gradient photovoltage have been described in Refs. 3–5, and a semi-quantitative analysis has been given in Ref. 5. An alternative explanation of the origin of the light-gradient photovoltage has been proposed in Ref. 6.

The early measurements in the 70's were all made with xenon flash excitation and were recorded on a micro- and millisecond time-scale. In the 80's a faster time resolution of 5 ns and later 40 ps was achieved by laser flash excitation and a high-frequency coaxial design of the electrodes [7,8].

The recent achievement of a picosecond time resolution of the light-gradient photovoltage made it possible to time resolve primary photosynthetic reactions in the reaction center (RC), to measure trapping times and yields under various experimental conditions that include exciton-exciton interactions [9–12]. However, for a quantitative evaluation of yields that are nonlinearly related to the light-gradient photovoltage a theoretical description of the effect is required.

The present theory aims particularly at the correlation between the amplitude of the photovoltage and the fraction of RCs closed by a flash. The equations are written in a form that allows incorporation of theories on exciton dynamics that take into account singlet-singlet annihilation and energy transfer between photosynthetic units (PSUs) like that in Ref. 13. Furthermore, the equations cover the general case in which part of the RCs are already in the closed state before the flash. Such conditions, that are favorable for gaining information on the organization of antenna systems, are usually achieved by preflashes or background light.

In this article, the amplitude in response to one flash, the preclosure of RCs, the effect of multiple flashes, the time-courses, and general aspects of the light-gradient are considered.

## Theory

In the following we consider an experimental arrangement in which two planar electrodes form a capacitor and the sample under consideration represents its dielectric. Light shall enter the capacitor from the top through a semi-transparent grid-electrode (Fig. 1).

The aim of the following theory is the derivation of equations that correlate the light-gradient voltage, the fraction of closed traps, and the energy of a monochromatic flash. A fraction of RCs may already be in the closed state before the flash. The global assumption made throughout this analysis is that the measured photovoltage, $V$, from a hypothetical, perfectly oriented single membrane is proportional to the transmembrane potential and this in turn is proportional to the fraction of traps closed by the excitation flash, $\Delta q_c$ ($0 \leq \Delta q_c \leq 1$):

$$V = V_o \cdot \Delta q_c \tag{1}$$

where $V_0$ denotes the effectively measured photovoltage when the maximal transmembrane potential is created

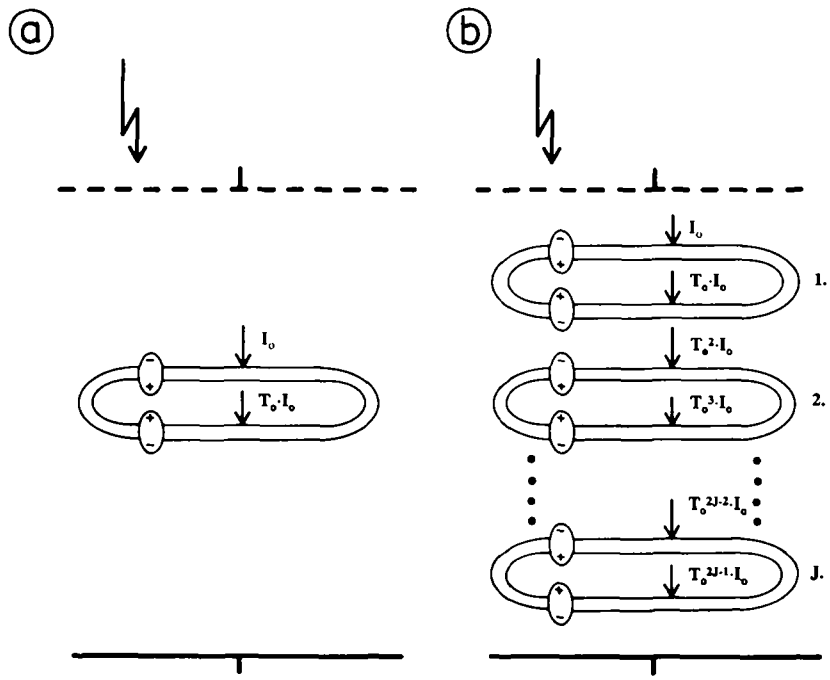


Fig. 1. (a) Schematic representation of a photosynthetic vesicle in a planar capacitor with a semi-transport upper plate. (b) Scheme of the series connection of many vesicles in a stack.

by closure of all RCs ($\Delta q_c = 1$). In fact, this first equation is the link with theories that describe trapping dynamics independent of a light-gradient. These theories must provide the value for $\Delta q_c$ as well as its time dependence and its dependence on the excitation energy of the flash.

In the examples – if not stated otherwise – we assume for a clearer presentation that the closure of traps occurs in PSUs independent of each other (separate units) or that the quenching rate constant of the RCs does not change during the time interval considered here, and that annihilation effects are negligible. Then the closure of traps is independent of the fraction of already closed RCs.

