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MONO- BI- TRI- AND POLYPARTITE MODELS IN PHOTOSYNTHESIS

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SUMMARY: It is shown how energy fluxes in mono-bi-tri- and polypartite photosystems can be described. The derivation of the energy distribution term α and the probability of spill over p_{21} as proposed by W.L. BUTLER are reviewed.

1. INTRODUCTION

1.1 Models

A model can be considered as a tool to give us a better understanding of the problem being investigated. The proposed model pursues two distinct goals:

- to connect and explain the known data within a common framework,
- to make predictions on the behaviour of a sample under new experimental conditions.

A tool is a very modest construction compared to a piece of art an artist can create with it. In analogy, a model is a very crude representation of the biological system a scientist tries to describe. Therefore, a model is a valuable tool for unifying and explaining data, as well as making predictions. These predictions encourage the development of new measuring techniques which enable us to control the validity of the model under new experimental conditions. Even if a model has to be modified or rejected due to new information, it is still considered to be an intellectual creation.

1.2 The presentation of a model

Every model can be presented as:

- **a pure verbal description**
- **an analogical graphic representation**
- **an analytical and mathematical formulation.**

These three forms of presentation carry identical information. That means e.g. a tripartite mathematical formulation and a tripartite verbal description both belong to a tripartite graphic presentation of a model. However, this is not strictly followed in the literature and may lead to confusion.

1.3 The theoretical information of a model

A paint brush can be used for drawing or for other purposes depending on the technique applied. The technique of handling a model, determines how it can be associated with the biological system under consideration. These abstract handling techniques of a model are **theories.** The verbal description, graphic representation and mathematical formulation of a model should be unambigously linked to well defined theories. Different theories for the same model may lead to similar, different or the same conclusions. Therefore, the reader can only understand what a model means if he knows which theories are being referred to by the author pertaining to a model.

1.4 The practical information of a model

There is no limit as far as formulation of fantastic and very sophisticated models are concerned. However, the terms needed for the description of a model increase exponentially with its complexity. A model of practical usefulness should be measurable, which means that a correlation between the experimental signal and each theoretical term is needed. The inability to associate an experimental signal with each term (variable or constant) in a model forces us to formulate an assumption of its value like zero, infinite constant or negligible. Therefore, the information supplied by a model depends on the amount of experimental information which can be associated with it. There is a constant battle to find an optimum compromise between theoretical and practical information which a model supplies. The more complex the model, the better it helps to understand biological systems. More speculations and assumptions however, are needed to associate it with the experimental data. The simpler the model, the more rigid it is and the less it represents the biological system. But it can be strictly associated with the experimental data. W.L. Butler and his colleagues have been always aware of the highly **multipartite** structure of any photosystem. But the availability of independent experimental data forced one to formulate the photosynthetic apparatus as a **bipartite, tripartite** or **polypartite** model. All these formulations are in fact, consequent extensions of the formulation of a **monopartite** photosystem.

2. THE MONOPARTITE PHOTOSYSTEM

The model of a monopartite photosystem developed by W.L. Butler (1) and M. Katajima supports a concept which is able to explain principal activities such as photochemistry and fluorescence emission of PSII. It uses the biochemical terms of pigment concentration and first order rate constants for the de-excitation events of excited pigments. The static concept of the model is based on the established opinions that a pigment pool acts as an antenna to absorb photons and that some of the absorbed photons are trapped by the reaction centers. W.L. Butler introduces a dynamic concept in his model as follows: The energy which flows from the excited antenna pigment pool to the reaction center has several options:

1st **The excited reaction center transforms its excitation energy into photochemistry by reducing an electron acceptor while the reaction center gets oxidized.**

2nd **The excited reaction center is unable to perform photochemistry. The excitation energy of such a closed reaction center migrates back to the antenna pool.**

The rate constants of the de-excitation of the excited reaction center of PSII are k_F, k_D, k_T (fluorescence, heat dissipation or energy transfer). The rate constants of the de-excitation of the excited reaction center are k_p, k_d, k_t (photochemistry, heat dissipation, energy transfer from the reaction center back to the antenna pool). A reaction center which is able to perform photochemistry upon excitation is named **open**, otherwise **closed**. The energy migrates back and forth from a closed reaction center to an antenna pool. The concept of **energy cycling** between the antenna pool and the reaction center was introduced this way. The equations and concept of energy cycling between the antenna and the reaction center are identical to the equation for the energy cycling between the antenna pools of neighbouring photosynthetic units described much earlier by A. Joliot and P. Joliot (2).

