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Review

The absolute size of a photosynthetic unit *

David Mauzerall and Nancy L. Greenbaum

The Rockefeller University, New York, NY (U.S.A.)

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Key words: Optical cross section; Poisson distribution; Trap; Reaction center; Single turnover flash; Quantum requirement; Energy transfer; Fluorescence induction; Antenna, light-harvesting; Pigment protein

Contents

* This review is dedicated to William Arnold who, together with Ralph Emerson, led this field of research into the new world of biophysics.

Abbreviations: RC, reaction center; PSU, photosynthetic unit; PSU(z), photosynthetic unit defined by product (z); RC I (II), reaction center of Photosystem I (II); PS I (II), Photosystem I (II); NI (NII), number of pigment molecules in PS I (PS II); nI (nII), number of RC I (RC II); Chl, chlorophyll; $\sigma(z)$, optical cross section of PSU (z) measured via component z; σ(pig), optical cross section of pigment in vivo; Q_A, quinone with maximum absorption at 325 nm, primary electron acceptor of reaction center II; P-700, chlorophyll with maximum absorption at 700 nm, primary electron donor of reaction center I; $Y(z)$, yield of product z per hit of an RC; F_0, initial fluorescence (fluorescence induction curve); F_v, variable fluorescence (fluorescence induction curve); F_{max}, maximal fluorescence (fluorescence induction curve); LHC II (I), light-harvesting complex of Photosystem II (I); DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichloroindophenol; ATP, adenosine 5′-triphosphate; $\Delta\epsilon$, difference extinction coefficient.

Correspondence: D. Mauzerall, The Rockefeller University, 1230 York Avenue, New York, NY 10021, U.S.A.

Summary

The photosynthetic unit is a complex of pigments and proteins coupled to a reaction center where the initial light-driven charge separation of photosynthetic reactions takes place. The 'size' of the photosynthetic unit is defined as the number of light-absorbing pigment molecules contributing excitation energy to a reaction center. It is an important parameter in the study of the structure and function of photosynthetic membranes. Several approaches used for relative and absolute measures of photosystem stoichiometry and size are evaluated, and results achieved by these methods are compared. The concept of the absolute size of a photosynthetic unit leads to a simple relation between the size of the oxygen forming unit, the total chlorophyll per O_2 (the classical Emerson-Arnold unit), and the quantum requirement for O_2 formation. These measurements lead to further understanding of the mechanisms of energy transfer and of the adaptive mechanisms of plants to varied environments.

I. Introduction

Light is the ultimate energy source of all life on earth. Photosynthetic organisms transform the energy of light into chemical activity: virtually each photon absorbed leads to excitation of a specialized chlorophyll molecule in the reaction center (RC), which then donates an electron to an acceptor. Subsequent electron-transfer reactions form O_2, reduce CO_2, and generate ATP. This photochemistry and its coupled dark reactions occur within milliseconds, after which the system is restored to its original state and is ready to 'turn over' again. In green plants and algae, the overall process involves two kinds of RC's coupled in tandem: activation of RC II generates a weak reductant and a strong oxidant which oxidizes H_2O to O_2. The weak oxidant formed by RC I excitation is in turn neutralized by the weak reductant from RC II via a pool of quinones. Electron transfer in RC I also forms a strong reductant which reduces pyridine nucleotides for subsequent CO_2 fixation. The charge and proton gradient across the photosynthetic membrane formed by these reactions is used to drive ATP synthesis.

The solar energy incident on the surface of the earth is quite dilute, roughly 2 $mE \cdot m^{-2} \cdot s^{-1}$ of photosynthetically active radiation at maximum. A hypothetical primitive *Chlorella* cell filled with RC's, each having a single pigment and the associated photosynthetic machinery, in a sea brimming with nutrients, would have a duplication time of about a month. This is a bit slow even for the assumption, probably mistaken, that life proceeded at a less hectic pace in the early days of evolution. If, however, our hypothetical cell's photosynthetic apparatus were peppered with 300 or so pigment molecules, each capable of transferring energy to the reactive chlorophyll molecule, then the duplication time would fall to hours, as is presently observed. The cell receives a relatively higher return on the synthesis of pigment protein complexes than on the synthesis of complete photosynthetic complexes. A photon absorbed anywhere in the 'antenna' surrounding an RC can be used to initiate the photochemical reactions of photosynthesis, thereby greatly increasing the flux of chemical free energy obtained from the flux of solar photons. A continuous flux of free energy is required to support the organized structure of a living cell.

There are theoretical limits to the size of a photosynthetic unit (PSU), that is, the number of pigment molecules transferring energy to the RC. Because the energy transfer in a homogeneous antenna is random in direction, the mean time before trapping at an RC increases with the size of the antenna. The functional dependence of this time on size depends on the dimensionality or connectivity of the antenna [1], which may be fractal [2]. The finite radiative lifetime dictates an upper limit to the size of a PSU, beyond which there is a decrease in the efficiency of trapping. The largest size is found experimentally to be about 400 chlorophylls (Chl) in *Chlorella* [3]. Nature takes advantage of several methods to minimize this loss in efficiency. By adding an absorber at higher energy (e.g., Chl *b*, phycobilins, carotenoids) adjacent to Chl *a*, nonrandom downhill energy transfer occurs towards the RC. Within a homogeneous cluster of these pigments, reduction of the dimensionality of the energy transfer, such as is done in

the phycobilisomes of cyanobacteria and red algae, can dramatically decrease the time before trapping [4]. By these means the size can be increased to 1000 molecules and still be efficient. The limit now may be set by the required structural stability of the large complex rather than by photophysics of the molecules.

The minimum size of a PSU is that of the RC itself. The bacterial reaction center establishes the canonical number of pigments as 6 [5]. The increasing purification of RC I and II should soon establish their minimal numbers. A preparation of RC II from spinach thylakoids contains 7 ± 1 pigments [6]. The theoretical reasons for more than one pigment incorporated into an RC are fascinating. Among them are ultrafast charge separation in the initial photoact and rapid long distance, transmembrane charge transfer via interpigment electron tunneling [7]. The record for the in vivo minimal size of PSU II has been set by a phycocyanin-minus mutant of *Cyanidium caldarum* which has 20–40 Chl [8,9]. A mutant of barley lacking Chl *b* has 95 Chl *a* in PS I and 37 in PS II [10].

While it is known that over 99% of the color we observe in modern photosynthetic systems is that of antenna pigments, there is still much speculation concerning its structural organization and its adaptation to environmental changes. In this review we will describe the various approaches used to measure the size of PSU's and the distribution of pigments between them. We will limit our discussion to organisms containing the two photosystems. The size of a PSU enters into many aspects of photosynthesis. It can relate classical variables of photosynthesis: the number of quanta required to make oxygen and the total chlorophyll per oxygen. Determination of the absolute size of a PSU allows a more detailed understanding of the adaptation of photosynthetic organisms to changes in their environment.

II. The Emerson-Arnold unit

The concept of the photosynthetic unit originated in the 1930's with the extraordinary experiments of Emerson and Arnold [11], who found that only one molecule of oxygen was produced per 2500 chlorophyll molecules when *Chlorella* cells were illuminated with brief (less than 10 μs) saturating flashes of light. The prescient calculations of Gaffron and Wohl [12] demonstrated that a steady-state production of oxygen could be reached well before each chlorophyll molecule in the sample absorbed a photon in weak continuous light. These insights led to the view that many chlorophyll molecules contribute absorbed light energy to a reaction center where the photochemistry takes place [13]. The isolation of the bacterial reaction center by Reed and Clayton [14] verified this concept. Although the 'size' of the Emerson-Arnold PSU could be definitely stated as 2500 Chl/O_2, its meaning was vague, as it was recognized that the multi-step reaction sequence could be composed of smaller units which cooperated to make an O_2 molecule.

The discovery by Kok [15] of the absorption changes at 700 nm identified the reaction center of Photosystem I (PS I), P-700. The finding of this and other components of the photosystems present in small ratio to chlorophyll such as the quinone Q_A, the first stable electron acceptor of RC II, allowed division of the total chlorophyll by the number of these unique components. In general, for component z, one divides the total number of chlorophylls by the number of molecules of component z to obtain pigment size of a unit associated with z. As with the Emerson-Arnold unit size, these total numbers are useful as a relative internal measure of changes caused by adaptation or development, but do not provide information concerning the functional distribution of the total chlorophyll between activities of Photosystems I and II.

The term 'PSU size' was therefore redefined to specify the number of pigment molecules contributing energy to a particular photochemical reaction. The following sections will discuss approaches currently used to determine absolute sizes of PSU I or II based upon: (1) the kinetics of photoreduction or oxidation of RC II or RC I components; (2) the optical cross sections of the photosystems; and (3) biochemical assays of isolated preparations.

III. Kinetic approach to PSU size

The rate of formation of intermediates specific to each photosystem allows the distributions of excitations to the two photosystems to be determined. The initial rate of formation of a photosynthetic intermediate or product z in an optically thin sample is given by the number of units times the effective photons absorbed:

$$\frac{dz}{dt} = n(z)\int \phi(z)\sigma(z)I(\lambda)\,d\lambda = n(z)N(z)\int \phi(z)\sigma(\text{pig})I(\lambda)\,d\lambda \tag{1}$$

where $n(z)$ is the number of units in the sample, $\sigma(z)$ is the optical cross section of the PSU forming z, $I(\lambda)$ the light intensity as a function of wavelength λ, assumed to be uniform and constant across the sample, and $\phi(z)$ is the quantum yield of the intermediate z. The integral is over the wavelength distribution of the light source. The term $\sigma(z)$ can be replaced by the product of $N(z)$, the numbers of pigments or size of a unit which forms z, and σ(pig), the in vivo optical cross section of the pigment molecule(s) (see subsection IVB). There will be two such equations: one for each photosystem. Each equation contains three unknowns (assuming $I(\lambda)$ and σ(pig) at the excitation wavelength(s) are known), re-

quiring four additional pieces of information to solve the problem. Usually the unstated assumption is made that the pigment cross sections are the same in the units and that the quantum yields $\phi(z)$ are all equal, or even more restrictive, that they are unity. This assumption may be appropriate if z is P-700 and inappropriate if z is an extraneous acceptor or donor. In the latter case $\phi(z)$ should be determined separately. Through these assumptions the integral in Eqn. 1 becomes a constant, equal for both photosystems, thereby reducing the number of additional pieces of information required to 3. If the number of units, $n(z)$, is determined as described in subsection IIIA, the problem can be solved by one further equation, that of conservation:

$$\text{total Chl} = N(\text{I}) \cdot n(\text{I}) + N(\text{II}) \cdot n(\text{II}) \quad (2)$$

Thus to determine the relative size of the two PSU's, a minimum of five measurements is required.