Under the above assumptions the fraction of RCs closed by a flash of the energy, $E$, can be calculated according to the cumulative Poisson distribution [13–15]:

$$\Delta q_c(E) = Q_o \cdot (1 - \exp(-\Gamma \cdot \sigma \cdot N.E)) \tag{2}$$

or by introducing the average number of excitons created per RC, $z = \sigma \cdot N \cdot E$:

$$\Delta q_c(z) = Q_o \cdot (1 - \exp(-\Gamma \cdot z)) \tag{2a}$$

where $Q_o$ is the fraction of RCs open before the flash, $\Gamma$ the quantum yield of the primary photosynthetic charge separation, $\sigma$ the absorption cross section of an antenna pigment, and $N$ the number of antenna pigments per RC ($N$ = photosynthetic unit size).

*The elementary two-membrane model*

In this part we resume an equation by Fowler and Kok [5] that correlates the light-gradient photovoltage to the transmembrane voltage for the simplest case of only one vesicle in the direction of the light (Fig. 1a).

Each vesicle shall consist of two membranes, the upper and lower membrane with the indices 1 and 2, respectively. If the pigmented membrane has the transmission $T_o$, the light-gradient photovoltage follows from the difference (i.e., antiparallel orientation) of RCs closed by a flash in the upper and lower membrane according to:

$$V(z) = V_o \cdot [\Delta q_{c,1}(z) - \Delta q_{c,2}(T_o \cdot z)]$$

$$= V_o \cdot [Q_{o,1} \cdot (1 - \exp(-\Gamma \cdot z)) - Q_{o,2} \cdot (1 - \exp(-\Gamma \cdot T_o \cdot z))] \tag{3}$$

If all RCs are open before the flash (dark-adapted case) Eqn. 3 simplifies to:

$$V(z) = V_o \cdot [\exp(-\Gamma \cdot T_o \cdot z) - \exp(-\Gamma \cdot z)] \tag{3a}$$

This last equation already accounts for the basic feature of the light-gradient photovoltage, namely an asymmetric bell-shaped energy dependence as experimentally found [5,12].

*A multi layer model*

The transmission of a photosynthetic membrane can be estimated to be between $T_o = 0.990$ and $T_o = 0.999$, depending on the wavelength. With these transmissions Eqn. 3a would predict much smaller light-gradient photovoltages than actually measured. Therefore, the assumption of single vesicles in the light path is not given. In chloroplasts, for instance, the lamellar stroma and grana membranes would indicate several tens of membranes connected in series and more than one organelle is in the volume under investigation. This will be accounted for in the following by introducing $J$ vesicles with $2 \cdot J$ membranes forming stacks with respect to the light axis (Fig. 1b).

The energy falling onto the upper membrane of the vesicle $j$ is given by

$$E_{j,1} = T_o^{(2j-2)} \cdot E_o \tag{4a}$$

and onto the lower membrane

$$E_{j,2} = T_o^{(2j-1)} \cdot E_o \tag{4b}$$

with $j \in (1,2,\ldots J)$.

The total light-gradient photovoltage from the stack then follows as the sum over the photovoltages from all vesicles connected in series. When a fraction of RCs is already closed before the flash and if $E_o$ is normalized to the hits per RC ($z = \sigma \cdot N \cdot E_o$), the photovoltage can be expressed as follows:

$$V(z) = V_o \sum_{j=1}^{J} \{ Q_{o,2j-1} \cdot [1 - \exp(-\Gamma \cdot T_o^{(2j-2)} \cdot z)]$$

$$- Q_{o,2j} \cdot [1 - \exp(-\Gamma \cdot T_o^{(2j-1)} \cdot z)] \} \tag{5}$$

When all RCs are open before the flash:

$$V(z) = V_o \sum_{j=1}^{J} [\exp(-\Gamma \cdot T_o^{(2j-1)} \cdot z) - \exp(-\Gamma \cdot T_o^{(2j-2)} \cdot z)] \tag{5a}$$

A similar equation (Eqn. 1 in Ref. 5) has been used to fit the data in [5].

As can be easily shown, there exist a good approximation by setting the exact solution for the photovoltage, $V$, of stacks containing $J$ vesicles in series, proportional to the photovoltage, $V_{eff}$, of one hypothetical 'effective' vesicle with an effective transmission, $T_{eff}$:

$$V(z) = f \cdot V_{eff}(z) \tag{6}$$

in which $T_{eff}$ is related to $T_o$ by:

$$T_{eff} = T_o^{2J-1} \tag{7}$$

The effective photovoltage for the general case where a fraction of – or the special case where all – RCs are

open before the flash then is:

$$V_{eff}(z) = V_o \cdot [Q_{o,1} \cdot (1 - \exp(-\Gamma \cdot z)) - Q_{o,2} \cdot (1 - \exp(-\Gamma \cdot T_{eff} \cdot z))] \quad (8)$$

$$V_{eff}(z) = V_o \cdot [\exp(-\Gamma \cdot T_{eff} \cdot z) - \exp(-\Gamma \cdot z)] \quad (8a)$$

or more generally

$$V(z) = f \cdot V_o \cdot [\Delta q_{c,1}(z) - \Delta q_{c,2}(T_{eff} \cdot z)] \quad (9)$$

These last equations may be considered to describe an 'effective' vesicle representing the uppermost and lowermost membrane of a stack. The proportionality factor, $f$, can be shown to be

$$f = \frac{J}{2J-1} \quad (10)$$

For stacks with a large number of cascaded vesicles $f$ approaches 0.5 (for $J \rightarrow \infty$) and $T_{eff}$ approaches zero. This is equivalent to single oriented membranes. Since the factor $f$ affects only the photovoltage amplitude and since the absolute amplitude is an adjustment parameter to experimental data, which does not depend on the fraction of closed RCs, $V_{eff}(z)$ is generally sufficient for analysis.