3. ENERGY CYCLING DETERMINES THE COMPLEXITY OF A MODEL

Energy cycling is nothing else but a flux of energy which repeatedly moves back and forth from one location to another. Each exciton has the probability to migrate in one direction. The energy cycling degenerates into an irreversible one way energy migration if the probability of energy transfer back to its origin is zero. Therefore, real energy cycles or one way energy

transfers (like spill over) can be formulated using the same equations. The complexity of a photosynthetic model is determined firstly, by the number of pigment pools and reaction centers assumed and secondly, by the energy cycles one attributes to the model. The following different types of energy cycle can be defined. Their distinctions are crucial inasmuch as each type influences the experimental data differently:

1. Trapping is the energy migration from the antenna pool of a photosystem to its reaction center (e.g. trapping in PSI RC or trapping in PSII RC).
2. Coupling is the energy migration from one antenna pool to another antenna pool of the same photosystem (e.g. energy cycling between the core antenna and the light harvesting complex of PSII).
3. Spill over is the energy migration from one antenna pool of a reaction center to an antenna pool of another reaction center (e.g. core antenna of PSII to antenna of PSI or LHC of PSII to core antenna of PSI etc.)
4. Grouping is the energy migration from one antenna pool of a photosystem to an antenna pool of a neighbouring photosynthetic unit (e.g. LHC of PSII to the LHC of a PSII of a neighbouring unit).

A monopartite model includes only trapping.
A bipartite model includes trapping and spill over in its simplest form, or trapping, spill over and grouping if unit-unit energy transfer is considered.
A tripartite model includes trapping, spill over, coupling with or without grouping.
A polypartite model is the general description of a model which includes all biochemically known pigments.
Every model can be arranged as a separate pack model (no grouping) or as a grouped pack model (allowing grouping to occur).

The models used and proposed by different authors can all be classified according to the above expressions. Each author, however, uses different terminologies to describe the energy migration in his model so that it is often difficult to compare one model with another. Nevertheless, it is possible to formulate all models using the same terminology provided that the following rules (generally accepted in Biochemistry and Photochemistry) are taken into consideration:

1) To each pigment pool an index number is given. e.g. 1 for core antenna of PSI, 2 for core antenna of PSII, 3 for the light harvesting Chl a/b complex etc.

2) Each reaction center is labeled with an index letter indicating that it is a single molecule and not a pigment pool. e.g. for the reaction center of PSI index a and for the reaction center of PSII index b.
3) The non-defined location where the dissipated energy goes are labeled with an index e.g. F for fluorescence and D for heat dissipation.
4) The absorption fluxes of each pigment pool are represented by J_1, J_2, J_3 etc, the excitation rates by E_1, E_2, E_3 etc and the energy fluxes by E_{21}(spill over from pigment pool of PSII to pigment pool of PSI) or by E_{2b} (energy flux from pigment pool 2 to the reaction center of PSII) or by E_{2F} (the total fluorescence emission of the pigment pool 2). The measured fluorescence signal is labeled F_2 and it is proportional to the total flux E_{2F}. The same is valid for F_1 and E_{1F} etc. (All energy fluxes or rates are determined by the amount of photons or excitons moving per time).
5) The rate constants are designated by the term k_{ij}, the probability that an exciton goes from the location i to the location j is p_{ij} and the lifetime of an excited pigment P_i^* is τ_i etc.
6) All other terms can be derived by applying these rules. e.g. the quantum yield of photochemistry of PSII is $\psi_{2b} = E_{2b}/J_2$. In the case of PSI, the quantum yield of photochemistry is $\psi_{1a} = E_{1a}/J_1$.

