Chapter 11

Action Spectrum and Quantum Yield of Photosynthesis

It was suggested in Chapter 9 that the function of certain accessory pigments (for example, phycoerythrin in red algae, and fucoxanthol in the brown ones), is to permit the utilization for photosynthesis of light in the middle part of the visible spectrum, not absorbed efficiently enough by chlorophyll *a*.

In 1883–85, Th. W. Engelmann, a German physiologist, placed cultures of red, brown, and green algae on the microscope stage located in the focal plane of a spectroscope. Using motile bacteria as oxygen indicators, he found that red algae produced most oxygen in the green part of the spectrum, brown algae in the blue-green part of it, and green algae in the blue-violet and the red region. Every time, the peak of photosynthetic activity coincided with the region of maximum absorption. Engelmann then offered a "law," $E_{\text{abs}} = E_{\text{photosyn}}$, suggesting that all light *absorbed* by chloroplast pigments is also light *used* for photosynthesis.

Engelmann's interesting results were forgotten when Richard Willstätter and Arthur Stoll in Munich carried out their famous investigations of chlorophyll and photosynthesis, published in two volumes in 1913 and 1917, respectively. Using green leaves exposed to incandescent light, they concluded, rather incidentally, that light absorption by carotenoids

did *not* contribute to photosynthesis. This erroneous conclusion may have been due to several reasons, beginning simply with the extreme weakness of blue-violet rays in the light of an incandescent bulb. However, the authority of Willstätter (who was awarded the Nobel Prize in 1915), and general concentration on the study of chlorophyll as "the" photosynthetic pigment, made most students of photosynthesis reluctant to admit a possible contribution to photosynthesis of pigments other than chlorophyll. When a German plant physiologist, C. Montfort, concluded, from crude measurements, that light absorption by fucoxanthol contributes significantly to the photosynthesis of brown algae, little if any attention was paid to his claims.

Since 1940, however, more reliable measurements, first by W. M. Manning, H. J. Dutton, and co-workers at the University of Wisconsin, and then by Robert Emerson and co-workers (first in California and then at the University of Illinois), brought general confirmation of qualitative validity of Engelmann's law. In fact, only light quanta absorbed by the pigments dissolved in the cell sap or bound in cell walls (such as the anthocyanins) are totally lost for photosynthesis. All quanta absorbed by pigments located in the chloroplasts (which includes the carotenoids, the phycobilins, and the chlorophylls) contribute, to some extent, to photosynthesis. However, the effectiveness of this contribution varies between 20 and 100 percent of that of the quanta absorbed by chlorophyll *a* (or, more exactly, by the most effective of the several forms of chlorophyll *a*).

The question naturally poses itself: do accessory pigments carry out photosynthesis, as it were, on their own? If this were so, it would be difficult to understand why all plants capable of photosynthesis contain chlorophyll *a*, including the deep-sea red algae, in which chlorophyll *a* has little chance to absorb directly a significant number of light quanta. An alternative interpretation is offered by the observations of *sensitized fluorescence* of chlorophyll *a* in vivo (to be described in Chapter 12): it is that quanta taken up by accessory pigments are transferred by a so-called resonance mechanism to chlorophyll *a*, and contribute to photosynthesis only after this transfer. They act as if they were servants who keep their master, chlorophyll *a*, well supplied with quanta, so he can keep up his photocatalytic trade.

It is thus legitimate to postulate that one function of the accessory

pigments in photosynthesis is to supply quanta to chlorophyll *a*. In the case of yellow carotenoids, where the efficiency of this transfer is low, it is logical to surmise that these pigments have also another and more significant function, either in photosynthesis or in some other metabolic process. What that function is, we do not yet know.

THE ACTION SPECTRA OF PHOTOSYNTHESIS

We shall now consider the problem more quantitatively. Light of a single wavelength, λ (or a single frequency, ν) is called *monochromatic*. (In practice, "monochromatic" light always covers a finite band of wavelengths.) In photochemical research, it is often desirable to measure the chemical effect produced by monochromatic light. This requires the use of powerful light sources, and of monochromators of high resolving power. The term "action spectrum" is used to describe a plot of the intensity of some phenomenon (for example, the rate of photosynthesis) produced by monochromatic light as function of the wavelength (or frequency) of this light. More specifically, one measures the rate of O_2 evolution (or of CO_2 uptake), P, produced by a known number of *incident* quanta, I_i—or, better, by a certain number of *absorbed* quanta, I_a—of a given wavelength. Both plots:

$$\frac{P}{I_i} = f(\lambda) \tag{11.1}$$

and

$$\frac{P}{I_a} = f(\lambda) \tag{11.2}$$

have occasionally been described as "action spectra." We shall use this term for the more significant plot (11.2).

