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Minireview

On some aspects of photosynthesis revealed by photoacoustic studies: a critical evaluation

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Abstract

Photoacoustic techniques have been widely developed in photosynthesis research since the 1970s. We can divide the progress in this field into three periods. In the first period, a pioneer, William W. Parson (and his co-workers) discovered that the photochemical charge separation is accompanied by a conformation change. In the second period, the technique was essentially used to measure the two components of photochemical activity detected in the gas phase: energy storage (photothermal effect) and gas exchange (photobaric effect). In the third period, the time resolution and sensitivity of detection in liquid phase were significantly improved. In reviewing this last period, we shall focus on three aspects: conformation changes, thermodynamic parameters, and quantum yield spectra.

Abbreviations: A_1 – the phylloquinone electron acceptor of PS I; BBY – Berthold, Babcock and Yocum; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DEAE – diethylaminoethyl ether sepharose; F_A,F_B – iron-sulfur electron acceptors of PS I; LHC – light-harvesting complex; LIOAC – laser-induced optoacoustic calorimetry; LIOAS – laser-induced optoacoustic spectroscopy; P – the primary electron donor of the bacterial reaction center; P_{680} – the primary electron donor of PS II; P_{700} – the primary electron donor of PS I; PAC – photoacoustic calorimetry; PAS – photoacoustic spectroscopy; PS I – Photosystem I; PS II – Photosystem II; Q_A – the first quinone acceptor of bacterial and PS II reaction center; PTRPA – pulsed time-resolved photoacoustics; S states – successive steps of positive charge accumulation in the oxygen evolving complex of PS II; Y_Z – tyrosine electron donor to P_{680}^+

Introduction

Although described by some authors as 'an increasingly popular method in photosynthesis research' (Fork and Herbert 1993), the photoacoustic technique is by far not as popular as optical (absorption or fluorescence) photometry. However, this technique gives a unique insight into the energetic balance of the photochemical processes, and also into some other aspects such as the conformation change or gas exchange (oxygen release by leaves) associated with photochemistry.

Many photoacoustic studies of photosynthesis have been obscured by extensive theoretical and mathematical developments. Sometimes they are not immediately essential and mask the main thing, when an intuitive approach would be sufficient and more fruitful. Other useless complication has resulted from the number of different names (PAC or PAS, LIOAC or LIOAS, PTRPA: see list of abbreviations for their full forms) assigned to the same technique, using one and the same physical principle: the photoacoustic effect. Inappropriate or non-standardized language also contributed to confuse the reader. As an example, some authors named *photochemical loss* the amount

of absorbed light energy that is converted into chemical energy, meaning that it *decreases* the thermal emission. This rather confusing name is designated, roughly speaking, the *photochemical energy storage*, i.e., the exact opposite of an *energy loss*.

The principle

The photoacoustic effect is *the production of sound by light*, according to the very terms of its discoverer Alexander Graham Bell (1880). Upon excitation of an absorbing sample by pulsed or modulated light, volume changes occur in the sample and the surrounding medium, generating pressure waves. Practical use of Bell's discovery for studying condensed materials had to wait almost one century, until suitable measuring techniques were developed in the 1970s. These techniques use either continuous modulated light or flash excitation, and the pressure changes are detected by a microphone or a piezoelectric transducer (for the basic experimental devices, see Malkin and Canaani 1994).

The pioneers: William W. Parson and his co-workers (1972–1981). Detection in the liquid phase under flash excitation; discovery of conformational volume changes

The use of the photoacoustic effect to measure flash-induced volume changes in photosynthetic materials was introduced by Callis et al. (1972) using a suspension of *Chromatium* chromatophores; it was developed further by Arata and Parson (1981a) using reaction centers of *Rhodopseudomonas (Rhodobacter) sphæroides*. The volume changes were measured by a capacitor microphone in direct contact with the liquid phase, on a time scale from 100 μs to 1 s following the flash (see Figure 1 for a photograph of Bill Parson). An attractive feature of the flash detection used was the possibility of analyzing the complete relaxation kinetics of the light-induced volume changes (Figure 2). The response time of the capacitor microphone was limited to 100 μs, and the large volume of the measuring cell ($\approx$ 15 ml) required the use of a large amount of biological material. However this technique, when suitably improved, would still have its full potential for kinetic studies.

Callis et al. (1972) clearly stated that the flash-induced volume change (ΔV) was composed of two

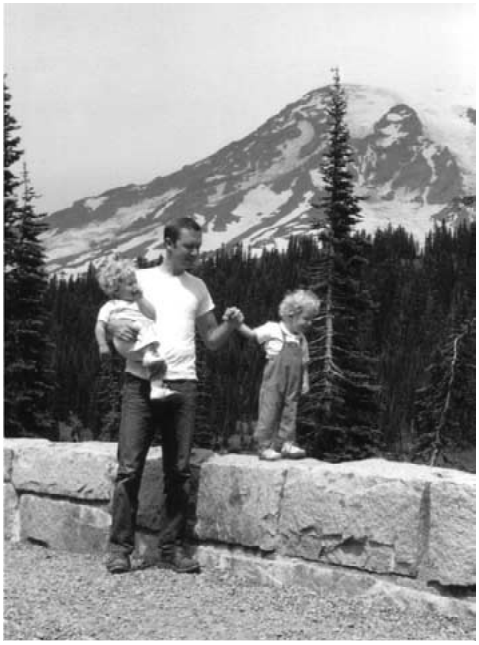

Figure 1. A 1968 photograph of Bill Parson with daughters Wendy and Christy in Mt. Ranier Park. Photo by Polly Parson.

terms: the thermal expansion of the medium through heating, and the volume difference between reactants and products. The first term (ΔV_{th}) results from thermal conversion of part of the absorbed light energy (photothermal effect), and the second one (ΔV_{conf}) reflects molecular conformation changes associated with the photoreaction. Thus:

$$\Delta V = \Delta V_{th} + \Delta V_{conf}$$

These two components of the photoacoustic signal could be resolved by measuring the volume changes at two different temperatures, assuming that only ΔV_{th} is temperature dependent. In addition to the thermal *expansion* (ΔV_{th}), the authors calculated a *contraction* (ΔV_{conf}) of approx. 33 Å^3 per electron transferred, likely due to local electrostatic interactions between the photo-induced positive and negative charges and the surrounding medium (electrostriction) (Arata and Parson 1981a). However, the enthalpy change calculated from ΔV_{th} was inconsistent with those obtained by the same authors using delayed luminescence (see below).