IIIA. Stoichiometries of RC I and RC II

Numbers of the two reaction centers per sample (nI, nII) are generally determined via the absorption spectra of an RC component present in assumed small integer ratios to the RC. While the use of P-700 absorbance changes ($\Delta\epsilon = 64\ \text{mM}^{-1} \cdot \text{cm}^{-1}$; Ref. 16) as a measure of nI is universally accepted, the measurement of quinone (Q_A) reduction as a measure of n(II) is more difficult. While most authors now agree upon an extinction coefficient between 11 and 13 mM^{-1} at 325 nm [17–19], the measurement is complicated by considerable heterogeneous absorption, light scattering, and optical filtering by other components. For example, the 'flattening factor' [20] used to correct the observed absorbance changes for heterogeneous absorption can be very large, up to 50% of the measured value, requiring care in its application (e.g., Refs. 21–25).

The number of RC II's has also been estimated by the yield of O_2 from repeated saturating single turnover flashes. The total chlorophyll per oxygen evolved per flash (Emerson-Arnold PSU size) is usually divided by four to obtain nII because of the assumed four light-driven one-electron oxidation steps required to form O_2 from H_2O [26–29]. This number does not take quantum losses into account, which may be as high as 25%, since it is found that 10 quanta are required to form O_2 [30–32], not 8 (the two sequential photoreactions). We show below (subsection IVD) that the Emerson-Arnold PSU size must be divided by the quantum requirement to obtain the absolute size of PSU(O_2). The Chl/O_2 (divided by four) ranges from approx. 200 in cyanobacteria (Table I) to about 800 in green algae (Table II).

Numbers of total Chl per RC II approximately agreeing with those measured by the absorption change at 320 nm have also been determined by several other methods in spinach thylakoids. These measurements include absorption changes at 540–550 nm [33], an

TABLE I

Relative measurements: cyanobacteria

Tables I–V are a representative, but not exhaustive, listing of experimentally derived values for parameters relating RC stoichiometry and size (relative or absolute) of various photosynthetic organisms. Methodology for determination of stoichiometries has been abbreviated as follows: $O_2/4$ total Chl per oxygen per flash divided by four; ΔA_{320} or ΔA_{325}, absorption change upon reduction of Q_A, the primary acceptor of PS II; A_{700}, absorption change upon chemical or photooxidation of P-700, the primary donor of PS I. The RC II values assume one Q_A per RC II. Total ΔChl/RC (Tables I–III) are relative values of stoichiometry: the total Chl in the sample divided by the measured number of RC (subsection IIIA). NII and NI are the number of Chl specifically associated with PS II or PS I, respectively (Tables IV and V), and are therefore absolute values (subsections IIIC and D; IVC and E; and section V). For clarity, Tables IV and V have been separated according to methodology used. Further details concerning each of the methods can be found in the text.

Material	Method		Growth conditions	RC II/RC I	RC II/cell ($\times 10^5$)	Total		Total		Ref.
	II	I				Bilins / RC II	Chl / RC II	Bilins / RC I	Chl / RC I	
Anabaena variabilis	$O_2/4$	ΔA_{700}	500–1500 lx	2.12	6.0	202	293	95	138	
			4000–6000 lx	1.26	4.8	95	178	75	141	26
Synechococcus lividus	ΔA_{325}	ΔA_{700}	(not stated)	0.43			308		133	180
Synechococcus 6301 *(Anacystis nidulans)*	ΔA_{320}	ΔA_{700}	5000 lx	0.4			384		166	196
	$O_2/4$	ΔA_{700}	10 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	0.44		219	330		145	28
	ΔA_{320}	ΔA_{700}	white light	0.43		200	370	85	160	
	ΔA_{320}	ΔA_{700}	yellow light	0.27		240	575	65	156	24
	ΔA_{320}	ΔA_{700}	red light	0.71		300	230	212	163	
	$O_2/4$	ΔA_{700}	gold light	0.40		207	360	82	143	27
	$O_2/4$	ΔA_{700}	red light	1.0		680	180	696	186	

TABLE II

Relative measurements: algae

Material	Method		Growth conditions	RC II/RC I	RC II/cell ($\times 10^5$)	RC I/cell ($\times 10^5$)	Total Bilins / RC II	Total Chl / RC II	Total Bilins / RC I	Total Chl / RC I	Ref.
	II	I									
Chlorophyta:											
Dunaliella tertiolecta	$O_2/4$	ΔA_{700}	45 $\mu E \cdot m^{-2} \cdot s^{-1}$	0.89	32.5			830		730	29
			600 $\mu E \cdot m^{-2} \cdot s^{-1}$	0.86	11.4			710		610	
Chlamydamonas reinhardtii	$O_2/4$ atrazine binding		(not stated)					830 440			22
	ΔA_{320}	ΔA_{700}	47 $\mu E \cdot m^{-2} \cdot s^{-1}$	1.35				672		905	25
			400 $\mu E \cdot m^{-2} \cdot s^{-1}$	2.65				380		1008	
Chlorella pyrenoidosa	$O_2/4$ atrazine binding		(not stated)					380 220			22 22
Rhodophyta:											
Neoagardhiella bailyei	$O_2/4$	ΔA_{700}	110–180 $\mu E \cdot M^{-2} \cdot s^{-1}$ (natural enviroment)	0.44			550	315		140	28
Porphyridium cruentum	$O_2/4$	ΔA_{700}	750 lx	2.22			700	240	70	108	26
Phytoplankton:											
Skeletonema costatum	$O_2/4$	ΔA_{700}	30 $\mu E \cdot m^{-2} \cdot s^{-1}$	2.24	6.6			605		1360	29
	$O_2/4$	ΔA_{700}	600 $\mu E \cdot m^{-2} \cdot s^{-1}$	1.06	4.4			590		623	
Gonyaulax		ΔA_{700}	8–10 W/m^2			750				600	184
Glenodinium		ΔA_{700}	8–10 W/m^2			14				600	

EPR-detected donor to P-680 [34], Mn^{2+} associated with the water-splitting complex [35], and pheophytin content [36]. Jursinic and Dennenberg [22], by using five different observables, attempted to reach a definitive value for the number of total Chl per RC II in pea thylakoids. While the number of binding sites for atrazine (an inhibitor of RC II) was one per 320 Chl, 600 Chl per observable were measured by: reduction of Q_A, flash yield of O_2 divided by four, reduction of 2,6-dichloroindophenol (DCIP, an artificial acceptor for RC II), and release of protons (attributed to water oxidation by a complex procedure). The similarity in stoichiometries measured by the latter four observables in pea thylakoids conflicts with the findings by Whitmarsh and Ort [37] and McCauley and Melis [33]. In these latter studies, 630 total Chl/RC II were measured by H^+-extrusion [37] compared with 380 Chl/RC by Q_A reduction in spinach thylakoids [33].

Representative data for measurements of the total Chl per RC II and I and the RC II/RC I ratio are presented in Tables I (cyanobacteria), II (algae), and III (higher plants). Large variations among values, even within a single species, can be seen. While some of the variations may be caused by different growth conditions

TABLE III

Relative measurements: higher plants

Materials	Method		Growth conditions	RC II/RC I	Total Chl / RC II	Total Chl / RC I	Ref.
	II	I					
Spinach chloroplasts	ΔA_{320}	ΔA_{700}	unfractionated thylakoids		380	650	33
			grana partitions		230	–	
	ΔA_{325}	ΔA_{700}	summer grown	1.43	356	508	180
Pea chloroplasts	ΔA_{325}	ΔA_{700}	developing	3.3	102	338	180
	ΔA_{325}	ΔA_{700}	mature	0.77	669	513	
Pea thylakoids	ΔA_{325}	ΔA_{705}	45 W/m^2	1.44	574	825	
	$O_2/4$			1.34	616		22
	DCIP reduction			1.41	586		
	H^+ evolution			1.39	592		
	atrazine binding			2.56	322		

(see subsection VIC), some of the differences may be the result of arbitrary corrections or assumptions made in the calculations.

IIIB. Distribution of energy between the photosystems

In order to translate determinations of total chlorophyll and numbers of reaction centers into the size of the PSU, i.e., the number of Chl contributing energy to a RC, one must also determine the fraction of excitations going to Photosystem I and II for a given wavelength distribution of the actinic source (Eqn. 1). If one has a measure of the distribution of excitations into RC I and RC II, then the total pigment is divided proportionately. Butler [38] used the excitation spectra of fluorescence at 77 K, where PS I has a strong emission at 730 nm, and a model of energy transfer to accomplish the separation. This method is not ideal because of the unphysiological conditions and the as yet unknown origin of the 730 nm emission. Wang and Myers [39,40] used the effect of modulated and continuous light of differing wavelengths on the measured modulated and continuous O_2 evolution. Malkin et al. [41] used a similar approach but with photoacoustic and fluorescence detection of the same Emerson enhancement, the non-linear interaction of excitation of the two photosystems by a combination of light of particular wavelengths. The rate of oxygen production (or of fluorescence yield) by far red light (greater than 700 nm) is markedly enhanced by addition of shorter wavelength light [42]. The effect is explained by the larger absorption cross section of PSU I than of PSU II in the far red and thus an imbalance in the electron flow between the two photosystems, with the one receiving less light becoming the limiting reaction. Since Emerson enhancement is a strictly non-linear effect (the sum of the separate effects is not superposable on the measured combined excitation), some care is required in its use. Wang and Myers [39] have discussed the important effect of light intensities on these measurements.

IIIC. Measurement of the size of PSU II by fluorescence induction

One of the most commonly used measures of PSU II is the increase in fluorescence yield upon illuminating a photosynthetic system with saturating light. Kautsky and Hirsch [43] first observed the increase of Chl *a* fluorescence following initiation of illumination to dark-adapted plants. It is useful to consider an RC as a trap for excitations, which can be open or closed. As RC's are closed by illumination, the fluorescence yield increases some 3-fold from F_o (open RC's) to F_{max} (closed RC's). This increase in yield is called F_v and its measurement is termed fluorescence induction. It was assumed that an RC with reduced Q_A (a primary acceptor in PS II), i.e., a 'closed trap' would rebuff an excitation, allowing it to wander further until trapped by an open center, i.e., one having oxidized Q_A [44]. It is implicitly assumed that all RC's are connected to an infinite sea of Chl. The enhanced lifetime would lead to an increased yield of fluorescence. The detailed mechanism of the process is still under discussion [45–47].

