Laser Chem. 1986, Vol. 6, pp. 233–252
0278-6273/86/0604-0233$12.00/0

Printed in Great Britain

PRIMARY PROCESSES OF PHOTOSYNTHESIS STUDIED BY FLUORESCENCE SPECTROSCOPY METHODS

A. FREIBERG
Institute of Physics
Estonian SSR Acad. Sci.
Tartu 202400

1. INTRODUCTION

Photosynthesis is one of the superior accomplishments in the evolution of Nature. It is thanks to photosynthesis that the human race ixists and has reached its present stage of development. The amount of energy annually utilized by photosynthesizing organisms ($\sim 3 \cdot 10^{21}$ J) exceeds by about an order the amount of energy consumed by mankind[1] ($\sim 4 \cdot 10^{20}$ J in 1980). These global as well as some purely cognitive factors urge the elucidation of photosynthesis mecanisms

The utter complexity of the problem has necessitated an incoporation in the photosynthesis science a number of other sciences, such as physics, chemistry, biology, and some interdisciplinary sciences as well. Exact physical methods have proved their actuality in a number of cases. Among them a more informative one is the fluorescence spectroscopy method. Its use is based on a circumstance that after absorbing the light by photsynthesizing organisms, because of the interaction with the electromagnetic field, a part of the absorbed energy is indispensably emitted in the form of photons (in the particular case of singlet-singlet transition it is fluorescence). The fluorescence intensity in each moment of time is proportional to the concentration of excited

species. The light-excited molecule in a photosynthetic unit (PSU) (the definition of PSU, see part 3 of this paper) can lose energy via various channels like light quantum emission, radiationless dissipation at the expense of nonadiabaticity of electronic states or in the process of transferring energy to other molecules. The "aim" of the so-called primary processes of photosynthesis is to bring the absorbed energy with possibly smaller losses (i.e. as quickly as possible) to a special molecular complex, a photochemical reaction centre, where it is stabilized for a sufficiently long time (for a fraction of second). Fluorescence decay after a short pulse excitation gives, in the real time scale information on the balance between different relaxation channels and allows the efficiency of the so-called photochemical relaxation channel, which is the most important among the others, to be measured directly.

The duration time of primary processes, characteristically less than 10^{-8} s, asserts high demands to the experimental technique, particularly excitation sources, spectral devices, recording systems and data processing. To describe thoroughly the situation by means of fluorescence a full set of its parameters, such as decay time τ, yield ϕ, polarization, excitation and emission spectra, etc., to be measured.

This paper does not aim at a comprehensive survey of the fluorescence studies of primary processes because of its limited content. The rather that the relevant surveys can be found in literature[3-10]. Instead we shall focus our attention on some actual problems related with the study of energy transfer processes by fluorescence kinetics of basic photosynthetic pigments chlorophyll (Chl) and bacteriochlorophyll (BChl) in vivo. Among these a special consideration is given to methodological problems of picosecond measurements, as a number of inconsistent (or seemingly inconsist-

ent) experimental data have appeared recently. To introduce the subject we should immediately point at an important methodological evidence. The τ of the emission of chloroplasts and chromatophores (and also their fragments) is known to be a function of several parameters: $\tau = \tau(A,B,C,D,..?)$, where A is the sample and its state (the following aspects are of special importance here: method of preparation, dilution medium, temperature, state of the photochemical apparatus, etc.); B are the excitation conditions (intensity, duration, spectral composition, excitation homogeneity, etc.); C are the recording conditions (spectral composition of the radiation recorded, presence of spurious radiation, etc.); D is the method of recording (meaning that systematic errors and uncertainty of results peculiar to various methods, vary)[2]. Unfortunately, not always the whole set of factors listed is duely taken into account. Then some experimental results obtained in the Institute of Physics of the Estonian Academy of Sciences (in collaboration with Moscow State Univerity) on the primary processes of photosynthesis in bacteria are discussed. Our approach proceeds from the desire to study most important natural phenomena in the conditions possibly closer to the natural ones. A spontaneous (in contrast to a stimulated) spectral and time response of the sample to a weak (average intensity $\lesssim$ the average day-time solar radiation intensity on the Earth's surface) picosecond excitation at the ambient medium temperature is recorded.