If P is expressed as the number of O_2 molecules evolved per second, and I_a as the number of quanta absorbed per second, P/I_a is the *quantum yield* (Φ) of photosynthesis. If all pigments that absorb light were equally effective in photosynthesis, in all parts of their absorption bands, the plot of Eq. 11.2 would be a horizontal line. It is the deviation from the horizontal that is of interest.

Light of a certain wavelength is often absorbed by several pigments present in the cell. If the *action spectrum* of photosynthesis, obtained by systematic measurements of P in monochromatic light throughout the visible spectrum, is compared with the *absorption curves* of the several pigments present, conclusions can be drawn as to the relative efficiency with which different pigments contribute to photosynthesis. Such measurements require certain precautions. We know that the pigments participate in the primary photochemical reaction, which determines the rate of photosynthesis in *weak* light; while in *strong* light (in the "light-saturated state"), the overall rate is determined by a dark enzymatic reaction. To give significant results, action spectra must be measured in the light-limited state, that is, at low light intensities. In strong, saturating light, all the revealing features may be smoothed out.

One practical problem in photochemistry is to obtain sufficient light energy (that is, a sufficient flux of light quanta) within a narrow "monochromatic" band to produce a measurable chemical effect. When the effect of a single light quantum can be multiplied thousands of times, as in the development of a photographic plate, the problem is relatively easy; but when Einstein's law applies, that is, when one absorbed quantum changes only one molecule, energy limitations become bothersome. Furthermore, in radiation from the incandescent lamp, the intensity drops rapidly from the red to the blue end of the spectrum. This is why we have much better data on the dependence of the yield of photosynthesis on wavelength in the long-wave, orange-red region, than in the short-wave, blue-violet spectral region. (Xenon lamps may be used in the blue region, but, unfortunately, their emission is rather unsteady.)

Very crude action spectra can be obtained by means of colored glasses, or colored gelatin filters, which transmit broad spectral bands. Much better are the so-called interference filters, which isolate, from a continuous spectrum, bands about 10 nm (or even 5 nm) wide. With a set of such filters, one can explore the whole visible and near ultraviolet spectrum. Still better, however, is to use a *monochromator*—a powerful spectroscope—isolating from a continuous spectrum nearly monochromatic spectral bands.

Truly significant action spectra are plots of the *maximum quantum yield* (Φ) of photosynthesis, in monochromatic light, as function of the wavelength (or frequency) of this light. The most reliable and systematic measurements of this type have been made by Robert Emerson and

co-workers, with the green alga, *Chlorella pyrenoidosa* (Fig. 11.1), the red alga, *Porphyridium cruentum* (Fig. 11.2), the blue-green alga, *Chroococcus,* and the diatom, *Navicula minima* (Fig. 11.3). These measurements were made with precision manometers and a large grating monochromator.

One fact is immediately obvious: light absorbed in the blue-violet region of the spectrum is relatively ineffective in bringing about photosynthesis. In the face of such deficiency, one can first postulate that all quanta absorbed by a certain group of pigments (such as the carotenoids), do not contribute to photosynthesis, or contribute to it with low efficiency. In a second approximation, one has to consider the possibility that some pigments of a given group are less efficient than the others (carotenes may be less efficient than carotenols, or chlorophyll *b* less efficient than chlorophyll *a*). Finally, one has to consider the possibility that quanta of different size, absorbed by one and the same pigment (for example "blue" and "red" quanta absorbed by chlorophyll) may differ in efficiency. Forgetting for a moment this last possibility and considering action spectra only as evidence of differences in photo-