More details are supplied by the energy flux theory in bio-membranes (3).

4. EXPERIMENTAL BASIS OF THE MODELS

The monopartite model of Butler explains the two extreme points of a fluorescence induction curve at room temperature or low temperature in the presence of DCMU. The two extreme points are the initial fluorescence F_o and the maximal fluorescence F_M. The maximal variable fluorescence is defined as $F_v = F_M - F_o$. The fluorescence rise from F_o to F_M can be attributed to the energy cycling between the antenna and the reaction center of PSII (trapping). The shape of the fluorescence induction kinetics at room temperature is either exponential or sigmoidal. The unit-unit transfer model of Joliot and Joliot associates the sigmoidal form of the curve with the existence of energy cycling between several photosynthetic units (grouping). At low temperature the shape of the fluorescence curve is never sigmoidal. A model has been developed by Strasser which allows the conversion of low temperature induction curves to room temperature curves and vice versa (4).

The low temperature (-196°C) emission spectra of green chloroplasts show two to three emission peaks. Their position vary from organism to organism. The long wavelength peak (in higher plants at 735 nm) is attributed to the emission of PSI, the middle peak (nearly in all plants at 695 nm) is attributed to the emission of core antenna or the phaeophytin of PSII. The short wavelength peak (between 680 and 695 nm) can be mostly attributed to the core chlorophyll (CP 43)(CP 47) and to the light harvesting chl a/b complex. However, some antenna of PSI and some early chlorophyll forms emit in this region as well. At all wavelengths where fluorescence emission occurs, an intact photosynthetic apparatus exhibits variable fluorescence at low, as well as at room temperature due to the redox state of the reaction center of PSII. In most cases, the observed variable fluorescence does not depend on the redox state of P700. However, some PSI particles and some algae do show some variable fluorescence which is dependent on the redox state of P700.

W.L. Butler concluded (at least in the case of higher plants and most green algae) that the variable fluorescence observed on the emission band of PSI (735 nm) is entirely due to energy transfer from PSII to PSI. This statement predicted that the excitation spectrum of the variable fluorescence at 735 nm and 695 nm would be proportional to one another. This prediction was confirmed experimentally (5). A second prediction of the energy transfer

concept by Butler was that the excitation spectra for the variable fluorescence of the PSII would be proportional to the excitation spectra for initial and maximal fluorescence of PSII. A set-up which allows simultaneous measurements of the fluorescence induction kinetics at two or three different wavelengths (e.g. at 685, 695 and 735 nm) was used to confirm this prediction (6). (This experimental set-up was developed by the author in 1970 in the Photobiology Laboratory of the University of Liège, Belgium, using multibranched fibreoptics and a HeNe-laser for excitation) (7).

It is an experimental fact that at 77K the plot of the time dependent fluorescence rise signal of PSI (signal F_1) versus the time dependent fluorescence signal of PSII (signal F_2) is a straight line.

Therefore, we can correlate the two signals empirically as follows (see Fig. 2):

$F_{1(t)}$ = Intercept on F_1 axis + slope . $F_{2(t)}$

In cases where the redox state of P700 influences the variable fluorescence at low temperature, it is necessary to pre-illuminate the sample with far red light in order to oxidize the reaction center of PSI.

All models (bi-tri-poly-partite, with or without grouping, separate pack or grouping pack formulation) which include the energy cycle of trapping and spill over from PSII to PSI in any form, predict a straight line plot of F_1 versus F_2.

The empirical description of this plot with its experimental terms as indicated in Fig. 2 is:

$$F_{1(t)} = \underbrace{(F_{1(M)} - F_{2(M)} \cdot F_{1(v)}/F_{2(v)})}_{\text{Intercept on } F_1\text{-axis}} + \underbrace{\frac{F_{1(v)}}{F_{2(v)}}}_{\text{slope}} \cdot F_{2(t)}$$

The above equation of a straight line can be plotted if two points are given. The first point has the coordinates $F_{2(o)}$, $F_{1(o)}$ while the second point has the coordinates $F_{2(M)}$, $F_{1(M)}$. The maximal variable fluorescence are $F_{2(v)}$ and $F_{1(v)}$. They are calculated as $F_{2(v)}=F_{2(M)}-F_{2(o)}$ and $F_{1(v)}=F_{1(M)}-F_{(o)}$. The biophysical meaning of the two constants (intercept and slope) depends on the model applied.