Detection in the gas phase: energy storage and gas exchanges (1978–1994)

Photoacoustic detection in the gas phase under *modulated light excitation* has been applied extensively to

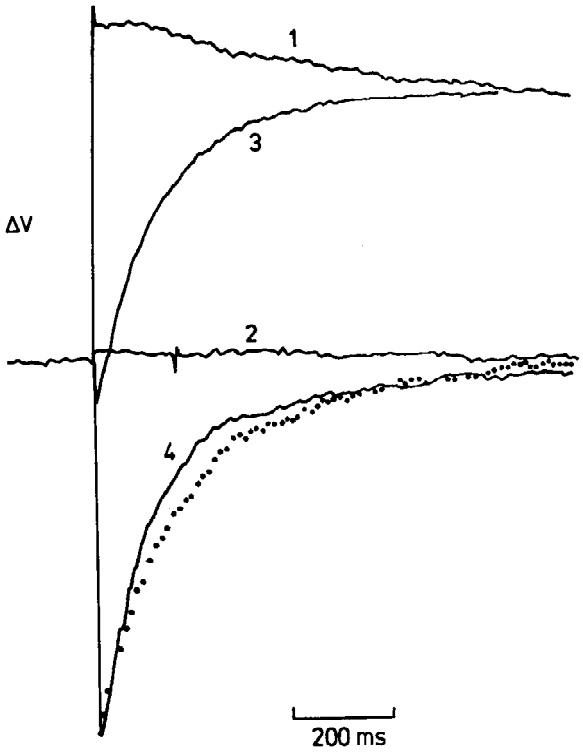

Figure 2. Flash-induced volume changes of *R. sphæroides* reaction centers containing a single quinone acceptor (Q_A). Temperature: 22 °C (trace 3) and 3.6 °C (trace 4). Traces 1 and 2 are the volume changes of a bromocresol purple solution (photochemically inactive) of the same absorbance. Temperature: 22 °C (trace 1) and 3.6 °C (trace 2). The dotted line (trace 3 minus trace 1) represents the volume change due to the charge separation at 22 °C. Excitation: dye laser flash of 0.5 μs, wavelength 588 nm. From Arata and Parson (1981a).

Figure 3. A photograph of Shmuel Malkin, playing the piano.

the evaluation of photosynthetic energy storage and oxygen evolution *in vivo*, especially in the laboratory of Shmuel Malkin at the Weizmann Institute in Rehovot (see Figure 3 for a photograph of Malkin, who is also an accomplished musician). Modulated heat is emitted by the illuminated sample at the same frequency as the excitation light, and the thermally induced pressure wave is detected by a gas-coupled microphone (Cahen et al. 1978). Note that the gas microphone measures primarily thermal expansion (ΔV_{th}) in the gas phase (owing to the large expansion coefficient of air), without any noticeable contribution of conformation changes (ΔV_{conf}) (Lasser-Ross et al. 1980). In addition, Malkin and Cahen (1979) pointed out that gas exchanges could also give rise to modulated volume changes superimposed on the photothermal changes. Studying tobacco leaves, Bults et al. (1982) showed that at low modulation frequency (approx. 100 Hz and below), a considerable fraction of the photoacoustic signal results from direct pressure modulation by modulated oxygen evolution (photobaric effect), whereas at high frequency (above 200 Hz), the main contribution is from conversion of modulated heat to modulated pressure.

The modulated technique was also adapted to *pulsed light excitation*. In leaves illuminated by single turnover flashes, Canaani et al. (1988) and Mauzerall (1990) were able to observe photoacoustic pulses of oxygen evolution, oscillating in accordance with the S states. From a single measurement, the complete relaxation kinetics could be analyzed in the time range of 30 μs to 100 ms following the flash.

Detection in the liquid phase under laser pulse excitation (1985–2002)

A much higher time resolution (in the nanosecond to microsecond range) was reached by *laser optoacoustic spectroscopy*, in which the pressure changes induced by a laser pulse are detected in the liquid phase by a piezoelectric transducer (Patel and Tam 1979). In the classical version, the acoustic wave is detected at right angles from the laser beam. The time resolution of heat detection is restricted by the duration of the laser pulse, the time response of the piezoelectric detector, and the transit time of the acoustic pulse across the diameter of the laser beam. The latter (about 0.7 μs per mm) is usually limiting. Application of this technique to highly scattering materials such as intact plant tissues required a special optical arrangement to cancel

Figure 4. Photograph of (left to right) Pierre Joliot, Daniel Béal and René Delosme (June 2002). Photo by Richard Kuras.

the scattered-light induced signals. Following the first application *in vivo* by Jabben and Schaffner (1985) on intact leaves, a number of studies appeared in subsequent years, especially from Silvia E. Braslavsky and colleagues (Braslavsky 1986; Nitsch et al. 1988, 1989; Braslavsky and Heihoff 1989; Mullineaux et al. 1991), joined later by Shmuel Malkin and colleagues (Malkin et al. 1994; Puchenkov et al. 1995).