2. EXPERIMENTAL METHODS

Almost all methods used in pico-nanosecond nonstationary fluorescense spectroscopy can be distinguished by their high (10^4-10^8 Hz) and low (0.1-10 Hz) repetition rates of operation cycles. The former case allows the use of efficient signal averaging and noise suppression methods, in the latter case, the insufficient signal--to-noise ratio is, as a rule, compensated by the growth of excita-

tion power (proceeding from a rough calculation that the excitation energy average in macroscopic time lapse be preserved).

In high repetition rate mode operate phase fluorometers (the present-time limit resolution 10-50 ps), single-photon counting systems (50-100 ps), synchroscan streak camera systems (1-5 ps), and also some systems based on up-conversion technique and fluorescence correlation function measurements (in these methods time resolution is determined by the excitation pulse duration). For high-frequency excitation source serve the electrical spark discharge, various continuous-light sources whose light flux is chopped by an external modulator (mainly in phase fluorometers) and, since 1969[11], continuous-wave (CW) mode-locked picosecond lasers (HeNe, Ar^+, Kr^+, and dye lasers), which by the present moment are in the most widely extended use. (Regardless the sufficiently high time resolution of phase fluorometers and their monentuous processes it should be noted that their results are reliable and unambiguous only in case of single exponential decay. Therefore, more direct measurement techniques are to be preferred.)

With low repetition rates work fluorometers based on solid state mode-locked picosecond lasers and optical parametric oscillators, and also on nitrogen- or excimer-laser-pumped dye lasers. As emission registers high-speed photodetectors together with oscillographs, the Kerr cells, and traditional single-shot streak cameras are used. Their common drawback is a relatively low dynamic range, also difficulties in synchronizing the moment of detection with excitation. Nevertheless, the introduction of streak camera systems in 1975[12-15] denotes a big onward leap in the development of the experimental technique for fluorescence studies of the primary processes in photosynthesis. Contemporary streak cameras allow the whole fluorescence decay curve to be recorded within a single laser shot, they have high sensitivity and excellent

time resolution (~0.5 ps).

Experiment shows[16] that the combination of picosecond time resolution with high spectral resolution ($\lesssim$1 nm) meats great difficulties, which, as shown in[17], to a certain limit $\Delta\omega\Delta t \approx 1$ ($\Delta\omega$ and Δt resp. the spectral and time resolution) is reduced to the technical perfectness of separate units. In real spectral devices the area of the resolution element $\Delta\omega\Delta t$ exceeds the theoretical (diffraction) limit by several orders because of the presence of inescapable aberrations and other faults. This is immaterial when low-spectral-resolution devices (prism and also interference filters, etc.) are used, as then the time resolution is limited by the detector response. In case a better spectral resolution is needed, i.e. when grating spectrometers are used, the excess broadening of their time response (up to 10^3 ps) is removable, as shown in[17], by using double monochromatization in subtractive dispersion scheme. In conformity with[18], if the product of experimental $\Delta\omega$ and Δt tends to the theoretical limit, a spectrochronogram is measured. It is possible to demonstrate that the information content of the spectrochronogram is maximal.

An approach based on picosecond spectrochronography was used in investigating the fluorescence kinetics of pigments in vivo in 1981[2]. In a standard version our picosecond spectrochronograph consists of three basic parts (Figure 1).

(1) a picosecond CW (pulse repetition rate 82 MHz) synchronously-pumped dye laser (tuning range 685-850 nm operating on three dyes: oxazine 1, oxazine 750, and styrile 9; maximum average power up to 300 mW; pulse duration $\lesssim$ 3 ps). To obtain excitation in the near UV region of 345-420 nm the second harmonic generation in $LiIO_3$ crystal is used with the conversion efficiency up to 1%;
(2) subtractive dispersion double monochromator mounted from two