Eqns. 8 and 8a show that the photovoltage amplitude depends strongly on the effective transmission. It should be noted here, that the effective transmission is not identical to the transmission of the sample, which consists of strongly pigmented organelles surrounded by nearly transparent medium. The absorption of such a suspension is 'flattened' as compared to the absorption of a corresponding solution [16]. The light-gradient photovoltage, however, is not correlated to the average transmission, but rather samples the 'worst' case, a local light-gradient in this inhomogeneous system. This is best seen assuming a very dilute suspension, where the probability of one organelle shading another one is negligible and $T_{eff}$ is determined by the absorbance of one organelle. In this case the average transmission, but not $T_{eff}$, can be increased by further dilution. In addition, not only absorption, but also (multiple) scattering and reflexion in the suspension could contribute to the different light intensities that make up $T_{eff}$. The above equations describe the basic principles of the light-gradient irrespective of the real, complex origin of the light-gradient. The different light intensities making up $T_{eff}$ could be due to absorption, but also to multiple scattering and reflection in the suspension.

As obvious from the foregoing, the effect of the light-gradient is completely described by only one parameter, $T_{eff}$. For data analysis, the knowledge of its value is usually necessary. The capacity of Eqn. 8a to evaluate $T_{eff}$ will be demonstrated by a parameter fit to

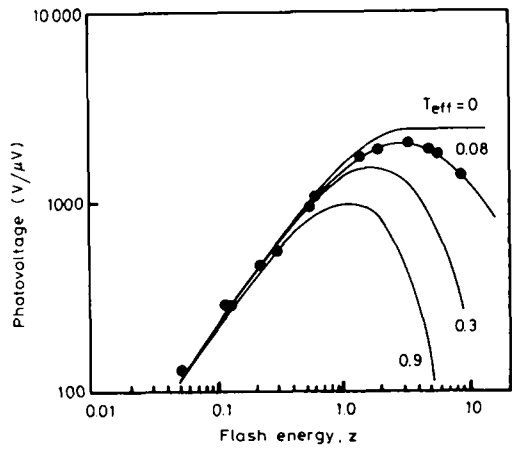


Fig. 2. Light-gradient photovoltage due to PS I from dark-adapted, stacked pea chloroplasts (•) evoked by 12 ns $Q$-switch flashes at 532 nm. Absorbance: $A_{532} = 0.2$. For further experimental details see Ref. 10. The drawn lines were calculated for different $T_{eff}$ using Eqn. 8a. The following fixed parameters were chosen: $\Gamma = 0.95$; $\sigma = 1.6 \cdot 10^{-17}$ cm$^2$; $N = 280$. The curves were adjusted at low energies with the parameter $V_o$.

experimental data from PS I, for which the quenching rate constants of open and closed RCs ($P^+$) are known to be equal [17]. The light-gradient photovoltage upon 12 ns excitation (i.e., negligible annihilation) from pea chloroplasts due to the charge separation in PS I is shown on a double-logarithmic plot in Fig. 2. A group of curves calculated by Eqn. 8a for different $T_{eff}$, but constant $\Gamma \cdot N$, was adjusted to the data by varying $V_0$ as to match the data at low energies. It can be seen that the position of the maximum and the decaying slope at high energy allows the evaluation of $T_{eff}$. The best fit yields $T_{eff} = 0.08$. However, the macroscopic transmission of the sample was $T = 0.65$ at the excitation wavelength. This large difference is an example for the arguments given above. For the concentration used, it can be estimated that several chloroplasts in series in the light path of 100 $\mu$m are involved in building up the effective transmission. Assuming that $T_{eff}$ is solely determined by absorption, the transmission of one single chloroplast as the largest undilutable absorption unit must be in the order of 0.5 at 532 nm. This high value could be found only in the grana stacks (the chlorophyll concentration in the membrane is about 0.1 M).