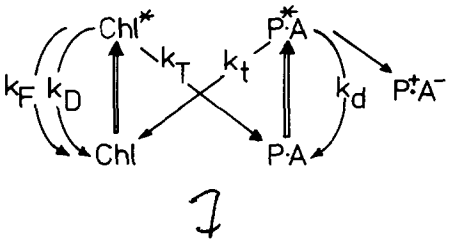

Figure 1:
Energy fluxes as proposed by W.L.Butler for a monopartite photosystem as PSII. The term Chl symbolizes the antenna pigment of PSII and P.A. represents the reaction center of PSII. This model is identical with the model of Joliot and Joliot for energy cycling between photosynthetic units if the term P.A is replaced by Chl. For details see text.

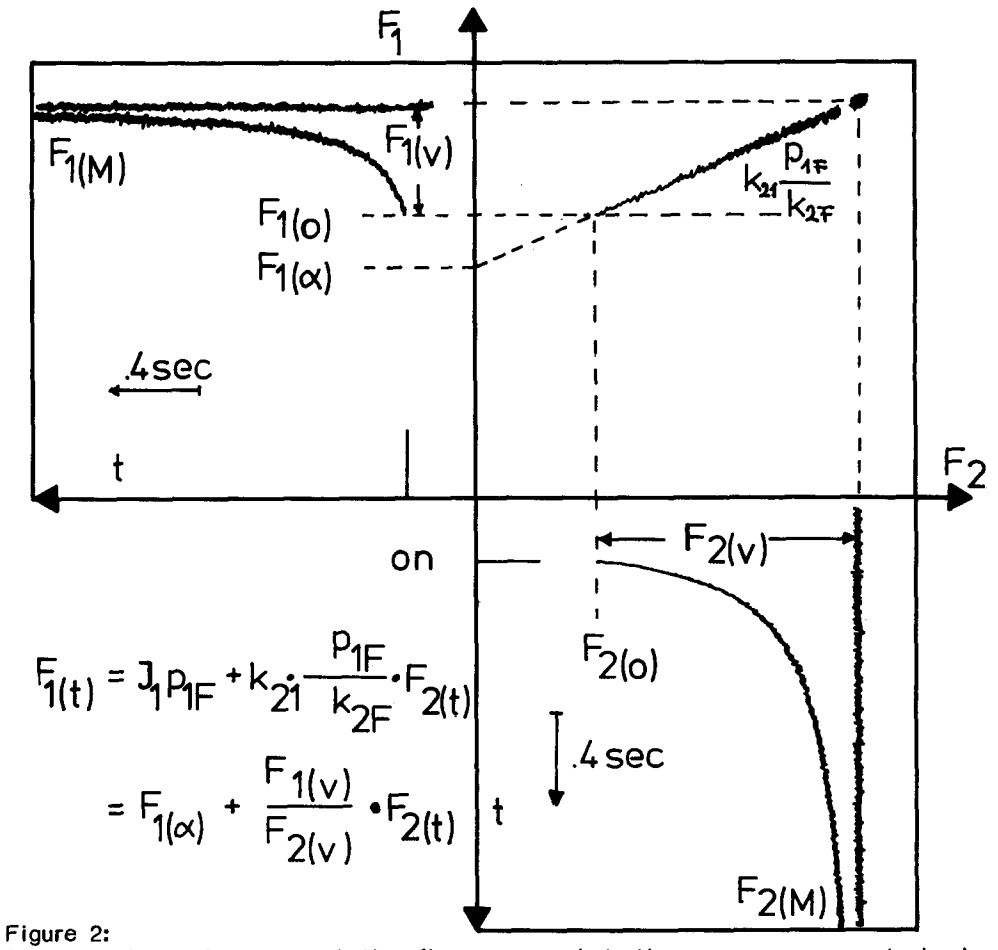

Figure 2:
The experimental traces of the fluorescence induction curves measured simultaneously at 735 nm for F_1 and 695 nm for F_2 at low temperature using chloroplasts of higher plants or leaves. The three dimensional square with the axis F_1, F_2 and time shows fluorescence induction at 77K. The fluorescence signals F_1, F_2 are given in relative intensities and the time is indicated. ON means the moment when light was switched on. Both kinetics F_1 vs time, F_2 vs time were measured simultaneously and stored in the computer.