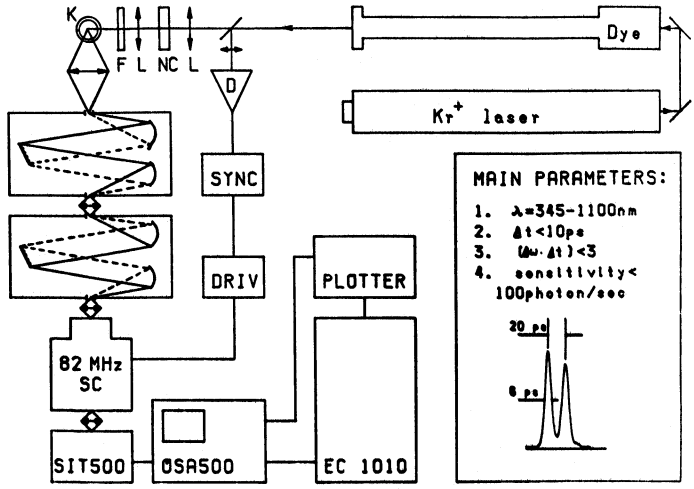

FIGURE 1 Picosecond spectrochronograph for investigating nonstationary spectra[19]. In the insert two 3 ps laser pulses mutually shifted by 20 ps, measured by means of a spectrochronograph, are depicted.

high-luminosity (1:2.5) single-grating monochromators (reverse dispersion 2 nm/mm with the grating 600 grooves/mm);

(3) a recording system based on a streak camera in synchroscan operation mode, which records the fluorescence excited by each laser pulse. The accumulation of successive traces on the streak--camera screens enables the investigation of extra weak signals with high dynamic range (~3 orders of magnitude). A single count level of the SIT vidicon videosignal from the streak camera screen is provided by a flux of 100-1000 incident photons on the streak camera photocathode depending on the type of the cathode used. High precision of synchronization by laser light pulses[19] ensures the overall time resolution of the spectrochronograph 1-5 ps. For collecting and operative processing of a huge mass of data the spectrochronograph is connested to a central computer complex.

It is to the purpose here to compare, though briefly, the fluorescence method with another equally widespread absorption method. We would like to underline three essential aspects:
(1) in a differential absorption method by means of a weak probing beam the change in the optical density of the sample as a result of interaction with a comparatively powerful pumping pulse is measured

$$\Delta D(t) = \lg(I_o^c/I_o) - \lg(I^c(t)/I(t)) = \lg(I(t)I_o^c/I^c(t)I_o)$$

where I_o and I_o^c are the probing beam intensities in the channels of measurement and comparison, respectively, before the arrival of the pumping pulse; $I(t)$ and $I^c(t)$ are the same after pumping. The measurement error depends on the relative errors of measuring all the four values and it cannot be made infinitely small. The mean square error of $\Delta D(t)$ is

$$\Delta[\Delta D(t)] = [(\Delta I^c(t)/I^c(t))^2 + (\Delta I(t)/I(t))^2 + (\Delta I_o^c/I_o^c)^2 + (\Delta I_o/I_o)^2]^{1/2}.$$

It is, as a rule, larger than that on measuring an essentially background-free fluorescence singal, the rather that the relative light-induced absorption changes in photosynthetic systems are $\gtrsim 100$ times less than relative fluorescent changes;
(2) the relaxation investigation method based on absorption measurement requires, unlike the fluorescence method, an application of complementary probing actions, which in principle can distort the course of the processes studied. In this sense one can refer even to a forced character of the system response;
(3) due to the population of the excited states by the pumping pulse, the time-dependent absorption from these always exists. It is difficult to decide, however, at what relaxation stage and between which states the absorption occurs. These difficulties become explicit if, instead of the common scheme of energy levels, a more realistic picture of potential energy curves is used. In contrast,

the interpretation of the spontaneous fluorescence signal, as a rule, is unambiguous, including the interpretation of weak transient spectral components emitted before the thermal equilibrium has been established[20].

The statements on literature[21,22] as if fluorescence does not render adequately the processes occurring in PSU or as if its data are interpreted incorrectly, must be considered an exaggeration. On the other hand, the absorption method has its indisputable advantages over the fluorescence one, as it is correctly referred to in[21,22]. The only constructive approach here lies joining the strong sides of both methods to solve such difficult problem like elucidating the details of the primary processes in photosynthesis.

