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Photoacoustic detection of flash-induced charge separation in photosynthetic systems. Spectral dependence of the quantum yield

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Abstract

A new high-sensitivity photoacoustic spectrometer is described. The volume changes occurring in a thin layer of photosynthetic suspension during the first microsecond following a monochromatic laser flash (3 ns duration) are detected by a piezoelectric ceramic. These volume changes result from two components of opposite signs: (1) thermal expansion due to the release of heat in the medium, and (2) contraction occurring in the neighbourhood of the charged photoproducts. The expansion signal measures the thermal losses in the light-conversion process. Under weak flash excitation, the difference between the amounts of heat released by an active and an inactive sample measures the energy stored by the photoreactions. As the contraction signal is also linearly related to the number of flash-induced charge separations, the overall volume change, when compared between the active and inactive states of the sample, gives a relative measure of the quantum yield. In the spectral range investigated (630–705 nm), the quantum yield is practically independent of wavelength in purified PS I and in PS II (BBY) particles. In spinach chloroplasts, a depression of the quantum yield in the absorption region of LHC II suggests that a significant fraction of the light quanta absorbed by LHC II are not transferred to open reaction centres. In intact cells of *Chlamydomonas reinhardtii* with closed PS II reaction centres, the quantum yield spectrum of the PS I centres was compared in state I and state II. Upon transition from state I to state II, a large proportion of the quanta absorbed by the PS II antenna was found to be transferred towards PS I reaction centres. When measured at two different temperatures, the overall volume change can be deconvoluted in its two components (expansion and contraction), and the absolute value of energy storage calculated. In purified PS I and in PS II (BBY) particles, this value was found close to 1 eV per absorbed quantum, which is approximately the energy required for the formation of the radical pairs $P700^+(F_A,F_B)^-$ and $Z^+Q_A^-$.

Key words: Photoacoustic spectroscopy; Quantum efficiency; Excitation transfer; State transition

1. Introduction

The use of photoacoustic spectroscopy to measure flash-induced volume changes and thermal conversion in photosynthetic materials was introduced by Callis et al. [1] in 1972, and developed by Arata and Parson [2]. The volume and enthalpy changes accompaning photoreaction in chromatophores of *Chromatium vinosum* [1] and reaction centres of *Rhodopseudomonas sphaeroides* [2] were measured by a capacitor microphone in close contact with the sample suspension, on a time scale from 100 μs to 1 s following the flash.

A much higher time resolution was introduced using 'laser-induced optoacoustic spectroscopy', in which the heat emitted by a sample after absorption of a laser pulse is detected as an acoustic signal by a piezoelectric transducer [3]. This method was applied by Nitsch et al. [4] to the energetics of processes occurring in Photosystem (PS) I and PS II within a time window of 1.4 μs. These authors used aqueous suspensions of PS I and PS II particles from the cyanobacterium *Synechococcus*, and intact cells of the same species. Using the same method, Mullineaux et al. [5] measured the energy storage in cyanobacterial cells adapted to light-states I and II. In all cases, the authors found a very high fraction of energy stored, especially in PS I.

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Abbreviations: PS, Photosystem; LHC, light-harvesting complex; DMSO, dimethyl sulfoxide; DCPIP, dichloro-2,6-phenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DAD, diaminodurene or 2,3,5,6-tetramethyl-*p*-phenylenediamine; MES, 2-(*N*-morpholino) ethanesulfonic acid.

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In the present work, we will describe a new high-sensitivity photoacoustic spectrometer designed for spectral measurements of volume changes and energy storage in photosynthetic materials on the microsecond time scale.

2. Materials and methods (Fig. 1)

Measuring light

The monochromatic source of measuring light is a grating-tunable dye laser module LSI (DLM-120) pumped by a pulsed nitrogen laser (LSI, VSL-337, 120 μJ, 3 ns). The dye that we used (7 mM DCM in DMSO) covers the wavelength range from 620 to 720 nm. The proportion of broadband dye fluorescence in the detecting light (less than 0.5% of the emission in the 630–695 nm range) was lowered by increasing to 40 cm the distance from the laser source to the entry of the optical fibre leading to the measuring cell (no collimating lens was used at the output of the laser source). At wavelengths above 695 nm (where the output energy of the dye strongly decreases), most of the broadband fluorescence light was eliminated by a far-red glass filter. The geometrical distribution of the measuring light was homogenized by a glass optical guide (4 mm diameter, 40 mm length) before entrance into the optical fibre. This precaution is essential for good correlation between the simultaneous measurements of light energy and light-induced volume changes.

