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RELATIONSHIPS BETWEEN THE PHOTON DISTRIBUTION BETWEEN THE TWO PHOTOSYSTEMS, THE CONCENTRATION OF SYSTEM II REACTION CENTERS AND THE INTERSYSTEM EQUILIBRIUM CONSTANT IN *CHLORELLA PYRENOIDOSA*

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SUMMARY

From Emerson enhancement measurements of O_2 evolution in *Chlorella pyrenoidosa*, it was possible to establish a relationship between the concentration of photosystem II open reaction centers (E) and the distribution of photons between photosystems I and II $[(1-\alpha)/\alpha]$ during steady state. The superposition of lights of two different wavelengths (1 and 2) gives concentrations of E and α intermediate between those obtained with light 1 and 2 separately. This relationship extends a previous one based on quantum yield measurements. It has been expressed here by a curve corresponding to a fixed value of the intersystem apparent equilibrium constant (K). Up to 700 nm, K remains equal to 6. Above this wavelength, although the margin of error is rather great, K apparently increases to 12 or more.

The possibility of "spill-over" of light absorbed by System II to System I was studied. There is no probability that this spill-over, if any, exceeds 25 % in Chlorella.

The apparent equilibrium constant is decreased by 3(3,4-dichlorophenyl)-1,1-dimethylurea. This is not in favor of the hypothesis of fully independent electron-transfer chains in photosynthesis; it is therefore likely that some communication between those chains exists.

INTRODUCTION

JOLIOT *et al.*[1] have found that it was possible to determine an equilibrium constant (from 3 to 10) between the concentration of photochemical centers of System I (P_{700}) and System II (E or Q) in isolated chloroplasts. Likewise with Chlorella, quantum yield values (from 600 to 680 nm) could be interpreted by assuming an equilibrium constant equal to 6 (ref. 2) which suggests the occurrence of a back flow of electrons.

As a matter of fact, however, the equilibrium constant K so defined probably does not describe a true thermodynamic situation. Redox potential determination

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

of fluorescence[3] and P_{700} (ref. 4) suggest a much higher equilibrium constant ($K \simeq 2000$).

On the other hand, MALKIN[5] has shown that the observed K can be accounted for by a model of independent electron-transfer chains. This allows the simultaneous coexistence of reaction centers in oxidized and in reduced forms; they do not react with one another because they belong to different chains. We consider the intersystem equilibrium constant to be a characteristic of the relationship between the two photochemical centers that does not necessarily explain their coupling mechanism.

The quantum yield experiment, published in a preceding paper[2], allowed us to determine the equilibrium constant with precision in a small range of wavelengths, in which the two systems absorb in nearly equal quantities (between 600–680 nm). We propose to determine K at longer wavelengths from the Emerson enhancement values and in particular to study whether or not it remains constant.

MATERIAL AND METHODS

The biological material is the unicellular green alga *Chlorella pyrenoidosa* Chick, Emerson strain, grown under controlled conditions on synthetic mineral medium (macroelements of TAMIYA *et al.*[6], microelements A_4 and B_7 of ARNON[7] *plus* $6 \cdot 10^{-5}$ M EDTA).

O_2 exchange was measured using a bare concentration-type electrode (the cathode and anode are directly in contact with the medium). This electrode was similar to that of JOLIOT[8] but adapted here for quantum yield measurements by placing an annular photoresistor directly under the cuvette containing the electrodes.

The cuvette (Fig. 1) is cylindrical (25 mm in diameter, height 4 mm). It includes in its inner surface a thin layer of platinum, the edge of which forms the cathode, and the reference electrode is a thick layer of silver, plated with chloride. These electrodes are separated by Plexiglas. The medium is stirred with a Plexiglas disc, the diameter of which is 0.2 mm less than that of the cuvette. Its shaft passes through a Teflon bearing in the bottom of the cuvette; it is driven by a synchronous motor at 3000 turns/min. The equilibration of the disc must be excellent, so that the liquid in the cuvette runs in a laminar manner along the electrodes (vibration creates whirls and noise in the recording). The cathode is polarized at -0.7 V. The current variations are amplified and registered with a "Graphispot" (Sefram) recording galvanometer (period: 0.45 sec).

The cuvette can be illuminated with two 500-mm Bausch-and-Lomb monochromators.

The rates of O_2 evolution are measured by the differences in the recorded slopes, just before and just after the end of illumination. The rapid response of the galvanometer eliminates in these measurements, interference caused by "respiration changes" of the algae.

The standard experimental conditions are the following: Chlorella at about 200 μg chlorophyll per ml (always the same concentration for a given set of experiments), suspension equilibrated with air, at 20°, illumination over 1000 ergs$\cdot$cm$^{-2}\cdot$sec^{-1}, depending on the experiment and the wavelength (but always well below the saturation); average half band width, approx. 6.5 nm.