The fluorescence induction curve is measured by the increase in fluorescence with time following sudden illumination of a sample with saturating light. The fluorescence induction curve is often not the complement of an exponential, but is sigmoidal. Since the increase of fluorescence yield occurs only from closed traps, the induction curve of a dark-adapted system requires two or more hits to an RC. Thus a lag phase should always be visible unless there is either inadequate time resolution in the measurement or a sizable fraction of previously closed traps. The presence of a variable fraction of closed traps can cause an error of up to 100% in the apparent 'size' of the fluorescence induction unit. Christoffers [48] has provided a description of the kinetics and quantum efficiencies of fluorescence induction by computer simulation of the fluorescence emission process controlled by the redox state of Q_A. Renger and Schulze [49] modeled the kinetics of fluorescence induction curves as a function of actinic light intensity and degree of Q_A inhibition. They concluded that the Q_A redox state is the only relevant factor in determining the fluorescence quantum yield. However, numerous assumptions were required to fit their data to their model. Moreover, their calculations could not fit the data above 10^{-7} M DCMU, i.e., above 75% inhibition, whereas 10^{-5} M DCMU is typically used in these measurements. Thus their conclusion may be overstated.

Malkin and Kok [50] established the use of the complement of the area over the fluorescence induction curve in a sample poisoned with DCMU as a measure of the fraction of RC's with reduced Q_A. DCMU is a herbicide which blocks electron transfer between Q_A and Q_B, the secondary acceptor of PS II. Its usefulness is dependent upon the assumption that it transforms RC II into a single turnover system even in continuous light. Plots of the log of the complement of the area of the fluorescence induction curve vs. time have been resolved into two components by Melis and coworkers [23–25,51–58] and Thielen and Van Gorkom [21,21a]. This biphasicity has been interpreted as evidence for two discrete populations of PSU II, termed α and β [55–57,21a]. The α centers are postulated to be fully active in electron transport and O_2 production, and the β centers are proposed to have 3-fold smaller antennae and little or no O_2 production [54,59]. Chylla et al. [60] and Graan and Ort [61] have similarly provided evidence for two populations of RC II. The population active in oxygen evolution comprises approx. 60% [61]

to 68% [60] of RC II centers and has a turnover time of 50 ms, in contrast with 2–3 s for the other type [60]. Evidence that α centers are preferentially situated within the appressed membrane regions of stacked thylakoid membranes while β centers (and RC I) are mostly located in the stromal regions has been obtained [23,33,51,59]. Melis [53] has speculated that the β centers are precursors of mature, or α, centers. Evidence for heterogeneity in PS II has also been obtained from the rate of reduction of Q_A [51,52].

A number of observations have emphasized the complexity of the fluorescence induction curve. Bell and Hipkins [62] and Butko and Szalay [63] noted that the slow increase of the fluorescence yield creates uncertainty in the precise measurement of F_{max}. This causes errors in measurements of the complementary area over the induction curve, affecting the ratio of presumed α/β centers dramatically, as an increase of 2% in F_{max} increases the resultant area by 150%. These authors also found an accurate determination of α to be extremely difficult because of the nonlinear nature of the plot of area growth vs. time. Extending the fluorescence measurement does not solve the problem, since the increase in the fluorescence yield soon decreases [45]. Indeterminacy of the base line is also indicated by the negative curvature (concave downwards) on the log plots [51–54,57,58]. Such a curvature implies a greater degree of nonlinearity of the photoreaction than is known to exist, and is likely caused by systematic baseline errors. Additionally, Hodges and Barber [64] found that varying [DCMU] between 0.2 and 100 μM altered the slow part of the fluorescence induction curve, but had little or no effect on the fast phase. Low [DCMU] may result in some electron transport between Q_A and Q_B, preventing saturation of Q_A^-. These authors concluded that the apparent heterogeneity in PS II may therefore be a function of the degree of DCMU-inhibition.

Schreiber and Neubauer [65,66] have investigated the complex kinetics of the fluorescence induction curve by use of intense actinic light. They confirm Deslosme's [67] result that the increase of fluorescence yield is composed of a fast photochemical phase and a slow (approx. 0.1 s) thermal or dark reaction of about equal amplitudes. The dark reactions may include fluorescence yield changes caused by transmembrane potentials [68], ion concentrations (see Ref. 69 for discussion), and other effects. The addition of DCMU appears to speed up or eliminate the thermal phase, but the mechanism remains obscure. Until this change is understood, conclusions based upon fluorescence induction should be treated with caution.

The ambiguities encountered with measuring the increase in fluorescence in continuous light can be avoided by use of single-turnover flash measurements of fluorescence. Mauzerall [70] used the pump-probe method [71] to measure the laser flash energy saturation of the fluorescence increase in *Chlorella* at 337 nm in the absence of inhibitors. The shape of this curve is the complement of an exponential, i.e., a simple Poissonian, which indicates a single population of PSU II. Geacintov et al. [72] obtained similar results in spinach chloroplasts following single-turnover actinic flashes at 337 nm, 530 nm and 650 nm. The shape of the saturation curve may be sharper than Poissonian [73], suggesting the influence of energy transfer between units (subsection VA) and possible cycling of electrons within PS II.

IIID. Measurement of the size of PSU I

The kinetic approach has also been used for the determination of the size of PSU I. The plot of the log of the change in absorbance at 700 nm associated with P-700 photooxidation vs. time is linear, indicating a single size of the antenna [23,24,51,52,54,58,59,74]. This measurement has been combined with rates derived from fluorescence induction curves to assign distributions of energy and thus of chlorophyll between the two photosystems.

Representative values for the sizes of PS II and PS I determined by kinetic approaches are listed in Table V. Note that the sizes quoted are a function of the light source used for measurement via the integral in Eqn. 1. With broad band illumination, one measures a weighted average of the absorbances and yields. Thus variations are to be expected, particularly if one compares measurements made with blue light with those made with white or red light including wavelengths greater than 700 nm. Because of Emerson enhancement, the integral is most variable in this wavelength region in green photosynthetic systems, and this variation occurs over a far greater wavelength range in red algae and cyanobacteria.

IV. Optical cross-section approach to PSU size

The ideal experiment for measurement of the functional distribution of pigments is done under physiological conditions, minimizes perturbation of the system, and is rapid enough to prevent changes taking place during the experiment. A working example of this approach utilizes the light-saturation behavior of the activities specific to the two photosystems following single-turnover flashes of nearly monochromatic light. In favorable cases an absolute measure of the two PSU's is obtained simultaneously in living cells without added mediators or poisons.

IVA. Light saturation of photosynthetic systems

The light saturation curve contains much of the information we wish to obtain in order to understand

photosynthesis. This curve is a graph of the rate of a specific photosynthetic process plotted vs. the incident light intensity, or energy for the case of light pulses. Saturation occurs because the rate of absorption of quanta must, at some photon flux, become equal to the turnover rate of the photosynthetic system. For the case of light pulses, saturation occurs because all possible RC's have absorbed a photon. The slope of the linear portion of the curve measures the product of the quantum yield for the process and the absorption cross section of the incident light (Eqn. 1). It is, however, the nonlinear part of the light saturation curve which contains unique information relating light absorption to the photosynthetic system. The curvature of the graph for continuous light is a balance between light absorption and the complex turnover time(s) of the photosynthetic apparatus. The complexity is caused by the two serial photosystems and by the interconnecting electron storage (quinone) pool. There are thus several turnover times to the system. For example, input from PS II when the quinone pool is completely oxidized occurs within 300 μs, but output from the pool when it is reduced requires up to 17 ms [75,76]. This latter time may be the carbon fixation time [75]. These multiple and illumination-dependent turnover times complicate the analysis of continuous light saturation curves of photosynthesis – and of fluorescence induction curves. By contrast, the curvature of the graph for single turnover flashes is largely determined by a single parameter: the absorption of all the pigments, and only of those pigments, which contribute energy to the observed process. The optical cross section for this process is the most straightforward operational definition of a PSU. It is for this reason that we believe the analysis of the light saturation curves with pulsed light is most useful. Based upon known turnover times, we can optimize the flash length time for a single turnover flash. It must be at least 10 times shorter than the fastest turnover time to avoid more than one turnover of the system, and it must be several times longer than the photochemical processes to avoid deleterious effects of multi-photon annihilation in the PSU. In these systems, 1 μs is ideal.

IVB. Measurement of the optical cross section and the absolute size of a PSU

Absorption of light in solution is described by Beer's law, which relates the concentration of molecules, path length, and extinction coefficient to the absorbance of the solution. The uncritical transfer of this description to heterogeneous systems, such as algae, leads to considerable confusion in the definition and determination of 'concentration' and 'path length'. Such difficulties are avoided by the use of optical cross sections, σ, with units of area [71]. This is the area which, when multiplied by the fluence, gives the probability that the molecule will absorb a photon. In solutions, i.e., with randomly oriented molecules, σ per molecule in Å^2 is equal to the decadic extinction coefficient times $3.82 \cdot 10^{-5}$, which converts Avogadro's number, decadic logarithms, and liters-cms to square Ångströms. Because of heterogeneous absorption and light scattering, the cross section of a pigment in a cell is unlikely to be equal to that in solution, particularly in regions of strong absorption (see subsection IVB2). Therefore, the operational cross section of a pigment must be obtained by measuring the in vivo absorption of a sample and obtaining the number of pigment molecules present by extraction. Assuming the sample is isotropic, the above factor can be used to convert the absorbance per molecule in vivo to the pigment cross section in vivo. Dividing the cross section of a PSU by the in vivo cross section of the pigments at the same wavelength results in the absolute size of a PSU: the number of pigments per RC. If there are n pigments one must use n wavelengths, chosen to maximize differences, to obtain n cross sections.

IVB-1. Optical cross section of a PSU, $\sigma(z)$

The cross section of a PSU is measured by determining the relative yield of a product or intermediate (with due care for kinetics) as a function of the energy of a homogeneous single turnover flash of monochromatic light on an optically thin sample. Since even a single cell of a photosynthetic organism contains a very large number (10^5) of photosynthetic units, the specific number of hits, n, following a pulse of light of duration T is given by the Poisson distribution:

$$P_n = \frac{x^n e^{-x}}{n!} \tag{3}$$

where x = average hits per PSU $= \sigma(z)\int_0^T I \, dt = \sigma(z)E$ where $\sigma(z)$ is the optical cross section for absorption of a photon by a unit forming z, I is the instantaneous intensity of the light, assumed to be spatially uniform, the integral is over the duration of the light pulse, and E is the fluence: quanta per unit area [77]. For a simple system with one cross section where one or more hits to the system produce the same effect, the yield, $Y(z)$, of a photoproduct, z, is given by the cumulative one-hit Poissonian:

$$Y(z) = Y_0(z)\left(1 - e^{-\sigma'(z)\phi(z)E}\right) = Y_0(z)(1 - e^{-\sigma(z)E}) \tag{4}$$

where $Y_0(z)$ is the yield of product per hit or closing of an RC. Since $e^{-\sigma(z)E}$ is that fraction of targets not hit (see Eqn. 3), Eqn. 4 follows. The Poisson statistics are mandated by the experiment itself: a very large number of targets each of area much smaller than the illumination area, so that the probability of a given RC being hit

by a given photon is very small. It is assumed that E is uniform and constant across the sample, i.e., the sample is optically thin. A 'thick' sample requires integration of Eqn. 4 over the optical path [78]. Nonuniform illumination will broaden the saturation curve, as will scatter or strong absorption in the sample.