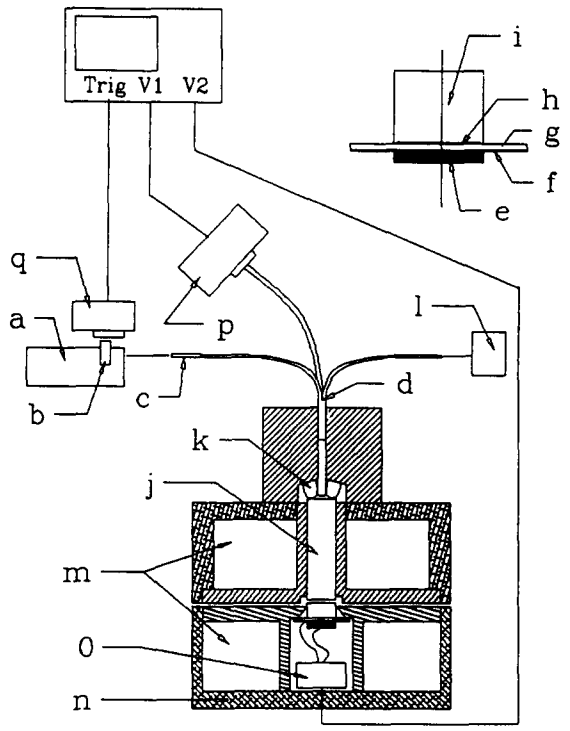

Fig. 1. Diagram of the measuring device. a, laser; b, dye cuvette; c, glass optical guide; d, optical fibres; e, piezoelectric ceramic; f, mirror; g, glass protection of the mirror; h, sample; i, glass cylinder; j, glass light-guide; k, light-emitting diodes; l, halogen lamp; m, thermostated chamber; n, insulating walls; o, preamplifier; p, measuring photodiode; q, triggering photodiode.

Measurement cell

The volume changes are detected by a piezoelectric ceramic (RTC, PXE 5, 1 MHz resonance frequency) of 16 mm diameter and 1 mm thickness, glued to the back (lower) surface of a horizontal aluminium mirror of 30 mm diameter. The reflecting (upper) face of the mirror is protected by 1 mm glass. A drop of photosynthetic material is laid down on the upper glass face of the mirror, and then enclosed in a limited volume (14 mm diameter, 50 μm thickness) which has been hollowed in the lower part of a glass rod of 16 mm diameter and 10 mm height. The measuring (pulsed) light beam, and an optional continuous actinic beam, are directed on the measuring cell through a glass light guide of 16 mm diameter and 9 cm length, which distributes illumination evenly on the surface of the sample. Continuous

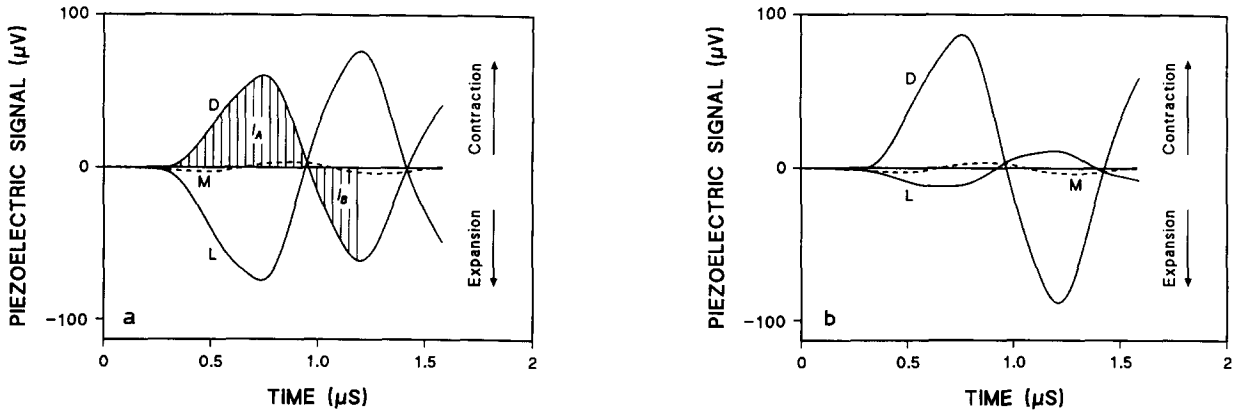

Fig. 2. Photoacoustic waves generated by a weak laser flash of 680 nm. M is the wave generated in the mirror (its shape was measured by filling the cell with water). In D and L, the measuring cell was filled with a suspension of purified PS I from *Synechocystis*. D: dark-adapted sample, with addition of 60 μM DAD, 10 mM sodium ascorbate, 0.2 mM methylviologen. L: saturating background illumination, without any addition. Temperature, 23°C in a, and 0°C in b.

actinic light is available from different sources: a ring of eight light-emitting diodes (Toshiba TLRA 180, wavelength 660 nm) on the upper face of the glass light guide, and a halogen lamp (Schott KL 1500, 150 W, anticaloric filter Schott KG 1) equipped with an optical fibre.

The volume changes induced within the light absorbing sample by the laser pulse generate a pressure wave, which is detected by the piezoelectric ceramic after propagating through the sample and the mirror. The type of piezoelectric transducer that we chose combines an appropriate acoustic impedance with a fast time response. The small volume and thickness of the measuring cell (with a high ratio surface area/thickness) favour a high time resolution, and a large amplitude of the pressure wave relative to the light energy absorbed in the sample. The proximity between the ceramic and the illuminated surface improves the efficiency of acoustic energy collection by the ceramic. The fraction of incident light which is not absorbed by the sample is mainly reflected by the mirror, and in this case does not induce any signal in the ceramic. Only a small amount of light is absorbed in the aluminium layer of the mirror. A small fraction of light could be scattered sideways, and absorbed by the stainless steel walls of the chamber. In this case, the acoustic waves generated in the steel do not directly reach the ceramic (because the measurement cell is surrounded with air). They are delayed by a few microseconds, beyond the detection window.