MATHEMATICAL ANALYSIS

(*A*) *The model used*

(*a*) *Definitions.* e_I, e_{II}, fractions of the traps of photosystem I and photosystem II in the open form; (E = photosystem II traps); k_I, k_{II}, quantum yield of photoreactions I and II, respectively; i, total light quanta absorbed; α, fraction of the total absorbed light which is absorbed by photosystem II; i_I, i_{II}, light absorbed by photosystem I and photosystem II: $i_I = (1-\alpha)i$, $i_{II} = \alpha i$, thus

$$\frac{i_I}{i_{II}} = \frac{1-\alpha}{\alpha} \tag{1}$$

v_I, v_{II}, rates of photoreaction I and of O_2 evolution (photoreaction II), respectively.

(*b*) *Relationship between* v_{II} *and* e_{II}. For photoreaction II, JOLIOT AND JOLIOT[9] found a relationship between the rate of O_2 evolution, v_{II}, and the concentration of photosystem II open centers, e_{II}. This relationship is expressed by:

$$v_{II} = k_{II} i_{II} \frac{e_{II}}{1-p+e_{II}p} \tag{2}$$

(p is the probability factor of energy transfer between System II traps $\simeq 0.5$). We set:

$$u = \frac{e_{II}}{1-p+e_{II}p} \tag{3}$$

so we obtain:

$$v_{II} = k_{II} i_{II} u \tag{4}$$

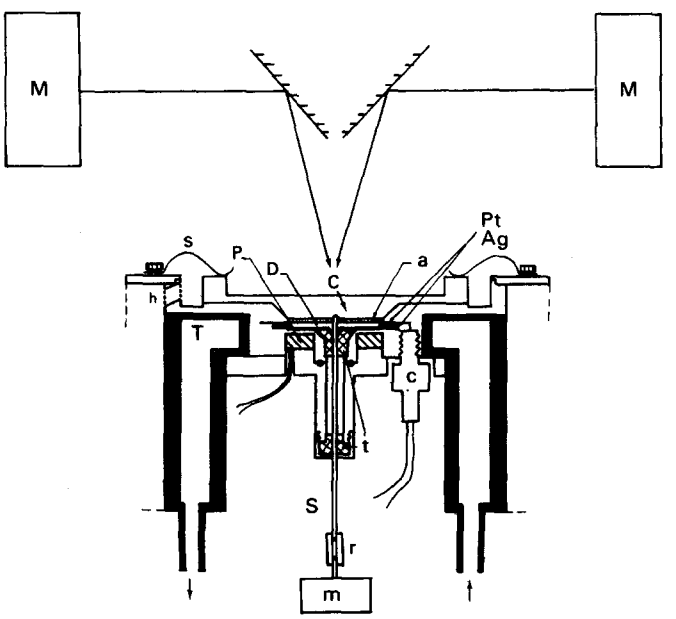


Fig. 1. Diagram of the apparatus for measuring O_2 exchanges and light absorption by algae. Pt, Ag, platinum and silver (chloride plated) electrodes; a, algal suspension; C, cover; D, stirring disc; S, shaft; P, photoresistor; s, spring; h, hole for evacuating the used suspension; T, thermoregulated bath; t, Teflon bearing; c, coaxial connector; m, synchronous motor: r, rubber tubing (connecting stirring disc and motor shaft); M, monochromator.

(*c*) *Equilibrium between photoreactions I and II active centers.* Photoreaction I re-creates open centers of photosystem II (*i.e.* E), with a rate,

$$v_I = k_I i_I e_I \tag{5}$$

Eqn. 5 is based on the assumption that no energy migration exists between System I traps (see ref. 1).

During steady-state O_2 evolution, under light-limiting conditions, the variation of e_{II}, which is equal to its rate of formation by photoreaction I *minus* its rate of destruction by photoreaction II, is zero (neglecting possible dark redox reactions on E):

$$k_I i_I e_I - k_{II} i_{II} u = 0 \tag{6}$$

From Eqn. 1 we find therefore:

$$\frac{k_I}{k_{II}} \cdot \frac{1-\alpha}{\alpha} = \frac{u}{e_I} \tag{7}$$

We suppose that e_I and e_{II} are linked together by an equilibrium constant K:

$$\frac{(1-e_I)(1-e_{II})}{e_I e_{II}} = K^{-1} \tag{8}$$

so,

$$e_I = \frac{1-e_{II}}{1+(K^{-1}-1)e_{II}}$$

and with Eqn. 3:

$$e_I = \frac{1-u}{1+Au} \tag{9}$$

where $A = K^{-1}(1-p) - 1$. Therefore Eqn. 7 becomes:

$$\frac{k_I}{k_{II}} \cdot \frac{1-\alpha}{\alpha} = u \frac{1+Au}{1-u} \tag{10}$$

(*d*) *Effect of spill-over.* The above calculation is developed assuming that there is no "spill-over"[10] from photosystem II to photosystem I. It is more complete if we try to introduce a simple model of direct excitation transfer from System II to System I. This model does not involve the physical nature of the medium in which the excitation diffuses (effect of three-dimensional space[11], heterogeneity, *etc.*) and implies that all other types of energy dissipation (such as heat) remain unchanged.