3. ENERGY TRANSFER IN PHOTOSYNTHESIS

At present the following construction and functioning model of PSU is generally accepted. The unit contains at least two types of Chl-protein (or BChl-protein) complexes. The first-type complexes are the so-called light-harvesting antenna complexes (LA). These serve to collect light and transfer excitations to the second-type complexes - reaction centers (RC) where a multistage rediationless energy transformation occurs in the form of charge separation in RC. In this scheme the RC appears as a trapping center for excitations in LA. The typical ratio of the number of Chl molecules in LA and the number of RC in bacterial photosynthesis is 50 and 100-400, in the photosynthesis of higher plants. It is shown that the Chl molecules in RC and LA are identical and therefore their ability to perform various functions should rather be explained by different arrangement of Chl molecules in pigment-protein complexes and by the peculiarities of interaction between the complexes. This explains also the absence of fluorescence self-quenching due to high concentration of Chl *in vivo* molecules ($\sim 0.1 M \simeq 6 \cdot 10^{20}$ cm^{-3})[4]. Within the given simplifications, which are more justified on

case of bacterial photosynthesis, the energy transfer problem divides into two: energy transfer along LA to RC and its trapping on RC. In more realistic models the heterogeneity and anisotropy of the antenna are to be taken into account, also, the matter that in reality various PSU are connected at the level of energy transfer, forming the so-called domains (see, e.g. [23-26] and below), and other complicating factors[27].

The common viewpoint is also that the absorbed energy in LA is localized on separate pigment molecules (or small molecular aggregates) and is transferred in the form of singlet excitation via inductive resonance[23-28]. Indeed, one can hardly expect that in a disordered or only partially arranged conglomerate of molecular complexes, yet more at room temperature, there could exist non-localized excitations and, consequently, an essentially coherent energy transfer[29,30]. In conformity with[30,31] the excitation migration along LA can be considered coherent only during the phase memory time $1/\Gamma$, where Γ is the width of the pigment molecule spectrum (in the case of BChl it is $\sim 10^{-14}$ s at room temperature).

In incoherent approximation and at low level of excitation intensity I(t) the probability of the i-th molecule excitation ρ_i is determined by the solution of a system of kinetic equations (which inexplicitly contain also equations for the trapping centre):

$$\frac{\partial \rho_i}{\partial t} = -\frac{\rho_i}{\tau_{oi}} - \sum_{j=0}^{N} (F_{ji}\rho_i - F_{ij}\rho_j) + I_i(t) \qquad (1)$$

where $1/\tau_{oi}$ is the monomolecular decay rate of i molecule, that takes into account fluorescence and intramolecular conversion; F_{ji} is the rate of energy transfer from i molecule to j molecule (usually only the transfer between nearest neighbours is taken into account); N is the number of molecules (compexes) in PSU. In the approximation of weak dipole-dipole coupling

$$F_{ij} = (1/\tau_{oi})(\bar{R}_o/R_{ij})^6 \qquad (2)$$

where $\bar{R}_o$ is the distance between the energy donor and acceptor, where the transfer rate equals $1/\tau_{oi}$. The critical distance $\bar{R}_o$ is related with the overlapping of the donor fluorescence spectrum $F(\omega)$ and the acceptor absorption spectrum with the cross-section $\sigma(\omega)$ as follows[32]:

$$\bar{R}_o = [3\phi\alpha c^4/(4\pi\omega^4 n_o) \int F(\omega)\sigma(\omega)d\omega]^{1/6}$$

α is the multiplier allowing for the anisotropy of dipolar interaction, n_o is the refration index of the solvent , and c the light velocity. In more exact version of the theory possibility of energy transfer from the vibrationally non-equilibrium excited electronic state is also taken into account[33] and then, instead of $F(\omega)$, the formula of $\bar{R}_o$ should include the whole spectrum of the donor resonance secondary emission which, besides the ordinary luminescence, contains also hot luminescence and resonant Raman scattering.

