BBA 45629

A COMPARISON OF THE SEPARATE PACKAGE AND SPILL-OVER MODELS OF PHOTOSYNTHESIS FOR THE ALGA *CHLORELLA PYRENOIDOSA*

W. P. WILLIAMS*

School of Molecular Sciences, University of Warwick, Coventry (Great Britain)

(Received June 19th, 1967)
(Revised manuscript received September 11th, 1967)

SUMMARY

1. A general expression for the fractional absorption of the pigment systems associated with the two photosystems of Chlorella, was derived on the basis of the changes in the action spectrum of O_2 evolution on partially poisoning the alga with 3-(4-chlorophenyl)-1,1'-dimethylurea. Two solutions of this expression were calculated, using the experimental data of GINGRAS, LEMASSON AND FORK, one corresponding to the separate package and the other to a perfect spill-over model. The values predicted on the basis of the separate package model were in much the better agreement with earlier results.

2. The assumption of such a model seemed, however, to be irreconcilable with earlier observations on the wavelength dependence of the relative quantum yield of O_2 evolution. Two methods of determining the fractional absorptions incorporating these latter results were outlined. They gave differing results suggesting that the available data were inadequate. An attempt was made, therefore, to assess its reliability.

3. The balance of evidence seemed to favour the separate package formulation but it was concluded that no final decision could be made until the relative quantum yield data have been carefully rechecked for the wavelengths of special interest.

INTRODUCTION

Light energy absorbed by the photosynthetic pigments of green plants and algae is thought to be utilised in driving two photosystems coupled in series by an electron transport chain. One of these, often referred to as Photosystem I, is thought to mediate NADP reduction, whilst the other, often referred to as Photosystem II, is thought to mediate O_2 evolution. Schemes incorporating these general principles have been proposed by several authors[1]. Most of these schemes visualise no direct interaction between the pigment systems associated with the two photosystems, and are, therefore, termed separate package models.

Abbreviations: CMU, 3-(4-chlorophenyl)-1,1'-dimethylurea; DCMU, 3-(3,4-dichlorophenyl)-1,1'-dimethylurea.

* Post-doctoral Research Fellow, University of Warwick. Present address: Biophysics Department, Chelsea College, Manresa Road, Chelsea, London, Great Britain.

Discrepancies between the measured wavelength dependence of the quantum yield of O_2 evolution[2] and the values calculated on the basis of such models[3] led to the suggestion that there might be a possibility of energy transfer from Photosystem II to Photosystem I when the fractional absorption of Photosystem II is greater than that of Photosystem I. Modified schemes incorporating such a transfer, often termed spill-over models, have been proposed by KOK[4] and CLAYTON[5].

The action spectra of the two photosystems of Chlorella have been calculated by DUYSENS[6], from the O_2 evolution data of FRENCH, MYERS AND MCCLEOD[7], and by WILLIAMS, MURTY AND RABINOWITCH[8], from fluorescence data. The former derivation assumed the existence of separate packages and the latter was independent of the type of model. The results were very similar, tending to give support to the separate package model.

In this investigation the absorption spectra of the photosystems have been calculated, on the basis of the two types of model, from the experimental results of GINGRAS, LEMASSON AND FORK[9] on the effect of the addition of 3-(4-chlorophenyl)-1,1'-dimethylurea (CMU) on the action spectrum of O_2 evolution from Chlorella.

RESULTS AND DISCUSSION

GINGRAS, LEMASSON AND FORK[9] observed that the addition of small amounts of CMU (10^{-7}–10^{-5} M), to Chlorella resulted in a partial inhibition of O_2 evolution. They found, in agreement with analogous measurements made by DUYSENS, AMESZ AND KAMP[10] on Phorphorydium, that the action spectrum of O_2 evolution changed on poisoning so that it resembled the absorption spectrum of Photosystem II.

This change indicates that Photosystem II is preferentially inhibited. DUYSENS AND SWEERS[11] have suggested that one molecule of inhibitor is sufficient to completely inhibit photosynthesis in a photosynthetic unit consisting of Photosystems II and I. In the case of partial poisoning this would mean that photosynthesis should be completely stopped in a fraction of the units, *i.e.*, an equal inhibition of both systems. This is not observed. The preferential inhibition of Photosystem II could be due to:

(1) An intermittent inhibition of a unit, *i.e.*, the unit is 'sick' not 'dead'. (2) The existence of more than one electron transport pathway between Photosystem II and Photosystem I, possibly as recently suggested by MALKIN AND KOK[12]. (3) The possibility of energy transfer between Photosystem I reaction centres but not between Photosystem II reaction centres.