The excitation is created in System II by photons with the rate αi. According to JOLIOT AND JOLIOT[9], each excited closed reaction center, the total concentration of which is $(1-e_{II})$, has a probability p to transfer its excitation to another open or closed center in System II. The probability for n transfers between $(n+1)$ centers is $p^n(1-e_{II})^{n+1}$. But, competitively, the energy may also be transferred from a closed photosystem II center to System I; the probability of this spill-over is defined as r (since p was found $\simeq 0.5$, ref. 9, $r \leqslant 0.5$). Then, the probability that System I will receive an excitation from System II after n transfers in System II is $rp^n(1-e_{II})^{n+1}$. Once the excitation is transferred to System I, the probability of its utilization is $k_I e_I$. Therefore, the part of the System I rate that would be due to spill-over is equal to:

$$v_{I}(II \rightarrow I) = \sum_{n} \alpha i p^{n} (1 - e_{II})^{n+1} r k_{I} e_{1}$$
$$= k_{I} \alpha i e_{I} r \frac{1 - e_{II}}{1 - p + e_{II} p} \quad (11)$$

At the steady state, v_{I}, the total photoreaction I rate due to the absorption of photons by System I and to the spill-over, is equal to v_{II}, the photoreaction II rate:

$$k_{II} \alpha i \frac{e_{II}}{1 - p + e_{II} p} = k_{I}(1 - \alpha) i e_{I} + k_{I} \alpha i r e_{I} \frac{1 - e_{II}}{1 - p + e_{II} p} \quad (12)$$

Our model does not imply that the transfer of excitation to photosystem I has an effect of decreasing the rate of photoreaction II by the effective diminishing of α as was supposed by MALKIN[12]. We consider instead that the effect of spill-over in adjusting the rates of the two photoreactions is through the adjustment of the concentrations of e_1 and e_2 (*i.e.* u). Therefore, with Eqn. 3, Eqn. 12 becomes:

$$\frac{k_{I}}{k_{II}} \frac{1 - \alpha}{\alpha} = u \frac{1 + Au}{1 - u} - \frac{k_{I}}{k_{II}} \cdot \frac{r(1 - u)}{1 - p} = D(u) \quad (13)$$

The higher the number of inactive centers of System II $(1 - u)$, the greater is the effect of the spill-over (by comparing with Eqn. 10, r and p being constant). So at 700 nm, $(1 - u)$ is small and the effect of spill-over is negligible; on the contrary, at 650 nm it will be maximal (if it exists).

We want to obtain the experimental curve $D(u) = [k_{I}/k_{II}]\,[(1 - \alpha)/\alpha]$ and compare it to the preceding theoretical relationship (Eqn. 13).

(B) Relationship between the curve $D(u)$ and the Emerson enhancement effect.

(I) Expressions of Emerson enhancement

If algae are illuminated with two light intensities, i_1 and i_2, of wavelengths λ_1, λ_2, we obtain: v_1, steady-state rate of O_2 evolution with $i_1(\lambda_1)$; v_2, steady-state rate of O_2 evolution with $i_2(\lambda_2)$; v_{12}, steady-state rate of O_2 evolution with $i_1 + i_2(\lambda_1, \lambda_2)$.

We can define the enhancement by:

$$E_1 = \frac{v_{12} - v_2}{v_1} = \frac{v_{12} - v_1 - v_2}{v_1} + 1 \quad (14)$$

$$E_2 = \frac{v_{12} - v_1}{v_2} = \frac{v_{12} - v_1 - v_2}{v_2} + 1 \quad (15)$$

$(E_1 - 1)v_1 = (E_2 - 1)v_2$, therefore

$$E_2 - 1 = \frac{v_1}{v_2}(E_1 - 1) \quad (16)$$

(see Fig. 2). We may also define enhancement in another manner by:

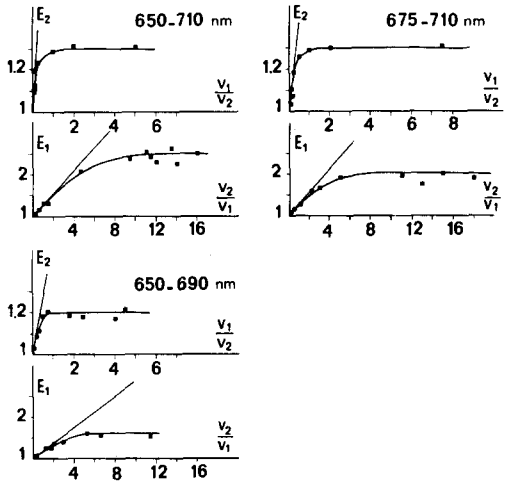
$$E_{12} = \frac{v_{12}}{v_1 + v_2} \quad (17)$$

$$E_{12} - 1 = (E_2 - 1)\frac{v_2}{v_1 + v_2} = (E_1 - 1)\frac{v_1}{v_1 + v_2} \quad (18)$$

If we set

$$x = \frac{v_2}{v_1 + v_2} \quad (19)$$

plotting $E_{12} - 1$ as a function of x, the slope of the curve at $x = 0$ is $E_2^{max} - 1$, and for $x = 1$ it is $1 - E_1^{max}$ (see Fig. 3).



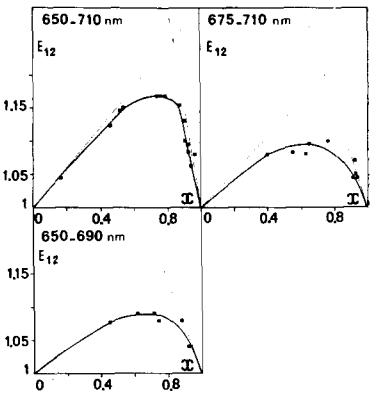


Fig. 2. Enhancement calculated as E_1 *versus* v_2/v_1 ratio and E_2 *versus* v_1/v_2 ratio for the given pairs of wavelengths. Standard experimental conditions (see text).

Fig. 3. Enhancement as E_{12} *versus* $x = v_2/(v_1 + v_2)$ for the given pairs of wavelengths. Same experiment as in Fig. 2.

(II) Construction of the curve D(u) with the Emerson enhancement

The O_2 rate when the algae are illuminated with two wavelengths, λ_1 and λ_2, separately is:

$$\text{For } \lambda_1\text{: } v_1 = k_{II}\alpha_1 i_1 u_1$$
$$\text{For } \lambda_2\text{: } v_2 = k_{II}\alpha_2 i_2 u_2 \qquad (20)$$

(Eqn. 12 shows that these expressions are true even if spill-over exists.) If we use the two lights simultaneously ($i_1\lambda_1$, $i_2\lambda_2$) the new rate v_{12} is proportional to the new concentration of u. Since the total of photons absorbed is $\alpha_1 i_1 + \alpha_2 i_2$, $v_{12} = k_{II}(\alpha_1 i_1 + \alpha_2 i_2)u$. From Eqn. 20,

$$k_{II}\alpha_1 i_1 = \frac{v_1}{u_1} = \rho_1 \text{ and } k_{II}\alpha_2 i_2 = \frac{v_2}{u_2} = \rho_2 \qquad (21)$$

Therefore:

$$v_{12} = (\rho_1 + \rho_2)u \qquad (22)$$

Since $i = i_1 + i_2$, the new value of α, the fraction of photons received by pigment System II, is:

$$\alpha = \frac{\alpha_1 i_1 + \alpha_2 i_2}{i_1 + i_2} \qquad (23)$$

(the general relationship $v = k_{II}\alpha i u$ is always true).

The expression $[k_I/k_{II}] \cdot [(1 - \alpha)/\alpha] = D(u)$, which is the curve to be studied, becomes, in the case where both wavelengths are used simultaneously:

$$\frac{k_I}{k_{II}}\cdot\frac{1-\alpha}{\alpha}=\frac{D_1\rho_1+D_2\rho_2}{\rho_1+\rho_2}=\frac{D_1\dfrac{1-x}{u_1}+D_2\dfrac{x}{u_2}}{\dfrac{1-x}{u_1}+\dfrac{x}{u_2}}=D(u) \tag{24}$$

(Knowing Eqns. 23, 21, 19 and that

$$D_1=\frac{1-\alpha_1}{\alpha_1}\cdot\frac{k_I}{k_{II}} \text{ at } \lambda_1 \text{ and } D_2=\frac{1-\alpha_2}{\alpha_2}\cdot\frac{k_I}{k_{II}} \text{ at } \lambda_2.)$$

The different geometrical and mathematical demonstrations are based only on an experimental relationship, Eqn. 2. They do not contain any physical hypothesis of the process which gives the function $D(u)$.