Pierre Joliot and Daniel Béal designed a new high-sensitivity photoacoustic spectrometer operating in the same time window of 1 μs, but using a quite different geometry (Delosme et al. 1994; see a photograph of the authors in Figure 4). The measuring pulsed light, in this instrument, is distributed evenly on a thin layer of photosynthetic material (50 μm thickness). The total volume of the measuring cell is less than 8 μl. The fraction of incident light which has not been absorbed by the layer is reflected backwards by a mirror, and a piezoelectric ceramic detects the pressure waves propagating in the direction of the laser beam. Note that another front-face illumination cell has been described by Melton et al. (1989). The group of David Mauzerall used a similar principle, according to a design of Arnaut et al. (1992). In the technique used by Delosme et al., the theoretical response time corresponds to the transit time (about 30 ns) of the sound wave across the 50 μm thickness of the sample. However, the instrument was adapted for detection in the μs range, using a ceramic of 1 MHz resonance frequency. The high signal-to-noise ratio of the method allows detection of signals from samples exposed to very weak monochromatic flashes, which do not induce any significant actinic effect (about 1 photon per 400 reaction centers).

All the various applications of the photoacoustic method could not fit into the limited space of this minireview. In the following, we shall focus on three of them: measurement of the conformation changes, determination of the thermodynamic parameters, and quantum yield spectra.

Absolute value of the conformational volume change

Curiously, the pioneering work of Callis et al. (1972) seems to have been widely ignored for a number of years (see, however, Lasser-Ross et al. 1980). Some reviews (Fork and Herbert 1993) quoted this work, but overlooked the major discovery of a conformational volume decrease, and considered that the photoacoustic signal in liquid phase was purely thermal. Others (Braslavsky and Heibel 1992; Malkin and Canaani 1994; Braslavsky 1994) correctly abandoned this view, and recognized that the conformational change should by no means be neglected.

Delosme et al. (1994) observed a conformational change in both PS I and PS II of photosynthesis, and almost at the same time Malkin et al. (1994) confirmed its occurrence in reaction centers of *R. sphæroides*. Further studies followed rapidly: Puchenkov et al. (1995) and Mauzerall et al. (1995) attempted to determine more precisely its absolute value.

Photosynthetic bacteria

Puchenkov et al. (1995) found a photoinduced contraction of -32 ± 1 Å^3 per reaction center of *R. sphæroides*, in very close agreement with the value of ~ -33 Å^3 (or 20 ml mol^{-1}) reported by Arata and Parson (1981a). Puchenkov et al. attributed to an inaccurate extrapolation procedure the smaller value (-12 Å^3) reported in a previous work by Malkin et al. (1994). Halfway between these two values, Mauzerall et al. (1995) found -20 Å^3, and Edens et al. (2000) considered that a value of -28 Å^3 was more accurate. These results are summarized in Table 1.

Cyanobacteria, green algae and plants

Delosme et al. (1994) observed that a conformational volume change occurred in purified PS I from *Synechocystis*, and estimated its value to be about -20 Å^3 per absorbed quantum. A somewhat larger value of -26 Å^{3}was found recently in the group of David Mauzerall, by Hou et al. (2001a). According to Hou

Table 1. R. sphaeroides

Reference	Volume change
Arata and Parson (1981a)	~ -33 Å^3 [a]
Malkin et al. (1994)	-12 Å^3
Puchenkov et al. (1995)	-32 Å^3
Mauzerall et al. (1995)	-20 Å^3
Edens et al. (2000)	-28 Å^3

[a]The same value was found by Callis et al. (1972) in *Chromatium* chromatophores.

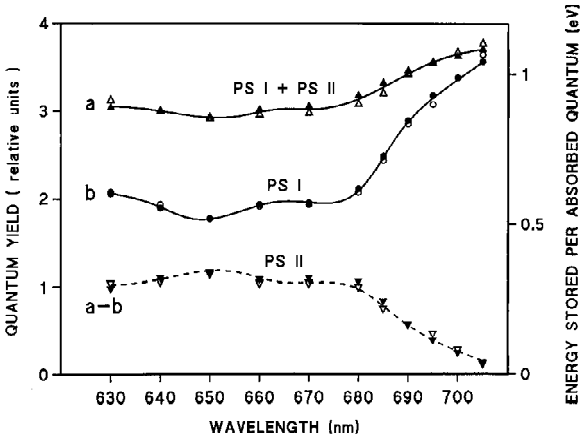

Figure 5. Photoacoustic quantum yield spectrum of spinach chloroplasts in the red region. (a) control (dark-adapted): PS I and PS II together. (b) PS II was inactivated by addition of 40 μm DCMU plus 4 mm hydroxylamine and 30 s of continuous light. Then the chloroplasts were left in the dark before the set of measurements. (bottom curve): PSII alone (a-b). Temperature: 23 °C (solid symbols) and 0 °C (open symbols). The volume changes at 0 °C were multiplied by the factor 1.3. From Delosme et al. (1994).

et al. (2001b), the contraction is much less in PS II: -9 Å^3 (at pH 6) and -3.4 Å^3(at pH 9) in manganese-depleted PS II core complexes (a value of -9 Å^3 was also found recently by A. Boussac and R. Delosme (unpublished data) in purified active PS II cores from *Thermosynechococcus elongatus* (Roncel et al. 2002). But the most surprising result was obtained by the group of Mauzerall in intact cells of *Synechocystis* (Boichenko et al. 2001): while the contraction of PS I was -27 Å^3, that of PS II was only -2 Å^3.