To find the relation between the fraction of closed traps and the amplitude of the light-gradient photovoltage, we use an approximation by defining the fraction of closed traps as the mean value of closed RCs in the upper and lower membrane of the 'effective' vesicle according to:

$$\Delta q_c(z) = \tfrac{1}{2} \cdot [\Delta q_{c,1}(z) + \Delta q_{c,2}(T_{eff} \cdot z)] \quad (11)$$

With this definition, Fig. 3 shows for the dark-adapted case the dependence of the fraction of closed

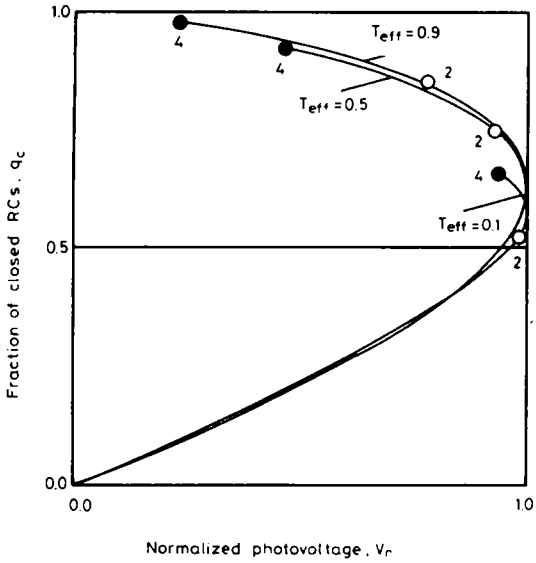


Fig. 3. Relation between the fraction of RCs closed by a flash and the light-gradient photovoltage for three different values of $T_{eff}$ as indicated. The end points (filled circles) of the curves indicate the fractions of RCs closed by four hits per RC ($z = 4$) and the corresponding photovoltage. The open circles indicate the fractions of RCs closed by two hits per RC ($z = 2$).

traps, $\Delta q_c(z)$, on the photovoltage, $V(z)$, as calculated by Eqn. 8a for three values of $T_{eff}$. Marked by open circles are the photovoltage and the fractions of RCs closed by a flash with an energy of $\Gamma \cdot z = 2$. The end-points of all curves are at $\Gamma \cdot z = 4$. Three main features are noticed: (i) The functions $V(\Delta q_c)$ are not unique; (ii) with decreasing $T_{eff}$ the energy needed to close all RCs increases drastically; and (iii) the energy required to evoke the maximal photovoltage corresponds to about half-saturation, and is only slightly dependent on whether the transmission is high, medium, or low.

*Pre-closure of RCs*

Up to this point, it has not been specified how in the non-dark-adapted case the pre-closure of RCs is accomplished. With regard to the states of the system, two limiting cases may be considered.

Firstly, if the closure occurs chemically, all membranes contain the same fraction of closed RCs and $Q_o = Q_{o,1} = Q_{o,2}$ (isotropic closure). Then in Eqns. 5 and 8 the term $Q_o$ becomes a prefactor and the photovoltage is just proportional to the fraction of RCs open before the flash. ($Q_o$ has to be determined by independent measurements.)

Secondly, if the closure occurs by an actinic preflash of an energy $z_a$, the upper and the lower membranes contain different fractions of closed RCs (anisotropic closure, prepolarization) that are given by:

$$Q_{o,1} = \exp(-\Gamma \cdot z_a); \quad Q_{o,2} = \exp(-\Gamma \cdot T_{eff} \cdot z_a) \tag{12}$$

The validity of these equations is given when the asymmetry induced by the preflash does not change during the time interval, $\Delta t$, between the preflash and the probe flash. This may lead to a negative (or opposite) polarity of the photovoltage, when the preflash leaves more RCs open in the lower than in the upper membrane.

Smaller degrees of anisotropy may be present at the instant the probe flash is given, when the closure is made by continuous light. This case is treated in the following section.

*Multiple flashes*

In the following we will consider a train of picosecond flashes (spaced by several nanoseconds), that consists of $i$ flashes having the energies, $z_i$, which are Gaussian-distributed, according to:

$$z_i = z_t \cdot \frac{1}{\sqrt{\pi} \cdot \Delta i} \cdot \exp -(1/\Delta i)^2 \tag{13}$$

where $z_t$ is the total energy of the train (which is an easily measurable quantity) and $\Delta i$ is the width of the train (in general $\Delta i$ is not an integer).

If the total energy suffices to close most of the RCs (which is usually a given experimental condition), the photon density in single picosecond flashes may be high enough to cause singlet-singlet annihilation. Furthermore, if the PSUs are connected, excitation energy may be transferred from RCs closed by preceding flashes to open ones. The only theory presently available that accounts for these effects, is found in Ref. 13. We therefore combine in the following this theory, written for the lake model of antenna organization, with the light-gradient theory.

For a train of picosecond flashes the light-gradient photovoltage evoked by flash number $i$ depends on the prepolarization by the preceding flashes. Hence, the photovoltage evoked by the $i$th flash, $V_i$, can be calculated as the difference between the polarization states created by flash number $i-1$ and flash number $i$. Modifying Eqn. 9,

$$V_i = f \cdot V_o \cdot (\Delta q_{c,1,i} - \Delta q_{c,2,i}) \tag{14}$$

The fractions of still open RCs in the upper and lower membranes, $Q_{o,1}$ and $Q_{o,2}$, have to be calculated in a successive manner by applying Eqn. 11 in Ref. 13, using $z_i$ and $T_{eff} \cdot z_i$ as excitation energy in the upper and lower membrane of the effective vesicle, respectively.

$$V_i = f \cdot V_o \cdot [(Q_{o,1,i-1} - Q_{o,1,i}) - (Q_{o,2,i-1} - Q_{o,2,i})] \tag{14a}$$

The two equations can also be used for double flash experiments by setting $i = 2$.

