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THE QUANTUM YIELD OF REACTION CENTER PHOTOOXIDATION IN SUBCHLOROPLAST FRAGMENTS ENRICHED WITH PHOTOSYSTEM I*

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SUMMARY

Suspensions of light particles sedimented at $165000 \times g$ from pea subchloroplast preparations were investigated. The exciting light readily caused the photooxidation of P_{700} in a fraction of active particles. The effective energy transfer has been shown to occur between individual P_{700}. The quantum yield of P_{700} photooxidation was estimated by means of the relative method to be $\geqslant 0.75$.

INTRODUCTION

Determination of the quantum yield of the primary photosynthetic process has been performed by many authors (see for example refs 1 and 2). The initial slopes of photoinduced kinetics have usually been measured for reaction center oxidation. Cytochrome oxidation[3], $NADP^+$ and quinone reduction or redox transformations of exogenous compounds have also been studied, as they reflect the efficiency of the primary charge separation process. The quantum yield can then be calculated as the ratio of the photoconverted molecules to the light quanta absorbed within the same time.

However, this direct, absolute technique includes a number of measurements and calculations that cannot be made with a sufficient accuracy: (1) it is difficult to obtain the absolute number of light quanta with an accuracy better than 10–20%; (2) determination of the amount of light absorbed cannot be accurately measured in turbid media; (3) determination of the volume of the cuvette illuminated by both exciting and measuring beams can be done with a precision hardly better than 10–30%, without using lasers; (4) the *in vivo* values of differential extinction coefficients cited by various authors for the above mentioned compounds sometimes differ by 30–50%; (5) it is difficult to measure the slope of the kinetic curve with an accuracy better than 5%, without using a derivative method; (6) the process of

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reaction center, cytochrome *etc.* recovery must be taken into account; (7) the calibration of differential spectrophotometer sensitivity is usually done with an accuracy of 3–10%.

All the above considerations show that the absolute method can provide results whose precision is hardly better than 20–50%, even if several tens of individual experiments were subjected to a statistical treatment.

Hence other methods involving measurements of relative parameters may prove fruitful for investigating primary photosynthetic events.

For example, a fluorescence method has been described for purple bacteria[4]. Measurements of relative fluorescence yield (with an accuracy of 5–10%) against the portion of active reaction centers (relative value) make it possible to obtain the quantum yield of reaction center photooxidation with an accuracy of $\pm$ 3%. But this method has a limitation in that it can only be applied to fluorescing photosystems that are characterized by the multicentral (statistical) type of photosynthetic unit organization. Photosynthetic units of plant Photosystem II has been shown[5] to be of not purely multicentral but of connected type and Photosystem I does not fluoresce noticeably.

In this contribution an approach is described for obtaining the quantum yield of reaction center photooxidation in pea subchloroplast particles enriched with Photosystem I.

METHODS

Chloroplasts were obtained as described elsewhere[6]. The material was then suspended in 0.4 M sucrose, 0.35 M NaCl, 0.05 M Tris–HCl buffer (pH 7.8) and disrupted by incubation with 0.5% digitonin. The suspension was centrifuged for 30 min at $50000 \times g$ and light subchloroplast particles were then sedimented from the supernatant at $50000 \times g$ by centrifugation for 60–90 min at $165000 \times g$. Light particles suspended in a minimal volume of 0.4 M sucrose + 0.01 M NaCl were kept in a Tumberg vessel under argon at 0 °C during 2–3 weeks. Their photochemical activity did not change during the storage. According to the fluorescence method[6], light particles were enriched with Photosystem I and the degree of enrichment was more than 2 as compared to the chloroplasts.

The photoinduced changes in the absorption around 700 nm were measured with a double-beam differential spectrophotometer[7], sensitivity up to $5 \cdot 10^{-5}$ absorbance units. The exciting light from a 170-W tungsten lamp was filtered with interference (434 ± 4 nm) and additional optical cut-off ($\lambda > 500$ nm) filters. The energy of the exciting light was measured by a calibrated photocell of the S-1 type. A red glass filter fixed between the cuvette and the measuring photomultiplier protected the photomultiplier from the scattered exciting light. The fluorescence emission induced by the exciting light could pass through this red filter, but it was not amplified by the electronic circuits that were sensitive only to the signals induced by the modulated measuring light. The corresponding modulated fluorescence emission was negligible because its maximal possible level was less than 0.005% of the measuring beam with light particles[8].

