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USE OF LASERS IN PHOTOPHYSICAL RESEARCH OF PHOTOSYNTHESIS

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During the past decade, extensive research has been carried out to utilize the unique characteristics of laser light. Mainly the high intensity, short pulse and small divergence of the laser beam have been made use of, and some of the results are briefly summarized here. The effect of the coherence of actinic light on the primary photochemical charge separation is discussed in some detail.

INTRODUCTION

A brief survey of the most recent reviews, monographs and congress reports on the rich and complex subject of the photophysics of photosynthesis reveals that the use of lasers has opened up new and highly exciting avenues in this fiels[1-5]. Many of the topics of current interest owe their existence to the application of laser techniques providing characteristics of actinic and measuring light which are not available with conventional light sources. Because of the vast number of relèvant papers, we shall confine ourselves to selected topics and to a reasonable amount of the available information.

1. ULTRAFAST AND MULTIPHOTONIC PROCESSES

The successful production of shorter light pulses and higher powers, especially after 1975, encouraged several laboratories to

carry out research into hitherto unexplored regions relating to primary events. With very short single exciting pulses, it is possible to study time-resolved kinetics of fluorescence rise time and decay (the dynamics of exciton transfer) and the dynamics of the early steps of charge separation. At very high exciting light intensities, a number of different non-linear phenomena appear, which depend among others on the pulse duration; an example is bimolecular exciton-exciton annihilation.

Let us consider a few examples. Fluorescence spectroscopic studies were surveyed quite recently[5]. Energy transport characteristics have been determined from fluorescence life time and quantum yield[6], and from delayed luminescence[7]. The use of shorte flashes led to successful study of the primary photochemical charge separation in photosystem-2, and to the establishment of the role of pheophytin-a as primary acceptor in the reaction center[8]. Photosystem-2 reactions have been studied by laser flash-induced 150 ns luminescence[9]. Since the processes in the reaction centers are very fast, 5-50 ps pulses should be used in order to prevent (or at least reduce) the change of the photochemical state of the centers during the flash excitation[10]. With very intense exciting pulses, a "photosynthetic reaction center parameter" has been suggested for discrimination between "photosynthetic" and "non-linear" regions[11]. The non-linear chlorophyll-a absorption has been found to depend on the organization of the chlorophyll-a[12]. The yields of both fluorescence and photochemical reaction products should depend on the excitation intensity[13]. It must be borne in mind that the effects of ultrafast and extremely intense pulses are not directly related to in vivo processes which occur with continuous excitation and moderate light intensity under physiological conditions.

2. RE-REDUCTION OF THE PRIMARY DONOR IN PHOTOSYSTEM-2 IN THE NANOSECOND TIME RANGE FROM ABSORPTION CHANGE

The very small divergence and effective focusing of the laser beam opened up the way for the study of very fast absorption changes. A brief description of the problem may be given. In recent years, sound evidence has accumulated in favor of the role of pheophytin-a (Pheo) as the electron acceptor between P_{680} (a special chlorophyll-a) and Q (a plastoquinone molecule)[8]. According to the new concept, the primary charge separation is $P_{680}Pheo \xrightarrow{h\nu} P_{680}^{+}Pheo^{-}$, followed by the re-reduction of P_{680}^{+} to P_{680}. The main phase of this process at physiological temperatures is in the ns time range. Until a few years ago there was only indirect evidence of the existence of the ns phase, based upon the hypothesis that chlorophyll-a fluorescence is quenched by P_{680}^{+}. It has long been known that the absorption spectrum of P_{680} depends on its redox state (its name is derived from the location of the absorption peak exhibiting maximum change on oxidation-reduction). Direct evidence of the ns re-reduction of P_{680}^{+} requires determination of the absorption kinetics at 680 nm in the ns time range, in contrast to the indirect evidence from fluorescence measurements. The absorption changes cannot be measured without the use of special lasers, for the following reasons. In order to obtain well-defined initial conditions, saturating actinic flashes of high intensity should be used, which lead to intense prompt fluorescence with maximum intensity at around 680 nm (and are also highly scattered by the sample), a region where the absorption changes are to be measured. No optical filtering is possible, and therefore the absorption changes cannot be measured in the ns time range. Even modulation of the measuring light allows measurements only in the μs range[13]. There is another maximum change in P_{680} absorption at around 820 nm. However, even in this spectral region ns time resolution cannot be