To demonstrate the sensitivity of 'train' experiments for the evaluation of molecular parameters like the

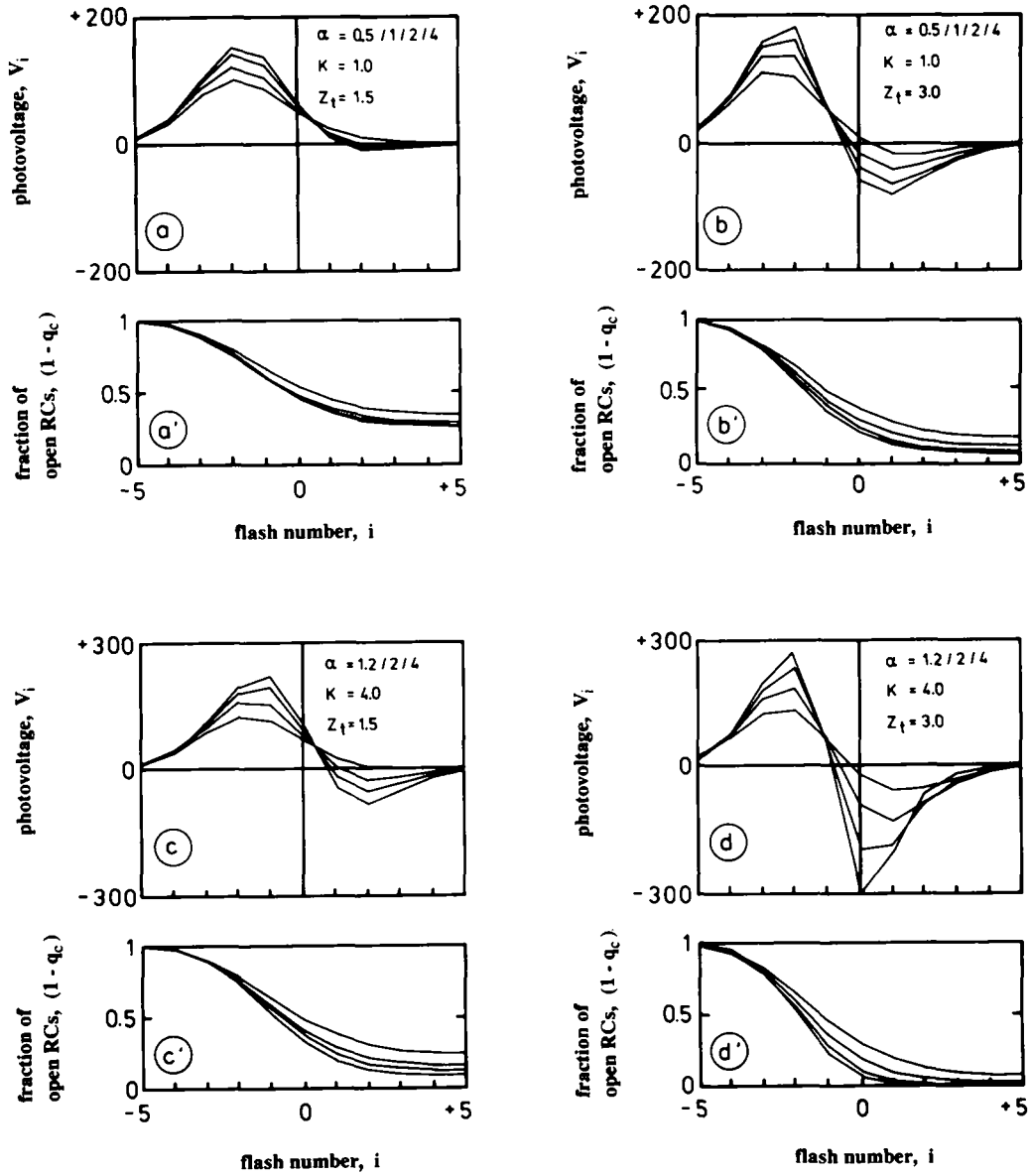


Fig. 4. Peak photovoltage from a train of picosecond flashes as calculated by Eqn. 14a, when all RCs are open before the flash train. The peak photovoltages of the individual flashes are connected by straight lines. The energy distribution of the flashes in the train was taken Gaussian, according to $E_i \approx \exp -(i/2.6)^2$. The halfwidth of $\Delta i = 2.6$ is a typical value for Nd-YAG lasers [10,12]. The calculations were made for an optically thin sample, taking $T_{eff} = 0.9$. The total energy of the train was either $z_t = 1.5$ (left-hand side) or $z_t = 3.0$ (right-hand side). The ratio of the quenching rate constants for open and closed RCs was either $K = k_o/k_c = 1$ (a, b) or $K = k_o/k_c = 4$ (c, d). The parameter $\alpha$ in the upper four figures represents the competition between trapping and annihilation according to Ref. 13. (The larger $\alpha$, the higher the annihilation losses.) The progressive closure of RCs by the successive flashes is shown beneath each figure, by plotting $(1 - q_c)$.

parameter $\alpha$, which describes the competition between trapping and annihilation, and the ratio of quenching rate constants of open and closed RCs, $k_o/k_c$, we applied Eqn. 14 to a Gaussian flash train (Eqn. 13). We assume that in the time interval between two successive flashes the excitons have completely decayed and the closed RCs do not re-open during the train.