(*a*) *Relationship between* E_{12} *and the shape of the curve* $D(u)$. In Eqn. 24, $D(u)$ is the average of D_1 and D_2 weighted, respectively, with ρ_1 and ρ_2 (see Eqn. 21) and therefore Eqn. 24 is equivalent to:

$$\frac{D(u)-D_1}{D(u)-D_2}=-\frac{\rho_2}{\rho_1}=\frac{u_c-u_1}{u_c-u_2} \tag{25}$$

where u_c refers to the abscissa of the chord joining D_1 to D_2 (see Fig. 4). From Eqn. 22:

$$v_{12}-v_1-v_2=(E_{12}-1)(v_1+v_2)=(u-u_1)\rho_1+(u-u_2)\rho_2 \tag{26}$$

This expression, which represents the increase above the sum of rates v_1+v_2, is the average of $(u-u_1)$ and $(u-u_2)$ weighted by the same respective factors ρ_1 and ρ_2.

If the curve $D(u)$ is a straight line (the chord) joining D_1 to D_2, Eqn. 26 is equal to 0. So, the Emerson effect is a measure of the curvature of the curve between D_1 and D_2.

If we set:

$$\delta u=u-u_c \tag{27}$$

the difference in the abscissa at the ordinate $D(u)$ between the curve and the chord joining D_1 and D_2, we find from a simple geometrical consideration of Fig. 4:

$$\begin{aligned}&(E_{12}-1)(v_1+v_2)=\delta u(\rho_1+\rho_2)=\delta u\left(\frac{v_1}{u_1}+\frac{v_2}{u_2}\right)\\&\text{and } E_{12}-1=\delta u\left(\frac{x}{u_2}+\frac{1-x}{u_1}\right)\end{aligned} \tag{28}$$

Knowing x, u_1 u_2 and E_{12} by experiment, one may therefore obtain δu, with which the curve between u_1 and u_2 may be constructed (if D_1 and D_2 are known).

(*b*) *Relationship between* E_1-1 *and the curve* $D(u)$. From Eqns. 18 and 28 we have:

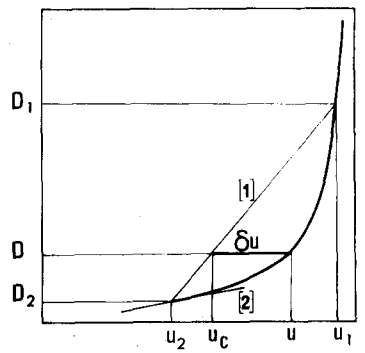
$$E_1-1=(E_{12}-1)\frac{v_1+v_2}{v_I}=\delta u(\rho_1+\rho_2)\frac{1}{v_1}$$

in the limit, $v_1\to 0$ (this is taken generally in the experiments to find E_1: $v_1 \ll v_2$ and $\rho_1 \ll \rho_2$ therefore $u\to u_2$).

Recalling u_c, the abscissa of the chord corresponding to the ordinate $D(u)$; St_2, the slope of the tangent at $D_2=[(D(u)-D_2)/(u-u_2)](u\to u_2)$; Sc_{12}, the slope of the chord from $D(u)$ to $D_2=[(D(u)-D_2)/(u_c-u_2)]$; we find:

$$(E_1 - 1)v_{1\to 0} = \frac{u_1 - u_2}{u_1}\left(\frac{Sc_{12}}{St_2} - 1\right) \qquad (29)$$

(*c*) *Experimental construction of the curve* $D(u)$. At a first approximation, u_1 is considered as being equal to 1 (λ_1 close to 700 nm). The values of u_2 were measured precisely in the range $0.73 < u < 0.84$ in a previous work[2]; experimentally these values were obtained by the ratio $v_s/v_{i\ preil}$ at a given wavelength (v_s, steady-state rate of O_2 evolution; $v_{i\ preil}$, initial rate after a preillumination near 710 nm, $v_s = k_{II}\alpha i u$, $v_{i\ preil} = k_{II}\alpha i$).



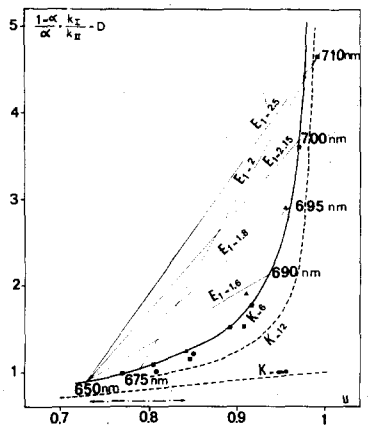


Fig. 4. Schematic representation of the curve $D(u)$. 1, chord joining D_1 and D_2; 2, tangent at D_2; δu, difference in the abscissa at the ordinate D between the curve $D(u)$ and the chord joining D_1 and D_2.