attained with a conventional measuring beam. Mathis and van Best[14] suggested the use of a CW-operated gallium aluminium arsenide injection laser as measuring light source. The very thin beam of this laser allows decrease of the solid angle of light collection by the photodetector, so that only a very small amount of fluorescence and scattered light reaches the detector. The (10 mW) measuring light does not disturb the dark adaptation of the sample, but its intensity is high enough to ensure the low photon noise level of the beam. The avalanche photodiode detector meets the requirements of the measurement: it has a high quantum efficiency (85% at 820 nm), wide-band amplification, low noise and a fast rise time, without a post-impulse tail. A block diagram of the apparatus (after[14]) is shown in Figure 1. The main phase of the re-reduction of P^{+}_{680} (with 30 ns half time) has been observed with this apparatus. A similar set-up was later used with repetitive actinic flashes and the results led to the assumption that the rate of reduction of P^{+}_{680} is different in the different S states of the O_2-evolving complex[15]. This assumption was corroborated by

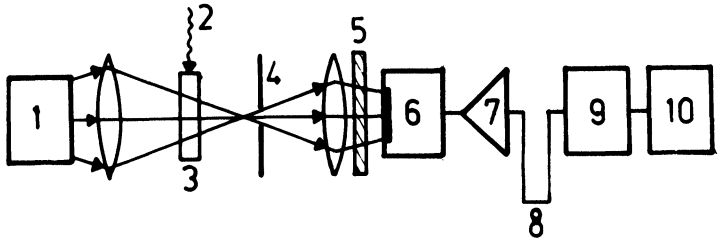

FIGURE 1 Apparatus for the measurement of absorption changes after laser flash excitation in the 20 ns - 10 μs time range (from[14]). 1 - laser diode (820 nm), 2 - exciting laser flash, 3 - cell, 4 - diaphragm, 5 - interference filter, 6 - avalanche photodiode, 7 - 30 MHz amplifier, 8 - delay line, 9 - digitizer, 10 - averager

the results of direct experiments[16]. For measurement of the dependence of the reduction kinetics on the number of actinic flashes, the signal to noise ratio and other characteristics were improved. The kinetics in the S_0 and S_1 states is monophasic (half time 20 ns), and in S_2 and S_3 states biphasic (half times 50 and 300ns). All these new measurements contributed to a better understanding of the function of the O_2-evolving system.

3. COHERENT EXCITATION AND THE UTILIZATION OF LIGHT ENERGY IN THE PHOTOSYNTHETIC PRIMARY PROCESSES

The most significant property of laser light is the coherence, since high intensity, a short pulse, a small devergence and a high monochromaticity can in principle be produced with conventional light sources (though with much difficulty in practice), whereas coherent light can never be. Any specific biological effect of laser light can therefore be attributed to the coherence. We decided to study the effect of the coherence of light on the utilization of light in photosynthesis[17]. We do not know of any other attempt to investigate the biological effects of coherence, and we therefore report our studies in some detail. We presume that the ability of a photosynthetic organism to differentiate betweer coherent and incoherent light is based upon the coherence-dependent photon distribution of actinic light. Since the pthotosynthetic units (PSU-s) (fairly well separated parts of the pigment system as concerns the uptake of light energy) cannot use more than one photon during their turnover time (about 1 ms), independently of the actual number of incident photons (i.e. the function of a PSU is similar to that of a photon counter), it can be assumed that the utilization of light depends on the photon statistics. For differences in photon statistics to be attained within the space--time volume determined by the size and the turnover time of the PSU (or the time of illumination, if this is shorter), we need

