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Minireview

Excitation transfer between photosynthetic units: the 1964 experiment

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Abstract

We review here the background and the experiments that led to the concept of excitation energy transfer among photosystem (PS) II units. On the basis of a kinetic analysis of oxygen evolution and chlorophyll *a* fluorescence yield, the authors showed, in 1964, that the PS II photochemical reaction involved in the formation of oxygen is not a first-order process. We concluded that excitation energy localized in a 'photosynthetic unit' including a reduced primary acceptor is transferred with a high probability to neighboring PS II units. Here, the beginnings and the original data of this topic are presented.

Abbreviations: Chl – chlorophyll; DCMU – 3,(3,4-dichlorophenyl)-1,1-dimethylurea; PS – photosystem; RC – reaction center

Introduction

Modern studies in photosynthesis begins with the classical experiments of Emerson and Arnold (1932) who established that the maximum amount of oxygen evolved by a short saturating flash is much smaller than the amount of chlorophyll (Chl) present. These authors concluded that the oxygen yield per flash is limited by an enzyme, E, present at a concentration much lower than that of Chl ($\sim$ 1/2500). (Emerson's photograph can be seen in Govindjee and Gest 2002.)

Gaffron and Wohl (1936) introduced the concept of the '*photosynthetic unit*,' in which the excitation energy is rapidly exchanged within a closely packed ensemble of Chl molecules. Excitation energy is finally trapped at the level of a reaction center (RC), where the primary charge separation occurs (see Clayton 2002). (Hans Gaffron's photograph appears in a paper by Homann, this issue.) This interpretation was based on the concept of excitation transfer between pigments, proposed by Perrin (1932) and further developed by Förster (1948).

In agreement with the hypothesis of Gaffron and Wohl, Duysens (1952) established the occurrence of efficient excitation transfer from the many accessory pigments to chorophyll (in algae) and to bacteriochlorophyll in photosynthetic bacteria. Moreover, Duysens discovered a small absorption change that he ascribed to a pigment P, present at low concentration. P, which later turned out to be the reaction center, was assumed to be photochemically active and able to trap efficiently the excitation energy. Further, Duysens and Sweers (1963) observed that a quencher (Q, later called Q_A) present at low concentration modulates the fluorescence yield of Chl *a*. They proposed that this quencher, in its oxidized form, is the 'primary' acceptor of Photosystem II (PS II) reaction center. Only photoactive reaction centers including an oxidized primary acceptor Q are able to quench efficiently the fluorescence. Figure 1 shows a photograph of Duysens with late Jan Amesz. (Their work on antagonistic effect of light 1 and 2 on the redox level of cytochrome f in 1961 proved the 'Z'-scheme of photosynthesis; see Duysens 1989.)

Figure 1. L.N.M. Duysens (right) and the late Jan Amesz at the retirement celebration of Amesz in 2000. Photograph by Howard Gest.

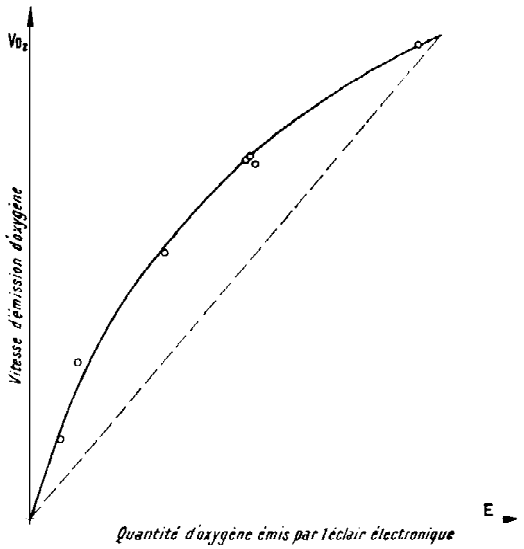

Figure 2. Rate of oxygen evolution (ordinate) as a function of the concentration of the active enzyme E (measured by quantity of oxygen evolved in a light flash, ordinate) in the green alga *Chlorella pyrenoidosa* (reproduced from the original French paper by A. Joliot and P. Joliot 1964).

Pierre Joliot (1965a, b) established that the quencher Q and the enzyme E, which limits the oxygen yield per flash, are at the same concentration. Taking into account that four positive charges are required to form one oxygen molecule, the concentration of the enzyme E was estimated to be 0.004 of the concentration of the Chl (also see Joliot, this issue).