It is also assumed that no photoselection occurs, i.e., that the sample absorbs isotropically. If this is not so, integration over the angular distribution must be carried out, as has been done for the case of bacteriorhodopsin in purple membranes [79]. However, photosynthetic systems are largely isotropic at most wavelengths because of repeated energy transfers between pigments of varying orientation.

The overall yield of a product in a photosynthetic system is determined by a photochemical yield, $\phi(z)$, and a product yield, $Y_0(z)$. The former refers to the efficiency of trapping absorbed quanta and the latter to the efficiency of forming product once the energy is trapped. The former process occurs on the nanosecond time scale while the latter is on the millisecond time scale. Thus it is relatively easy to distinguish these processes. The measured cross section $\sigma(z)$ is the effective cross section as it contains the photochemical yield, $\phi(z)$. Since the quantum yield of elementary reactions in photosynthetic systems is greater than 0.95 [80,81], the distinction can often be ignored.

The above results hold for a multistep reaction, such as O_2 formation, if the photochemical yield is the same for all the steps. If it is not, then Eqn. 4 must be replaced by a less simple form [82].

There are several unique properties to the measurement of the size of a PSU via its optical cross section. Nowhere does the total chlorophyll content of the sample enter into the calculation. In fact, the presence of 'dead' cells or 'inactive' chlorophyll has no effect as long as the sample is optically thin. One measures only those pigments which contribute an excitation to the yield of product. If a chlorophyll molecule delivers a fraction of its excitation to the O_2 system, only that fraction of a molecule is measured. The measured σ is dependent only on absorption and the quantum yield of trapping. It is independent of the O_2 yield, $Y_0(O_2)$. The efficiency of O_2 evolution is in the $Y_0(z)$ term in Eqn. 4, which is cancelled in the normalization of the measured O_2 yields. The cells must be in a definite state (either dark adapted or in steady state by a sequence of flashes) and the response to a single flash of variable energy be measured in order to obtain an accurate flash saturation curve [83].

It may seem strange that a process which requires four 'hits' or ten quanta is characterized by a one hit Poisson distribution (Eqn. 4). The explanation lies in the extraordinary efficiency of the photosynthetic system. If the oxidizing intermediates which accumulate to form O_2 are not lost during the time between the flashes, then for a linear photochemical sequence (such as the S-state cycle which forms O_2; Refs. 84 and 85) in which σ is constant, one obtains (in the steady state) the usual single hit Poisson saturation curve. Essentially, all hits are equal, since every absorbed photon contributes equally to product evolution. The product yield observed, $Y(z)$, will be one-fourth that of a single unit if there are four steps in the cycle and if the yield of each step in the cycle is 100%. A loss of intermediates is readily detected by varying the interflash time interval.

With continuous light this loss of intermediates leads to a light saturation curve which initially depends on the square of the light intensity [86–88]. This quadratic dependence (as opposed to a possible fourth-power dependence) indicates that the losses occur chiefly at a single step, since each step involving a loss contributes an independent probability to the formation of O_2.

IVB-2. In vivo optical cross section of a pigment

To determine this parameter the absolute light absorption by a suspension of cells in a known volume is measured under conditions which cancel the effects of light scatter [89,83] *. The pigment in this sample is extracted with any suitable solvent in which the pigment extinctions are known. The number of molecules of pigment so determined is used to obtain an in vivo extinction which on multiplying by $3.82 \cdot 10^{-5}$ gives σ(pig) at that wavelength. Note that nowhere in this procedure are there assumptions regarding either the shape of the absorption in vivo versus in vitro or the equality of extinction coefficients. The optical cross section of a pigment at a wavelength of large absorption will decrease with increasing cellular content of this pigment because of heterogeneous absorption, and thus must be determined under the same conditions as the PSU measurement. The cross section is more constant in regions of low absorption [83]. For each pigment contributing energy to a unit, an in vivo cross section at a wavelength chosen for maximal differential absorption must be determined. Together with measurements of the cross sections of the unit at these wavelengths, assignment of the number of specific pigments to specific PSU's can be made. This procedure can be applied to bulk antenna pigments which are uniform within a class, such as Chl *a* or phycocyanin. It must be stressed that the cross sections determined in this way are the

* This can be done by use of an integrating sphere to capture randomly scattered transmitted light, or by inclusion of particles in the reference cuvette which mimic the scattering of the cell suspension [83]. Recent measurements in our laboratory have made use of a highly scattering white filter introduced between the sample and reference cuvettes and the measuring beam: the Shibata technique [89]. Linearity of the absorbance of the suspension at the measured wavelength with respect to cell concentration and extrapolation of the plotted data through the origin are confirmation that the effects of light scattering on the cell suspension have been cancelled.

actual probabilities of light absorption by pigments that are used by the cell in carrying out photosynthesis. This direct approach is highly preferable to indirect methods by which the in vivo absorption is circuitously corrected to an in vitro absorption by arbitrary empirical factors.

IVC. Measurements of the optical cross section for O_2 production

Much of the above was clear to Arnold in the 1930s but conclusive experiments had to await the availability of pulsed lasers. Kohn [90] attempted the cross section measurement using a flash lamp and filters but the results were uncertain. Myers and Graham [91] determined the flash saturation curve for O_2 in *Chlorella*, but the use of a broad wavelength distribution prevented the calculation of a useful cross section. Flashlamp-pumped dye lasers are ideal light sources because they combine monochromaticity with 1 μs pulse width of sufficient energy.

Sorokin [92] measured the size of a PS II unit from the increase in pulsed fluorescence yield of pea chloroplasts, obtaining 150 Chl molecules, with a $\sigma(F)$ at 436 nm of 300 Å^2 (Table IV). Mauzerall used the fluorescence yield of a 7 ns probe flash 30 μs after a 7 ns pump flash of varying intensity to obtain a $\sigma(F) = 50$ Å^2 at 337 nm in *Chlorella* [70]. Geacintov and Breton [47] obtained 17 Å^2 at 530 nm by the same method. These cross sections were not reduced to numbers of pigments, but the results of the two studies are consistent with the in vivo absorptions.

Ley and Mauzerall [83] measured the optical cross section for oxygen production in *Chlorella* vulgaris. The conditions were highly favorable: less than a monolayer of photosynthetically competent cells on a bare platinum electrode were illuminated by less than 1 μs flashes at 596 nm. The single cell is the ultimate in optical thinness for an intact biological system, and the actinic wavelength is in a region weakly absorbed by the pigments; the σ(Chl) was thus independent of cellular chlorophyll content at this wavelength. For *Chlorella* grown under normal light conditions, $\sigma(O_2)$ was 70 Å^2 at 596 nm (Table IV). This corresponds to approx. 240 Chl ($a + b$), since the optical cross section of both Chl *a* and Chl *b* molecules (σ(Chl)) in *Chlorella* at 596 nm is 0.29 Å^2. This is known because changing the ratio of Chl *b* to Chl *a* from 0.4 to 0.15 by increasing growth irradiance did not change (Chl) at 596 nm in vivo [83].

The fit of the data on O_2 formation in *Chlorella* to Eqn. 1 was good ($\pm 2\%$) and $\sigma(O_2)$ can be determined to about $\pm 5\%$, part of the error being that of estimating illuminated area and light energy. However, this does not mean that each oxygen unit in a Chlorella must have precisely this $\sigma(O_2)$. A square distribution of σ which varies by a factor of 3 may also be an acceptable fit to the data, but the same distribution over a factor of 10 clearly is not [83]. Similarly, a bimodal distribution of equal amplitudes and $\sigma_2 > 3\sigma_1$, could be detected,

TABLE IV

Absolute sizes by cross sections and by biochemical analysis

Pulsed-light cross sections were detected by O_2 (σ_{O_2}), H_2 (σ_{H_2}), respiratory oscillation (σ_{osc}), EPR (σ_{EPR}) or fluorescence yield (σ_{fluor}). Extraction refers to biochemical analysis.

Material	Method		Growth	RC II/RC I	*N*II	*N*I	Ref.
	II	I					
Cyanobacteria:							
Synechococcus	action	action	gold light	0.40	50	123	27
6301	spectra	spectra	red light	1.0	73	111	
	–	extraction				130	125
Chlorophyta:							
Chlorella							
vulgaris	σ_{O_2}	σ_{osc}	0.1–0.2 W/m^2		390		
			8 W/m^2		240	265	83, 103
			200–300 W/m^2		140		
	σ_{O_2}	σ_{H_2}			193 *	117 *	118
Chlamydamonas reinhardtii	σ_{O_2}	σ_{H_2}			115 *	125 *	
Higher plants:							
Spinach subchloroplast particles		σ_{EPR}				120	102
	extraction				220		123
	extraction				230		124
Pea thylakoids	σ_{fluor}				150		92

* We have included only the major components of the PS II and PS I sizes measured by Boichenko and Litvin [118]. Minor components of approx. 10-fold smaller size were also reported to comprise 12–35% of the units.

but a 15% contribution with $\sigma_2 = 2\sigma_1$, could not. Therefore, it is questionable whether or not bimodality such as that proposed by Melis (β centers, which comprise 25% of PS II, are 1/3 the size of PS II α centers) would be observable by this method. The much slower turnover time (on the order of 1–2 s^{-1}) and apparent lack of O_2 production by the proposed β centers would be in itself sufficient reason to make them invisible by this approach. The above data is consistent with a narrow, but not with a broad or extensive, bimodal distribution of PSU sizes *.