The last result contrasts strongly with those published by Delosme et al. (1994, 1996) on green algae and plants. In view of the experiments of Delosme et al., the contraction in PS II cannot differ by an order of magnitude from that of PS I in these materials. Although not specified by these authors, the following values can be estimated from their photoacoustic measurements at two temperatures: about -11 Å^3 in DEAE (diethylaminoethyl ether sepharose) PS II core particles from *Chlamydomonas reinhardtii* (de Vitry et al. 1991), -16 Å^3 in BBY (Berthold–Babcock–Yocum) PS II particles from spinach, and -13 Å^3 in spinach isolated chloroplasts (versus about -23 Å^3 for PS I in the same material). Together with these data, the photoacoustic quantum yield spectra of spinach chloroplasts (Figure 5) and of whole cells of *C. reinhardtii* (in state 1) imply that in both materials the contraction of PS II approaches 60% of that of PS I (see the discussion of this point in Delosme et al. 1994).

Thus, the quasi-absence of contraction of PS II in *Synechocystis* cells, as it was reported by Boichenko et al. (2001), would mean that the electrostatic properties of PS II *in situ* differ significantly between cyanobacteria and green organisms. This interesting discovery requires confirmation. Especially, one would like to be sure that PS II was fully active in the photoacoustic experiments of Boichenko et al., a requirement very difficult to satisfy in dark-adapted cells of cyanobacteria (see below the section 'Quantum yield spectra').

Thermodynamic parameters

Photosynthetic bacteria

Parson and his coworkers were the first to consider the thermodynamics of photosynthetic electron transport not only in terms of free energy (determined by redox titrations of the electron carriers), but also in terms of the underlying enthalpy and entropy changes. Studying the primary photochemical reaction in the photosynthetic bacterium *Chromatium vinosum*, Case and Parson (1971) resolved the free energy changes into enthalpy and entropy changes, by measuring the midpoint redox potentials of the electron donors and acceptors as a function of temperature. Unexpectedly, they found that the charge separation did not cause a significant enthalpy change, and thus concluded that an entropy *decrease* accounted for all of the free energy stored.

This unexpected result required confirmation by an independent approach. Measurement of heat released or absorbed during a reaction is the most direct method to determine the enthalpy change (ΔH) of the reaction. Thus the photoacoustic technique is ideally suited for the determination of ΔH, provided that the *thermal* contribution (ΔV_{th}) can be resolved from the overall

Table 2. Reaction $PQ_A \rightarrow P^+Q_A^-$ in reaction centers of *R. sphaeroides*. All the data are expressed in eV

Reference	ΔG	ΔH	TΔS
Arata and Parson (1981a)	+0.65	+0.05	−0.6
Arata and Parson (1981b)	+0.52	+0.63	+0.11
Nitsch et al. (1989)	+0.62	+0.62	∼0[a]
Malkin et al. (1994)		+0.83	[+0.31][b]
Puchenkov et al. (1995)		+0.56	[+0.04] [b]
Edens et al. (2000)		+0.94	+0.42[b]

[a]Intact cells of *R. rubrum.*
[b]Assuming ΔG = +0.52 eV.

signal. Only in this case one can do photoacoustic *calorimetry*, properly speaking.

Using this approach, Callis et al. (1972) confirmed the surprising result of Case and Parson (1971): they found that the charge separation did not cause any significant enthalpy change in chromatophores of *C. vinosum*, and this finding was once more confirmed by Arata and Parson (1981a) for the reaction $PQ_A \rightarrow P^+Q_A^-$ in reaction centers of *R. sphaeroides* (ΔH = +0.05 to 0.13 eV, depending on the type of centers used): obviously, there is no room in Figure 2 for a temperature dependent component (ΔV_{th}) in the flash-induced volume change.

All these results disagreed seriously with those of Carithers and Parson (1975) indicating a ΔH of +0.7 eV (and TΔS = +0.11) in chromatophores of *R. viridis*, from measurements of the temperature dependence of delayed fluorescence. Arata and Parson (1981b) repeated the same type of delayed fluorescence measurements in reaction centers of *R. sphæroides*, and found ΔH = +0.63 eV, inconsistent with their earlier calorimetric determinations. This major discrepancy has never been convincingly resolved. All the later photoacoustic studies concluded that there is a positive enthalpy change of at least +0.5 eV for the formation of $P^+Q_A^-$ from the ground state PQ_A, and thus a *null or positive* entropy change. The data are summarized in Table 2, and call for the followings comments:

(1) The conclusions of Nitsch et al. (1989) might be unreliable, because the authors have ignored the conformation changes (these are probably not negligible, although 33% ethylene glycol was added to enhance the thermal part of the signal: cf. Callis et al. 1972; Delosme et al. 1994; Malkin et al. 1994).

(2) The data of Malkin et al. (1994) differ strongly from those of Puchenkov et al. (1995) collected later in the same laboratory. The last ones resulted from a more precise analysis, and thus were considered as more reliable by the authors.

(3) Edens et al. (2000) assigned the positive sign of the entropy change to the release of counterions from the surface of the reaction center when the charge transfer cancels the dominant opposite charges.

Table 3. Purified PS I (cyanobacteria). Reaction $P_{700}(F_{A,}F_B) \rightarrow P_{700}^+ (F_{A,}F_B)^-$

Reference	ΔG	ΔH	TΔS
Nitsch et al. (1988)[a]		+1.52[a]	[+0.52][a,b]
Delosme et al. (1994)		∼ +1	[∼0][a]
Hou et al. (2001a)	+1.03	+1.38	+0.35

[a]The final state considered was $P_{700}^+A_1^-$.
[b]Assuming ΔG ≈ +1 eV.