The reaction center concentration was calculated using the value for the extinction coefficient of P_{700} equal to $7.2 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ that was obtained by averaging data from contributions[9,10].

THEORETICAL ANALYSIS

It seems likely that the primary photoprocess in Photosystem I is an electron donation by reaction center P_{700} (as in purple bacteria). It is evident, that in the case of exciting light strong enough for the P^+_{700} recovery process to be neglected, the rate of the P_{700} photooxidation (dP^+/dt) is proportional to the quantum yield of this process (φ_P):

$$dP^+/dt = I_a \cdot \varphi_P \tag{1}$$

I_a, light absorbed in einstein$\cdot s^{-1}$; P^+, concentration of reaction centers in oxidized state.

The monocentral type of photosynthetic units organization predicts a linear dependence[11] of φ_P (and hence dP^+/dt) against the fraction of active P_{700}, whereas the multicentral type of it is characterized by a non-linear dependence[11,12]:

$$dP^+/dt \sim \varphi_P = \frac{K_P \cdot P/P_0}{K_\Sigma + K_P \cdot P/P_0} \tag{2}$$

K_P, the rate constant depending on efficiency of energy migration in photosynthetic units and trapping in active reaction centers (P); K_Σ, the sum of rate constants for all competing processes (fluorescence, internal conversion, intersystem crossing and quenching in wasteful centers); P_0, full concentration of reaction centers (in our case $P_0 \cong P + P^+$).

Formula 2 may be easily transformed to:

$$\varphi_P = \frac{\varphi_P^{max} \cdot P/P_0}{1 - \varphi_P^{max} \cdot (1 - P/P_0)} \tag{3}$$

where $\varphi_P^{max} = \varphi_{P \to P_0} = K_P \cdot (K_\Sigma + K_P)^{-1}$.

Corresponding theoretical dependences are shown in Fig. 1 for some φ_P^{max} values treated as a parameter. It is seen that the non-linearity of the curves increases with φ_P^{max}, especially in the $\varphi_P^{max} \to 1$ region. We suggested the use of the latter for determining φ_P^{max}. In fact ,the relative experimental values of dP^+/dt as well as P/P_0 may be used. The experimental errors of the initial slope determination for kinetic curves may be easily reduced to less than 10% and that of P/P_0 to less than 3% of P_0. These errors are shown in Fig. 1 as rectangles. The following table shows the relative accuracy of the φ_P^{max} determination.

RESULTS

Light particles have been investigated. The efficiency of electron transport in this object was much lower than in chloroplasts. The absorbed light with an intensity of $\geqslant$ 100 erg$\cdot cm^{-2} \cdot s^{-1}$ ($\geqslant 4 \cdot 10^{-11}$ einstein$\cdot cm^{-2} \cdot s^{-1}$) readily causes oxidation of nearly all active reaction centers ($\Delta A_0 \sim P_0$ on Fig. 2). The value of $\tau_{1/2}$ was inversely proportional to exciting light intensity and equal to 3.5 s for 100 erg$\cdot cm^{-2} \cdot s^{-1}$. The slopes of the kinetic curves for different values of $P \sim \Delta A$ were averaged from 3–4 individual measurements. These values were the same

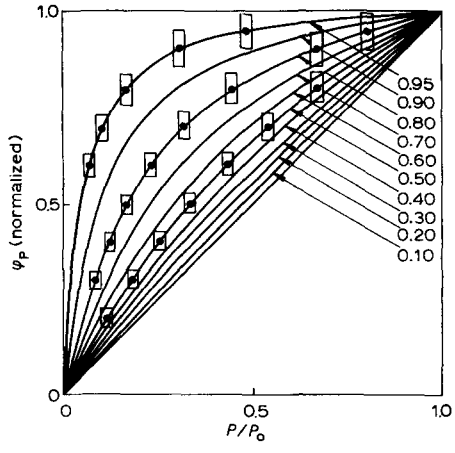

Fig. 1. The theoretical dependences of normalized φ_P against the fraction of active P_{700} for the monocentral type of photosynthetic unit organization (the diagonal line), for the multicentral one (curves, corresponding to φ_P^{max} values equal to 0.1–0.95). The rectangles represent the experimental errors, corresponding to the line A of Table I.