IZAWA AND GOOD[13] have calculated that a relatively large fraction of the inhibitor, within spinach chloroplasts, may exist in the free state. This suggests that the enzyme–inhibitor complex is weakly bound allowing the unit to be considered as sick. However, whatever the reason for this preferential inhibition, the data can be used to calculate the absorption spectra of the two photosystems.

Consider an ensemble of N photosynthetic units, each consisting of Photosystems I and II. Let the rate of photosynthesis in the ensemble be R_0. Let it be reduced to R_p on poisoning. Let the total flux of absorbed quanta be Q, of which a fraction x is absorbed in Photosystem II and a fraction $(1 - x)$ in Photosystem I. Allow a spill-over of quanta absorbed in Photosystem II to Photosystem I when the fractional absorption in Photosystem II is greater than in Photosystem I. Let the efficiency of this transfer be γ. Then, assuming the intrinsic efficiencies of Photo-

systems I and II are the same, the series model predicts that for $\lambda < 685$ nm (the region in which absorption in Photosystem II is usually larger than in Photosystem I):

$$R_0 = \begin{bmatrix}\text{Rate limited by} \\ \text{absorbance in I}\end{bmatrix} + \gamma \begin{bmatrix}\text{Rate limited by energy} \\ \text{transfer from II to I}\end{bmatrix} \quad (1)$$

The value of the second term should be determined by the amount by which x exceeds the optimal distribution of equal absorption in each photosystem, so:

$$R_0 = [\text{const. } NQ(1 - x)] + \gamma\,[\text{const. } NQ\,(x - 0.5)] \quad (2)$$

On poisoning, Photosystem II is preferentially inhibited and absorption in Photosystem II becomes rate determining at all wavelengths. If the fractional efficiency of Photosystem II is reduced to y:

$$R_p = \begin{bmatrix}\text{Rate limited by} \\ \text{absorbance in II}\end{bmatrix} = \text{const. } NQxy \quad (3)$$

The value of y can be obtained by measurement of the fractional inhibition of photosynthesis in the range $\lambda > 685$ nm, in which absorption in Photosystem II is rate limiting for both the poisoned and unpoisoned algae.

If the fractional inhibition of photosynthesis, z, is defined as $1 - R_p/R_0$:

$$z = 1 - \frac{\text{const. } NQxy}{[\text{const. } NQ(1 - x)] + \gamma\,[\text{const. } NQ(x - 0.5)]} \quad (4)$$

which simplifies to:

$$x = \frac{(1 - z) + 0.5\,\gamma(z - 1)}{y + 1 - z + \gamma(z - 1)} \quad (5)$$

Substituting $\gamma = 0$ and $\gamma = 1$ in Eqn. 5 (the conditions of separate package and perfect spill-over):

$$\text{Separate package } x = \frac{1 - z}{y + 1 - z} \quad (6)$$

$$\text{Perfect spill-over } x = \frac{0.5(1 - z)}{y} \quad (7)$$

The wavelength dependencies of x obtained on substituting the values of y and z obtained by GINGRAS, LEMASSON AND FORK[9] into Eqns. 6 and 7 are shown in Fig. 1. The value of y was taken as 0.525, the fractional efficiency at 690 nm. The same wavelength dependencies calculated from the O_2 evolution data of FRENCH, MYERS AND MCCLEOD[7], and the fluorescence data of WILLIAMS, MURTY AND RABINOWITCH are included in this figure for comparison. The corresponding absorption spectra are shown in Fig. 2. The absorption spectrum from the data of FRENCH, MYERS AND MCCLEOD was taken from a paper by CEDERSTRAND, RABINOWITCH AND GOVINDJEE[14].

The data calculated using the separate package equation are in better agreement with the data from the other investigations than that calculated using the perfect spill-over equation. This suggests that the separate package model gives the more accurate description of the actual system. The assumption of such a model is, however, difficult to reconcile with EMERSON AND LEWIS's observation[2] that the relative quantum yield of O_2 evolution for Chlorella is practically constant in the region 570–685 nm.