5. BIOPHYSICAL SIGNIFICANCE OF EXPERIMENTAL EXPRESSIONS

5.1 The ratio $F_{2(v)}/F_{2(M)}$

Each fluorescence induction curve measured at an emission wavelength of PSII (at low temperature or room temperature, in the presence of DCMU) exhibits the two distinct signals, $F_{2(o)}$ and $F_{2(M)}$. Therefore, $F_{2(v)}=F_{2(M)}-F_{2(o)}$.

The fluorescence of a PSII with open reaction centers (no LHC and no grouping) is equal to the absorption rate (J_2) of PSII times the probability that an absorbed photon gets emitted (p_{2F}). As soon as a light harvesting complex or energy transfer from unit to unit (grouping) is taken into account, then the excitation rate of the antenna of PSII is equal to the sum of all three energy fluxes which have reached the antenna pool of PSII after the occurrence of absorption, grouping and coupling of the LHC. The fluorescence of a PSII with closed reaction centers is equal to the product of the photon flux absorbed by PSII and the gain factor (due to the energy cycling between the antenna and the reaction center of PSII) times the probability p_{2F}. The fluorescence of PSII with open or closed reaction centers of any model is equal to the excitation rate E_2^{op}(open centers) and E_2^{cl}(closed centers) of the antenna times the probability p_{2F} (that an exciton is dissipated as fluorescence).

$$F_2 = E_2 \cdot p_{2F} \text{ or } F_2^{op} \cdot p_{2F} \text{ or } F_2^{cl} = E_2^{cl} \cdot p_{2F}$$

op and cl refer to open and closed reaction centers. If all reaction centers are open, the fluorescence signal corresponds to $F_{2(o)}$ and if all reaction centers are closed, the fluorescence signal corresponds to $F_{2(M)}$. The ratio therefore is:

$$F_{2(v)}/F_{2(M)} = (F_{2(M)}-F_{2(o)})/F_{2(M)} = (E_2^{cl}-E_2^{op})/E_2^{cl}$$

In the case of a bipartite model $E_2^{op} = J_2$ and $E_2^{cl}=J_2 \cdot (1-T)^{-1}$

J_2 is the absorption flux of the antenna of PSII and T is the trapping product of $p_{2b} \cdot p_{b2}$ (p_{2b} is the probability that an exciton from the antenna reaches the reaction center of PSII, p_{b2} is the probability that the exciton at the reaction center goes back to the antenna pool). The energy cycling between two types of antenna of one photosystem is described in analogy to the trapping product T and the coupling product C. An energy cycle between units is de-

scribed by the overall grouping product G. In the literature, G is indicated as p_{22} or p_{2G} depending on the model discussed (2)(8). The biophysical meaning of the ratio $F_{2(v)}/F_{2(M)}$ of a real tripartite model with trapping T, coupling C and grouping G can be formulated in a general way ref (3)(8) namely:

1) $F_{2(v)}/F_{2(M)} = T/(1-C).(1-G)$ Tripartite model (grouped)

A tripartite model without grouping (G=O):

2) $F_{2(v)}/F_{2(M)} = T/(1-C)$ Tripartite model (separate pack)

A bipartite model with grouping (C=O):

3) $F_{2(v)}/F_{2(M)} = T/(1-G)$ Bipartite model (grouped)

A bipartite model without grouping (G=O and C=O):

4) $F_{2(v)}/F_{2(M)} = T$ Bipartite model (separate pack)