The baseline noise of the ceramic is 2 μV rms. Under the conditions of Fig. 2 (low flash intensity in the linear region of the light saturation curve of the photoreactions), the piezoelectric signal generated by the photosynthetic sample peaks at about 100 μV. The signal is amplified by a preamplifier (and impedance adaptor) with a gain of 25 and a bandwidth from 10 kHz to 6 MHz. The measuring cell, ceramic and preamplifier are enclosed in a thermostated chamber, and shielded from electrical noise by electrostatic screening.

Signal processing

The piezoelectric signal is amplified and filtered, with a total gain of $2 \cdot 10^4$ and a bandwidth from 100 kHz to 3 MHz. This signal must be corrected for the fluctuations of the flash energy (about 8%). For this purpose, the energy of the laser flash is measured by a photodiode (EG&G, UV 444 BQ) and an amplifier-integrator. The amplified signals (from the measuring cell and the photodiode) enter the two channels of a digital oscilloscope (LeCroy 9400 A, 100 MHz, with averaging function). A photodiode excited by the fluorescence of the laser dye triggers the oscilloscope. In the present work, 10 to 100 pulses were averaged for each measurement, depending on the intensity of the signals. The digitized averaged waves are treated by an AT microcomputer (OPS 386, 33 MHz), and the signal from the measuring cell (volume change) is divided by the signal from the photodiode (light energy). We checked that in the presence of a solution of dye (DCPIP) in the measuring cell, the amplitude of the acoustic wave was proportional to the energy of the flash.

When the measuring cell is filled with photosynthetic material, the electric signal generated by the ceramic (D or L in Fig. 2) results from two different causes: (a) the pressure wave generated within the photosynthetic sample; (b) the pressure wave generated by the small fraction of light absorbed within the aluminium layer of the mirror (M in Fig. 2). A third component of the signal is the electric artefact generated by the nitrogen laser. This artefact has been reduced by electrostatic screening of the laser and the measuring cell, and allowing sufficient space (60 cm) between the laser and the measuring device. As the residual electric artefact lasts 0.5 μs, most of it occurs before the time window of measurement (see below).

The acoustic wave generated within the biological sample ('sample signal') has to cross the glass protection (1 mm thickness) of the mirror before it reaches the ceramic. This is not the case for the acoustic wave (M) generated within the mirror ('mirror signal'). As a result, the wave generated within the sample is delayed by about 200 ns (corresponding to the propagation of a longitudinal acoustic wave through 1 mm glass) and lengthened, in comparison with the wave generated in the mirror. The ceramic and filtering have been chosen so that the two waves are nearly 90° out of phase. Under these conditions, the contribution of the mirror component (M) may be eliminated from the overall signal (D or L) by the following calculation: in a convenient time window (from 0.4 to 1.2 μs after the laser flash) which covers essentially the first microsecond of the 'sample signal', the computer calculates the integral I_A of the acoustic wave from 0.38 to 0.96 μs, and the integral I_B from 0.97 to 1.18 μs. The difference $I = I_A - I_B$ (represented by the total hatched area in Fig. 2a) is a selective measure of the pure 'sample signal', because it corresponds to the highest contribution of the 'sample signal' relative to that of the 'mirror signal' (the integrated contribution of M over the considered time window is practically zero). In what follows, $I_{\theta,D}$ will refer to I measured at θ°C in dark-adapted material, and $I_{\theta,L}$ to the corresponding measure under saturating actinic light.

3. Results and discussion

Absorption of light from the laser flash by the photosynthetic pigments results in a release of heat in

the suspension medium. The corresponding volume increase ΔV_{th} induces a pressure surge, which is converted into electric voltage by the ceramic. The quantity of heat released – and consequently the amplitude of the pressure wave generated – increases when the photochemical activity of the reaction centres decreases. The signal is maximal when the reaction centres are fully inactive, for example upon saturation by a strong light background, as in L of Fig. 2a. By comparing the amount of heat evolved from a photoactive sample to that from the inhibited sample, one could theoretically measure the fraction of absorbed light energy which is stored by the photoreaction.

However, conversion of light energy into heat is not the only source of photoacoustic signal. Conformation changes associated with the photoreaction give rise to a volume change ΔV_{conf}, which can be detected as a pressure wave by the transducer [1]. The overall photoacoustic signal (D in Fig. 2) measures the sum of the two types of volume changes [1,9]:

$$\Delta V = \Delta V_{th} + \Delta V_{conf} \quad (1)$$

Except those from Parson and coworkers [1,2], most of the photoacoustic studies on photosynthetic systems have neglected this second type of acoustic signal [4–8].