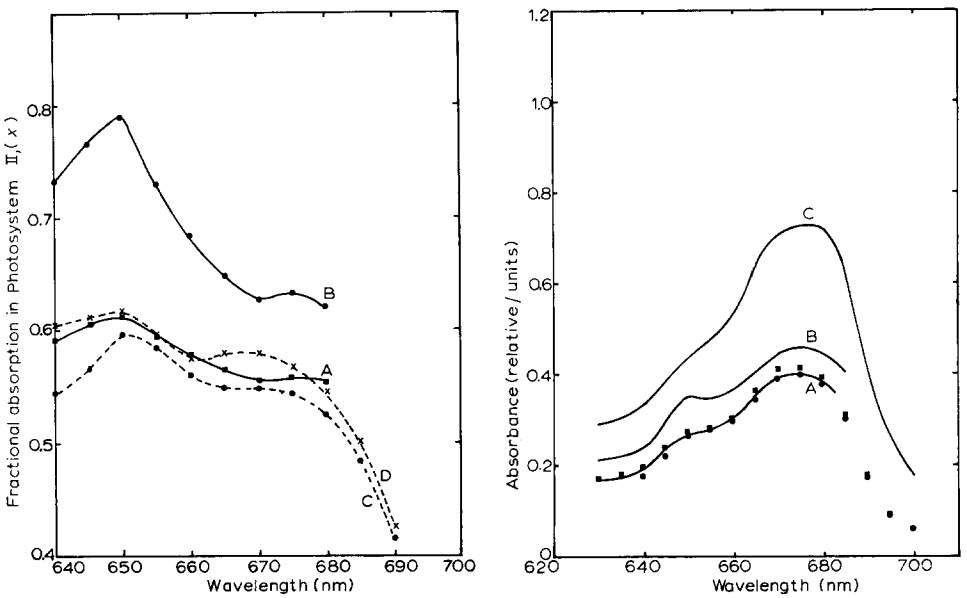

Fig. 1. Fractional absorption of Photosystem II as a function of wavelength. Curves A and B correspond to the CMU poisoning data, calculated on the basis of the separate package and spill-over models, respectively. The corresponding values calculated from the O_2 evolution measurements[7], Curve C, and the fluorescence measurements[8], Curve D, are shown for comparison.

Fig. 2. Absorption spectrum of Photosystem II in the red spectral region corresponding to the fractional absorptions given in Fig. 1. Curves A and B, CMU poisoning data calculated on the basis of the separate package and the spill-over models, respectively. Values derived from O_2 evolution (●) and fluorescence data (■) are also shown. Curve C corresponds to the total absorption of the alga in this region.

The relative quantum yield Φ_r, can be derived in terms of γ and x. Φ_r is taken as the ratio of the quantum yields at a given wavelength and at 685 nm (the point of onset of the red drop). This is equal to the ratio of the rates of O_2 evolution of the unpoisoned algae for these wavelengths, as given by Eqn. 2. If the rate at 685 nm is taken as that corresponding to an equal distribution of the absorbed quanta, then Φ_r for $\lambda < 685$ nm is given by:

$$\Phi_r = \frac{R_{0\lambda<685}}{R_{0\lambda=685}} = \frac{[\text{const. } NQ(1-x)] + \gamma\,[\text{const. } NQ(x-0.5)]}{\text{const. } NQ\ 0.5} \tag{8}$$

which simplifies to:

$$\Phi_r = \frac{(1-x) + \gamma(x-0.5)}{0.5} \tag{9}$$

For the conditions $\gamma = 0$ and $\gamma = 1$:

$$\text{Separate package } \Phi_r = \frac{(1-x)}{0.5} \tag{10}$$

$$\text{Perfect spill-over } \Phi_r = 1 \tag{11}$$

To obtain a complete description of the system, we need to know both γ and x. Using the equations derived above this can be done in either of two ways: (i) The

values of x can be obtained from the fluorescence data[8], and the value of γ can be calculated using the values of Φ_r from EMERSON AND LEWIS's results[2] by use of Eqn. 9. (ii) The values of both x and γ can be derived from the CMU inhibition data and the EMERSON AND LEWIS data by combination of Eqns. 5 and 9.

Eqns. 5 and 9 can be simplified to give the simultaneous equations:

$$x(y + 1 - z) - 1 + z - \gamma x(1 - z) + 0.5\,\gamma(1 - z) = 0 \tag{12}$$

and

$$0.5\,\Phi_r - 1 + x - \gamma x + 0.5\,\gamma = 0 \tag{13}$$

Multiplying Eqn. 13 by $(1 - z)$ and simplifying:

$$x = \frac{(1 - z)\,0.5\,\Phi_r}{y} \tag{14}$$

resubstituting for x in Eqn. 13

$$\gamma = \frac{0.5\,\Phi_r\,(y + 1 - z) - y}{0.5\,\Phi_r\,(1 - z) - 0.5\,y} \tag{15}$$

Consider the two wavelengths 685 and 650 nm, the latter being characteristic of the dip in EMERSON AND LEWIS's curve. By method (i) given that $x = 0.616$ (see Fig. 1), if $\gamma = 0$ (separate package) $\Phi_r = 0.768$, *i.e.* the quantum yield at 650 is about 77 % of that at 685 nm. This is in good agreement with MYERS's value[3]. If, however, we take $\Phi_r = 0.95$ (EMERSON AND LEWIS's value), $\gamma = 0.785$. By method (ii) taking $\Phi_r = 0.95$ (ref. 2), $z = 0.17$ and $y = 0.525$ (ref. 9); $x = 0.75$ and $\gamma = 0.91$.