Fig. 5. Curve $[k_I/k_{II}]\,[(1-\alpha)/\alpha] = D(u)$. The slopes of the chords joining D_2 and D_1 are obtained from the E_1^{max} data (see text). The experimental points are obtained from the E_{12} data (●, 650–690 nm; ▲, 675–710 nm; ■, 650–710 nm). The curves are calculated for different values of the equilibrium constant K (←·—·→, range of wavelengths previously studied from quantum yield measurements.[2])

From Eqn. 29, knowing u_1, u_2, St_2 and $E_1^{max} - 1$, we can find the value of Sc_{12}. This allows us to draw the chord joining D_1 to D_2. (St_2, the slope at the tangent at D_2, is measured graphically on the curve; the data pertaining to the curve $D(u)$ for $0.73 < u < 0.84$ have been previously obtained[2]. In Fig. 5, one can see that the intersection of two chords corresponding to two different values of u_2 (λ_2 being 675 nm instead of 650 nm for instance) permits the calculation of a more precise value of D_1.

Knowing the chord, with Eqn. 28, one may build the whole curve between D_2 and D_1; the values of E_{12} as a function of x give a series of values of δu that are drawn from the chord at the corresponding values of the ordinate given by Eqn. 24.

RESULTS AND DISCUSSION

(*A*) *Emerson enhancement measurements*

The Emerson enhancement values obtained with Chlorella are given in Figs. 2 and 3. We first tried to use the results from the literature[10,13], but they were not complete enough to be used with confidence in this work. These authors did not

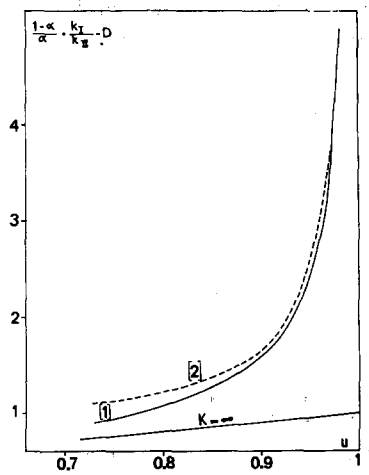
measure the ratio $v_s/v_{i\ preil} = u$ (for the concentration of E) for the wavelengths λ_1 and λ_2. It is therefore not possible to verify the similarity of our material with that of MYERS AND GRAHAM[10] for example. The curve $E_{12}(x)$ was drawn only for 700 nm by ELEY AND MYERS[13] and should be subject to caution in interpretation. For instance, the absorptions of lights 1 and 2 being different ($A_{650\ nm} \simeq 3 \times A_{700\ nm}$) the intensities of λ_1 and λ_2 do not vary in the same ratio through the thickness of the layer if a rate electrode is used. The same phenomenon can also occur with the concentration electrode, although it is minimized by the rapid stirring of the suspension.

Fig. 5 gives the curve obtained by the method described above from the experimental results of Emerson enhancement alone. Therefore it gives the distribution of photons $(1-\alpha)/\alpha$ between System I and II (assuming $k_I = k_{II}$) as a function of the steady-state concentration of the photosystem II traps. The experimental points fit on a theoretical curve obtained using an equilibrium constant K equal to 6 and a probability of spill-over equal to 0 (identical therefore to that obtained with the quantum yield values). However, the fit is not precise for $\lambda > 700$ nm. The equilibrium constant seems to vary here (it may be for instance 12 at 710 nm), but the margin of error for its determination is great (see Fig. 5).

(B) Study of the possibility of excitation transfer from System II to System I

We want to see if we can find agreement with $r > 0$ between the experimental curve and theoretical curves. For low values of $r(\leqslant 0.1)$ it is obvious that within the experimental errors, the agreement remains rather good. But if we try to find the value of K which fits the experimental curve for a higher value of r (r = 0.3, for example) it is impossible.

Fig. 6 shows this result. If $r = 0.3$, we may with Eqn. 13 substract from the experimental Curve 1 the quantity, $[k_I/k_{II}]\,[r(1-u)/(1-p)]$; we then obtain Curve 2, which would be the experimental curve if there was no spill-over. The curve thus



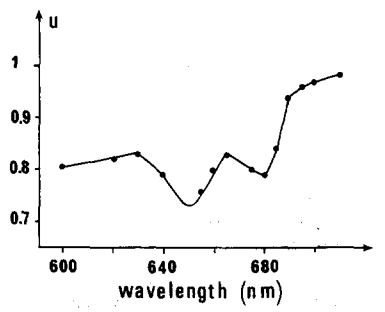


Fig. 6. Spill-over possibility. 1, experimental curve $D(u) = [k_I/k_{II}]\,[(1-\alpha)/\alpha]$; 2, theoretical curve without spill-over if it is supposed that for Curve 1 a spill-over of 30% exists.

Fig. 7. Variation of u (which measures the concentration of photosystem II traps) as a function of illumination wavelength $[u = e_{II}/(1-p+e_{II}p) = v_s/v_{i\ preil}]$.

obtained no longer corresponds to any positive equilibrium constant at $u = 0.73$ (650 nm) because the slope of Curve 2 at this point becomes less than the slope of the curve corresponding to $K = \infty$.