System (1) is not solvable in its general form. It depends on the arrangement of LA as well as on initial excitation conditions. Therefore one is usually satisfied with computer calculations of various definite models[27,34,35]. Yet, in a particular case of a regular lattice an analytical solution of system (1) exists, that helps one to a deeper understanding of the processes under investigation. The soulution is given in the terms of the effective lifetime of the excitation T and of the full probability P(t) that excitation is still in LA (and not trapped by RC or decayed by other reasons)[36,37].

$$T = \int_0^\infty P(t)dt \qquad (3)$$

$$P(t) = \sum_m^{N-1} C_m \exp[-(\gamma_m + 1/\tau_{om})t] \qquad (4)$$

γ_m and C_m depend on F_{ij}, C_m depends also on initial excitation conditions. Qualitatively, the time T is the sum of two times: the

average time t_a for excitation to reach at RC, if it was generated somewhere in the antenna, and the time t_t, if RC was excited directly[36-38].

$$T \simeq \langle n \rangle / \langle F \rangle + [1 + (F_D/F_T)(N-1)]/k_p \qquad (5)$$

where $\langle n \rangle$ is the average number of jumps in LA (in the case of two-dimentional quadratic lattices, it is $N \ln N/\pi + 0.2N$ [39]); $\langle F \rangle$ is the average over LA transfer rate constant, F_D/F_T is the ratio of some average rate constants of excitation detrapping and trapping, respectively, at the RC, k_p is the charge separation rate constant in RC. It is taken here that in the conditions of photosynthesis $\tau_o \gg T$, that is usually well fulfilled. In the case of $t_a \gg t_t$ one can speak about the transfer where its rate is limited by the excitation diffusion in LA, in the opposite case, about the transfer that is limited by excitation trapping on RC. As maintains the author of[37], the fluorescence decay law in the case of a regular matrix arrangement is practically always exponential and the detection of nonexponentiality indicates deviations from regularity. The opposite statement is certainly not valid.

The above said immediately suggests an experimental scheme to elucidate the limiting stages of the primary energy transfer rate in photosynthesis. For that a successive selective excitation of LA and RC is needed and the obtained lifetimes should be compared[26,36,40].

So far nothing has been said about the influence of the RC state upon fluorescence. Until recently for bacteria the dependence on the RC state was quantitatively established only for the fluorescence yield[23]. The ϕ and τ dependences on the RC state can be explained by the following simplified scheme of processes in RC

$$DPQ \xrightarrow{h\nu} DP^*Q \rightarrow DP^+Q^- \rightarrow D^+PQ^-$$

where P are the photochemically active RC molecules (considered to be $(BChl)_2$ in bacteria); P* is the photoexcitated state of P; D

and Q are respectively the donor and acceptor of the electron. In the so-called closed RC state, P^+Q^-, the fluorescence yield exceeds several times (approaching ~5%) that in the open or photochemically active state, PQ, whereas, as shows experiment, the dependence in the share of closed centres P^+/P_o in the amount of the culture studied is smooth. The definite form of this dependence, however, is determined by the structure and build-up of the pigment system as well as by the details of energy transfer process. The influence of the pigment system build-up can be elucidated by considering two simple physical models: (1) different PSU in chromatophores or chloroplasts do not exchange mutually energy; (2) several PSU constitute a unit system at the level of energy transfer (a multicentre or lake model). In the first case, the fluorescence yield is a linearly growing function of P^+/P_o, $\phi = \phi_o + aP^+/P_o$, where ϕ_o corresponds to the fluorescence yield in case all RC are in the state PQ and a is a constant. In the case of heterogeneous PSU with various number of LA molecules on RC, the dependence is superlinear[41]. In the simplest version the fluorescence decay is a two-component one, where the short time corresponds to the PSU emission with RC in the PQ state and the long one, in the P^+Q^- state. In the second case, a hyperbolic equation $\phi = a/(1 - pP^+/P_o)$ [23,41] is observed, where p is a constant which depends on k_p. The fluorescence decay in a trivial version is single-exponential and the course of τ is proportional to that of ϕ. In formula (5) the change of RC redox state shows itself through the change of N. (In a multicenter case N is redetermined as a number of LA molecules per RC in PQ state.)