We have, therefore, two conflicting results: (a) Ignoring EMERSON AND LEWIS's results, all three types of measurement, measurement of O_2 evolution with a strong background light[7], inhibition of O_2 evolution by CMU and fluorescence measurements[8], give similar spectral distributions for the two photosystems (see Fig. 2). The former two were calculated assuming the operation of a separate package mechanism and the last is independent of the model. (b) If EMERSON AND LEWIS's curves are taken into account, the fluorescence results predict an approx. 60:40 distribution between Photosystems II and I at 650 nm and a transfer efficiency of about 79 %, whilst the inhibition results predict a 75:25 distribution and a transfer efficiency of 91 %.

Any decision as to which result is the more credible must depend on an assessment of the reliability of the various measurements. This is difficult. The inhibition results predict values of Φ_r in good agreement with those of MYERS[3] and those calculated from the data of FRENCH, MYERS AND MCCLEOD[7]. This consistency suggests that these results (though not necessarily the separate package interpretation of them) are reliable.

The discrepancy between the calculated and experimental values of Φ_r lie mainly in the region 680–690 nm. A 10 % change in these experimental values would make them much more compatible with the theoretical calculations. Much larger changes, of the order of 25 % at some wavelengths, would be needed to reconcile the fluorescence results with the other data. The quantum yield of fluorescence as a function of wavelength has been carefully measured using two different instrumental systems in RABINOWITCH's laboratory[8,15]. A high degree of consistency was obtained.

It is possible that the error lies in the interpretation of fluorescence data rather than in its measurement. The absorption spectra of the two photosystems were calcu-

lated on the basis of two assumptions: (i) The action spectrum of a dilute suspension of 3(3,4-dichlorophenyl)-1,1'-dimethylurea (DCMU)-poisoned algae, measured at 750 nm, is practically equivalent to the absorption spectrum of Photosystem II. (ii) The fractional absorptions of the two photosystems are equal at the point of onset of the red drop in O_2 yield.

Assumption (ii) is central to the series hypothesis of photosynthesis. If it is wrong the whole analysis must collapse. Assumption (i) assumes that the fluorescence action spectrum of Photosystem II is not influenced by the possibility of spill-over. This was thought unlikely as the quantum yield curves of poisoned and unpoisoned algae (conditions under which the degree of spill-over should greatly vary) were very similar.

The balance of the evidence seems to support the view that the separate package model gives the better description of the photosynthetic mechanism. However, this conclusion depends on an appraisal of the reliabilities of three sets of data, all based on practically difficult measurements. In the author's opinion no definite decision can be made until the relative quantum yields of O_2 evolution for the wavelengths of special interest have been carefully rechecked.

REFERENCES

1 L. VERNON AND M. AVRON, *Ann. Biochem.*, 34 (1965) 269.
2 R. EMERSON AND C. M. LEWIS, *Am. J. Botany*, 38 (1943) 165.
3 J. MYERS, *Natl. Acad. Sci.-Natl. Res. Council Publ.*, 1145 (1963) 301.
4 B. KOK, *Natl. Acad. Sci.-Natl. Res. Council Publ.*, 1145 (1963) 45.
5 R. K. CLAYTON, *J. Theoret. Biol.*, 5 (1963) 497.
6 L. N. M. DUYSENS, *Natl. Acad. Sci.-Natl. Res. Council Publ.*, 1145 (1963) 1.
7 C. S. FRENCH, J. MYERS AND G. C. MCCLEOD, in M. B. ALLEN, *Comparative Biochemistry of Photoreactive Systems*, Academic Press, New York, 1960, p. 361.
8 W. P. WILLIAMS, N. R. MURTY AND E. RABINOWITCH, *Photochem. Photobiol.*, submitted for publication.
9 G. GINGRAS, C. LEMASSON AND D. C. FORK, *Biochim. Biophys. Acta*, 69 (1963) 438.
10 L. N. M. DUYSENS, J. AMESZ AND B. M. KAMP, *Nature*, 190 (1961) 510.
11 L. N. M. DUYSENS AND H. E. SWEERS, *Microalgae and Photosynthetic Bacteria*, Jap. Soc. Plant and Cell Physiology, Tokyo, 1963, p. 353.
12 S. MALKIN AND B. KOK, *Biochim. Biophys. Acta*, 126 (1966) 413.
13 S. IZAWA AND N. E. GOOD, *Biochim. Biophys. Acta*, 102 (1965) 20.
14 C. N. CEDERSTRAND, E. RABINOWITCH AND GOVINDJEE, *Biochim. Biophys. Acta*, 126 (1966) 1.
15 L. SZALAY, E. RABINOWITCH, N. R. MURTY AND GOVINDJEE, *Biophys. J.*, 7 (1967) 137.