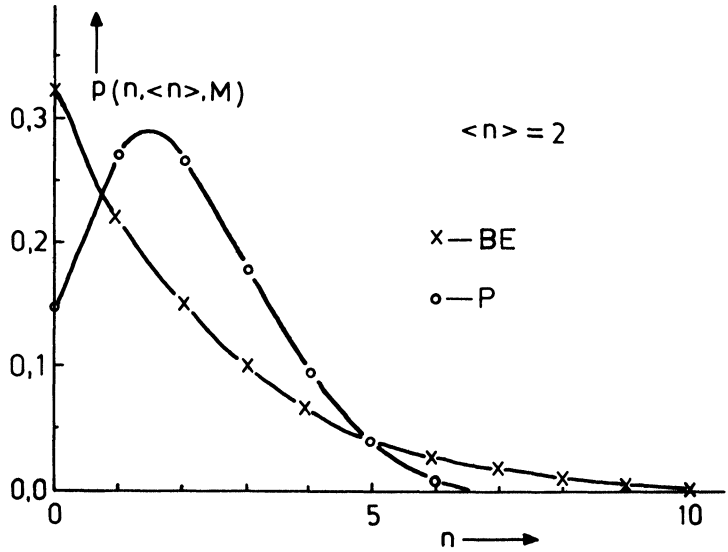

FIGURE 2 Probability of detection of n photons for M = 1 (BE distribution) and M = ∞ (P distribution). Average intensities are equal ($\langle n \rangle = 2$)

light with a phase cell (coherence volume) including this space--time volume. This condition is easily met for the spatial coherence, as the PSU is several orders of magnitude smaller than the volume relating to the spatial coherence. However, the temporal coherence can be ensured with lasers only.

a) Photon distribution and coherence. For a closer consideration of the conditions of finding the expected effect, we summarize the properties of photon distribution in relation to the coherence.

Mandel[18] states that the probability of finding n bosons (photons) over a number of phase cells M ($M \gg 1$) is

$$p(n,\langle n \rangle,M) = \frac{\Gamma(n + M)}{n!\,\Gamma(M)}\left(1 + \frac{M}{\langle n \rangle}\right)^{-n}\left(1 + \frac{\langle n \rangle}{M}\right)^{-M} \qquad (1)$$

where $\langle n \rangle$ is the expectation value (average number) of the number of photons. This equation gives Bose-Einstein (BE) distribution for $M = 1$, and Poisson (P) distribution for $M = \infty$. These distributions are shown for $\langle n \rangle = 2$ (for the same average intensity) in Figure 2. It is worth remembering that both for few and for many (e.g. $n = 0.1$ and 7) photons the probability is higher when $M = 1$. This fact is important in the photon counter model of the PSU.

A more practical form of equation (1) is obtained if time parameters are introduced. Bedard[19] gives

$$M = \frac{1}{2}\left(\frac{2T}{\tau_c}\right)^2 / \left[\frac{2T}{\tau_c} - 1 + \exp\left(-\frac{2T}{\tau_c}\right)\right] \qquad (2)$$

i.e. M depends on the exposure time T, and the coherence time τ_c. If this expression is substituted into equation (1), we obtain the relation between photon distributions and coherence.

To find the range of T/τ_c values in which the BE distribution goes over to P, the standard deviation of (1)

$$\sigma^2 = \langle n \rangle \left(1 + \frac{\langle n \rangle}{M}\right)$$

should be plotted versus T/τ_c. If $T/\tau_c \ll 1$, virtually BE distribution is observed whereas when $T/\tau_c \gg 1$ the distribution is virtually P. Figure 3 shows that the transition of the distribution from BE to P is practically complete when T/τ_c is varied within 1-2 orders of magnitude on either side of 1. In other words, a photodetector having a surface area smaller than the coherence area will "feel" BE or P photon distribution for coherent or incoherent light, respectively.

b) A photon counter model of the photosynthetic unit. For the sake of simplicity, let us suppose that there is no transfer of electronic excitation energy between the PSU-s. As mentioned earlier, a PSU cannot utilize more than one photon until its turnover time is completed. If it absorbs n photons, n-1 photons will be reemitted as fluorescence; if the probability of this event is p(n),

