THE EFFECT OF TIME-DEPENDENT COHERENCE OF EXCITATION ON THE PRIMARY PROCESSES OF PHOTOSYNTHESIS

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A coincidence counter model for the photosynthetic unit is elaborated from theoretical and experimental point of view. It is established that the photosynthetic units of the investigated systems (green plant leaves and spinach chloroplast suspension) do not act as coincidence counters above 50 μsec, i.e. they are indifferent to the coherence properties of the exciting ligh during an illuminating time longer than 50 μsec.

Introduction

Lasers as powerful light sources are commonly used in optical investigations of biological systems. In contrast to classical light sources, most lasers have large energy and small divergence and bandwidth. In addition they possess a fundamental feature originating from the principles of their operation, as their electromagnetic fields may be described by fairly large degrees of temporal and spatial coherence [1, 2]. Although this subject has been investigated widely recently, we have no information on its applications for biological purposes.

Let us illuminate a photosynthesizing system, considered as a compact mass of photosynthetic unists (PSU-s), for different durations. If T, the exposure time, is low enough to be of the order of magnitude of τ_c, the coherence time of the incident electromagnetic wave, the statistical properties of the field should be taken into account, as they are sensitive in this range to the ratio T/τ_c. The stream of incident photons is no longer uniform, but will exhibit the fundamental property of clumping. Let us consider the PSU as a coincidence counter. In this case, its response (*e.g.* the number of split water molecules) should depend on the photon distribution of the illuminating field. A similar effect was predicted for photographic processes by ROSENBLUM [3].

The aim of our study was to investigate the theoretical and experimental aspects of the coincidence counter model of the PSU.

Theory

1. *Pseudo-thermal light source*

As the coherence time of classical thermal light sources is very short ($\tau_c \cong \cong 10^{-15}$ sec), the condition that the exposure time must be of the same order as the coherence time is extremely difficult to satisfy. A light source with longer coherence time is needed, and the time should be changed over a wide range without altering the intensity and the geometry of the illumination. These conditions may be fulfilled by passing the light of a He-Ne laser operating in a single TEM—OO mode through a moving ground glass disc [4]. It can be shown [1, 5, 6] that the monochromatic laser beam undergoes a Gaussian spectral broadening ($\Delta\nu$) due to the scattering on the randomly-distributed grains of the ground glass. This spectral profile is characteristic of the thermal light. The coherence time of the scattered field may be deduced from $\Delta\nu$:

$$\tau_c = \left(\frac{1}{\Delta\nu}\right) = \frac{r_0}{v} \frac{4\pi}{\sqrt{2 \cdot \ln 2 \left(1 + \frac{4k^2 r_4^0}{f^2}\right)}}, \tag{1}$$

where r_0 is the radius of the incident beam, v is the velocity of the illuminated area on the disc, k is the wavenumber and f is the focal length of the focusing lens. Equation (1) shows that the coherence time of this special light source may be varied in a rather simple way: using an arrangement with constant k, r_0 and f, τ_c can be varied by altering the angular velocity of the rotating disc.

2. *Photon distributions*

The main question from our purpose is how the photon distribution of the pseudo-thermal light depends on T and τ_c.

It is well known that the fluctuations of boson (photon) numbers within one cell of phase space, which is the equivalent of $T \ll \tau_c$, are described by Bose–Einstein distribution. The validity of this for an arbitary time interval T was extended by MANDEL [7]. According to Mandel, the probability of finding n bosons (photons) over a number of cells M ($M \cong 1$) of phase space is

$$p(n, \langle n \rangle, M) = \frac{\Gamma(n+M)}{n!\,\Gamma(M)} \left(1 + \frac{M}{\langle n \rangle}\right)^{-n} \left(1 + \frac{\langle n \rangle}{M}\right)^{-M}, \tag{2}$$

where $<n>$ is the expectation value of the photon numbers. This generalization of Bose–Einstein distribution should hold for light of arbitrary spectral density. Eq. (2) gives Bose–Einstein distribution for $M=1$ and Poisson distribution for $M=\infty$. These cases are plotted for $<n>=2$ in Fig. 1. Thus, the distribution given by Eq. (2) is the transition from Bose–Einstein to Poisson statistics. T and τ_0 can be introduced by evolving the degrees of freedom, M:

$$M = \frac{T^2}{2 \int_0^T (T - \tau) |\gamma(\tau)|^2 d\tau}, \tag{3}$$

where $\gamma(\tau)$ is the normalized autocorrelation function of the optical field amplitude, and can be approximated by

$$\gamma(\tau) = \exp\left(-\frac{2\tau}{\tau_c}\right). \tag{4}$$

Introducing (4) into (3) the analytical expression

$$M = \frac{1}{2} \frac{\left(\frac{2T}{\tau_c}\right)^2}{\frac{2T}{\tau_c} - 1 + e^{-\frac{2T}{\tau_c}}} \tag{5}$$

is obtained. Fig. 2 shows log M vs. log T/τ_c. $M \cong 1$ if $T/\tau_c < 10^{-1}$, and may be approximated by $\frac{T}{\tau_c}$ if $T/\tau_c > 10$.