To conclude this chapter it is to be noted that PSU represents a model system with essentially nonlinear properties, which appear already at a rather delicate level of illumination ($\sim 10^{-2}$-10^{-1} W/m^2 instead of usual MW/m^2 in physics).

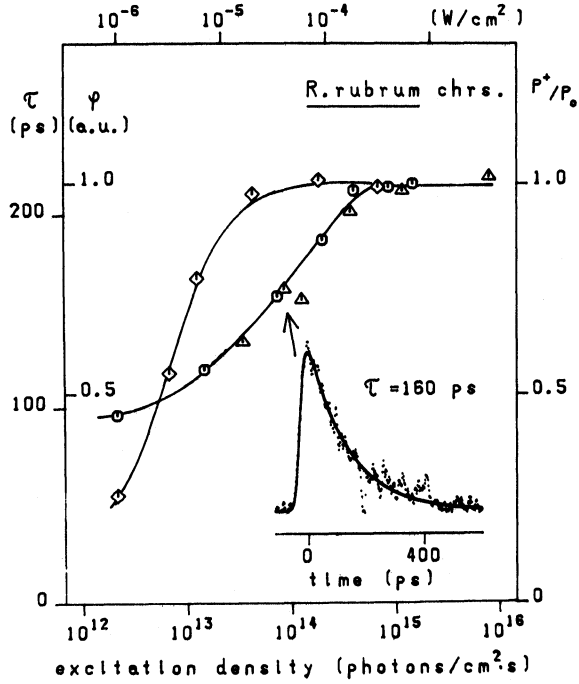

FIGURE 2 R. rubrum chromatophotes fluorescence lifetime τ (Δ-Δ-Δ) and yield ϕ (o-o-o) as a function of excitation density. (◇-◇-◇) - the normalized fraction of photooxidized RC, P^+/P_o. In the insert: example of fluorescence kinetics at excitation density of ~10^{14} phot/cm^2·s [42].

4. INVESTIGATION OF BACTERIAL PHOTOSYNTHESIS

In the light of what is said above the dependence of picosecond fluorescence kinetics on the RC state of cells and chromatophores of the purple bacteria Rhodo spirillum rubrum and Rhodopseudomonas sphaeroides was investigated[26,40,42]. The measurements, performed by the spectrochronograph described in part 2 (Figure 1), were carried out with the average excitation density from 10^{13} phot/cm^2s to 10^{17} phot/cm^2s. The maximum energy of each pulse was 10^{-14} -

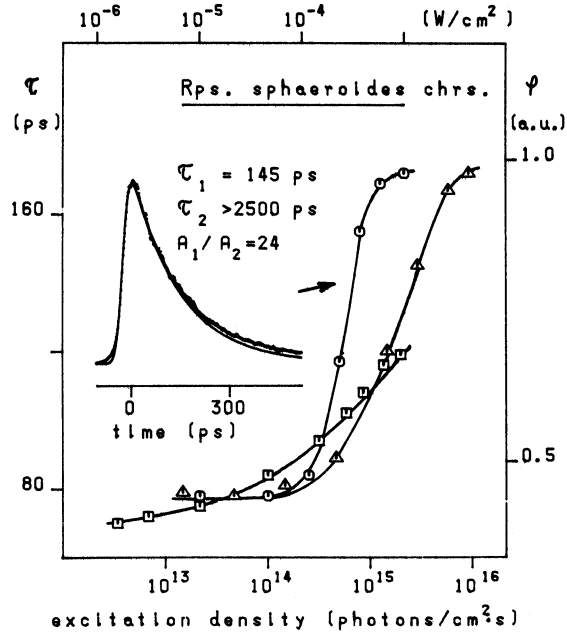

FIGURE 3 The same (excluding P^+/P_o curve) as in Figure 2 for Rps. sphaeroides chromatophores with a $5 \cdot 10^{-5}$ M TMPD addition[42]. The curve (□-□-□) represents ϕ for chromatophores without additions.