Several other organisms have been examined by the same method (Table IV). Spinach chloroplasts and *Scenedesmus* D3 have a $\sigma(O_2)$ similar to that of *Chlorella vulgaris* at 723 nm (2.5 $Å^2$; Ref. 93; Greenbaum N.L. and Mauzerall, D., in preparation). Since the size of a PSU is independent of wavelength, the result at 596 nm allows us to calculate the optical cross section of a Chl molecule associated with PS II at 723 nm. It is about 0.01 $Å^2$ in these organisms, a value which would be very difficult to measure directly. The cross section of a Chl molecule associated with PS I is, by contrast, about 10 times larger (see subsection IVE). The long-wavelength tails of absorption bands are highly sensitive to their environment. Note that excitation at these very long wavelengths is fully capable of forming O_2 with the same product yield (O_2 per RC hit) as at shorter wavelengths. Oxygen evolution is often not seen at these 'PS I' wavelengths because its cross section is small and thus very large flash energies or light intensities are required for its observation. Saturation of O_2 production with continuous light of 10 nm bandwidth at 723 nm would require a light source equivalent to about 5000 suns.

These directly measured PSU sizes can be compared with values achieved by other, less direct, methods. The total Chl/O_2 of pea thylakoids measured by Jursinic and Dennenberg [22], if divided by the quantum requirement of 10 instead of 4 (see subsection IVD), results in 280 Chl/RC II. This is the same as the size of PSU II α in mature pea chloroplasts measured by Melis (Ref. 23; Table V), and not significantly different from the sizes measured for *Chlorella* (Ref. 83; Table IV), *Scenedesmus*, and spinach chloroplasts (Greenbaum, N.L. and Mauzerall, D., in preparation). However, the O_2 flash yields for *Chlorella pyrenoidosa* measured by Jursinic and Dennenberg (Ref. 22; Table II), again divided by 10 instead of 4, result in a PSU(O_2) of 150. This is half the size of that obtained via the cross section measurement in *Chlorella vulgaris* (Ref. 83; Table IV). The reasons for these discrepancies may be partially the result of varied growth conditions, real interspecies variation in $\sigma(O_2)$, or difference in the quantum requirement.

Concomitant with O_2 evolution, O_2 is consumed by photosynthetic cells to an extent which can be significant in overall oxygen exchange. Steady-state mitochondrial respiration is simply the baseline of flash-induced O_2 yields. Light-induced changes of this respiration occur on the minute time scale (see subsection IVE). Therefore, neither is a contributing factor to O_2 flash yields. Flash-induced oxygen uptake (chlororespiration; Ref. 94) can be observed with the first flash to dark-adapted algal cells or isolated chloroplasts (or with each flash in the presence of DCMU; Greenbaum, N.L., unpublished data). The cause may be a rapid, nonenzymatic photoreduction of O_2 by the reducing side of PS I [95]. This signal occurs on a timescale roughly similar to that of O_2 production. It ranges up to 10% of the magnitude of O_2 production in algae, and even higher under some conditions in spinach chloroplasts when measured at 655 nm (Greenbaum, N.L., unpublished observations). It may therefore present a negative contribution to the net O_2 production. If measurements are made at a wavelength where the cross section for O_2 uptake is similar to that for O_2 production (i.e., $\sigma_I \approx \sigma_{II}$), its contribution to the observed cross section will be cancelled, and only the total yield will be affected. If the cross sections are significantly different at a particular wavelength and photoreduction of oxygen represents a substantial negative contribution to O_2 exchange, the resultant light-saturation curve will be distorted by O_2 uptake. At 723 nm, however, where there is a ten-fold difference in the cross sections of the two photosystems (Ref. 93; see subsection IVE), this O_2 uptake was not observed. Further investigation into the mechanisms of O_2 uptake are required to understand the phenomena.

IVD. The quantum requirement for O_2 production

The quantum requirement for oxygen formation in photosynthetic systems is associated with the Emerson-Warburg controversy. Emerson found 10 quanta were required for O_2 formation, while Warburg claimed far less. Warburg's number shrank to 3 [96,97], which is less

* We prefer plotting the data as O_2 yield versus log of quantum fluence as in Fig. 2 because the most significant error is in the yield, and the linear scale adequately reflects this error. Curves can be visually fit by simply sliding standard curves along the log fluence axis. The common plots of log of the complement of the O_2 yield versus fluence (the so-called 'killing curves') have a strong nonlinear error characteristic as the yield approaches saturation since the log of a small number becomes very large. This introduces a large 'baseline error', since the numbers become very sensitive to this parameter. The effect is the same as is observed in the similar plot of fluorescence induction curves (subsection IIID). The use of honest error bars on such plots (especially on tails of 'second components') is mandatory. We have seen such 'tails' in our data, but since they are most pronounced in highly pigmented cells and in the region of strong absorption we assign them to internal filtering of the actinic light pulse within a single cell, i.e., to inhomogeneous actinic light distribution.

than the number of photons needed if one electron per photon is removed from water. Kok's measurements [98] and his thorough review of the literature [30] supported Emerson's results, but there are still claims of low quanta requirements for net photosynthesis [99]. For a recent discussion of the subject, see Osborne and Geider [100]. We have proposed a model whereby formation of O_2 could require from 3–5 quanta in RC II [101].

The concept of the absolute size of a PSU unifies two classical measurements of photosynthesis: the Emerson-Arnold unit size and the quantum requirement for O_2 formation, the inverse of the quantum yield. The concept of the absolute size of a PSU thus resolves a long-standing problem and indicates a coherent solution to the understanding of energy flow in photosynthesis.

Under optically thin conditions, the total quanta absorbed by a sample containing n chlorophyll molecules and illuminated by a single turnover flash of energy E is $n\,\sigma(\mathrm{Chl})E$. In steady state, the O_2 produced by each flash is:

$$Y(O_2) = \left[\frac{n}{\mathrm{PSU}(O_2)}\right](1 - e^{-\sigma(O_2)E}) \tag{5}$$

where $n/\mathrm{PSU}(O_2)$ is the maximum yield, $Y(O_2)$, for that sample: total Chl n divided by Chl per O_2 per flash, i.e., the Emerson-Arnold unit. The minimum quantum requirement (QR) occurs at low energy, when $(1 - e^{-\sigma(O_2)E}) \rightarrow \sigma(O_2)E$. Thus

$$QR = \frac{n\sigma(\mathrm{Chl})E}{\sigma(O_2)E \cdot n/\mathrm{PSU}(O_2)} = \frac{\sigma(\mathrm{Chl})}{\sigma(O_2)}\mathrm{PSU}(O_2) \tag{6}$$

This equation allows us to obtain the true size of the unit for O_2 production, $\sigma(O_2)/\sigma(\mathrm{Chl})$, by dividing the Emerson-Arnold unit size by the quantum requirement for O_2. Note how the total chlorophyll in the Emerson-Arnold Unit and in the quantum requirement cancel when the absolute size of the O_2 unit is calculated. Eqn. 6 summarizes the relationship between parameters which have been the subject of 50 years of research and controversy. While it may be overly optimistic to predict the end of the controversy, it is nevertheless encouraging that placing our values of $\sigma(\mathrm{Chl})$, $\mathrm{PSU}(O_2)$ and $\sigma(O_2)$ into Eqn. 6 gives a quantum requirement of 10 ± 1 [83], the same number Emerson and Lewis [31] obtained for *Chlorella* some 50 years ago.

IVE. Measurements of the optical cross section of PSU I

Unlike PS II, there is no readily quantifiable product of PS I which allows for a rapid and accurate determination of its optical cross section. Weaver and Weaver [102] measured the EPR signal arising from $P\text{-}700^+$ in spinach subchloroplast particles. They estimated an optical cross section of 30 Å^2 for PSU I at 694 nm, equivalent to about 120 Chl (Table IV).

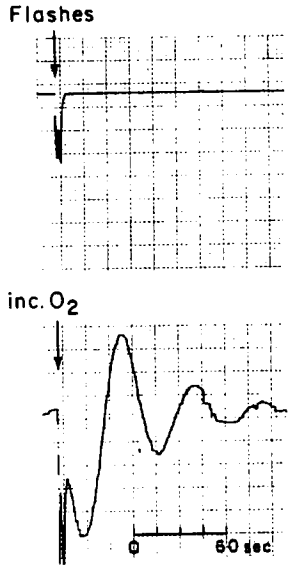

Fig. 1. Time-course of O_2 evolution and respiratory oscillation following laser flashes. The upper trace records the output of the thermopile used to monitor the total energies of the flashes (in this case, 13) before attenuation. The lower trace shows the output of the oxygen detection circuit. The baseline represents the rate of O_2 uptake by *Chlorella* in the dark.

A method has been developed in our laboratory which utilizes a flash-induced, time-dependent inhibition of respiration by intact algal or cyanobacterial cells [103]. Using this method, the cross sections of PSU II and I can be measured under identical conditions. The signal appears, after one or more flashes, as a net decrease in uptake of O_2 for approx. half a minute, followed by a series of damped oscillations before returning to baseline levels of respiration (Fig. 1). This oscillatory signal was initially assigned to PS I [104,105] because it was not inhibited by DCMU, but was blocked by millimolar concentrations of glucose or acetate (presumably by inhibiting carbon fixation). Additionally, the action spectrum of the oscillation was attributed to that of PS I [105,106].

Alternatively, these O_2 transients following changes in illumination have been attributed to oscillations in O_2 evolution [107–109]. This interpretation is unlikely, since O_2 is formed within 2 ms as a result of single-turnover flashes [110–112] and the subsequent respiratory oscillations occur in the dark for several minutes after O_2 evolution has ceased. O_2 evolution and the respiratory oscillation can be readily resolved (Fig. 1). Additionally, the two processes can be independently inhibited. The respiratory oscillation in *Chlorella* protoplasts is inhibited by carboxyatractyloside or atractyloside (Greenbaum, N.L., unpublished results), specific inhibitors of the mitochondrial adenine nucleotide translocase [113]. This is strong evidence that the oscil-

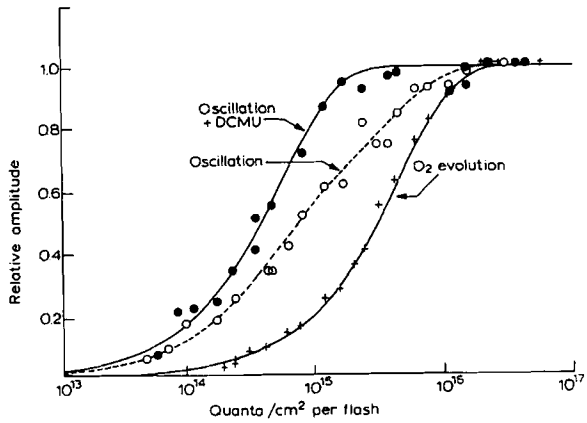

Fig. 2. Light-saturation behavior of O_2 flash yields (+) and respiratory oscillations (○, without DCMU; ●, with DCMU) in *Chlorella vulgaris* at 723 nm. The curve fit to the O_2 flash yield data is the cumulative one-hit saturation function calculated for a unique mean $\sigma = 2.5$ Å^2. The dashed curve fit to the oscillation data (○) is the sum of two Poissonians: equal contributions of curves with $\sigma = 3$ and 25 Å^2. The light-saturation behavior of the oscillation in the presence of DCMU (●) is fit with a Poissonian curve with $\sigma = 20$ Å^2. The absolute amplitude of the oscillation following saturating flashes is the same in the presence or absence of DCMU.

lation represents a transient inhibition of mitochondrial respiration in response to chloroplast-generated ATP (transported across the chloroplast membrane in the form of triosephosphates and subsequently oxidized in the cytosol) prior to the light-activation of ATP-requiring key enzymes of the carbon fixation cycle.