In all of the materials that we used, the conformational acoustic wave exhibits the same shape as the thermal wave, but with the opposite sign (as in D of Fig. 2). The sign of the conformation signal indicates that a contraction occurs in the sample, due to flash-induced charge separation. The contraction may arise from two different causes: (a) the reaction centre itself contracts when submitted to electrostatic interaction between the positive and negative charges generated by the laser flash, or (b) the electric charges cause a local ordering of the surrounding water molecules (electrostriction) [2]. A third possibility would be a contraction of the thylakoid membrane caused by the transmembrane electric field, but it seems to be ruled out by our observation of a large flash-induced contraction signal in purified PS I or PS II preparations, with a magnitude similar to that of membrane-containing materials (isolated chloroplasts or intact algae). Callis et al. [1] already discarded this last explanation in the case of *C. vinosum* chromatophores. With regard to hypotheses a and b, the same authors observed – and we confirm in the case of spinach chloroplasts – that the addition of 20% ethylene glycol to the suspension buffer does not affect the magnitude of the contraction signal, suggesting that the volume decrease originates in the reaction centre rather than in the external medium. However, more recently, Arata and Parson [2] favoured hypothesis b (electrostriction). The two possible origins are not mutually exclusive.

Whatever its exact physical origin, the contraction signal reflects a conformational change in the neighbourhood of the reaction centres, linearly related to the photochemical activity. This signal can be used as a relative measure of the number of flash-induced charge separations.

The thermal expansion of the medium (ΔV_{th}) is strongly temperature dependent, whereas the conformation (contraction) signal ΔV_{conf} depends little or not on the temperature of the sample [1]. A nearly pure conformation signal (D in Fig. 2b) is observed around 0°C in active photosynthetic particles or broken chloroplasts suspended in aqueous medium, because in this case the thermal expansion coefficient β of the medium (and consequently the thermal signal) is very low (the temperature at which $\beta = 0$ would be 4°C in pure water, but is slightly decreased in presence of salts, depending on the medium used). At room temperature (Fig. 2a), both components are of significant magnitude, but the contraction signal ΔV_{conf} remains predominant and accounts for the sign of the resultant signal (D in Fig. 2a). The absolute value of ΔV_{conf} may be estimated by comparing the pure conformation signal at O°C (D in Fig. 2b) to the pure thermal signal measured at 23°C in inactive material (L in Fig. 2a). The volume increase per absorbed light quantum of 680 nm is 16 Å^3 at 23°C, assuming that the thermal expansion coefficient of the suspension medium is that of water. Thus ΔV_{conf}, which exceeds by a factor of 1.3 the value of ΔV_{th} measured at 23°C in inactive material, corresponds to about 20 Å^3 per absorbed quantum.

Neglecting the contribution of ΔV_{conf} may have led some authors [4,5] to report surprisingly high values of energy storage: for example, Nitsch et al. [4] found that in PS I particles only 20% of the absorbed light energy was released within 1.4 μs following a laser flash of 677 nm, whereas 80% remained stored in the system. These authors may have underestimated the amount of heat released in the medium, because they did not take into account the conformational signal (ΔV_{conf}) occurring with the opposite sign. As they added 33% ethylene glycol to increase the thermal expansion coefficient of the suspension medium, the ratio $\Delta V_{conf}/\Delta V_{th}$ was probably smaller than in our experiments with aqueous medium. Nevertheless, we checked that in normally active spinach chloroplasts, the conformation signal ΔV_{conf} remained predominant at room temperature in the presence of 23% ethylene glycol. The sign of the resultant signal remained as in D of Fig. 2, a result difficult to reconcile with the above-mentioned publications.

When the reaction centres are in their dark-adapted, photochemically active state, the acoustic signal (I_D) induced by the laser flash is proportional to

$$\Delta V_D = \Delta V_{th,D} + \Delta V_{conf}$$

When the reaction centres are closed by saturating

background illumination, all of the flash energy absorbed by the sample is converted to heat, and the acoustic signal (I_L) is proportional to

$$\Delta V_L = \Delta V_{th,L}$$

From I_D and I_L, which are directly measured, one can deduce

$$E = \frac{I_L - I_D}{I_L} = \frac{\Delta V_L - \Delta V_D}{\Delta V_L}$$

$$= \frac{(\Delta V_{th,L} - \Delta V_{th,D})}{\Delta V_L} - \frac{\Delta V_{conf}}{\Delta V_L} \quad (2)$$

The term

$$\frac{\Delta V_{th,L} - \Delta V_{th,D}}{\Delta V_L}$$

is the energetic efficiency of the photoreaction, i.e., the fraction of absorbed light energy which remains stored in the sample at the time where the signal is detected. The second term

$$\frac{\Delta V_{conf}}{\Delta V_L}$$

also measures the efficiency of the reaction. But contrary to the first term, the absolute value of which is given by internal calibration using the inhibited sample, the second one cannot be easily calibrated. Consequently,

$$E = \frac{I_L - I_D}{I_L}$$

measures the photoreaction efficiency in relative units.