TABLE I

THE RELATIVE ACCURACY OF φ_P^{max} DETERMINATION

A, within the error of 10% and 3%; B, within the error of 5 and 3%, respectively, in dP^+/dt and P/P_0 determinations. These accuracies are not very difficult to obtain when working with a real object.

φ_P^{max}	: *0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*0.95*
A (%)	—	—	50	30	20	14	9	6	3	2
B (%)	—	50	25	15	10	7	5	3	2	1

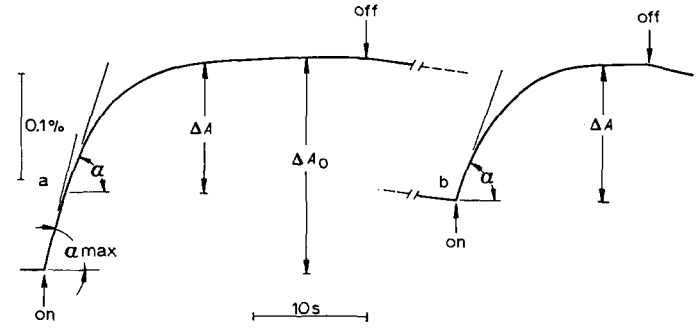

Fig. 2. Experimentally determined kinetics of the P_{700} photooxidation in light particles at $\lambda = 700$ nm (the maximum in the spectrum of photoinduced absorption changes). The reaction mixture contained: light particles (chlorophyll *a*, 4.5 μg/ml), 0.4 M sucrose, 0.01 M NaCl, 0.05 M Tris–HCl (pH 7.8). The intensity of exciting light absorbed was 175 erg·cm^{-2}·s^{-1}. Temperature was 18 °C.

$$\frac{\Delta A}{\Delta A_0} = \frac{P}{P_0} \text{ and } \frac{\mathrm{tg}\alpha}{\mathrm{tg}\alpha^{max}} = \frac{dP^+/dt}{(dP^+/dt)^{max}} = \frac{\varphi_P}{\varphi_P^{max}}.$$

within the error of 10% for the cases represented by points *a* and *b* on Fig. 2. The final data are depicted in Fig. 3. Experimental points are restricted by theoretical Curves 1 and 2, corresponding to $\varphi_P^{max}=0.32$ and 0.42, respectively. Therefore, the experimental φ_P^{max} value was equal 0.37 ± 0.05.

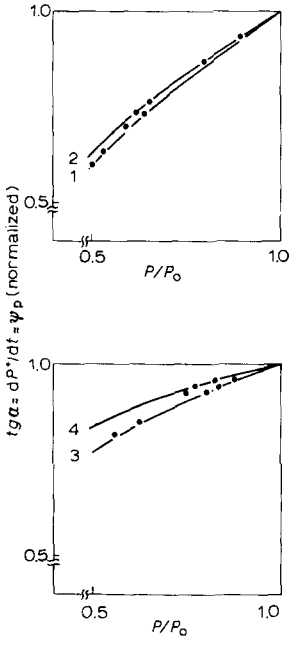

Fig. 3. The dependence of normalized tgα($\sim dP^+/dt \sim \varphi_P$) against the fraction of active P_{700} in light particles. A, without additions as in Fig. 2; B, in presence of ascorbate (10^{-3} M), *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (10^{-4} M), methyl viologen ($3\cdot10^{-4}$ M). Experimental conditions were the same as in Fig. 2. 1,2,3,4, the theoretical curves, corresponding to φ_P^{max} values equal to 0.32, 0.42, 0.71, 0.79.