The light-saturation behavior of the respiratory oscillation in *Chlorella* (Fig. 2), unlike that of oxygen evolution, is broader than a simple Poissonian [93,103], which generally indicates heterogeneity. At 723 nm, where PS I absorbs far more than PS II, the saturation curve of the respiratory oscillation can be fit by the sum of equal contributions of two Poissonian curves, one having a cross section similar to that of O_2 production (3 Å^2), and one approx. ten times larger (25 Å^2). This bimodality was initially interpreted as evidence for two populations of PSU I [93]. In the presence of DCMU, however, the curve is a single Poissonian with only the larger σ, suggesting that the composite curve measured in the absence of DCMU represents contributions by both photosystems, contrary to the original conclusion of Ried [105]. Because of the qualitative nature of Ried's action spectra for O_2 production and for the respiratory oscillation, the contribution of PS II activity to the oscillation may have been missed. The amplitude of the oscillation in the presence of the inhibitor following saturating flashes is not diminished from that of the control, as would be expected if the DCMU blocked half of the contribution to the signal. In the absence of significant electron transfer from PS II, it is possible that PS I-dependent ATP production is increased by cyclic photophosphorylation [114], thereby increasing the antirespiratory activity. The light-saturation curve of the respiratory oscillation in *Chlamydomonas* as 723 nm is fit by a single Poissonian with the same cross section as *Chlorella* (21 Å^2). The amplitude and cross section are the same in the presence or absence of DCMU. *Chlamydomonas* has a very small $\sigma(O_2)$ at this wavelength, thus providing a virtually PS II-free measure of PS I activity in the absence of an inhibitor.

The evidence is that PSU I has a single size, or narrow distribution of sizes, as does PSU II. Because the optical cross section for PS I and II are more similar in size at 596 or 655 nm than they are at 723 nm, the degree of broadening of the composite curve is less and it is therefore more difficult to resolve the curve into its components.

If the model described above for the respiratory oscillation is correct, and the behavior can be maintained by either cyclic or linear electron flow in PS I, the light-saturation curve may represent varying contributions by PS II and PS I. The light-saturation curve at 723 nm of the oscillation can be resolved into components which appear to be activities of PS II and PS I, and the composite curve therefore represents a simultaneous measurement of the optical cross sections of both photosystems. The ratio of the amplitude of these two contributions to the composite curve, however, may not reflect the ratio of the two photosystems in any simple way.

Another approach to obtaining σ_I has made use of the observation of Gaffron and Rubin [115] that many green algae adaptively induce a hydrogenase under anaerobiosis, thereupon forming H_2 as a stable photoproduct. Greenbaum [116] has shown that the ratio of total Chl to hydrogen ($2e^-$) is 1.9 times as large as that of total Chl to O_2 ($4e^-$) in *Chlamydomonas*. This is the expected ratio if almost all reducing equivalents are trapped by the hydrogenase. As in the case of the respiratory oscillation [103], the flash light-saturation curve for hydrogen production was broader than a simple Poissonian but had roughly the same σ as the O_2 curve when determined at 596 nm [117]. Hydrogen, unlike oxygen, is formed at the steady-state yield on the very first flash. This points out the large difference between the two photosystems. Whereas O_2 formation requires accumulation of redox equivalents, H_2 formation occurs from a common pool, probably ferrodexin.

Recent work by Boichenko and Litvin [118] has also used pulsed H_2 production to determine the optical cross section of PS I in various green algae. In their experiments, the cross section for PS II was measured by O_2 production with a platinum polarograph and that of PS I by H_2 under anaerobic conditions using the same electrode polarized for H_2 oxidation. The measured absolute PSU sizes in *Chlorella* and *Chlamydomonas* are given in Table IV. The optical cross section they quoted for Chl in *Chlorella* is about twice as large as that of Ley and Mauzerall [83], but the number of

pigments is similar to the latter determination. Boichenko and Litvin claim that there is heterogeneity in both photosystems, with a second class of PS II and PS I's having 10–20-fold smaller sizes and comprising 12% of PS II and 30–50% of PS I [118]. Apparent biphasicity in the 'killing curve' plots may be the result of baseline errors or of excess algae on the electrode (see footnote on page 129). It is our opinion that actinic light absorption at 674 nm in a single cell, a wavelength of maximal absorption, causes the apparent biphasicity. Our data with *Chlorella* or isolated spinach chloroplasts, if similarly plotted, appear biphasic at 655 nm, but not at 596 or 723, wavelengths with far less absorption per cell.

Boichenko and Litvin interpret their data [118] and those of Ley and Mauzerall [83] on the variation in PSU sizes with changes in growth irradiance conditions as occurring in steps of 40 Chl. The proposal is reminiscent of the claim by Schmidt and Gaffron [119] that Emerson-Arnold sizes in leaves occur in integral fractions of a canonical size. The occurrence of the Chl-protein complexes in units of small numbers of Chl provides too fine a gradation in PSU sizes. Only further investigation under carefully controlled conditions will provide more evidence for or against this hypothesis.

V. The biochemical approach to PSU size

With the increasing interest in the protein components of the reaction centers and their associated antennae, new approaches for the study of photosystem structure have been developed (see Refs. 120 and 121 for reviews). A photosystem is composed of (1) a core complex, which includes the RC and a small number of closely associated antenna Chl; and (2) the light-harvesting complex (LHC), or pigment-protein antennae. It is thought that every Chl *a* and *b* is noncovalently complexed with one of these numerous, specific, membrane-associated proteins. The relative amount of specific apoproteins of these complexes has been shown to change with varying growth irradiance conditions [122].

Much has been learned from isolation of intact complexes from surfactant extracts of thylakoids. Rapid and gentle fractionation maximizes Chl retrieval in the complexes. The best results are reportedly achieved with glycosidic surfactants such as octyl glucoside or dodecyl maltoside [120]. Murata et al. (123) and Lam et al. [124] have measured 220 and 230 Chl per RC II, respectively, in PS II particles prepared with Triton X-100 from spinach chloroplasts, and Lundell et al. have measured 130 Chl per P700 in Triton X-100 solubilized thylakoid membranes of *Synechococcus* 6301 [125]. Thus although the possibility remains that all Chl has not been recovered or that changes occur during the process of isolation, these numbers are consistent with values obtained by the other methods (see Table IV).

Interesting work by Evans and Anderson [126] has involved the study of absorption properties of gel slices of extracted LHC's. The weighted, summed absorption peaks of the separated complexes in vitro was close to that of chloroplasts in vivo. The resolution of the overall PSU sizes into contributions of the individual complexes will be a great advance in the study of the energy absorption and transfer in photosynthetic systems.

VI. Applications of optical cross sections and PSU size

VIA. Energy transfer between PS II units

Knowledge of the size of a PSU is crucial to the understanding of the efficient energy transfer from the antenna pigments to an RC. The organization and mechanism required can be very different depending upon whether a few or several hundred pigment molecules are involved. In the latter case, energy cascading or lower dimensionality (Section I) are required for efficient transfer to the trap. With the concept of a photosynthetic unit there arose a new problem. Are the units composed of one reaction center per antenna cluster (single trap or 'puddle' model) or are there many reaction centers per cluster (multi-trap or 'lake' model) [47]?

VIA-1. Analysis

It is worth analyzing the effects of excitations in multitrapped units, since the final results are not self-evident. For example, Eqn. 4 will not apply to multi-trapped units if the excitations escape from an encounter with a closed trap. Essentially, the cross section grows larger as traps are filled, and the saturation curve is sharper than Eqn. 4. However, if excitations also escape from open traps with equal probability, i.e., trapping is reversible, then a multi-trapped unit will show a simple Poisson saturation curve [82,127]. For a definition of the term 'escape', see Ref. 128.

To calculate the effect of escape of excitations from traps let us assume each large unit contains t traps or RC's. If one makes the assumption that the fraction of closed traps is constant irrespective of closing (or opening) of traps during the process of trapping, one can sum the geometric series describing the repeated trials and obtain [128]:

$$P = \frac{(1-A)c/t}{1-B+(B-A)c/t} \tag{7}$$

where P is the probability that an excitation is not trapped in a unit containing c closed traps out of t total traps, A is the probability of escape from closed traps,

and B is that from open traps. If $B = 0$, we recover Joliots' equation [129]. But, if $A = B$,

$$P_{A-B} = \frac{c}{t} \tag{8}$$

Thus, there is no observable effect of the 'escapes'! However, the measured cross section is the average per trap, i.e., σ/t, where t is the number of traps per connected unit [82,127]. Malkin obtained the latter result by an argument based on energy transfer with inefficient trapping [130]. We expect A to be less than unity because of quenching by the chlorophyll cation in the closed trap. Free radicals are excellent quenchers of excited states. Since the energy level of the RC II trap (P-680) is similar to that of the surrounding chlorophyll, we expect B to be greater than zero.

The restrictive assumption of constant fraction of closed traps does not take into account the stochastic, consecutive nature of trapping and escape. The fraction of closed traps, c/t, changes during the repeated trials by an excitation until it is 'fully' trapped or lost. This effect enters as more than one photon per trap hits each trap, which necessarily occurs when a flash (or continuous light) is saturating. We can assume multi-photon effects are not present, i.e., the excitation rate is less than 1 ns^{-1}. A three line algorithm [128,131] suffices to correct the calculation. The result is, again, that when $A = B$, there will be no effect of escapes on the observed shape of the saturation curve: it will be virtually indistinguishable from the behavior of Eqn. 4. When $A > B$, the saturation curve will be sharper, and when $B > A$ it will be more shallow, than the simple one hit Poissonian [82,128]. The shape of the curve is also a weak function of t, the number of traps per unit.