The absolute value of energy storage can be drawn from measurements at two different temperatures θ_1 and θ_2, taking advantage of the strong temperature dependence of the first term in expression (2), whereas the conformation change in the second term is assumed to be essentially independent of temperature [1]. In this case, the conformation signal is eliminated from the difference between the measurements at θ_1 and θ_2, and the energy storage efficiency is

$$1 - \frac{I_{\theta 1,D} - I_{\theta 2,D}}{I_{\theta 1,L} - I_{\theta 2,L}} \quad (3)$$

However, measurement of E at one temperature is sufficent – and gives a better signal-to-noise ratio –, whenever absolute values are not essential. Such will be the case in most of the following experiments, investigating the spectral dependence of the energy storage efficiency under different physiological conditions. I_L, which measures the amount of light energy absorbed by the sample, may be converted into a quantity proportional to the number of absorbed quanta, when divided by the energy of one quantum

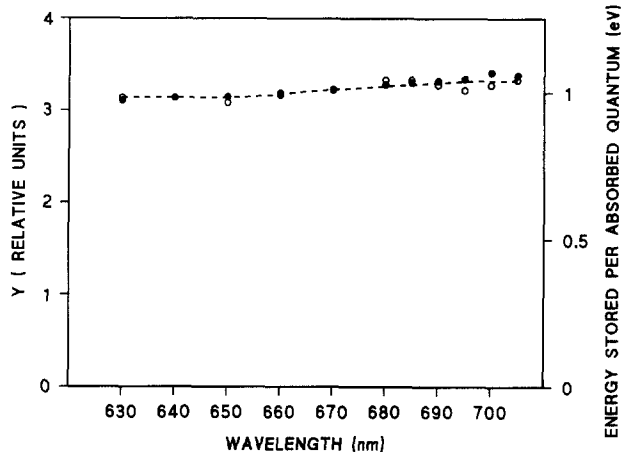

Fig. 3. Spectral dependence of the quantum yield in PS I preparations of *Synechocystis*. I_D was measured in a dark -adapted sample, with addition of 60 μM DAD, 10 mM sodium ascorbate, 0.2 mM methylviologen. The reference thermal signal (I_L) was measured without any addition, on a saturating light background. The quantity Y (left ordinate scale) is $E \cdot 1240/\lambda_{nm}$, with $E = (I_L - I_D)/I_{23°,L}$. E was measured at two different temperatures: 0°C and 23°C. The solid circles correspond to $E = (I_{23°,L} - I_{23°,D})/I_{23°,L}$, and the open circles to $E = (I_{0°,L} - I_{0°,D})/I_{23°,L}$, multiplied by the factor 1.35 for normalization.

(hc/λ). In this manner, the energy storage efficiency is converted to the quantum yield. In Fig. 3 and the following ones, we plotted on the left ordinate scale the quantity

$$Y = \frac{I_L - I_D}{I_{23°,L}} \cdot \frac{1240}{\lambda_{nm}}$$

which measures the quantum yield in relative units. The term $1240/\lambda_{nm}$ is the energy of one quantum, expressed in eV. At a given temperature, the value of Y depends on the thermal expansion properties of the medium, and is not directly comparable from one photosynthetic material to another.

In the following experiments, the chlorophyll concentration was typically 1 to 3 mg/ml. Such high concentrations, resulting in strong absorption of light by the sample, favour the sample signals at the expense of the mirror signal and the electric artefacts. Above 695 nm, however, the signal-to-noise ratio decreases markedly, due to the drop of the absorption spectrum of chlorophyll. Below 695 nm, the energy of the laser flash (about 15 μJ) was attenuated, so as to remain in the linear region of the light saturation curve of the photosynthetic reaction centres. We checked that an attenuation by a factor 2 did not change the photoacoustic signal (in dark-adapted material) normalized to the energy of the flash. We also checked that the spacing of the flashes (typically 1 s during each acquisition sequence) allowed complete dark-reactivation of the reaction centres closed by the flash.

Preparations containing a single Photosystem

Fig. 3 shows the spectral dependence of the quantum yield in PS I preparations from *Synechocystis* [10]. The signal I_D was measured on dark-adapted material, in the presence of an electron donor (DAD + ascorbate) and acceptor (methylviologen). The signal I_L was measured on a saturating light background, without any addition of electron carrier. The quantum yield is roughly constant over the explored wavelength range (630–705 nm), indicating that all the pigment molecules transfer with the same efficiency their excitation energy to the reaction centres. The absolute value of the energy storage efficiency, calculated according to expression (3), lies between 50% and 55%. Multiplying the energy storage efficiency by the energy of one quantum ($\mathscr{E}_{eV} = 1240/\lambda_{nm}$) gives the amount of energy stored per absorbed quantum. This quantity, as indicated on the right ordinate scale of Fig. 3, is close to 1 eV. It reflects the product $\rho\mathscr{E}_s$ where $\mathscr{E}_s$ is the energy stored by the reaction centre, and ρ, the quantum yield (number of charge separations per absorbed quantum). Then if we assume that the relevant state of the centre in the time-range of our measurement is the radical pair $P700^+(F_A,F_B)^-$, for which the midpoint-potential difference ΔE_m is expected to be close to 1 V [11], this would imply a quantum yield close to 1. However, we cannot exclude some contribution of the more primary radical pair $P700^+A_1^-$, that has a half-lifetime of 200 ns at room temperature (Sétif, personal communication). This would imply a slightly larger $\mathscr{E}_s$ (in the 1–1.2 eV range) and smaller ρ (0.8–1). Our main point here is that we obtain reasonable figures for the absolute value of $\mathscr{E}_s$. They differ significantly from those of Nitsch et al. [4], who found an energy storage efficiency of 83% in PS I particles from *Synechococcus*, corresponding to 1.5 eV per absorbed quantum. This value greatly exceeds what would be expected, even if the radical pair $P700^+A_1^-$ were stored with a quantum yield of 1 during the 1.4 μs time window specified by the authors.