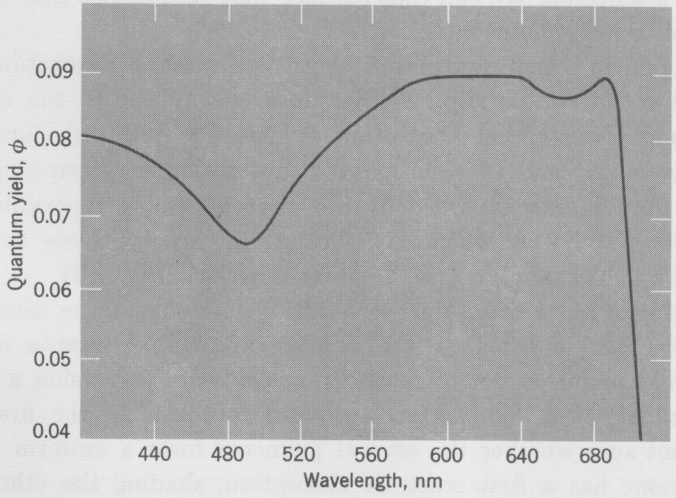

FIG. 11.1 Action spectrum of photosynthesis (quantum yield of oxygen evolution as a function of wavelength of light) in a green alga (*chlorella pyrenoidosa*). (R. Emerson and C. Lewis, 1943.)

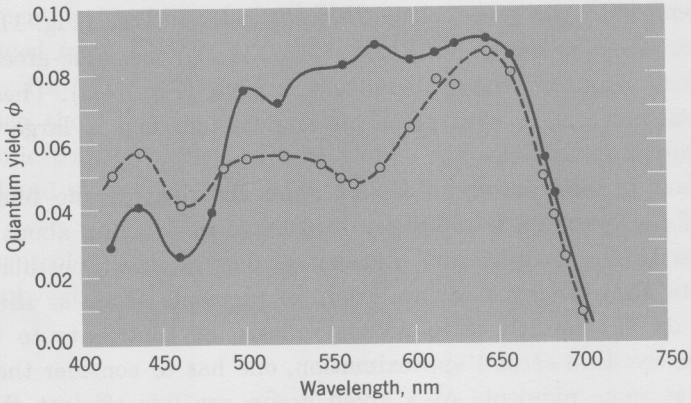

FIG. 11.2 Action spectrum of photosynthesis (quantum yield of oxygen evolution as a function of wavelength of light) in *Porphyridium cruentum* (a red alga). (Solid line–algae preilluminated with green light; dashed line–algae preilluminated with blue light.) (M. Brody and R. Emerson, 1959.)

synthetic efficiency of different pigments, we note that all action curves dip in the region (450–500 nm) where carotenoids contribute most to the total absorption. We can thus surmise that carotenoids are relatively poor sensitizers in photosynthesis.

The region in which phycobilins contribute most to absorption in red algae shows no similar dip. Neither does one appear in the region of predominant fucoxanthol absorption in diatoms (although fucoxanthol is a carotenoid), and of chlorophyll *b* absorption in green algae. This suggests that quanta absorbed by the phycobilins, by fucoxanthol, and by chlorophyll *b*, are about as efficient in photosynthesis as quanta absorbed by chlorophyll *a* (see, however, Chapters 9 and 13).

To estimate more precisely the relative efficiency of the several pigments, one has to calculate the relative number of quanta absorbed by different pigments out of monochromatic beam traversing a mixture of several of them. This is an awkward problem. In the first place, we are not sure whether the several pigments form a uniform mixture, so that none has a first crack at absorption, shading the others. If a homogeneous absorption is postulated, the allotment of quanta should be in proportion to the products, αc, of their absorption coefficients and their concentrations. The latter can be determined by extraction and

quantitative analysis; but the absorption coefficients in vivo are difficult to estimate precisely. We know that upon extraction, the absorption bands of the carotenoids and the chlorophylls shift (see Chapter 9) because they are affected in vivo by aggregation of pigment molecules and by their association with molecules of the medium. Precise determinations of these shifts are not easy, and the results not very reliable because of mutual overlapping of the several absorption bands. The allocation of absorbed quanta to different pigments is, therefore, fraught with considerable uncertainties. The relative efficiencies, calculated from Emerson's data on oxygen evolution, or from Duysens' data on fluorescence (see Chapter 12) are, therefore, only more or less satisfactory approximations.

Despite these uncertainties, one can definitely assert, from the analysis of the action spectra, that differences between the several pigments are more subtle than simple "effective" or "not effective." Even the yellow carotenoids are not *entirely* ineffective; if the best sensitizing efficiency of chlorophyll *a* is set as 100%, that of the carotenoids varies, in different plants, between 20% and 50%. The effectiveness of fucoxanthol in brown algae and diatoms seems to be about 80%.