10^{-10} J/cm^2, that fully excluded undesirable nonlinear excitation effects. In this intensity interval τ grows together with excitation intensity approximately from 60 ps to 210 ps (Figures 2 and 3). The fluorescence yield for all the bacteria studied, measured on continuous as well as on picosecond excitation, changed analogously. Parallel measurements of the ratio of photooxidized RC show that the increase of fluorescence is related with the RC passing into a closed, photooxidized state. The fluorescence decay in chemically pure chromatophores without any chemical additions was well approximated with a single exponential component at low as well as at saturating intensities. In the emission of intact cells and chromatophores with reductive substanc-

es, a nanosesond component appeared when a fraction of RC passed into the state PQ^-. At low excitation level the amplitude of that component amounted to several % of that of the picosecond component and grew by 2-3 times with the growth of exciting light density, while its lifetime probably remained unchanged. Thus, an explicit dependence of τ on the light intensity and its parallel behaviour with ϕ have been detected, that, together with the characteristic dependence of τ and ϕ on RC state, suggest the multicentricity of the build-up of the photosynthetic bacterial system.

These data allow numerical estimations of a number of important physical parameters. Thus, an estimation of the maximum charge separation quantum yield in RC $\phi_p^{max} = (\tau_a - \tau^{min})/\tau_a$ (τ_a is the excitation lifetime in isolated antenna complexes) gives 0.95±0.02 [42]. By assuming that RC is an absolute trap for excitations ($k_p \to \infty$), from formula (5) we obtain an estimation of the excitation stay-time on a BChl molecule of LA in a two-dimensional case, $(\langle F \rangle)^{-1} \gtrsim$ 1 ps and, respectively from formula (2), an estimation of the average intermolecular distance R,16-20 Å. Further, it is possible to calculate the coefficient of isotrópic excitation diffusion $D = R^2\langle F \rangle/4 \simeq 8 \cdot 10^{-3}$ cm^2/s and the diffusion path-length $L = (D\tau)^{1/2}$ $\simeq$ 70 Å [42]. The latter value agrees poorly with the above conclusion about the multicentre arrangement of the bacterial PSU. On these grounds the validity of the assumption of RC being an absolute trap is to be revised [40]. If the observed lifetime is limited by photoconversion rate in RC (the second term prevails in formula (5)) and not by diffusion along LA, all the constants obtain quite reasonable values $(k_p)^{-1} \simeq$ 3 ps, $(\langle F \rangle)^{-1} \simeq$ 0.1-0.5 ps, R ≃ 12-15 Å, $D \simeq 4 \cdot 10^{-2}$ cm^2/s, L ≃ 160 Å. Numerical values of L and R allow one to infer that in the domain 10-13 PSU may be connected on the level of energy transfer. Naturally, these estimations are too crude to make any far-reaching conclusions. Nevertheless, proceeding

from another type of experiments on R. rubrum, the authors of [43] reached qualitatively the same conclusion (the domain includes 14-17 PSU). It is interesting to note that analogous results have been obtained for chloroplasts by investigating excitation annihilation[44,45].

Some remarks on the differences between the single-pulse(SP) and CW picosecond excitation methods in the case of a multicentre PSU organization should be made[40,43]. In a SP mode, as a rule, it is difficult to work with the densities of $<10^{13}$-10^{14} phot/cm^2 per pulse. Considering the minimum antenna lifetime of 60 ps, all these quanta are virtually simultaneously absorbed, and, for instance, with the Chl (BChl) absorption cross-section of $\sim 10^{-16}$ cm^2, one molecule out of 1000 or 100 molecules, respectively, is excited. This means that even with minimum SP excitation densities there is at least one excitation per domain and on further density increase an appearance of nonlinear excitation interaction effects can be expected. It also becomes clear from here that the commonly used weak excitation criterion - one quantum per RC - does not suffice and, as a minimum, one must stick to a more strict condition - one quantum per domain. In our CW version, the average density of 10^{13}-10^{14} phot/cm^2·s makes only $\sim 10^5$-10^6 phot/cm^2 per pulse, that constitutes quite negligible excitation concentration in the domain and, consequently, an inappreciable probability of their meeting. However, as the pulse sequence period (~12 ns) is much less than the time of restoring RC into a state able to trap the next excitation, starting from a definite intensity, in full analogy with stationary excitation, there occurs an accumulation of RC in a closed state. This results in an observable growth and the subsequent saturation of τ and ϕ intensity dependences. The above-said is illustrated in Figure 4, where the expected dependences of τ