Fig. 4 shows the spectral dependence of the quantum yield in spinach BBY particles [12,13]. These membrane fragments have a highly active PS II, but little or no PS I. The quantum yield is roughly constant in the investigated wavelength range, with about 60% of energy stored, or 1.1 eV per absorbed quantum, which is close to the energy required for the formation of the radical pair $Z^+Q_A^-$ [14] with a quantum yield of 1. At first sight, these values seem in agreement with those of Nitsch et al. in [4] (65% of energy stored, or 1.2 eV per absorbed quantum in PS II particles from *Synechococcus*). However, the PS II particles used in [4] differ strongly from the spinach BBY particles (on the number of chlorophyll molecules per reaction centre, or the fluorescence parameters), and the comparison may not be relevant. The apparent agreement

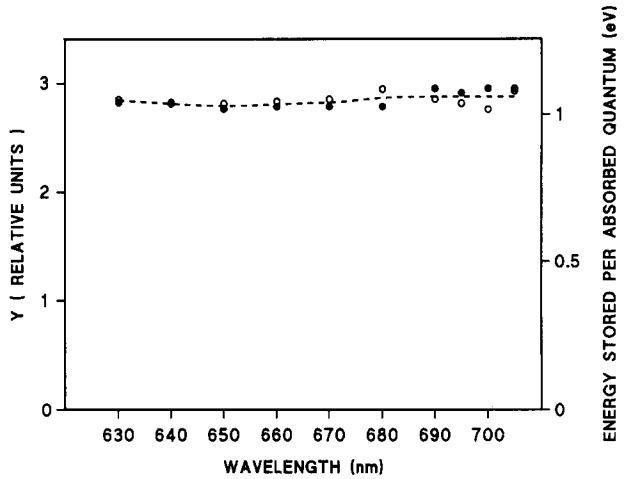

Fig. 4. Spectral dependence of the quantum yield in spinach BBY particles. No addition. The quantity $E = (I_L - I_D)/I_{22°,L}$ was measured at two different temperatures: 0°C and 22°C. The solid circles refer to $E = (I_{22°,L} - I_{22°,D})/I_{22°,L}$ and the open circles to $E = (I_{0°,L} - I_{0°,D})/I_{22°,L}$, multiplied by the factor 1.8 for normalization.

between our data on spinach BBY particles (which appear as a very efficient system), and those obtained by Nitsch et al. using possibly less active PS II particles but neglecting the conformation change, is very likely a fortuitous coincidence.

Fig. 5 shows a similar experiment with DEAE PS II particles prepared from *Chlamydomonas reinhardtii* according to Diner and Wollman [15]. These particles contain a small core antenna of around 40 chlorophyll *a* molecules per reaction centre. In the measurement of I_D, benzidine was added as electron donor and ferricyanide as electron acceptor. Only 40% of the absorbed light energy was stored. The amount of energy stored per absorbed quantum – about 0.7 eV –

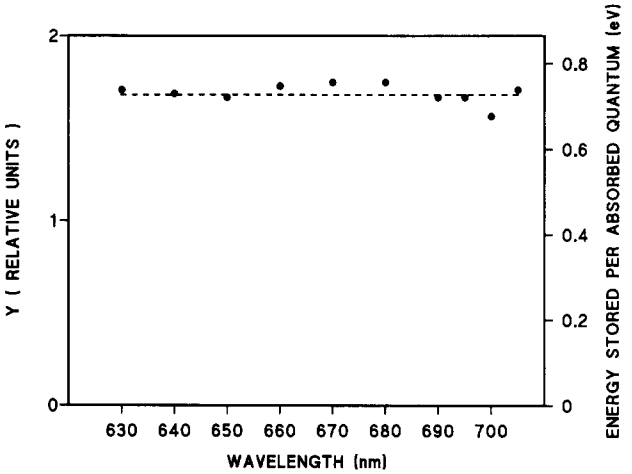

Fig. 5. Spectral dependence of the quantum yield in DEAE PS II particles from *C. reinhardtii*. Additions (dark-adapted sample): 10 μM benzidine and 10 μM sodium ferricyanide. The reference thermal signal (I_L) was measured without any addition, on a saturating light background. Temperature, 22°C.