Fig. 4 shows for two total energies of the train, $z_t = 1.5$ and $z_t = 3$ (left and right columns, respectively), characteristic photovoltage patterns, calculated for flash numbers $i = -5$ to $i = +5$. The energy in the earlier and later flashes is less than 1% and can thus be neglected. The case of negligible energy transfer between PSUs is approximated by $k_o/k_c = 1$ (a and b). The case of significant energy transfer is examplified by $k_o/k_c = 4$ (c and d). The progressive closure of RCs is depicted in the graphs beneath (a', b', c', d').

The following features can be seen in Fig. 4. An increase in the total energy is reflected by an earlier closure (compare a' – b' and c' – d') and a shift of the pattern to the left (compare a – b and c – d). An increase in the annihilation (larger $\alpha$), leaving all other

parameters constant, always counteracts the light-gradient because the losses are more pronounced in the vesicle membranes with the higher exciton density. Depending on the excitation energy this may lead to smaller or higher amplitudes. An increase of the energy transfer (larger $k_o/k_c$) is reflected by increasing amplitudes at earlier flash numbers (compare a – c and b – d).

These few examples show that the light-gradient photovoltage pattern from picosecond flashes displays substantial susceptibility to the annihilation and energy transfer parameters. This type of analysis for trains of picosecond flashes is used in one of the accompanying articles [12].

A more complicated but interesting case arises when a fraction of RCs is closed by applying dc-light. The asymmetry caused by the slowly incoming photons may leave a 'residual' anisotropy, if the rotational diffusion of the organelles in the capacitor, and the re-opening of RCs by the intrinsic electron transport chain is on the same time-scale.

These prepolarizing conditions can be quantitatively treated by assuming the closure of RCs to be governed by a simple steady-state saturation introduced by the dc-light intensity, $I_o$, reaching the upper ($I_o$) and lower ($T_{eff} \cdot I_o$) vesicle membrane. If the background light has the wavelength $\lambda$, the fractions of RCs still open in the upper and lower membrane are:

$$Q_{o,1} = 1 - \frac{I_o}{I_o + k/[\Gamma \cdot \sigma(\lambda) \cdot N]};$$

$$Q_{o,2} = 1 - \frac{T_{eff}(\lambda) \cdot I_o}{T_{eff}(\lambda) \cdot I_o + k/[\Gamma \cdot \sigma(\lambda) \cdot N]} \qquad (15)$$

where $T_{eff}(\lambda)$ and $\sigma(\lambda)$ are the effective transmission and the absorption cross-section at the wavelength of the dc-light, and $k$ is the rate constant for the decay of the closed state. Eqn. 15 describes the case of negligible energy transfer between PSUs ($k_o/k_c = 1$). The case of a lake model with energy transfer ($k_o/k_c > 1$) is treated in Ref. 11, Eqn. 4. If the tumbling rate of the organelles is on the same order of magnitude as $k$, the difference in the fraction of closed RCs in the upper and lower membrane will be smeared out. $T_{eff}(\lambda)$ may be estimated from $T_{eff}$ and the transmission spectrum of the sample, although wavelength-dependent light scattering might have a strong influence.

Using a prepolarization by background light as the starting condition in train experiments, Eqn. 14, predicts negative photovoltages for a wide range of $T_{eff}$ and degrees of closure. An experimental demonstration of the effect of pre-polarization by dc-background light is shown in Fig. 5. The experiment was made with *Rps. viridis* whole cells.

It is worth mentioning that the negative amplitudes in the presence of background light may be larger than the positive ones in its absence. This is true only when the absorbance of the sample is higher at the wavelength of the dc-light than at the wavelength of the flash.

*Time courses*

In the foregoing it was implicitly assumed that neither the electrogenic reaction nor the flash duration was time-resolved by the instrumental detection system. In the following, the case will be considered in which the detection electronics is sufficiently fast as to time-resolve the flash duration. However, the charge separation kinetics shall still be faster than the flash duration.