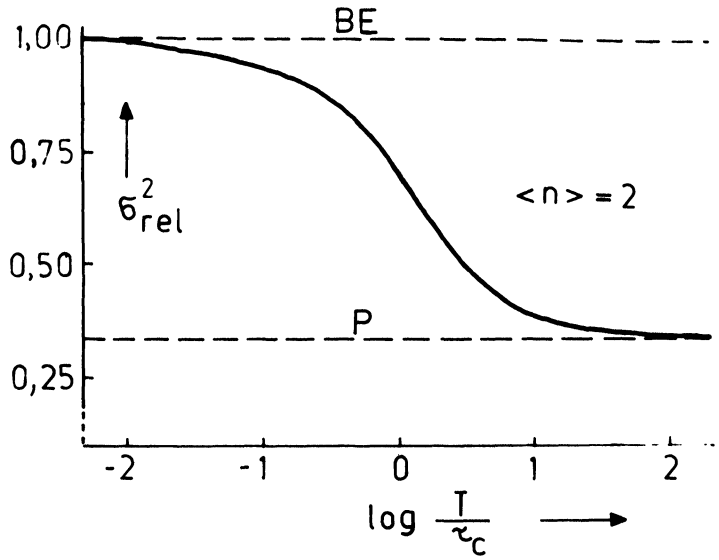

FIGURE 3 Dependence of the relative standard deviation of the distribution function (eq. (1)) on the ratio of the exposure time (T) and the coherence time (τ_c)

the total number of photons used in fluorescence is $F = \sum_{n=2}^{\infty}(n-1)p(n)$, or, after minor transformations, $F = p(0) + \langle n \rangle - 1$. The relative fluorescence yield is $\Delta\Phi = dF/d\langle n \rangle$. For a rectangular exciting light pulse, $\langle n \rangle = It$ (I is the rate of excitation in number of photons per unit time, while t denotes time). From equations (1) and (2), the time-dependence of the fluorescence yield (induction) can be obtained for BE and P statistics:

$$\Delta\Phi_P = 1 - \exp(-It) \quad \text{and} \quad \Delta\Phi_{BE} = 1 - 1/(1 + It)^2 \qquad (3a,3b)$$

As illustrated in Figure 4, the half rise time is about 60% greater and the initial slope is smaller for P statistics than for BE. A comparison of the complementary areas shows that in the range $It < 3$ the photosynthetic energy conservation is more effective in the case of P distribution.

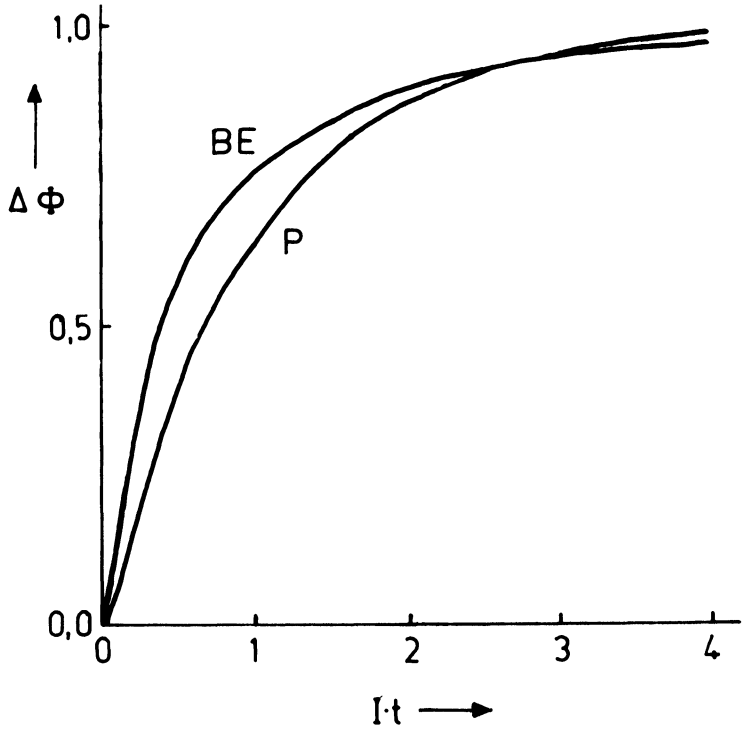

FIGURE 4 Fluorescence induction curves of the photosynthetic unit for Bose-Einstein and Poisson distributions of the exciting light. I is the rate of excitation; t denotes time.