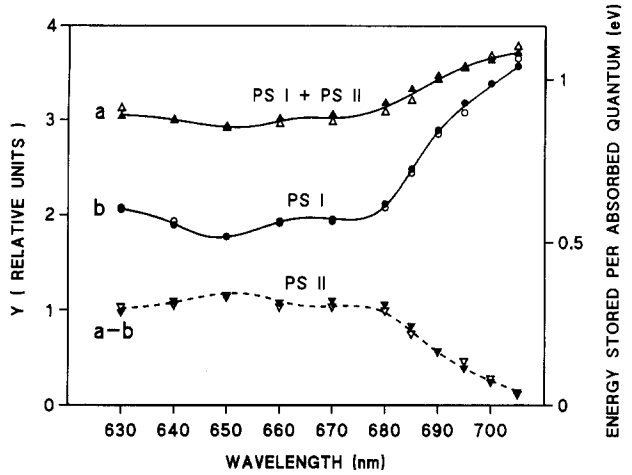

Fig. 6. Spectral dependence of the quantum yield in spinach chloroplasts (1.5 mg chlorophyll per ml, in 25 mM MES (pH 6.5), 10 mM NaCl, 5 mM $MgCl_2$) in the presence of 10 mM sodium ascorbate and 0.2 mM methylviologen. (a) Dark-adapted sample, no further addition. (b) The chloroplasts were treated with 40 μM DCMU plus 4 mM hydroxylamine, and saturated with continuous light during 30 s before the set of measurements. The quantity $E = (I_L - I_D)/I_{23°,L}$ was measured at two different temperatures, 0°C and 23°C. The solid symbols refer to $E = (I_{23°,L} - I_{23°,D})/I_{23°,L}$, and the open symbols to $E = (I_{0°,L} - I_{0°,D})/I_{23°,L}$, multiplied by the factor 1.3.

was much smaller than the energy required for the formation of the radical pair $P680^+Q_A^-$ (which is probably the species that we detected in this experiment, according to the lifetime reported in [16]), and would imply that about one-third of the absorbed quanta have not been transferred to active reaction centres. The quantum yield is constant in the explored spectral range, revealing no significant heterogeneity in the pigment organization. This suggests that the energy loss is attributable to inactive reaction centres, rather than to chlorophyll molecules more or less disconnected from the centres.

Preparations containing both Photosystems

In Fig. 6 (curve a), a similar experiment was performed using isolated spinach chloroplasts. A notable predominance of the PS I pigments – compared to those of PS II – appears in the quantum yield spectrum, characterized by a depression around 650 nm and an increase of the yield in the far-red region (beyond 680 nm). The shape of the spectrum is the same at 0°C and 23°C. Two different hypotheses could account for this shape:

(1) A significant fraction of the light quanta absorbed in the PS II antenna are not transferred to open reaction centres.

(2) The photoacoustic signal due to the charge separation occurring in a PS I reaction centre upon absorption of one quantum is larger than that originating from a PS II centre. In this case, the disparity should concern both thermal (energy storage) and conformation signal, because the shape of the quantum yield spectrum does not depend on temperature in the 0–23°C range, although the respective contributions of the two signals in the overall spectrum are highly temperature-dependent. In other words, the thermal and conformation signals have the same wavelength dependence. The possibility of both energy storage and conformation change in the PS I centre exceeding in the same significant proportion those observed in the PS II centre, although not entirely ruled out, would be a somewhat unlikely coincidence.

Hypothesis 1 seems more justified, and is reinforced by the unexpected observation that in chloroplasts (Fig. 6a) the energy stored per absorbed quantum seems to be significantly lower than in BBY particles (Fig. 4). The energy loss corresponding to the depression in Fig. 6a (about 15% of the maximal energy storage, or 30% when related to the sole PS II) is compatible with the ratio F_0/F_{max} (initial to maximal fluorescence yield) measured in fluorescence-induction experiments (20–25%), and with the relatively low quantum yield value of 0.7 reported by Thielen and Van Gorkom [17] for the reduction of Q_A in chloroplasts.

The data of Fig. 6a differ strongly from those reported by Nitsch et al. [4], Mullineaux et al. [5] and Bruce and Salehian [8] for intact cells of *Synechococcus*: the energy stored by absorbed quantum was 0.87 eV at 670 nm, 0.89eV at 677 nm and 1 eV at 690 nm in our spinach chloroplasts, corresponding to 47%, 49% and 56% of the absorbed light energy. In contrast, these authors found 74%, about 40% and more than 83% at these three wavelengths, respectively, in intact cells. Anyway, we never observed the apparent fall in energy storage near 677 nm reported in [4] and [8], and interpreted in [4] as the result of incomplete closing of the PS I centres during the internal heat calibration in presence of DCMU.

In Fig. 6 curve b, most of the PS II reaction centres were inhibited by addition of DCMU plus hydroxylamine and a few seconds of saturating preillumination before the experiment [18]. The spectrum of the relative quantum yield exhibits a substantial depression corresponding to the loss of PS II activity. The shape of this spectrum is very similar to that of the relative quantum yield spectrum of PS I (not shown) which may be calculated from the polarographic action spectra of Joliot et al. [19]. Likewise, the shape of the difference a – b (Fig. 6) matches reasonably that of the relative quantum yield spectrum of PS II calculated from [19]. The ratio b/a (not plotted on the figure) exceeds what would be expected, assuming with Joliot et al. that PS I and II absorb equally at 680 nm. This is probably due to the contribution, in spectrum b, of a significant fraction of PS II centres which recover in the dark in presence of DCMU plus hydroxylamine [18]. When

corrected for this proportion of active PS II centres (estimated to about 20% from fluorescence-induction measurements in situ), the amounts of energy stored by each of the two Photosystems are approximately equivalent in the 630–680 nm range. In separate experiments using optical spectrophotometry, we confirmed that the membrane potential induced by a weak light pulse of 660 nm (and measured by the electrochromic shift at 515–494 nm) originated almost evenly from both Photosystems, with a very small excess in favour of PS I (51–55% of the total signal).