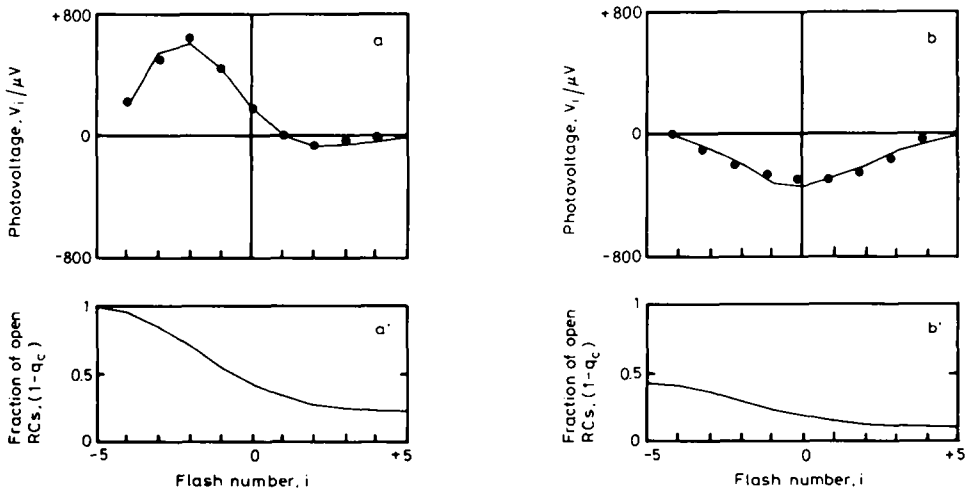


Fig. 5. The effect of prepolarization by dc-light on the peak photovoltages from *Rps. viridis* whole cells evoked by a train of 30 ps flashes (mode-locked Nd-YAG at 532 nm; total energy of train 1020 $\mu J \cdot cm^{-2}$). (a) Without background light. (b) With white background light. The progressive closure of RCs is shown in a' and b', respectively. The light-gradient fit parameters were: $\Gamma \cdot \sigma(532) \cdot N = 7.8 \cdot 10^{-17} cm^2$; $T_{eff} = 0.61$ and $T_{eff}(\lambda) = 0.34$. The prepolarization of the dc-light was $I_o/[k/(\Gamma \cdot \sigma(\lambda) \cdot N)] = 2.4$. The fit parameters of the theory of exciton dynamics [13] were: $\alpha = 1$ and $k_o/k_c = 1.35$.

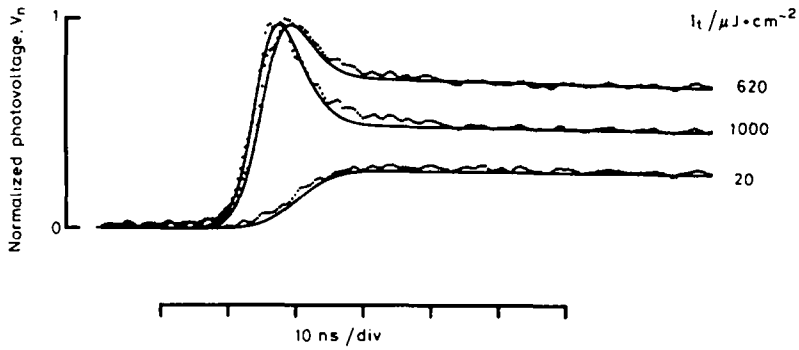


Fig. 6. Dependence of the time-course of the normalized light-gradient photovoltage due to PS I in stacked chloroplasts on the energy of a 12-ns flash from a Q-switched Nd-YAG laser at 532 nm (no annihilation, $k_o/k_c = 1$). The absorbance of the sample was $A_{532} = 0.2$. The total flash energy, $I_t$, follows from $I_t = \int I(t)$. The experimental traces were deconvoluted with an exponential decay time constant of $\tau = 80$ ns to account for the electrolytic self-discharge of the cell capacitor and ion fluxes around the chloroplasts. The fits were made assuming a flash with a Gaussian shape of $\tau_G = 10$ ns, an effective transmission of $T_{eff} = 0.08$ (according to Fig. 2), an antenna size of $N = 300$, a quantum yield of $\Gamma = 0.95$, and an absorption cross-section of $\sigma = 1.6 \cdot 10^{-17}$ cm$^2$.

These are the conditions in experiments with $Q$-switch flashes (about 12 ns) and high impedance detection (about 500 MHz bandwidth).

The photon density in these flashes is sufficiently small as to neglect singlet-singlet annihilation. A quenching by singlet-triplet annihilation shall not be considered here. (This is probably a good approximation if the excitation energy does not much exceed $z = 3$.) For the case that all RCs are open before the flash and without energy transfer ($k_o/k_c = 1$ or separate units), the time course of the photovoltage can be derived from Eqns. 2a and 9:

$$V(t) = V_o \cdot \int_0^t \{[\exp(-\Gamma \cdot z(\tau))] \cdot \Gamma \cdot \sigma \cdot N \cdot I(\tau) - [\exp(-T_{eff} \cdot \Gamma \cdot z(\tau))] \cdot \Gamma \cdot \sigma \cdot N \cdot T_{eff} \cdot I(\tau)\} \cdot d\tau \quad (16)$$

where

$$z(\tau) = \sigma \cdot N \cdot \int_0^\tau I(\tau') \cdot d\tau' \quad (17)$$

and $I(t)$ is the time-course of the intensity of the flash.

As an example, we use Eqn. 16 to fit time-courses of the PS I photovoltage from pea chloroplasts evoked by a 12 ns pulse from a $Q$-switched Nd-YAG laser. $I(t)$ is approximated by a Gaussian function.