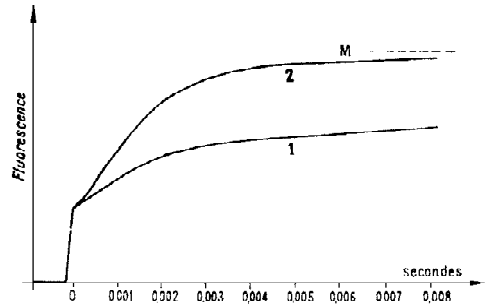

Figure 3. Chlorophyll *a* fluorescence induction in *Chlorella pyrenoidosa.* Abscissa shows time in seconds (commas, used in French, should be replaced by periods). Curve 1, no addition; curve 2, 0.1 mM orthopenanthroline. M stands for maximum fluorescence (reproduced from Joliot and Joliot 1964).

The 1964 experiments

According to the concept of photosynthetic units being separate structural entities, the rate of PS II reaction must be proportional to the concentration of the active photocenters. The rate of PS II reaction can be determined by measuring either the Chl fluorescence yield or the rate of oxygen evolution, as these parameters are linearly related (McAlister and Myers 1940; Delosme et al. 1959).

Using a highly sensitive oxygen electrode, the authors of this paper, Joliot and Joliot (1964) determined the steady-state rate of oxygen evolution under weak illumination and the concentration of active PS II centers by measuring the amount of oxygen evolved

Figure 4. A 1976 photograph in Leiden, The Netherlands, at a conference on photosynthesis. *From left to right*: Govindjee, G. Paillotin, Pierre Joliot and Anne Joliot. Behind Anne (to her right) is Reto J. Strasser. Paillotin and Strasser have contributed extensively to the topic of this paper.

by a saturating flash superimposed on the continuous illumination. The concentration of the photoactive reaction centers was modulated by the addition of various amounts of orthophenantroline, a specific inhibitor of PS II. As shown in Figure 2, the photochemical rate constant (ratio between the rate of oxygen evolution VO_2 and the concentration of active centers E) increases by about a factor three when the concentration of active centers decreases from its maximum value to zero (in Figure 2, compare the dashed line and the initial slope measured at E = zero). Similar conclusions can be drawn from the analysis of the Chl fluorescence kinetics measured in the presence of a saturating concentration of the electron transfer inhibitor orthophenantroline (Figure 3, curve 2). This kinetic displays a small lag phase at the onset of illumination, which implies that the Chl fluorescence yield is not linearly related to the concentration of active centers, in contradiction to it being a first-order process.

These data demonstrate that efficient transfer of excitation energy occurs between photosynthetic units, which are not separated one from the others.

Consequently, the cross-section of active photosynthetic units for the capture of light energy is an increasing function of the concentration of inactive units. On the basis of a simple mathematical analysis, the probability '*p*' of transferring the excitation energy from an inactive unit to neighboring units was estimated to be ~0.55.

Later data showed that in the absence of inhibitors, ~30% of Q_A is reduced under a weak continuous illumination that excites equally both PS I and PS II (Joliot 1965a). This implies that the hyperbolic function shown in Figure 2 is truncated and that the probability of excitation transfer between units (computed from Figure 2) is underestimated and closer to 0.7. The analysis of the Chl fluorescence kinetics in the presence of *o*-phenanthroline or 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) led to a similar underestimation of the probability of excitation transfer, due to a partial reduction of Q_A induced by these inhibitors. The fluorescence induction at low temperature (−52 °C) under conditions when the electron transfer from Q_A to the secondary quinone acceptor Q_B is blocked, displays a lag phase more pronounced than that observed in the presence of inhibitors (Joliot and Joliot 1972). A theoretical analysis of this curve again led to a *p* value ~0.7.

Concluding remarks

Later, Lavergne and Trissl (1995) have analyzed the theoretical relationship between the fluorescence, the photochemical yield of PS II and the fraction of open

Figure 5. A 1998 photograph at a dinner at the International Congress on photosynthesis research in Budapest, Hungary. Anne Joliot, Gyozo Garab, and Pierre Joliot. Photo by Govindjee.

RCs. On the basis of the analysis of the deviation from the linear Stern–Volmer dependence of $1/\Phi$ (where, Φ is fluorescence yield) on the fraction of open traps, the authors concluded in favor of a model of connected units, intermediate between a model of unrestricted exciton transfer (so-called 'lake model', a terminology used by G. Wilse Robinson during 1966–1967) and the isolated units (also called 'separate package') model, similar to that proposed by Joliot and Joliot in 1964. This area of research has been extensively studied, debated and commented on by Reto J. Strasser as well as by G. Paillotin. We leave these discussions to others who might write a more up-to-date review on this topic as our goal was to only describe mainly the 1964 experiment.

Finally, to make our narrative a bit more personal, we include two photographs. Figure 4 shows a photograph of the authors with Paillotin in the 1970s, whereas Figure 5 shows a photograph of the authors with Gyozo Garab (organizer of the 1998 International Congress on Photosynthesis Research, Budapest, Hungary).

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