One can obtain similar conclusions following the far more elaborate Master Equation, or micro-kinetic approach of Paillotin et al. [132], or by the macro-kinetic approach of Sonneveld et al. [133]. These treatments, when applied to yields, are complicated by a surfeit of parameters. The yields are given by ratios of sums and products of individual rate constants. The kinetics of the trapping and detrapping are almost certainly in the subnanosecond time range. Thus kinetics observed on the longer time scales are simply the convolution of the yield function with the time shape of the light pulse, and contain no novel information. Even the multi-photon effects on yield are readily treated by the statistical approach [82].

VIA-2. Results

A large probability of escape from closed traps will cause an increase in the cross section of a multi-trapped unit when many of the traps are closed, as more antennae pigments are made available to the remaining open traps. However, the cross section for O_2 production does not significantly increase on closing up to 92% of the traps in *Chlorella* with continuous light [128] or in *Chlamydomonas* at 75% closure, *Scenedesmus* at 50% closure, or isolated spinach chloroplasts at 85% closure (Greenbaum, N.L. Mauzerall, D., unpublished results). These results conflict with the common belief of a high probability of escape on hitting closed traps. The data are consistent only with a modest escape probability ($A \leqslant 0.25$ if $B = 0$), otherwise $(1 - B)/(1 - A) > 1.5$, assuming four traps per large unit. The usually quoted value of escape is greater than 0.5 [50,129]. Previous measurements, however, involve the use of the shape of the fluorescence induction curve to determine the fraction of closed traps, and the use of the Joliot's equation [129], both of which are subject to error as described above. Fluorescence yields measured in five species of eukaryotic algae by pump and probe flashes show a more complex function of irradiance than does the O_2 flash yield [134]. In these experiments, the optical cross section upon closing of traps was constant to $\pm 10\%$. The exponential shape of laser pump and probe fluorescence saturation curves [70] similarly indicates the low probability of energy transfer in *Chlorella* [70] and spinach [72]. Thus we believe there is no strong evidence that excitations have a much higher probability of escaping from closed than from open traps.

VIB. State transitions

With the discovery of two photosystems with different action spectra [135] operating in series to carry out oxygenic photosynthesis, the problem arose as to how serial electron transport was maximized when the illumination excited one photosystem more than the other. Mechanisms for equalizing energy flow by redistribution of excitations, termed 'state transitions', were therefore postulated. Myers and Graham [136] and Clayton [137] proposed that excess energy absorbed by the antenna of PS II could 'spillover' from closed RC II to open RC I, but not vice versa because the energy of the RC I trap (700 nm) is lower than that of the RC II trap (680 nm; see Ref. 138 for a review). Such a mechanism would necessitate both a sharing between the antennae of PS II and PS I, and significant escape from closed RC II. Evidence supporting spillover comes mainly from experiments on fluorescence, with a decrease in variable fluorescence yield interpreted as an increase in PS I activity. However, a change in the PS I-dependent electrochromic shift with closure of PS II traps, which would have been indicative of spillover from PS II to PS I, was not observed in the experiments of Joliot et al. [139] and of Trissl et al. [140]. It has recently been suggested [141] that the faster turnover time of PS I in isolated stromal lamellae following phosphorylation of 20–30% of LHC II [142] may be

limited to this preparation, as much smaller changes are observed in intact thylakoids [143,144].

Alternatively, a reversible shift in the fractional absorption of light by PS I and PS II (reviewed in Refs. 69 and 141), was postulated by Bonaventura and Myers [145]. State 1 occurs when excess light is absorbed by PS I and results in increased energy flow to PS II. Vice versa, in State 2, when light is preferentially absorbed by PS II, the energy flow to PS I is increased. The proposed migration of pigment-protein complexes between the different photosystems necessarily implies a measurable change in the optical cross sections of the photosystems [146,147].

The prevailing hypothesis concerning the mechanism of these changes in green plants and algae is that the transition to State 2 is the result of reversible phosphorylation of LHC II [148–151], signaled by changes in the redox level of the pool of quinones between the photosystems [148,149,152,153]. Experiments with mutants lacking either the LHC II or RC II implicate the light-harvesting protein complex itself, not the reaction center, in these changes [154–156]. Alternatively, a light-induced change in the ionic environment, particularly magnesium ions, has been proposed to play a role in the state transitions [157–159]. The data of Telfer et al. [160] on the flash yield of $\Delta A_{820\,nm}$ (PS I) in pea chloroplast membranes can be interpreted as a 70% decrease in $\sigma(I)$ on adding 5 mM $MgCl_2$. Fluorescence studies support the hypothesis that phosphorylation of LHC II in thylakoids of various algae and higher plants correlates with a decrease in the cross section of PS II [68,148,161,163]. Others, however, have found ATP-dependent phosphorylation to be independent of state transitions [164–167]. The optical cross sections for oxygen flash yields in *Chlorella* (Refs. 83 and 168; see also Greenbaum, N.L. and Mauzerall, D., unpublished results) and *Chlamydomonas* (Greenbaum, N.L. and Mauzerall, D., unpublished results) did not change ($\pm 5\%$) as a result of background far red or green light, as would have been expected if state transitions caused significant redistribution of antenna pigments. Conversely, illumination of spinach chloroplasts with 695 nm light resulted in a cross section for O_2 production at 655 nm approx. 30% larger than that measured with background light of 540 nm (Greenbaum, N.L. and Mauzerall, D., unpublished results).

Fluorescence changes at 735 nm following transitions to State 2 have been interpreted as an increase in the cross section of PS I. However, attempts to demonstrate an increase in PS I by other methods have been weak (see Ref. 141 for a discussion). Allen and Melis [141] and Haworth and Melis [74] have failed to detect a change in the rate of photooxidation of P-700 with exposure to light inducing States 1 or 2 in *Scenedesmus* and spinach thylakoids, respectively.

Distribution of energy between the two photosystems and state transitions have also been studied by photoacoustics [146,169]. Oxygen evolution is monitored directly by its escape into the gas phase from chloroplasts in intact leaves. This oxygen signal is distinct from the usual thermal photoacoustic signal [170]. Emerson enhancement (subsection IIIC) was used to determine the ratio of excitation of PS I and PS II. In tobacco leaves no evidence for spillover was obtained, but the reciprocal change in cross sections of the two systems was observed [146]. As an example, for the transition from State 2 to State 1, PS II absorption at 650 nm increased 40% and PS I decreased 20%. Use of the photoacoustic method on *Nostoc muscoreen* showed a 15% decrease in absorption of PS II at 580 and 620 nm with an equal increase in absorption by PS I on the State 1-to-2 transition [171].

State transitions have also been studied by time-resolved fluorescence spectroscopy in *Scenedesmus obliquus* [172]. A State-2-to-State 1 transition was interpreted as causing no change in absorption by PS I, but a 35% decrease in PS II_β and a 25% increase in PS II_α centers. Such an observation would be supportive of the recent suggestion that state transitions actually provide a means of energy dissipation by PS II (see Ref. 141).

It is clear that the various measurements have led to a variety of interpretations of the data. It is only by unambiguous, systematic measurement of the PSU sizes that a consistent picture may emerge. It should be emphasized that, in green plants and algae, changes in cross section reflecting state transitions, when observed at all, have been small. Growth of many organisms is often limited by nutrients, not light, so selection for these mechanisms would concentrate on shade species. Thus, we believe that the selection pressure for such mechanisms is questionable, considering the fluctuation in ambient illumination in the usual natural environment.

Changes in energy distribution in organisms having phycobilisomes as well as chlorophyll, e.g., cyanobacteria and red algae (see Ref. 173 for a review), are larger than in green algae and higher plants and may contribute to chromatic adaption (subsection VC2). For example, oxygen flash yields showed a 50% increase in the cross section of PS II measured by O_2 yield with light inducing State 1 in *Porphyridium* [174]. Most investigators do not attribute the changes in cross sections in these organisms to phosphorylation of pigment protein complexes (Refs. 74, 175 and 176; see, however, Ref. 177). They have alternatively been suggested to be the result of reorientation of phycobilisome core proteins [178].

VIC. Light adaptation

The size of a PSU is relevant to both the problem of light and shade adaption and the interrelated problem

TABLE V

Absolute sizes by kinetic approach

Material	Method		Growth conditions	RC II/RC I	N IIα	N IIβ	%N IIβ	N I	Ref.
	II	I							
Green algae:									
Dunaliella salina	ΔA_{320} and pheo content and fluorescence induction	ΔA_{700} rate P-700	80 $\mu E \cdot m^{-1} \cdot s^{-1}$	1.4	570	150		220	36
Chlamydomonas reinhardtii	ΔA_{320} and fluorescence induction	ΔA_{700} rate P-700	47 $\mu E \cdot m^{-2} \cdot s^{-1}$	1.35	620	200		370	25
			400 $\mu E \cdot m^{-2} \cdot s^{-1}$	2.65	460	200		170	
Higher plants:									
Pea chloroplasts	ΔA_{320} and fluorescence induction	ΔA_{700} and rate P-700	2 min light/ 98 min dark (immature)	2.9	0	56	100%	90	
			2 min light/ 48 min dark (developing)	2.0	170	28	58%	136	23
			continuous light (mature)	1.7	280	90	20%	220	
	ΔA_{320} and fluorescence induction	ΔA_{700} and rate P-700	far-red depleted	1.3	300	95		225	23
			far-red enriched	2.2	265	80		215	
Barley thylakoids	ΔA_{320} and fluorescence induction	ΔA_{700} and rate P-700	unfractionated thylakoids	1.8	260	170	30%	175	
			stroma		0	175	100%	185	51
			grana		250	0	0%	0	
Tobacco chloroplasts	ΔA_{325} and fluorescence induction	ΔA_{700} and rate rate P-700	4000–5000 lx	1.74	240	130	39%	190	21
Sugar beet	ΔA_{325} and fluorescence induction	ΔA_{700} and rate P-700	500 $\mu E \cdot m^{-2} \cdot s^{-1}$	1.6	248	111	24%	144	58

of chromatic adaption. It had long been observed that plants growing in the shade tend to be more deeply colored than those growing in bright light. Similarly, algae growing at different ocean depths have some tendency to stratify according to color. In order to maintain the efficiency of photosynthesis under varying environmental conditions, the numbers, sizes and ratios of PSU's may change. An unambiguous measure of PSU sizes in terms of numbers of specific pigments is of great value in understanding the mechanism of these adaptions. A representative range of the results from several laboratories is included in Tables I–V. The mechanism by which such changes are signalled and controlled is not well understood, but the redox level of the quinone pool between PS I and II and changes in ATP production have been postulated to play a role in the signalling process [24,179].