In addition, in order to find a theoretical curve fitting Curve 2 at points other than $u = 0.73$, it is necessary for the values of K to change continuously. For example, at 660 nm (corresponding to $u = 0.78$) K will be ∞, whereas at 700 nm $K = 6$. Thus a decrease of K is accompanying a decrease of α (*cf.* MALKIN[5]).

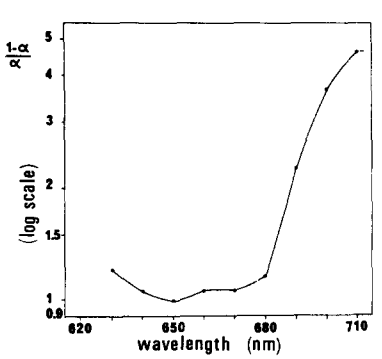
So our experimental results do not allow any possibility of spill-over greater than 25 % as it has been described here (we find a value of $K \leqslant \infty$ at $u = 0.73$ only when $r \leqslant 0.25$). However, even if, regarding the mechanism of energy transfer *per se*, the limit of 0.25 is not negligible with respect to the maximum possible value of $r(\simeq 0.5)$, the corresponding spill-over would have only a minor effect on the overall photosynthetic process.

(*C*) *Considerations of the results*

In Fig. 5, the values of the distribution of photons $(1-\alpha)/\alpha$ and of u are defined rather precisely at wavelengths between 685 and 710 nm, a range which it was not possible to study very well by direct quantum yield measurements[2]. Therefore, combining the results of these previous measurements (for $\lambda \leqslant 685$ nm) and of the present ones (for $\lambda \geqslant 685$ nm), one can rather accurately draw the curves u and $(1-\alpha)/\alpha$ *versus* λ (Figs. 7 and 8). From these curves, the quantum yield (Φ) values are readily obtained since $\Phi = k_{II}\alpha u$. In Fig. 9, the steady-state quantum yield spectrum obtained by EMERSON AND LEWIS[14] or SCHWARTZ[15] coincides with these values up to 700 nm. But the calculated value at 710 nm is distinctly higher than that measured experimentally by these authors. The reason for this discrepancy is not understood at the present time.

(*D*) *Interpretation of our results with respect to the* BONAVENTURA AND MYERS *effect*[16]

Continuous illumination by light absorbed mainly by photosystem I or II ("light 1" or "light 2") induces slow changes in the rate of O_2 evolution; in particular



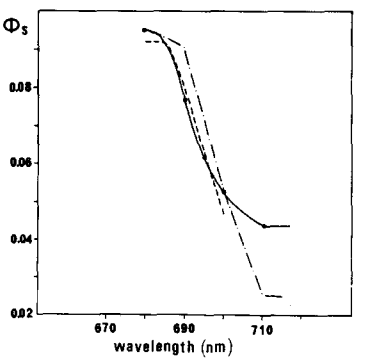


Fig. 8. Ratio between the fraction of light absorbed by System I $(1-\alpha)$ and the fraction of light absorbed by System II (α) *versus* wavelength.

Fig. 9. Wavelength profile of the quantum yield of steady-state photosynthesis from 680 nm. – – – –, O_2 evolution measured by EMERSON AND LEWIS[14]; –·–·–·–, $NADP^+$ reduction (relative quantum yields) measured by SCHWARTZ[15]; ———, calculated from u and α values obtained (see Figs. 7 and 8).

an increase of the quantum yield of about 15 % in 10 min when the algae, adapted to light 1 illumination (dark or prolonged illumination at 710 nm), are shifted to the light 2 state (by prolonged illumination at 650 nm)[16]. This phenomenon can be interpreted as a decrease of α in the transition from the light 1 state to the light 2 state; and inversely as an increase of α in the transition from the light 2 state to the light 1 state. It is thus necessary to define the conditions under which the experiments were made.

The measurement of u was made in state 1, the steady-state rate was measured after about 1 min of illumination. The value of u found here at 650 nm is therefore a true minimum; in state 2 it would increase like (E) or the quantum yield[16]. Emerson enhancement was also measured under the above conditions.

We can explain why, in the results of BONAVENTURA AND MYERS[16] during the transition to state 2 for example, the quantum yield (Φ_{O_2}) increases at 650 nm and decreases at 710 nm. By looking at Fig. 5 it can be seen that the decrease of α (increase of $(1-\alpha)/\alpha$) will result in a large increase of u (*i.e.* E) at 650 nm but a very small increase at 710 nm. Thus, if we consider the equation $\Phi_{O_2} = k_{II}\alpha u$, at 710 nm, α is the main factor which varies ($\alpha \downarrow, \Phi \downarrow$), whereas at 650 nm, u varies more than α, but in the opposite direction ($\alpha \downarrow, u \uparrow, \Phi \uparrow$).