Table 4. Purified PS II (cyanobacteria). Reaction $Y_ZQ_A \rightarrow Y_Z^+ Q_A^-$, or (at pH 6 in Mn-depleted cores) $P_{680}Q_A \rightarrow P_{680}^+Q_A^-$

Reference	ΔG	ΔH	TΔS
Nitsch et al. (1988)		+1.19	[+0.19][a]
Hou et al. (2001b)	+0.9[b]	+0.67[b]	−0.23[b]
Hou et al. (2001b)	∼+1[c]	+0.92[c]	∼ −0.1[c]

[a]Assuming ΔG ≈ +1 eV.
[b]at pH 9: the final state considered was $Y_Z^+ Q_A^-$.
[c] at pH 6: the final state considered was $P_{680}^+Q_A^-$, and the estimated ΔG could vary from 1.05 to 1.15 eV, depending on a possible deprotonation of histidine. Accordingly, TΔS could vary from −0.13 to −0.23 eV.

The values in Table 2 are spread over a considerable range. Especially the two more recent determinations lead to irreconcilable thermodynamic conclusions: the ΔH value measured by Puchenkov et al. is very close to ΔG and thus leaves no place for a significant entropy change. On the contrary, the very high enthalpy storage measured by Edens et al. largely exceeds the free energy of $P^+Q_A^-$ above the ground state. According to these authors, the difference results from a large *entropy increase* that a) has not been usually considered in the theories of electron transfer and b) is not expected to accompany electrostriction.

Table 5. Intact cells (cyanobacteria) (Boichenko et al. 2001)

	ΔG	ΔH	TΔS
PS I	+1.03	+1.44	+0.41
PS II	+1.05	+0.82	−0.23

Cyanobacteria

Tables 3 and 4 bring together the available data on purified PS I and PS II complexes, and Table 5 the recent data on whole cells of *Synechocystis*.

(1) Neglecting the conformation change may have led several authors to overestimate the energy stored in *Synechococcus*: Nitsch et al. (1988), and also Mullineaux et al. (1991) (near 1.6 eV in intact cells). Bruce and Salehian (1992), who found 1.26 to 1.37 eV, envisaged a possible contribution of conformation changes, but considered this contribution to be negligible.

Delosme et al. (1994) proved the last hypothesis to be far from being justified. They resolved the thermal component (ΔV_{th}) of the photoacoustic signal by measuring the volume changes at two different temperatures. They found that purified PS I of *Synechocystis* stored about 1 eV per absorbed photon of red light within the first microsecond following a laser flash. This estimation fits reasonably the free energy required for the formation of the radical pair $P_{700}{}^{+}(F_A,F_B)^{-}$ with a quantum yield of 1.

(2) However, Hou et al. (2001a), using purified PS I trimer complexes from *Synechocystis*, produced results similar to those obtained by Edens et al. (2000) in bacterial reaction centers: essentially a large enthalpy stored (+1.38 eV), and consequently a large *positive* entropy change (+0.35 eV). As in bacterial reaction centers, the unexpected sign of the entropy change was attributed to the escape of counterions from the surface of the particles.

(3) In manganese-depleted PS II core complexes from *Synechocystis*, Hou et al. (2001b) found a much lower thermal efficiency than in PS I, in spite of the fact that the calculated quantum yield was close to 1. According to Hou et al. (2001b) the ΔH values imply a *negative* entropy change (-0.23 to -0.1 eV), in contrast to the *positive* entropy change found in PS I and bacterial reaction centers. They explained this singularity of PS II by the absence of charge formation in the microsecond range. The above conclusion should be taken with great prudence. According to recent unpublished data of Alain Boussac and René Delosme, the thermal efficiency of purified active PS II cores from *Thermosynechococcus elongatus* depends strongly on the experimental conditions (such as the electron donor or acceptor used), and could be significantly higher ($\Delta H \geq +1$ eV) than those reported by Hou et al. for manganese-depleted cores from *Synechocystis*.

(4) The data of Hou et al. were confirmed by Boichenko et al. (2001) studying *intact cells* of *Synechocystis* (Table 5). A large *entropy increase* of +0.41 eV was found in PS I, contrasting with an *entropy decrease* of -0.23 eV in PS II. According to Boichenko et al., this last result is expected for charge formation in solution, considering that electron transfer in PS II (unlike PS I and bacterial centers) is associated with proton transfer.

In fact, the electrostatic events associated with the different steps of charge separation are not yet fully understood, and remain much debated. The various interpretations proposed by the group of Mauzerall in support of their experimental data seem rather obscure and apparently conflicting, and illustrate the complexity of this area of research.

Plants and green algae

Delosme et al. (1994) have shown that in isolated spinach chloroplasts, PS II stores less energy than PS I in the microsecond range (ΔH = +0.68 eV *versus* +1.06 eV). The same ratio applies to whole cells of *C. reinhardtii* in state 1, and also to tobacco leaves (Delosme 1998). This finding qualitatively agrees with the recent results of the group of Mauzerall on *Synechocystis* mentioned above, although the absolute values of ΔH reported by Delosme et al. are significantly lower (by 20%). Since the enthalpy stored in PS I (+1.06 eV) corresponded roughly to the free energy of the reaction $P_{700}(F_A,F_B) \rightarrow P_{700}{}^{+}\ (F_A,F_B)^{-}$, Delosme et al. did not call for an entropic term. Regarding PS II, they suggested that its relatively poor efficiency resulted from energy losses in the PS II antenna. Alternatively, the enthalpy change of the reaction $Y_ZQ_A \rightarrow Y_Z{}^{+}\ Q_A{}^{-}$ could be 60% lower than that of the reaction $P_{700}(F_A,F_B) \rightarrow P_{700}{}^{+}\ (F_A,F_B)^{-}$, as reported for *Synechocystis* by the group of Mauzerall, and in this case an entropy decrease in PS II should be worth considering.