VIC-1. Growth irradiance levels

There is a wide variety of responses to changing growth irradiance by photosynthetic systems (Tables I–V). Far from the assumed unit ratio of the photosystems, the RC II/RC I ratio in different photosynthetic membrane preparations (spinach chloroplasts, developing and mature pea thylakoids, and cyanobacteria) was found to vary from 0.43 to 3.3 [26,180,181]! The RC II/RC I ratio measured in several desert plants ranged between 0.5 and 3.0, with essentially all of the variation in the number of RC II [182]. Total Chl/RC II in leaf sections of a number of sun and shade plants grown in high-light environments ranged between 220 and 480; this stoichiometry increased with decreasing growth light to 630–940 total Chl/RC II [183].

Cyanobacteria and red algae grown in weak light had up to four times more RC I than RC II per cell [26].

The ratio approached unity in strong light (see *Anabaena* in Table I as an example). The number of RC I varied, while that of RC II was more constant. The numbers of RC I and RC II per cell increased 2.5-fold on growing the chlorophyte *Dunaliella tertiolecta* in high and low light, while their ratio remained constant [29]. The total Chl per RC increased only about 20% (Table II). However, the diatom *Skeletonema* costatum increased its number of RC II by 50% and decreased the number of RC I by about 30%, leading to a doubling of the ratio of RC II/RC I. The total Chl per RC II was constant while that of RC I doubled. There was no relationship between the variations in the number of RC II's and light-saturated rates of O_2 production in either of these organisms. However, in two species of marine dinoflagellates, the number of RC I per cell correlates with the amount of O_2 produced in saturating light [184], suggesting that RC I is the limiting factor in photosynthetic rates. More recent studies with *Dunaliella tertiolecta*, on the other hand, suggest that light-saturated photosynthesis is limited by carbon fixation rather than by electron transport [75].

Only in *Chlorella* do we have a quantitative dissection into absolute size and number of PS II. The absolute size of PS II increased from 130 to 400 Chl $a + b$ and the number of RC II increased 4-fold when *Chlorella vulgaris* was grown under decreasing light intensities [83]. This range was expanded to 70 and 390 Chl $a + b$ per RC II and a 7-fold range in the number of RC II by growth under extreme irradiance conditions [3]. Throughout the wide range of antenna sizes, the quantum requirement remained strikingly constant at 10 ± 1 quanta per O_2 with only two variants of 14 quanta at each end of the range. However, the quantum requirement for three species of phytoplankton increased from 9 to 20 quanta per O_2 with increasing growth irradiances of 70–600 $\mu E \cdot m^{-2} \cdot s^{-1}$ [185]. The authors suggest that either these complex organisms have an increased number of pigments not transferring energy efficiently or that there is an increase in cyclic phosphorylation at these high irradiances.

VIC-2. Chromatic adaptation

The amounts of Chl, phycocyanin, and phycoerythrin in the cell change according to the color of the growth light [186]. Growth of the cyanobacterium *Synechococcus* 6301 in red light, primarily absorbed by chlorophyll (mostly associated with PS I in red algae and cyanobacteria), produced more phycobilisome-PS II complexes than did growth in white light, thereby increasing the RC II/RC I and bilin/Chl ratios [24]. These adjustments in RC stoichiometry led to balanced absorption between the two photosystems. Adaptation of the organism to yellow light (primarily absorbed by the phycobiliproteins) by forming more PS I complexes, however, was insufficient to fully compensate for the lesser absorption by PS I. These conclusions are solely based on calculated absorption ratios of the two photosystems.

Myers et al. [27] measured similar changes in the RC II/RC I ratio in red-grown *Synechococcus* ($4 \times$ higher, compared with 3-fold higher in the above study [24]; see Table I). These authors found no differences in the cellular growth rate or in the sizes of PS I or PS II between cells grown in either light regime. The distribution of chlorophyll between the photosystems was calculated from the action spectra, in turn obtained from the effects of Emerson enhancement. There was, however, an increased bilin/RC II ratio in the red light grown cells and 5 times more total chlorophyll in the gold light grown cells.

There are definite limits to the usefulness of adapting to colors. Because of the broad and multiple absorption bands of molecules, in the words of Ramus et al. [187]: "If you are optically black, it does not matter what color you are". Thus under low light intensities the organism forms many and/or large-sized PSU's, and most incident photons are absorbed, irrespective of wavelength. At high light intensities some colors are preferentially absorbed, but light is then usually not limiting.

VIC-3. Development

The sizes of both photosystems increase with maturity in pea chloroplasts concomitant with a decrease in the ratio of RC II/RC I [23]. Thus the system makes RC's first and fills in the PSU size during development.

Relative sizes and number of RC II in synchronized cultures of *Chlorella pyrenoidosa* have been measured by changes in fluorescence induction [63]. Within the first 4 h of the cell cycle a 35% increase in the number of RC II was accompanied by a similar decrease in the size of the units. These values remained approximately constant for the next 6 h, after which the number of RC II decreased and their relative size increased. These data contrast with measurements of the Emerson-Arnold PSU [188] in *Scenedesmus obliquus* D_3, which remained constant at 1750 total Chl per O_2 per flash (Chl content and number of RC II increased proportionally) throughout the cell cycle.

VID. Bacterial reaction center

Cho et al. [80] used a fit to the entire Poissonian saturation curve to show that the quantum yield of charge separation in RC's from *Rhodobacter sphaeroides* R-26 mutant was 0.98. Gunner and Dutton [189] used measurements of optical cross sections to determine the photochemical yield of reduced quinone in photoexcited bacterial RC's. By substitution of the natural ubiquinone-9 with a large variety of synthetic quinones of varying midpoint potentials, they varied the free

energy of the electron-transfer reaction. Normalizing results from altered RC's to those of natural RC's eliminated the need for measurement of absolute light energies. The quantum yield was directly determined, since σ(RC) was constant and the measured σ is ϕ(quinone) · σ(RC). They concluded that the yield diminished with decreasing free energy of reaction.

VIE. Bacteriorhodopsin

Marinetti has recently applied the concept of the optical cross section of a photochemical unit to bacteriorhodopsin, the light-driven proton pump from *Halobacterium halobium* [190]. Transient proton, as well as nonproton, counterion movements were detected by changes in the bulk conductivity of a suspension of purple membrane following a light flash. The method has an internal actinometer, since the small amount of heating of the suspension by thermally degraded absorbed photons causes an observable conductivity change by the end of the photocycle. Marinetti found that the cross section for nonproton ion release is 3–4 times larger than that for proton uptake. Since the cross section for the latter process equals that of heating, i.e., of a single bacteriorhodopsin molecule, it follows that a hit to any of 3–4 bacteriorhodopsin molecules produces the nonproton ion movement, and further hits do not add to the observed transient. The large cross section implies that the nonproton movements exhibit cooperativity, whereas the proton movements do not. The cooperative nonproton ion release also correlates with the much higher quantum yield observed (up to 10 per photon [191]) compared with that for H^+ (0.4) [192]. Measurement of the optical cross section is a unique method of determining cooperative effects.

VII. The Future

The approach to the study of photosynthetic systems via their optical cross sections can be extended along several lines. Given the many absolute measurements on *Chlorella*, the *'Escherichia coli'* of photosynthesis, this organism can be used as a reference for relative measurements on other Chl $a + b$ systems. Such a benchmark simplifies further measurements of cross sections by avoiding the need for absolute calibration of the pulsed light incident on the sample.

Now that the outline of the measures of PSU II and PSU I on simple algae has been accomplished, more precise measures will produce answers to several of the problems raised in this review. The question of where does the 25% 'loss' in the idealized efficiency of photosynthetic O_2 formation (10 quanta required, not $8 = 4$ photoelectrons $\times$ 2 photosystems) occur may be answered. Is it in the product yield term Y or in the photochemical yield, ϕ (Eqn. 4, subsection IVB-1)? Given the absolute size of the units and their number, Eqn. 2 allows one to determine if all the chlorophyll has been accounted for. If not, a search for additional systems, contributing to activities other than the linear electron flow through PS II and I, must be made. One also has an objective method to group photosynthetic activities into discrete systems: measurement of the cross sections of several processes or intermediates should cluster into two (or more) categories. Several questions can be answered. How does the absolute size of a unit change with growth conditions of the plant or the algae? What is the connection of the phycobilisomes and RC's in red algae? Ley's estimate [174] of their giant cross sections (5000 Å^2, 1000 pigment molecules) is most provocative because of the limits on the maximum size of PSU's (Section I). Multi-wavelength excitation and analysis can be used to identify the contribution of the different classes of pigments. The chlorosomes of the 'green' photosynthetic bacteria [193,194] are particularly intriguing. These enormous structures are expected to have optical cross sections even larger than in the red algae. If the hypothesis of their structure is correct, the energy is transferred from these structures via 'funnels' to a collection of RC's. If the energy transfer to the RC is irreversible, a measurement may give the optical cross section per funnel rather than per trap. Again, analysis of multi-wavelength excitation will produce the contribution of each of these components to the total cross section of a trap. As the absolute absorptions and cellular content of the various Chl-protein complexes become known (see, e.g., Ref. 126) it will be possible to subdivide the size of a PSU further into its ultimate components.

The use of photoacoustic detectors opens a wide range of intact biological material to these studies. In leaves, the pulsed photoacoustic method has been shown to resolve readily the oxygen gas phase photoacoustic pulse from the usual photothermal effects [195]. The sensitivity allows measurement of single-flash response. The S-state oscillations of O_2 formation are readily measured. The connectivity of the PSU's may be deduced by measurement of the saturation curve for the oxygen yield on the third flash to a dark-adapted system. If the flashes have the same energy, the saturation function at low light energies will vary from linear (totally connected units) to cubic (totally independent units). Rough measurements by flashlamp-driven photoacoustics on a tobacco leaf showed a cubic dependence [195], but measurements by dye laser at 655 nm on a spinach leaf showed a more linear response (Mauzerall, D., unpublished results). The total photothermal effect itself gives the absorbance cross section of the leaf directly and can be used as an internal measure of the variation of the deposited energy with depth. It is possible that time-dependent energy deposi-

tion steps along the reaction sequences of PS I and II can be observed [195].

Thus the answer to the simple question 'how large is a PSU?' is rather complex and variable. This may be a reflection of its evolutionary origin and its important role in photosynthesis. The large number of molecules collaborating to gather photons for a reaction center allow photosynthesis to proceed rapidly and efficiently with the available flux of solar energy. Change in the size of the PSU allows a simple mechanism of adaptation to local light conditions. The study of the size of a PSU contributes to the understanding of many of these mechanisms which allow photosynthesis to support life on earth.

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