If energy transfer occurs (and in fact it does), the excitons originating from the excess number of photons $F = p(0) + \langle n \rangle - 1$ can migrate to neighboring PSU-s with probability η. A PSU having c neighbors receives no excitons from them with a probability $(1 - \eta)^{c[p(0) + \langle n \rangle - 1]}$, since the individual misses are independent events. Receiving at least one exciton via energy transfer results in a charge separation only if the reaction center of the receiver PSU is open, and therefore the probability of a charge

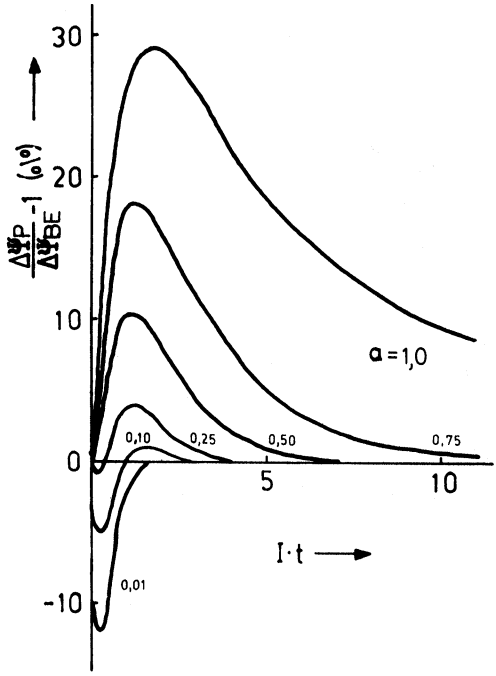

FIGURE 5 Relative photon utilization of the photosynthetic units for Bose-Einstein and Poisson distributions of the exciting light on different couplings of the units (a = 1: no coupling, no energy transfer; a < 1: coupling of neighboring units)

separation due to the energy transfer is $p(0)[1-(1-\eta)^{c(p(0)+\langle n\rangle-1)}]$ A PSU absorbs at least one photon with a probability 1 - p(0), and thus the overall photon utilization is

$$\Delta\Psi = 1 - p(0)(1-\eta)^{c[p(0) + It - 1]} \qquad (4)$$

Figure 5 shows the relative values of this function for BE and P distributions at several $a = (1-\eta)^c$ values as a function of It. For isolated PSU-s (when a = 1 and there is no energy transfer) at It =2, the difference is 30% for the two distributions; in coupled units the difference decreases and becomes less than 10% (independently of It) when $a < 0.5$.

c) Experimental. In order to check the above predictions, a light source is needed having variable coherence time (in the us-ms range) with constant intensity and geometry of illumination. These requirements are met if the light of a He-Ne laser operating in a single TEM-00 mode is passed through a rotating ground glass disc [20]. The frequency spectrum of the scattered laser light is Gaussian[21] and characteristic of the thermal light, but its coherence time is much longer. The coherence time of this pseudo-thermal light source can be varied by changing the linear velocity (v) of the illuminated area on the rotating disc[17]:

$$\tau_c = \frac{R\lambda}{a}\frac{1}{v}$$

Where R is the distance between the sample and the disc, λ is the wavelength of the light and a is the average diameter of the scattering grains of the ground glass. By measuring the average half-times of the intensity fluctuations, we found that the coherence time of our light source could be varied in the range 10^{-5} - 10^{-1} s. Using a phosphoroscope[17], we measured the chlorophyll fluorescence induction and delayed fluorescence of green plant leaves, green algae and spinach chloroplasts in the μs and ms time ranges. With a given illumination time, the coherence time of the pseudo-thermal

light source was varied between the two limiting photon distributions and the results were compared. The illumination time was decreased down to 50 μs, the lower limit of our instrument.

The experiments gave negative results: no significant differences could be observed. This may be ascribed to the coupling of the PSU-s, which leads to a decrease of the predicted effects of the different statistics. The experiments gave positive results too: from equation (4) we can estimate the value of η, the probability of transfer between the PSU-s. The statistical error of our experiments was about 5%; with this error, taking four neighbors into account (c = 4), the transfer probability is at least $\eta = 0.3$. Though this is indirect evidence of transfer, it may be of interest, for it is independent of the other (also indirect) evidence.

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