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The light saturation curve of photosynthesis

The concept of the photosynthetic unit first proposed by EMERSON AND ARNOLD[1] in 1932 has received much experimental support. Working with photosynthetic bacteria, REED AND CLAYTON[2] managed the complete separation of the bacteriochlorophyll of the antenna from that of the photoactive site. A biochemical isolation of two or more kinds of units from green plant material by ANDERSON AND BOARDMAN[3] has also been partially successful.

We have attempted to provide a biological separation of the antenna chlorophyll from the photoactive chlorophyll of Chlorella through the study of a mutant which becomes yellow when grown in the dark and may then become green under controlled[4] light conditions. In this way we have prepared cells which can carry out photosynthetic oxygen evolution at a normal rate if the light is sufficiently bright, although their chlorophyll content is only 10 % of that of normal cells. These cells are lacking a large part of their antenna chlorophyll and may be usefully compared to the normal cells during a study of the photoreactions of photosynthesis.

One aspect of such a study is the "light saturation curve" relating the rate of photosynthesis to the intensity of the light. The saturation of the rate at high intensities was the first indication of the slow dark reactions[5] of photosynthesis, and the linear part of the curve figured prominently in the long arguments[5] over the quantum yield. However, the lower end of the linear region has remained obscure. This is the region of the classical "compensation point", the light intensity at which the rate of photosynthetic oxygen evolution just equals the respiration rate of the cells. Further, in this region of the light saturation curve, an abrupt change of slope of the curve has been measured[5] (the Kok effect). Again, it is in this region that the effects of so-called "photorespiration" are relatively large. Oxygen isotope experiments have shown[6,7] that on illumination cells can change their rate of oxygen uptake. Since the observed oxygen evolution depends both on production by photosynthesis and on consumption by respiration, it would be worthwhile to adjust conditions so as to suppress respiration. Then it is possible to use the sensitivity and range of the oxygen luminometer[8] to measure the light saturation curve of photosynthesis over an extended range.

We have two independent arguments that respiration is not distorting the curves we will present here. First, we have made the measurements at an oxygen level (approx. 2 ppm) which severely limits respiration. We have further suppressed respiration in the mutant cells by starving them before the experiment and in the normal green Chlorella by growing them strictly autotrophically. Independent measures of the dark respiration in these cells (performed by Mr. Bruce Diner) show that we have suppressed respiration at least 100 times below that reported for normal ("fat") Chlorella in air. Second, we obtain the same light saturation curves when the oxygen level in the carrier gas is raised from about 2 ppm up to 60 ppm. This high carrier gas level is equivalent to the oxygen produced by the cells in the middle of the linear region of the light curve in the presence of the low oxygen carrier gas. This finding rules out oxygen level-dependent respiration as being of any crucial importance. It also suggests that any "photorespiration" that is present must be quite independent of a partial pressure of oxygen down to these low levels.

There may be objections that our special treatment of the cells and the extreme anaerobic conditions may make them inefficient or inactive. In fact, the light-saturated rate of photosynthetic oxygen evolution under our conditions equals that observed with Chlorella by other workers at ambient oxygen levels. Moreover, our rough measurements of quantum yields of oxygen evolution in the linear parts of our curves give values $\geqslant 0.1$, again in good agreement with values obtained under aerobic conditions. Thus the absolute values of our measurements show that our "prepared" cells are as active and efficient as ordinary Chlorella.

Fig. 1 shows some results of our measurements, comparing mutant Chlorella cells with normal chlorophyll content to cells depleted in chlorophyll. It is clear that below the linear region of the light saturation curve, the steady-state rate of photosynthetic oxygen production over two orders of magnitude is proportional to the square of the light intensity. The curves for both kinds of cells have precisely the same shape, but that of the partially greened mutant is displaced about one decade to higher light intensities. This shows that the shape of the light curve does not depend on the amount of chlorophyll per cell. It also indicates that the partially greened cells are depleted in their energy-capturing or antenna chlorophyll only; the loss of this chlorophyll can be compensated for simply by increasing the light intensity. A light saturation curve for the normal green (wild-type) Chlorella cells closely resembles the "continuous" curve in Fig. 1. On the other hand, the rate of oxygen production from a dichlorophenolindophenol or quinone Hill reaction seems to show a more linear dependence on light intensity below the saturation point. Sigmoid curves of photosynthetic rate of oxygen production *versus* light intensity have been previously[6,9,10] observed. Extrapolation of the data of SCHMID AND GAFFRON[11] on yellow

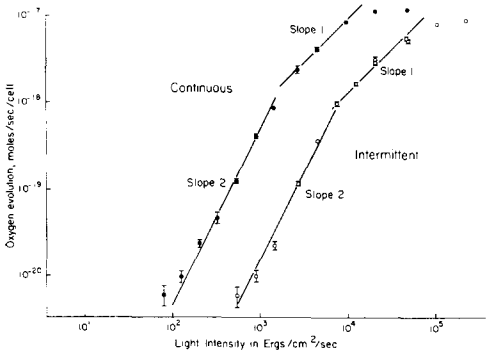

Fig. 1. Light saturation curves for photosynthesis of the mutant Chlorella. Photosynthesis is measured with the oxygen luminometer as the steady-state rate of oxygen evolved per cell when $1.1 \cdot 10^8$ cells (packed cell volume 0.010 ml) are illuminated in 5.7 ml of a solution containing 0.03 M phosphate and 0.01 M carbonate, buffered at pH 7.5. Light from a 750-W DDB projector lamp passes through heat filters and a chromate solution. The 10% intensity points of its spectrum are 500 and 840 nm, with a flat maximum from 580 to 700 nm. Intensity is measured with a YSI-Kettering Model 65 Radiometer and an ISCO Model SR Spectroradiometer. Schott neutral density glass filters decrease the light intensity in steps; the relative intensity of each point is measured by a photomultiplier. ●, dark-grown cells placed in continuous fluorescent light (intensity $4 \cdot 10^3$ ergs/cm^2 per sec) for 2 days before oxygen measurement, chlorophyll $1.9 \cdot 10^{-13}$ g per cell; ○, dark-grown cells placed in intermittent light (15 sec every 30 min, darkness in between) from a GE 93T incandescent lamp (intensity $2 \cdot 10^5$ ergs/cm^2 per sec) for 2 days before oxygen measurement, chlorophyll $0.22 \cdot 10^{-13}$ g per cell. The bars represent our estimated total error.

mutants of tobacco also results in a nonzero intercept of the linear part of the light saturation curve.

We thus conclude that the light curve of photosynthesis contains a nonlinear portion at low intensities and that the nonlinearity is inherent in the photoreactions of the complete system. We note that this nonlinearity in the steady-state response is quite compatible with the lags, steps and oscillations[9,12,13] observed when Chlorella are abruptly illuminated.

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