Thus, we know explicitly how the photon distribution depends on T/τ_c. In order to make clearer its variation, we calculate its standard deviation:

$$\sigma^2 = \langle n \rangle \left(1 + \frac{\langle n \rangle}{M}\right). \tag{6}$$

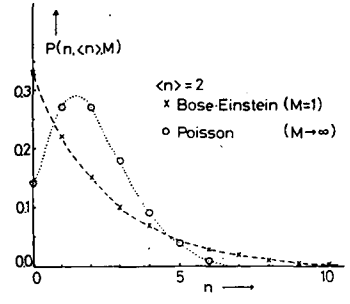


Fig. 1

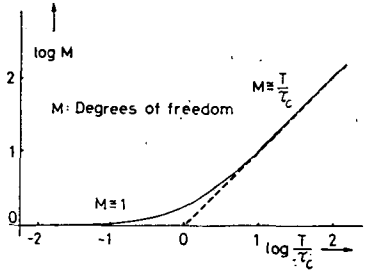


Fig. 2

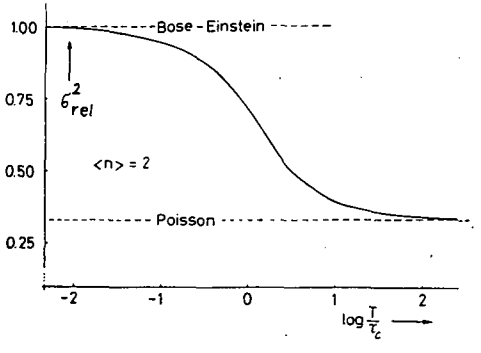


Fig. 3

This seems a suitable measure for comparing the different distributions on the time scale. This has been done in Fig. 3, where the standard deviations compared to that of Bose–Einstein statistics are plotted for $<n>=2$. Transition from one limiting distribution to the other occurs within one or two orders of magnitude of T/τ_c around 1 in practice.

3. *A Counter model for the PSU .*

Let us suppose that the PSU acts as a coincidence counter. If the PSU absorbs not fewer photons than a definite threshold (n_{th}) during a time interval T_a (accumulation time), then the primary photochemical processes will begin. In the opposite case, the energy of the absorbed photons will be dissipated by heat or emitted as luminescence. T_a is analogous to the resolution time of the multiplier in a coincidence counter circuit. The PSU is able to "remember" the absorption of the first photon until T_a. Let us assume that the accumulation time is equal to the exposure time. $P(<n, M)$ denotes the probability that any PSU receives $n \geqq n_{th}$ photons. It can be considered a relative measure of the photon energy utilization for photochemical purposes, and may be determined from the photon distribution given by Eq. (2):

$$P(\langle n\rangle, M) = 1 - \sum_{n=0}^{n_{th}-1} p(n, \langle n\rangle, M). \tag{7}$$

This equation is illustrated by Figs. 4 and 5 for $n_{th}=1$ and $n_{th}=4$, respectively. The applicability of the latter case may be supported by some of JOLIOT's papers *e.g.* [8]. Towards greater $<n>$ values, the differences between the curves increase

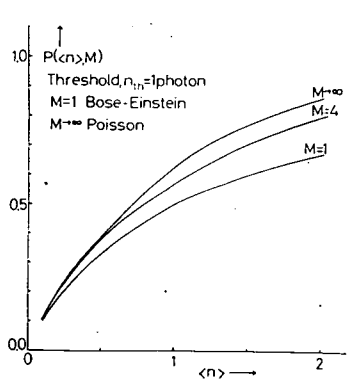


Fig. 4

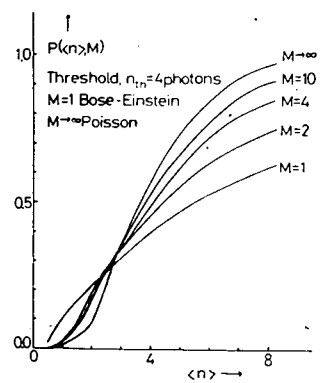


Fig. 5

and become monotonous functions of M. This can be interpreted by consulting Fig. 1. States of greater photon numbers are more probable in cases of small M (*e.g.* $M=1$) than in those of great M (*e.g.* $M=\infty$). This is in agreement with the clumping effect of photons described earlier. Fig. 6 illustrates the magnitude of the expected effect relative to the Bose–Einstein distribution for $\langle n\rangle = n_{th} = 4$. There should be a tendency among PSU-s to reach a uniform photon distribution (load of excitation energy) by energy transfer [9]. Let us suppose that PSU-s

with more than n_{th} photons are able to transfer their spare photon energy to units having fewer photons than n_{th}. (The case $n_{th}=1$ will be investigated in detail.) η denotes the probability of energy transfer between two PSU-s, one of which received 0, and the other more than 1 photon. From simple considerations,