(E) Action of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)

The inhibition site of DCMU is situated close to the photosystem II reaction centers[17]; DCMU prevents the oxidation of E by photoreaction I (refs. 17, 18) resulting in a decrease of E.

We have supposed that DCMU at the low concentration used does not affect the total absorption of photons (*i.e.* there is no change in the absorption spectrum[19]).

We assume that α and k_{II} (and more precisely the ratio $[k_I/k_{II}]$ $[(1-\alpha)/\alpha]$ stay identical to that of the uninhibited algae because it was shown[18] that the relationship $v_{O_2\uparrow} = k_{II}\alpha i u$ is the same whether a change of u is obtained by the action of 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU, an analog of DCMU) or by the action of different wavelengths of light.

We have obtained at different concentrations of DCMU ($1\cdot10^{-7}$–$4\cdot10^{-7}$ M) the

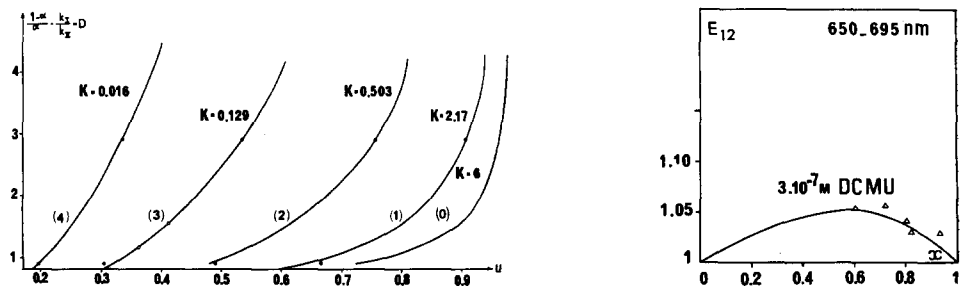


Fig. 10. Action of DCMU on the equilibrium constant K. 0, no DCMU (see Fig. 5); 1, $1\cdot10^{-7}$ M DCMU; 2, $2\cdot10^{-7}$ M DCMU; 3, $3\cdot10^{-7}$ M DCMU; 4, $4\cdot10^{-7}$ M DCMU. ●, direct measurements; ○, computed from Fig. 11. $u = v_8$ with inhibitor/v_1 prell without inhibitor. Standard experimental conditions.

Fig. 11. Enhancement E_{12} *versus* $x = v_2/(v_1 + v_2)$ with $3\cdot10^{-7}$ M DCMU. Standard experimental conditions.

new values of u when the algae are illuminated with 650- and 695-nm illumination [$u = (v_s$ with inhibitor)/($v_{i\,prel}$ without inhibitor]. The decrease of u by DCMU is different according to the wavelength of illumination. This result is in agreement with those obtained by GINGRAS AND LEMASSON[17] that the action spectrum of CMU inhibition of O_2 evolution is that of photosystem II.

In Fig. 10, the ordinate $[k_I/k_{II}]$ $[(1-\alpha)/\alpha]$ is known for 650 and 695 nm. So, for each concentration of DCMU we can put points on theoretical curves corresponding to decreasing equilibrium constants. Furthermore, some measurements of Emerson enhancement effect have been performed in the presence of $3 \cdot 10^{-7}$ M DCMU. The enhancements E_{12} are low (Fig. 11). From these results, values of δu allow us to put points on the corresponding Curve 3 in Fig. 10.

On the one hand, if the photosynthetic chains are fully independent, the action of DCMU at a given moment on one chain will be total, whether or not the following equilibrium exists:

active chain + DCMU $\rightleftharpoons$ inhibited chain

DCMU decreases the number of electron-transport chains, so the apparent equilibrium constant K should not change, since it is the result of statistical phenomena[5]. In this case k_I and k_{II} decrease in the same ratio: active chain/inhibited chain and there would be no change of k_I/k_{II}. (For the two photoreactions it is the same product, namely O_2, which is measured. Thus, blocking the chain at any point, the DCMU would affect in the same ratio the corresponding quantum yields k_1 and k_{II}.) The independent chain model is therefore in contradiction with the above experiment.

On the other hand, if there is a real equilibrium, as has been described in our model, there would also be no change of K by the addition of DCMU. The constant K would be independent of the total amount of one product of the chain as shown by Eqn. 8.

So, the relationship between the two systems is not rigorously an equilibrium. K is not really a constant; it is a function of the concentration of intermediate products (which are changed by DCMU). Therefore, we may propose, in conclusion, that a certain communication exists between the chains. It may be at the level of plastoquinone, as STIEHL AND WITT[20] have suggested to explain the mechanism of reduction or oxidation of plastoquinone.

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