There still remain many doubts and inconsistencies as to the thermodynamic parameters of charge separation in photosynthetic materials. As mentioned above, a major difficulty concerns the photoacoustic detection of PS II activity in intact cells of cyanobacteria, where the redox state of the plastoquinone pool regulates both electron transfer and excitation energy

distribution (state transitions). In this respect, green algae and plants are more easy to control.

Quantum yield spectra

The most successful application of the photoacoustic technique in photosynthesis is probably the measurement of action spectra and quantum yield spectra. Unlike the other applications discussed above, this type of study does not aim to determine *absolute* values, but only *relative* values of photochemical activity as a function of the wavelength of exciting light. Curiously, there are relatively few examples of such studies in the literature on photoacoustics in photosynthesis.

Modulated light excitation

The first photoacoustic quantum yield spectra of oxygen evolution and energy storage were measured under modulated light by Bults et al. (1982) and Canaani and Malkin (1984) in leaves, and Canaani et al. (1989) in *C. reinhardtii*. Herbert et al. (1990) published quantum yield spectra of energy storage in the red region for a wide variety of photosynthetic organisms in the presence or absence of DCMU (Figure 6). An interesting result was that no detectable energy storage occurred in C_3-type plants (*Oxalis*) when PS II was fully inhibited by DCMU. This could suggest that in C_3 plants a few electrons provided by PS II are required to compensate for the leaks of the cyclic process, and thus to maintain a noticeable electron flow around PS I. In contrast, DCMU-treated C_4 plants (*Sorghum*), algae and cyanobacteria showed significant energy storage, with a maximum in the far red region. The last point is a typical feature of PS I, and thus Herbert et al. (1990) must be credited for the first reliable photoacoustic spectra of PS I *in vivo*.

Veeranjaneyulu and Leblanc (1994) published quantum yield spectra of PS I and PS II (together and individually) measured in sugar maple leaves under modulated light (Figure 7). The overall spectrum (PS I + PS II) was nearly the same under state 1 and state 2 conditions, and showed the well-known 'red drop' discovered by Emerson and Lewis (1943), and also a depression in the region of carotenoids, separated in two parts by a small peak of chlorophyll *b* at 470 nm. However the red drop (due to the abrupt fall of PS II absorption above 680 nm) started from 670 nm instead of the expected wavelength of 680 nm, and also affected the PS I spectrum. Another unexpected drop occurred in the Soret band of chlorophyll *a*, below 430 nm. These anomalies, which could reveal some undesirable actinic effect of the detecting light, question the reliability of the spectra presented. Under state 1 conditions, PS II was found to be three times more efficient than PS I (even up to eight times in the Soret band of chlorophyll *b*): an unexpected imbalance when the LHC II connected to PS II should equilibrate, to a large extent, the optical cross sections of the two photosystems. Under state 2 conditions, where the connection of the mobile LHC II to PSI should favor PS I, the efficiency of both photosystems was nearly the same: just the situation that would be expected in state 1. Migration of LHC does not seem sufficient to explain these results, and two questions arise: (a) on the validity of the method using a 'saturating' far red background to resolve the PS I and PS II components of the photoacoustic signal, and (b) on the involvement of genuine state transitions in the changes observed.

Pulsed excitation: green algae and plants

All these earlier studies used modulated light excitation under steady state conditions, which implied the closure of a significant fraction of the reaction centers. Delosme et al. (1994) used a different approach. A monochromatic laser flash of very low energy sampled the photochemical activity in dark-adapted material, i.e., under conditions where the concentration of open reaction centers was maximal. Thus the measured quantum yield was not limited by the steady-state turnover of the centers, but only by the efficiency of excitation energy transfer. The efficiency of the different pigment-protein complexes was discussed on the basis of the quantum yield spectra measured in a variety of materials containing all or part of these complexes, and specially in whole cells and leaves (Delosme et al. 1994, 1996; Delosme 1998). Figure 8 illustrates the example of isolated spinach chloroplasts. The red part of the spectrum has already been shown in more detail in Figure 5. Note that no 'red drop' is expected here, since there is no steady-state linear electron flow, and thus underexcitation of PS II by far red light does not affect the PS I signal.

The above method proved to be particularly useful for the quantitative study of state transitions in *Chlamydomonas reinhardtii*, and solved the debate on the connection – or not – of the phosphorylated LHC II to PS I in state 2. It was demonstrated that about 80% of LHC II connects to PS I in state 2 (i.e., when the plastoquinone pool is fully reduced), increasing by