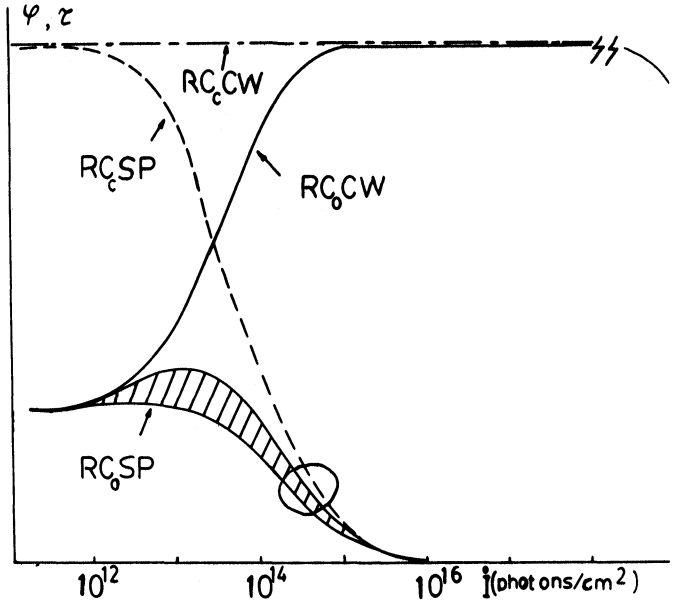

FIGURE 4 Model dependences of τ and ϕ on the incident light intensity. The initial state of RC(O - open, C - closed) and the picosecond excitation mode (SP - cingle pulse, CW - continuous-wave excitation) are denoted. The course of the curve in the striped area depends on the competition between the trapping on RC and annihilation. The latter inevitably prevails in the case of sufficiently high excitation densities. The course of ϕ under stationary excitation and in case of RC_O initial conditions corresponds to the curve RC_OCW. The approximate intensity scale is in phot/cm^2 per pulse for SP case and in phot /$cm^2 \cdot s$ for CW case.

and ϕ on the excitation nature and density are presented. Note that the measurements in the encircled area are rather insensitive to the RC state, that may explain the corresponding results of[22]. Due to the rather high density of SP excitation used there the influence of excited state absorption is also possible, causing the observed deformation of the absorption spectrum.

At high excitation frequency there is a danger of the triplete state accumulation and the following singlet-triplet annihilation (see, e.g.[8] and refernces therein). This should be highly important in the case of closed RC. No systematic investigations of this problem have been carried out yet. However, the fact that no decrease in fluorescence τ has been observed in R. rubrum chromatophotes up to average density of $\sim 5 \cdot 10^{18}$ phot/cm$^2 \cdot$s [40] suggests that it is negligible.

5. CONCLUSION

Our data as well as those of other authors are in qualitative agreement with the following simple model. Photons are absorbed by LA molecules and the excitations created reach RC in a very short time, ~1-10 ps. The irreversible trapping on RC, however, proceeds in 50-70 ps, since, figuratively speaking the excitation hits and jumps out of RC for several times. One may wonder what is the biophysical sense of such manifold detrapping? Calculations[35,46] show that the negative effect of detrapping on the photochemical yield is negligible and the only idea is that the Nature has probably failed to "design" a 100%-efficiency trap of the first hit. The rise in excitation intensity results in an ever-growing number of closed RC and, consequently, in an increase of excitation lifetime in the domain, where on the level of energy transfer about ten PSU have been connected. The present level of understanding of the problem is essentially due to the progress in experimental methods and tecnique.

The author is indebted to K. Rebane for his kind support and valuable advice, to A. Yu. Borisov and V. I. Godik for many useful discussions, to R. Avarmaa for reading and criticizing the manuscript, to V. I. Godik, K. Timpmann, and R. Tamkivi for cooperation in experiments and to T. Altukhova and E. Vaik for drawing up the manuscript.

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