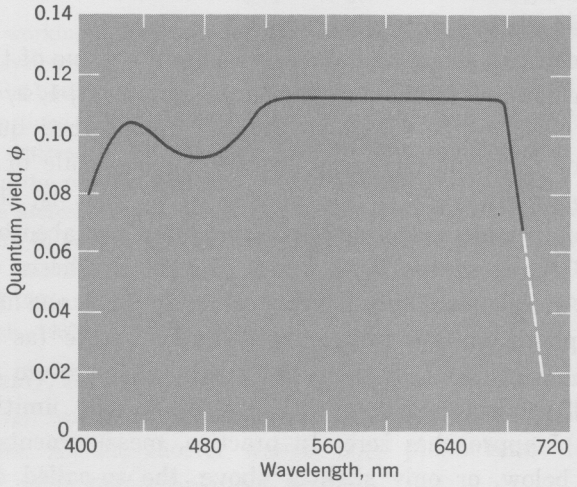

FIG. 11.3 Action spectrum of photosynthesis (quantum yield of oxygen evolution as a function of wavelength of light) in a diatom (*Navicula minima*). (T. Tanada, 1951.)

According to Marcia Brody and Robert Emerson, the relative efficiency of quanta absorbed (below 600 nm) by phycoerythrin in the red alga *Porphyridium* can vary from 1 to 0.6 of that of the quanta absorbed by chlorophyll *a* (at 650 nm), depending (see Fig. 11.2) on whether the cells had been preilluminated with bright green light (absorbed preferentially by phycoerythrin, and apparently stimulating its efficiency) or with blue-violet light (absorbed preferentially by chlorophyll *a* and apparently decreasing the effectiveness of energy transfer from phycoerythrin to chlorophyll *a*). Variations with wave-length in the efficiency of quanta absorbed in chlorophyll *a* itself, which is particularly strong in these algae, will be discussed in Chapter 13.

THE MAXIMUM QUANTUM YIELD OF PHOTOSYNTHESIS

We have dealt so far in this chapter with the *relative* quantum yields of photosynthesis in monochromatic light of different color. We found that this yield is approximately constant when light absorption occurs in chlorophyll *b* or *a* (however, see Chapter 13) and, under proper conditions, also in light absorbed by the phycobilins, but declines sharply in light absorbed by the carotenoids.

We shall now say something about the *absolute* value of the maximum quantum yield, Φ, or minimum quantum requirement, $1/\Phi$, of photosynthesis. The latter is the minimum number of absorbed quanta needed to reduce one molecule CO_2 and liberate one molecule of oxygen; the maximum quantum yield is the inverse of it, that is, the maximum fractional number of oxygen molecules produced per absorbed quantum.

From the shape of the light curve of photosynthesis (Fig. 6.1) it is clear that in order to determine the maximum yield, one has to measure the rate of photosynthesis in the "light-limited" state (as close to zero illumination as feasible). In fact, the maximum quantum yield can be defined as the initial slope of the light curve, the limiting value of $(\Delta P/\Delta I)$ as I approaches zero. In practice, measurements have to be carried out below, or only slightly above, the so-called *compensation point,* where photosynthesis just balances respiration so that the net gas exchange is zero. This means that the light intensity is such that it gives only one-tenth to one-fifth of the maximum rate of photosynthe-

sis of which the cells are capable. (It is these quantum yields in low light that have been plotted in Figs. 11.1 to 11.3.)

A landmark in the study of photosynthesis, and one of the first applications of quantum theory to photochemistry in general, had been the first determination, by Otto Warburg and E. Negelein in Berlin in 1922, of the maximum quantum yield of photosynthesis in *Chlorella*. This study first applied the manometric method to rate measurements of photosynthesis. It also first introduced unicellular algae (*Chlorella*) as objects for such studies. The result of this investigation appeared, at first, highly satisfactory: the minimum quantum requirement of photosynthesis was found to be 4, that is, four quanta absorbed for each oxygen molecule liberated. This seemed highly satisfactory because the reduction of one molecule of CO_2 by two molecules of H_2O requires, as we have repeatedly noted, the transfer of *four* hydrogen atoms. It seemed plausible that the transfer of each atom required one quantum. Since four mole einsteins of red light (680 nm) carry (see Table 10.1) about 172/Kcal, while about 120 Kcal of free energy are stored in photosynthesis per O_2 mole liberated, Warburg's figure suggested a remarkably high efficiency, $(^{120}/_{172} \simeq 70\%)$, with which plants can convert light into chemical energy.