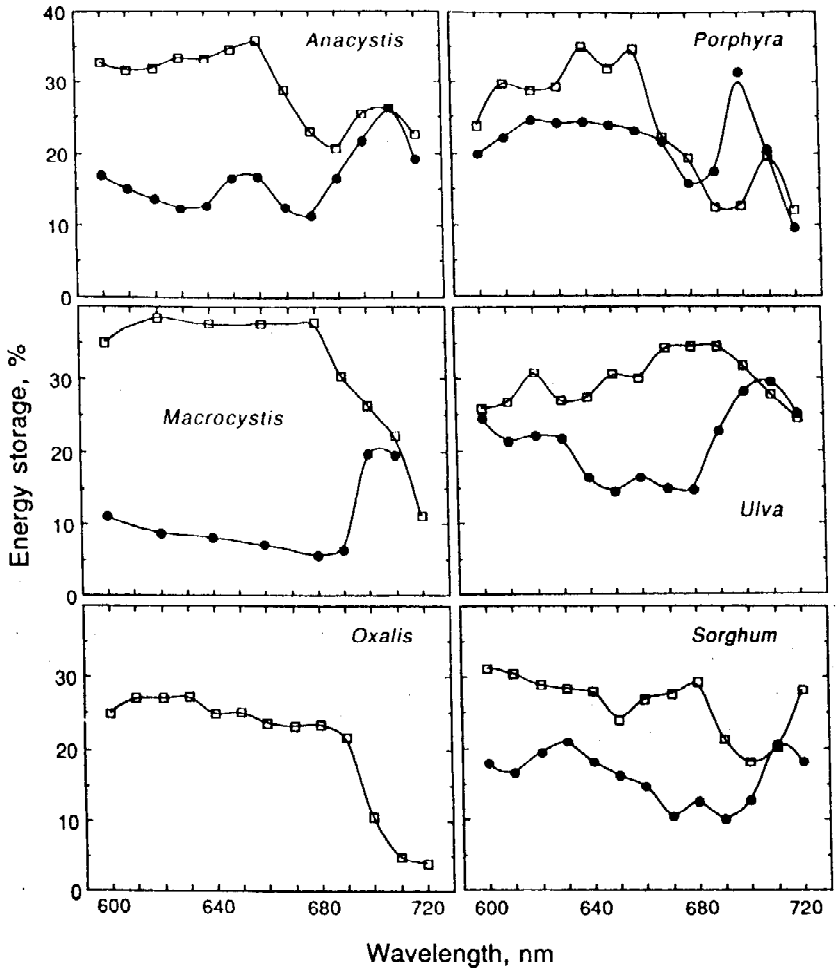

Figure 6. Quantum yield spectra of energy storage in the red region for controls (open squares) and samples in 25 μm DCMU (solid circles) in several species. Excitation: modulated light. The modulation frequency (20–215 Hz) was adjusted for each species so that the contribution of modulated oxygen evolution was negligible. From Herbert et al. (1990).

50–60% the antenna size of PS I at the expense of PS II. In state 1 (i.e., when the plastoquinone pool is fully oxidized), the antenna sizes of both photosystems are nearly equivalent. The last distribution prevails in plant chloroplasts (Figure 5) and leaves, where Delosme et al. did not succeed in detecting any transition to state 2. (For a historical minireview of state changes, see Allen 2002.)

Pulsed excitation: cyanobacteria

Figure 9 shows a quantum yield spectrum measured in the wild type of *Synechocystis* PCC 6803 (G. Ajlani and R. Delosme, unpublished data), with the following characteristics:

(a) As in green algae and plants, the maximal efficiency is observed in far-red light, which is absorbed preferentially by *chlorophyll a* of PS I;

(b) near 600 nm (where absorption by *phycocyanin* predominates), the quantum yield is relatively high but not maximal, indicating that phycocyanin is an efficient (but not perfect) light-harvesting pigment;

(c) the quantum yield is very low in the 460–530 nm range, where light is almost exclusively absorbed by *carotenoids*. This may be partly due to the presence of a significant amount of carotenoids outside the photosynthetic membranes: they obviously do not contribute to photochemistry. Further, there is a very low transfer efficiency of excitation energy from β-carotene to chlorophyll *a* in the PS II core (Delosme 1998), in contrast to the high ef-

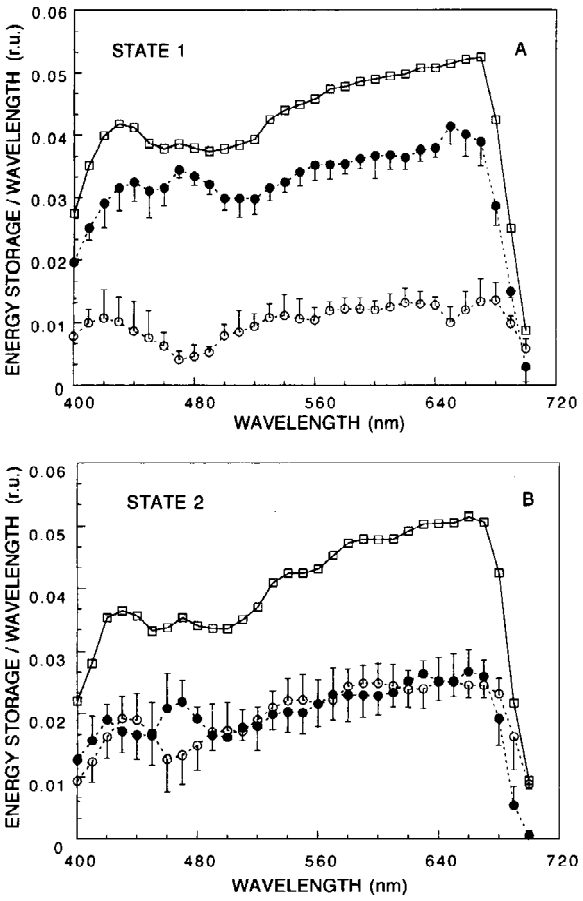

Figure 7. Quantum yield spectra of energy storage (in relative units) of PS I and PS II together (squares), PS I (open circles) and PS II (solid circles) in sugar maple leaves in state 1 (A) and state 2 (B). Excitation: modulated light (frequency: 100 Hz). From Veeranjaneyulu and Leblanc (1994).