In Fig. 6 are shown for three different energies the measured photovoltage and the theoretical fits using the same parameters, $V_o$ and $\Gamma \cdot \sigma \cdot N$, for all curves. As can be seen, at low energy the time-course of the photovoltage follows closely the integrated photon flux (error function), whereas with increasing energy the photovoltage rises faster and passes through a maximum.

The good general agreement between experiment and theoretical prediction demonstrates further the adequacy of the present light-gradient theory. The small deviations between the experimental and theoretical curves are due to a non-Gaussian shape of the laser flash. (Note that all photovoltage amplitudes in this and the accompanying articles refer to the level that remains after the flash.)

*Analysis with energy transfer between photosynthetic units*

The time-course of the photovoltage was calculated for vanishing energy transfer between PSUs ($k_o = k_c$), as probably is the case in PS I. However, in PS II and purple bacteria several units are known to be connected, forming a domain in which energy from closed RCs can migrate to open RCs, as the quenching rate constant for closed RCs is smaller than that for open ones, $k_o > k_c$.

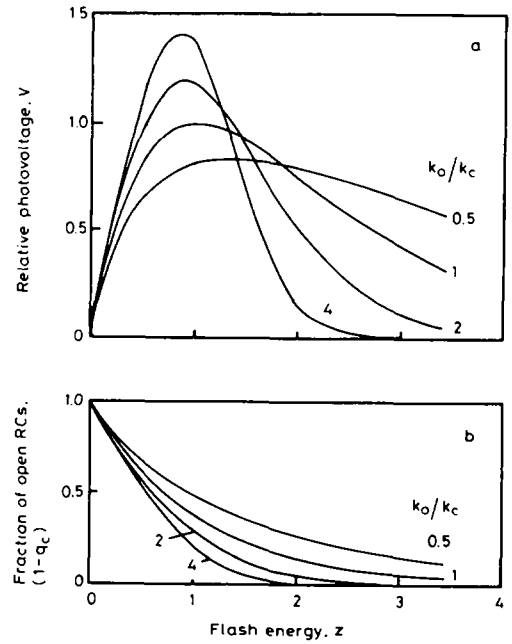


Fig. 7. Saturation curves without annihilation for optically thin samples ($T_{eff} = 0.9$) at different degrees of energy transfer assuming a lake model. The calculation was made with the identical set of parameters except $k_o/k_c$. The common amplitude for all curves was chosen as to yield a maximal photovoltage of one for $k_o/k_c = 1$.

If this connectivity is not taken into account this may lead to systematic errors in the evaluation of the parameters. In the following we estimated this effect for long (i.e., $Q$-switch) flashes where annihilation can be neglected.

Fig. 7 shows the effect of different ratios of the quenching rate constant for open and closed RCs, $k_o/k_c$, on the saturation curve (i.e., energy dependence) of the 'remaining' photovoltage (a) and on the fraction of open RCs (b). The calculation was made by introducing Eqn. 11 of Ref. 13 into Eqn. 9 and setting the 'annihilation constant' $\alpha = 0$. The calculation was made for optically thin samples and organelles.

With reference to the case of $k_o/k_c = 1$ or separate units (Fig. 7), an increasing energy transfer, $k_o/k_c > 1$, causes larger maximal photovoltages, a shift of the maximum towards lower energies, and a steeper decline at high energies. However, the initial slope at the lowest energies remains the same. Hence, the shape of the energy dependence of the light-gradient photovoltage contains information on the connectivity parameter, $k_o/k_c$.

*Other aspects*

Photoelectric measurements with the highest time resolution (presently 30 ps) are capable of resolving the trapping kinetics [9–12]. This case can be treated by considering the kinetics of the light-gradient photovoltage to be composed of the time-course of the concentration of electrogenic states in the upper and lower membrane. Hence, the fraction of closed RCs in Eqns. 1, 3 and 9 must be written as a function of time, $\Delta q_{c,1}(t)$ and $\Delta q_{c,2}(t)$. These time-courses follow from the specific kinetic model applied.

A further complication arises when two electrogenic steps of the primary charge separation fall into the resolvable kinetics. Then also these kinetics have to be put into the molecular model.

The present theory of the light-gradient can be used to characterize PSUs/domains in a quantitative manner. The quantities that are involved and that may become accessible even at low time resolution are: the unit size ($N$), the photosynthetic quantum yield ($\Gamma$), the ratio of quenching states ($k_o/k_c$), and a parameter that describes the competition between trapping and annihilation ($\alpha$). To extract by photovoltage measurements alone all four parameters, several sets of independent experiments are needed, as are, for example, saturation curves with long and short single flashes (without and with annihilation) and yield measurements by multiple flashes (picosecond trains) in which the fraction of open RCs varies. To reduce the number of parameters as well as to narrow their numerical values, it is most helpful to make a fluorescence yield measurement from which the ratio of the quenching rate constants, $k_o/k_c$, is determined.

The principles outlined here are of general relevance, since they may be involved in spectroscopic measurements when optically dense organelles are under investigation. For example, when optically dense organelles in flash spectroscopic measurements are excited perpendicular to the measuring beam, up to 50% of the RCs may be missed by the excitation and in turn also be missed at the detection side (Fig. 3).

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