Addition of exogenous ascorbate (10^{-3} M), *N′,N′,N,N*-tetramethyl-*p*-phenylenediamine (10^{-4} M) and methyl viologen ($3\cdot10^{-4}$ M) to the light particles suspension led to: (a) more than 2-fold increase in the "dark" concentration of active P_{700}; (b) an increase, up to 10–50 times, in the rate of the dark P^+_{700} recovery and hence an equal increase in the saturating light intensity; (c) an increase in the φ_P^{max} value (see Fig. 3, Curves 3 and 4) up to 0.75 ± 0.04. These facts indicate that exogenous compounds considerably improved photosynthetic electron transport. It is also important to point out that the value of the initial slope increased more (nearly 5 times!) than was expected from the data of Fig. 3. Moreover, we have performed the absolute φ_P^{max} measurements (the ratio of P_{700} molecules oxidized to light quanta absorbed) to find that: for light particles suspension without additional exogenous compounds $\varphi_P^{max}=0.090^{+0.045}_{-0.030}$ and for the one with ascorbate (10^{-3} M), *N′,N′,N,N*-tetramethyl-*p*-phenylenediamine (10^{-4} M), methyl viologen ($3\cdot10^{-4}$ M) $\varphi_P^{max}=0.43^{+0.22}_{-0.15}$.

The above difference between the φ_P^{max} values obtained by the two methods indicate, that there exists a part of light particles which are inactive in reaction

center photooxidation. This activity was restored in some of the light particles by addition of an exogenous compound. Nevertheless, part of the active particles was characterized by energy migration and trapping processes of high efficiency.

DISCUSSION

The non-linear dependence of dP^+/dt on P/P_0 demonstrated in this work for light particles appears to prove that reaction centers of Photosystem I are connected at the energy migration level, which is in agreement with the results of Amesz and Fork[2] for red alga cells. This conclusion seems to contradict the data obtained by Joliot *et al.*[5] for spinach chloroplasts by the fast amperometric method. These authors established that a linear dependence exists between the photoreduction rate of exogenous acceptor methyl viologen and P/P_0 in the conditions when Photosystem II was blocked. We can advance some possible reasons for this divergence:

(a) There is a possibility that separate monocentral photosynthetic units (if they really exist) associate more closely in the course of preparation of light particles (by decreasing the distances or improving the mutual intermolecular orientation). Thus the connected photosynthetic units in Lavorel and Joliot terminology[13] may be formed from separate ones.

(b) The dP^+/dt and P values were measured during the unsteady phase in this work whereas the corresponding parameters (the photoreduction rate of methyl viologen and the fraction of active centers) were obtained for the steady state conditions in ref. 5. But we have shown the dependence $dP^+/dt=f(P)$ to be the same within the experimental error for the moments represented by points *a* and *b* (Fig. 2). These moments are characterized by the same P_{700} amplitudes, although the exciting light was switched on about two seconds previously in *a* and simultaneously in *b*.

(c) The reaction center photooxidation and methyl viologen photoreduction may not be in rigid stoichiometry.

The multicentral type of photosynthetic unit organization advanced by us is not the only possible model that fits the non-linearity of $dP^+/dt=f(P)$ observed. Qualitatively similar dependences may be obtained for at least two alternative models of pigment complex organization. The first, developed by French scientists (Joliot, Delosme, Lavorel) is a connected model, where separate photosynthetic units may partly exchange by excitations. The second, treated theoretically by Clayton[14], is a model of isolated domains containing only a few (2–4) centers.

However, we prefer the multicentral model to the latter ones, because its validity has been proved for a purple bacteria photosystem[4,11,12] which in our opinion is rather similar to Photosystem I (considerable red absorption shifts reflecting the intermolecular aggregation and also low fluorescence lifetimes and high energy migration rate constants[15,16]).

Moreover, the calculations made in terms of this model produce φ_P^{max} values that would be lower than the true ones if either of the latter two models exist. Therefore, our data may be treated as $\varphi_P^{max} \geqslant 0.75$.

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