For almost twenty years, the four-quantum mechanism of photosynthesis was generally accepted and marveled at, although it placed speculations as to the possible detailed mechanism of photosynthesis into something of a straight jacket, because of the required high efficiency. Since 1938, however, attempts to repeat Warburg and Negelein's experiments with improved techniques led to the conclusion that the true maximum quantum yield is much lower—probably, by a factor of two. Robert Emerson and his co-workers in California, and then in Illinois, have carried out these experiments most extensively by so-called differential manometry (a method also first devised by Warburg, in which two manometers with two vessels of different size are employed). It permits separate determination of ΔCO_2 and ΔO_2 (without postulating $\Delta O_2 = -\Delta CO_2$, as in the original, one-manometer method). Other investigators, particularly Farrington Daniels, W. M. Manning, and co-workers at the University of Wisconsin, obtained similar results by various other methods, including polarography (potentiometric determination of oxygen), determination of synthesized combustible material with a calorimeter, and determination of the rate of carbon dioxide consumption

(by electrochemical analysis, by measurements of radioactive CO_2 uptake, of changes in the infrared absorption spectrum, or of changes in heat conductivity of the gas circulated over the plants).

In all these experiments, quantum requirements of the order of 10–12 have been usually found. The minimum values (which are the most significant ones) were close to 8. The conclusion that the normal minimum quantum requirement of photosynthesis is 8 appears compatible with the data of most researchers; scattering due to errors may account for the occasional finding of lower values. Recent (1968) precise studies on *Chlorella* sunspensions of different age, with different CO_2 concentrations, by Rajni Govindjee yielded no quantum requirements under 8. A quantum requirement of 8 suggests utilization of *two* quanta for the transfer of each hydrogen atom from H_2O to CO_2. It is significant that similar values (8–10 quanta per O_2 molecule) were found, in our laboratory, also for the Hill reaction (reduction of quinone by *Chlorella* cells, and reduction of different oxidants by chloroplast suspensions). The best available measurements of bacterial photosynthesis suggest that in this case, too, 8–10 quanta are required to reduce one CO_2 molecule, irrespective of the nature of the H-donor.

Warburg strongly opposed the findings that the minimum quantum requirement for photosynthesis is 8. Not only did he assert that his old findings, according to which four quanta are sufficient for photosynthesis, are still valid, but reported that *Chlorella* can carry out photosynthesis with a quantum requirement of only 2.8, corresponding to 100 percent efficiency in converting light energy into chemical energy! This, Warburg described as a result "he has always seen ahead," a proof that "in a perfect world, photosynthesis must be perfect."

Warburg found that minimum requirements of the order of 3 quanta per molecule O_2 could be observed for hours at a time, even in light strong enough to exceed compensation by a factor of 3 to 5.

These results contradict the findings of Emerson and co-workers, and the results obtained in other American laboratories, including a recent study by J. Bassham in Berkeley (1968). That photosynthesis can proceed with a 100 percent energy conversion yield is unlikely. It is hard to believe that a complex chemical reaction can operate, at high speed, with no "friction losses" whatsoever!

The value of 8, we believe, must be accepted at present as the most likely minimum quantum requirement of photosynthesis.

THE RED DROP

Figures 11.1 to 11.3 show one peculiarity that we have not yet considered. The action spectra drop not only at the short-wave end (where this can be attributed to the presence of carotenoids), but also at the long-wave end of the spectrum. This drop is particularly conspicuous in red algae, where it occurs at 650 nm, right in the middle of the main absorption band of chlorophyll *a* (Fig. 11.2). However, Emerson pointed out that the $\Phi = f(\lambda)$ curves in Fig. 11.1 (for *Chlorella*) and 11.3 (for *Navicula*) also show a drop, albeit only above 680 nm, that is, beyond the peak of, but still within, the chlorophyll *a* absorption band. These observations have become the starting point of an interesting development, including both experimental study and theoretical interpretation, with which we shall deal in Chapter 13.

The dip in the action spectrum of green algae in the neighborhood of 660 nm, seen in Fig. 11.1, also is reproducible and waits for an interpretation.