The last expression was derived from W.L.Butler (1). Based on the definition of $T=p_{2b}.p_{b2}$ and on the assumption that the probability of energy transfer from a closed reaction center of PSII to the antenna is almost unity, ($p_{b2}=1$) one can say that the experimental ratio $F_{2(v)}/F_{2(M)}$ is **proportional** to the trapping probability p_{2b}. In all models however, the quantum yield $\varphi_{2b(o)}$ of photochemistry when all reaction centers are open can be expressed by the following ratio:

$$\varphi_{2b(o)} = \frac{\text{initial excitation flux to the RC II}}{\text{light absorption flux by PS II}} = \frac{E_{2b(o)}}{J_2} = \frac{F_{2(v)}}{F_{2(M)}}$$

This expression shows that a change in the ratio $F_{2(v)}/F_{2(M)}$ can be attributed to a change in trapping, only if we are certain that our sample has no unit-unit (grouping), which is rarely the case under natural condltions. The correlation made by W.L. Butler (assuming $p_{2b}=1$) that $\varphi_{2b(o)} = p_{2b} = F_{2(v)}/F_{2(M)}$ is only valid if the sample is ungrouped and has no LHC. This expression does not allow calculations of the probability of photochemistry (p_{2b}) in any tripartite model or bipartite grouped model. The above equation also shows that the quantum yield of photochemistry is not equal to the ratio of the rate constants $k_{2b}/\Sigma k_{2x}$ as long as $E_2^{op} \neq J_2$.

5.2 The intercept $F_{1(\alpha)}$ of the plot F_1 versus F_2 (fig.2)

The intercept of the plot F_1 versus F_2 has the experimental description of $F_{1(M)}-F_{2(M)}\cdot F_{1(v)}/F_{2(v)}$ defined originally by Butler as a measure for the absorption energy distribution expression $\alpha(1)$. But later,[12] he defined it as a measure proportional to α. However, both statements are wrong. Butler defined α as the fraction of light which is absorbed by PSI.

$$\alpha = J_1/(J_1+J_2) = (1 + J_2/J_1)^{-1} \qquad \text{bipartite model}$$

where J_1 is the absorption flux of PSI and J_2 is the absorption flux of PSII. α **is a distribution term.** The shape of the absorption or excitation spectrum for α is a function of the ratio of the PSII and PSI absorption or excitation spectra.

The intercept $F_{1(\alpha)}$ is a fluorescence term for PSI.

This fluorescence term includes an excitation spectrum of PSI only and the unit: photons emitted by PSI per time and per cross-section. As a compromise, Butler agreed to call the intercept in the F_1 versus F_2 plot as $F_{1(\alpha)}$ which is that part of the PSI emission due only to photons absorbed by PSI. Therefore:

$$F_{1(\alpha)} = J_1 \cdot p_{1F}$$

The F_1 versus F_2 plot can now be written as: $F_{1(t)}=F_{1(\alpha)}+F_{1(\beta)}$
$F_{1(\beta)}$ is a function of the state of the reaction center of PSII and expressed as:

$$F_{1(\beta)} = F_{2(t)} \cdot F_{1(v)}/F_{2(v)}$$

$F_{1(\beta)}$ symbolizes an energy flux which was absorbed by PSII and partially spilled over (with the spill over energy transfer probability p_{21}) to PSI. The term $F_{1(\beta)}$ includes therefore, an excitation spectrum of PSII and an emission spectrum of PSI (due to spill over). $F_{2(o)}$ and $F_{2(M)}$ have the same excitation spectrum as $F_{1(\beta)}$ however, they have an emission spectrum of PSII. The three dimensional plot of fluorescence emission versus excitation wavelength and versus emission wavelength shows the terms F_2, $F_{1(\alpha)}$, $F_{1(\beta)}$ of a real biological bipartite system (in flashed bean leaves without LHC measured at 77K) (Fig.3).