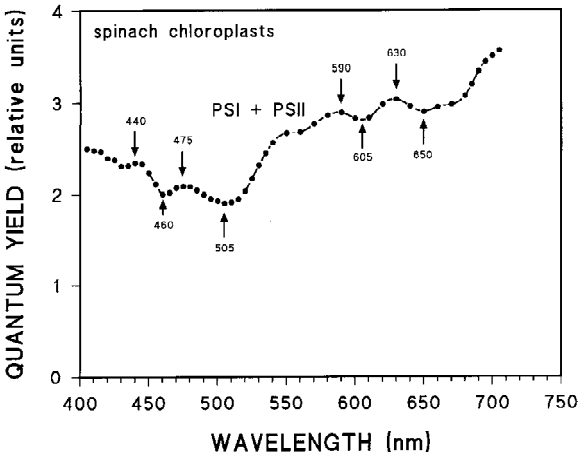

Figure 8. Photoacoustic quantum yield spectrum of spinach chloroplasts. The volume changes due to the charge separation was measured (in relative units) during the first microsecond following a laser flash of very low intensity (see Delosme et al. 1994, 1996, and Delosme 1998). From Delosme (1998).

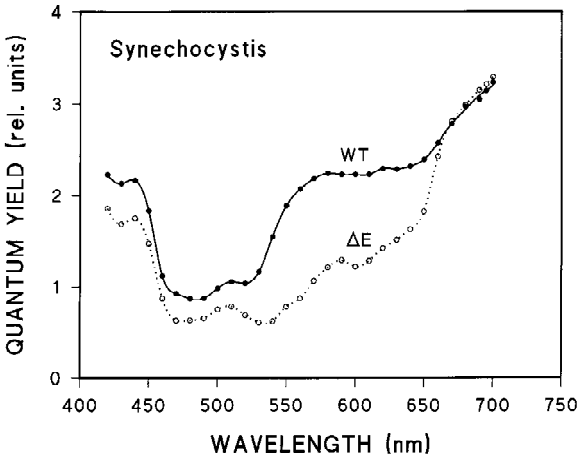

Figure 9. Photoacoustic quantum yield spectrum of *Synechocystis* (wild-type and ΔE mutant) (G. Ajlani and R. Delosme, unpublished [1997]).

ficiency of the energy transfer from xanthophylls to chlorophyll *a* in the LHC of plants and green algae (Siefermann-Harms and Ninnemann 1982; Delosme 1998). (For a historical discussion on energy transfer from carotenoids to chlorophyll *a*, see Dutton 1997.)

In the mutant ΔE of *Synechocystis* PCC 6714 (Ajlani and Vernotte 1998a), which contains phycobilins but no assembled phycobilisomes, the deep depression of the quantum yield in the 600 nm range means that the phycobilins cannot transfer their excitation energy to chlorophyll (Figure 9).

The PAL mutant of *Synechocystis* PCC 6803 (Ajlani and Vernotte 1998b) has no phycobilin, but synthesizes a large amount of carotenoids, noteworthy inefficient as light-harvesting pigments (Figure 10). The quantum yield is high and nearly constant in the red region, where chlorophylls are the unique absorbing pigments. Noteworthy, closure of the PS II reaction centers (by addition of DCMU plus hydroxylamine, not shown) scarcely decreases the quantum yield (only by a few per cent), despite the high PS II / PS I ratio detected in the PAL mutant by Ajlani and Vernotte (1998b). A plausible interpretation could be that these photoacoustic measurements were made in the dark, under anaerobic ('state 2') conditions allowing efficient energy transfer from PS II to PS I ('spill-over'). Thus both PS II and PS I antennae remained efficient when PS II was closed. The same observation applies to the other strains of *Synecho-*

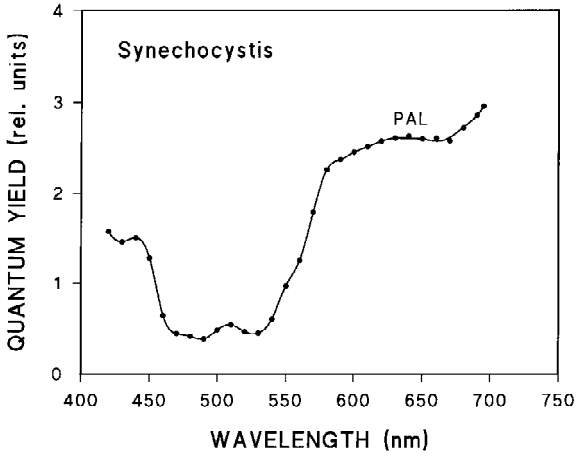

Figure 10. Photoacoustic quantum yield spectrum of *Synechocystis* (phycobilin-less PAL mutant) (G. Ajlani and R. Delosme, unpublished [1997]).

cystis. Most probably, the PS II centers were closed, even in untreated cells, because of the reduced state of plastoquinones in the dark. These experiments justify our reservations about the photoacoustic determinations of PS II parameters (under single flash excitation) published in the literature on intact cells of cyanobacteria. Conversely, the modulated light used by Herbert et al. (1990) probably favored state 1, with a partially oxidized plastoquinone pool and active PS II centers. Thus these authors were able to observe a pronounced contrast between the quantum yield spectra of *Anacystis* in the presence and in the absence of DCMU (Figure 6).

In a historical perspective, the quantum yield spectra of Figure 8 (spinach chloroplasts) and 9 (wild type of *Synechocystis*) and others published elsewhere (Delosme 1998) remind us of those published sixty years ago by Robert Emerson and Charlton M. Lewis (1942, 1943). These authors measured the steady-state rate of oxygen emission by a manometric technique, and light absorption by an independent method using a photronic cell. Despite a rather large half-bandwidth (6–20 nm), their quantum yield spectra of photosynthesis have hardly been surpassed in accuracy. They remain an excellent example of what *in vivo* experimentation can do in the hands of a genius scientist.

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Figure 11. René Delosme at the historic organ of Saint Félix Lauragais (International Festival 'Toulouse les Orgues,' October 2002). Photo by Claire Tardivel.

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