As already pointed out, isolated chloroplasts (Fig. 6a) seem to store less energy per absorbed quantum than BBY particles (Fig. 4) in the LHC II absorption region (around 650 nm). In contrast, the energy storage near 700 nm seems higher in chloroplasts (Fig. 6a) than in purified PS I (Fig. 3), suggesting a very efficient connection of the PS I antenna in chloroplasts.

Intact cells

In intact cells of plants and green algae, the distribution of excitation energy among the PS II and PS I reaction centres is regulated by the light-state transitions. Transition from state I to state II, which increases the excitation of the PS I centres at the expense of PS II, is favoured by reducing conditions and low concentration of ATP, whereas the opposite transition – from state II to state I – requires oxidizing conditions and high ATP level. In the experiment of Fig. 7 (a and b), we used cells of *C. reinhardtii* stabilized in state I by the following treatment, according to Bulté and Wollman [20]: the algal culture was aerated during 30 min under strong white illumination in the presence of 20 μM DCMU, before being fixed by 0.3 mM parabenzoquinone. The benzoquinone treatment prevents (1) reversion to state II, and (2) closure of the PS II centres by reduction of Q_A in the dark under anaerobic conditions. The quantum yield spectrum (Fig. 7) is similar to that of spinach chloroplasts (Fig. 6). Once again, we did not observe anything which could be compared to the apparent fall in energy storage near 677 nm reported in [4] and [8] for intact cells of *Synechococcus*.

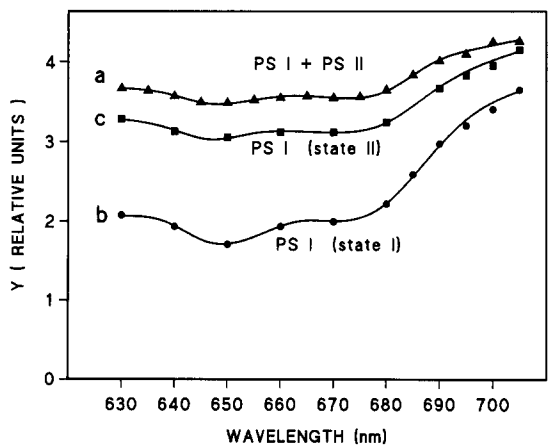

Fig. 7. Spectral dependence of the quantum yield in *C. reinhardtii*. Temperature, 22°C. In a and b, the cells have been fixed in state I. (a) Dark-adapted sample, no further addition. (b) The cells were treated with 40 μM DCMU plus 4 mM hydroxylamine, and saturated with continuous light during 30 s before the set of measurements. In c, the cells had stabilized in state II, before being treated as in b to block the PS II centres.

In curve b of Fig. 7, the spectrum was measured after addition of hydroxylamine and a few seconds of preillumination. In this case, (1) most of the PS II reaction centres are closed (to a larger extent than in spinach chloroplasts (Fig. 6b), in agreement with [18]); (2) the excitation energy of the PS II antenna cannot be transferred to the PS I centres. Only PS I is working, and the spectrum is similar to that observed in isolated spinach chloroplasts (curve b of Fig. 6), which are blocked in state I for lack of LHC-kinase activity. The difference a – b (not plotted on the figure) measures the efficiency of energy transfer to active PS II reactions centres in state I.

The same type of experiment was performed with untreated cells of *C. reinhardtii*. Under the anaerobic conditions prevailing in the measuring cuvette, the cells spontaneously stabilized in state II [21]. When all the reaction centres are open, the quantum yield spectrum (not shown) is similar to that of state I (Fig. 7, curve a). On the other hand, when the PS II centres are closed (curve c), the quantum yield is decreased only by about 10%, proving that a large part of the light energy absorbed in the PS II antenna is transferred to the reaction centres of PS I, as expected in state II. This situation contrasts strikingly with that of state I, depicted by curve b. The difference a – c (not plotted on the figure) measures the efficiency of the fraction of antenna which remains connected to PS II centres, whereas the difference c – b corresponds to the 'mobile' LHC II. The ratio (c – b)/(a – b) indicates that in state II, more than 70% of the quanta absorbed by LHC II are transferred to PS I reaction centres.

4. Conclusion

The photoacoustic method described above proved to be useful for measuring the efficiency of excitation transfer in photosynthetic materials. This technique applies to detecting the different light-absorbing fractions in a photosynthetic preparation, whenever these fractions are distinguishable by their excitation transfer properties. The different excitation transfer efficiencies appear as spectral variations of the quantum yield of charge separation. The method is of special interest in studying dynamic changes of pigment distribution in intact cells, such as those involved in the light-state

transitions. The present work should be regarded as a preliminary approach to more detailed investigations.

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6. References

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