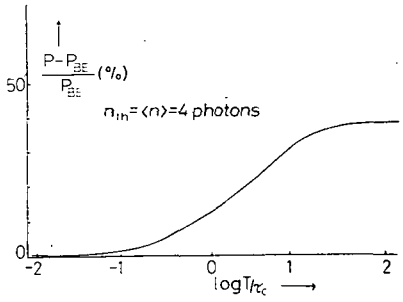


Fig. 6

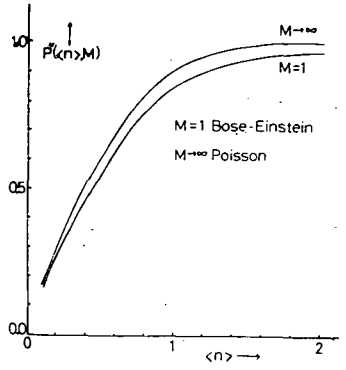


Fig. 7

the probability of utilization of the photon energy for photochemical reactions is:

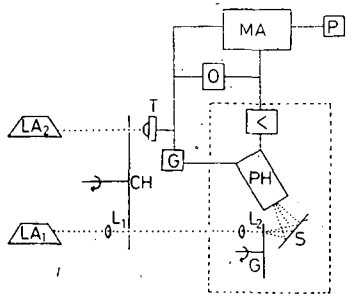
$$P^*(\langle n\rangle, M) = 1-p(n=0)\cdot\left[1-\eta\left(1-p(n=0)-p(n=1)\right)\right]^c \qquad (8)$$

where c is the number of neighbouring PSU-s. Fig. 7 shows Eq. (8) for $\eta=0.6$ and $c=8$. In spite of the rather effective energy transfer supposed between PSU-s in this case, the effects of different photon distributions on the first steps of photosynthesis remain identifiable.

Experimental

As the amounts of photochemical products in photosynthesis may be measured only with relatively great error, optical methods were used to investigate the predicted effect of coherence. Prompt and delayed fluorescence were measured because these are competing processes of the primary photochemical steps.

A laser phosphorescope was set up [10] according to the block scheme represented in Fig. 8. The exciting He-Ne gas laser (GL—8 MOM), operating in TEM—OO mode, had 5 mW output and about 1 μsec coherence time. The chopper runs at 3000 r.p.m., and the light/dark ratio can be changed from 1/30 to 1/00, which corresponds to exposure times of 600 to 50 μsec. The ground glass disc may be driven by different motors and the peripheral valocity (v) can be altered stepwise from 1 mm/s to 50 m/s. The luminescence of the sample is detected under a small an-



LA_1, LA_2	Lasers	PH	Photomultiplier
L_1, L_2	Lenses	G	Gate for PH
CH	Chopper	T	Triggering circuit
S	Sample	O	Oscilloscope
P	Printer	G	Rotating Ground Glass Screen
<	Amplifier		
MA	Multichannel Analyzer		

Fig. 8

gle by a photomultiplier (EMI 9558 A) protected by a filter against the intense laser light. In the case of measurement of delayed light emission, the photomultiplier will be gated electronically after a 50—100 μsec delay. The signal from the photomultiplier (1—10 kΩ anode resistance) is fed into a source-follower difference-amplifier and to a multichannel analyser (NTA 512) having a 20 μsec channel width. Because of the very small signal-to-noise ratio, the analyser works in averaging mode.

The theory discussed above assumes that the exposure time, the accumulation time and the time of coherence should be of the same order of magnitude if any role of the different photon statistics is to be expected to be revealed. For a given T value τ_c was changed around T, and the intensity of prompt fluorescence and the time-dependence of the delayed emission coming from different green plant leaves and spinach chloroplast suspensions were measured at room temperature. The same method was carried out with decrease of the exposure time to the smallest value which may be reached in our device (about 50 μsec). The measurement was repeated till the variance of each curve became less than 2%.

It was impossible to observe any difference between decay curves relating to the same exposure time. This means that the PSU-s of the investigated systems do not act as coincidence counters above 50 μsec, while if they do this lower, then their accumulation time must be less than 50 μsec. The PSU-s are indifferent to the coherence properties of the exciting beam above 50 μsec, i.e. the photons arriving "regularly" (coherently) have the same effect on the PSU as those coming "irregularly" (incoherently) during an illuminating time longer than 50 μsec. Further efforts will be made to lower these limits in order to be able to investigate the behavior of the PSU-s within a shorter time.

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Влияние зависимдй от времени когерентности возбуждения на первичные фотореакбии фотосинтеза

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На основании теоретических расчетов и экспериментальных данных разработана, модель, в которой фотосинтетическая единица рассматривается как счетчик совпадений. Установлено, что фотосинтетические единицы иследованных систем (зеленые листья и суспензии хлоропластов шпината) не являются счетчиками совпадений во временном интервале более 50 мксек, т. е. они индифферентны к когерентности возбуждающего света, если время освещения больше 50 мксек.