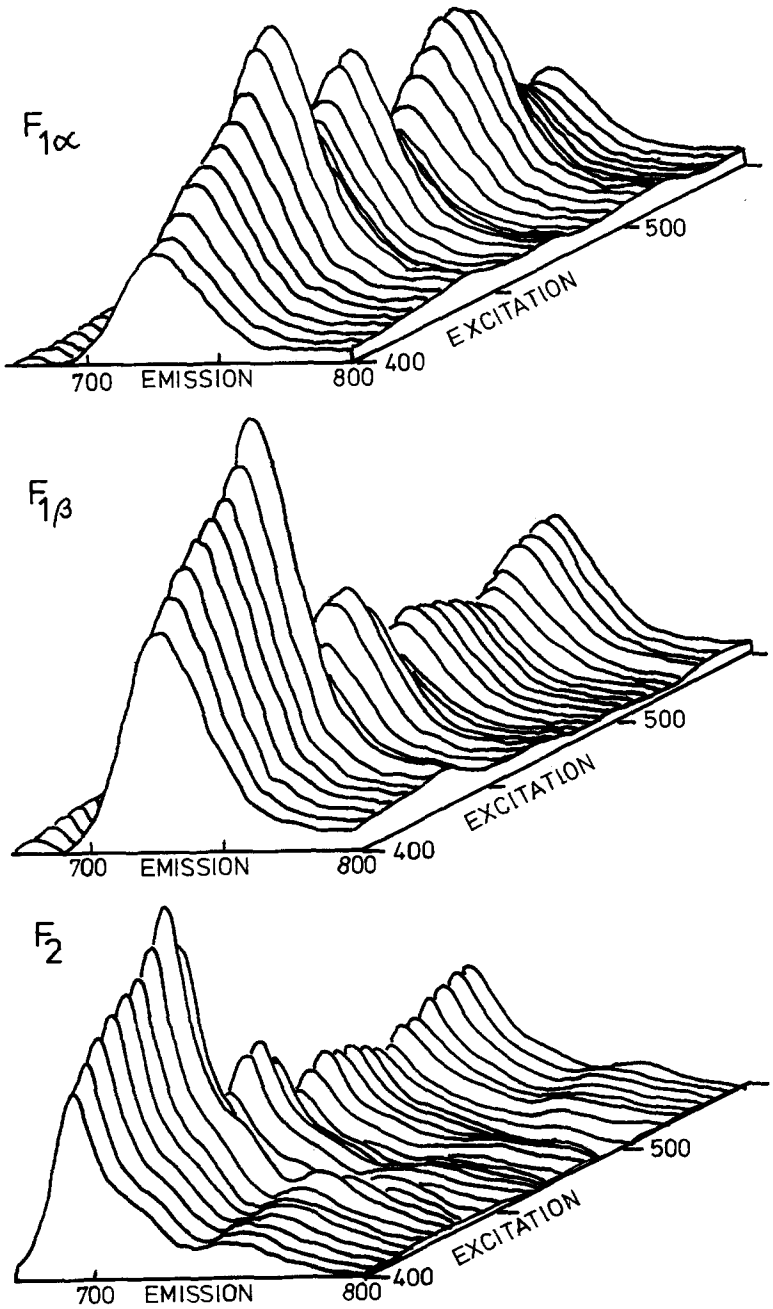

Figure 3: Excitation and fluorescence emission spectra of "pure" PSI (indicated as $F_{1(\alpha)}$) of "pure" PSII (indicated as F_2) and of the energy transfer flux from PSII to PSI (indicated as $F_{1(\beta)}$) in flashed bean leaves at 77K. Excitation and emission spectra were measured at the $F_{(O)}$ and at the $F_{(M)}$ levels, which allow the calculation of $F_{1(\alpha)}$ and $F_{1(\beta)}$.

5.3 The slope of the plot F_1 versus F_2 (fig.2)

The slope of the plot F_1 versus F_2 can be written empirically as:

$$\text{slope} = F_{1(v)}/F_{2(v)} = F_{1(\beta)(o)}/F_{2(o)} = F_{1(\beta)(M)}/F_{2(M)}$$

Its biophysical meaning is:

$$\text{slope} = p_{1F} \cdot p_{21}/p_{2F} = p_{1F} \cdot k_{21}/k_{2F} = F_{1(v)}/F_{2(v)}$$

where k_{21} and k_{2F} are the rate constants of spill over or fluorescence emission of PSII respectively and $p_{ij}=k_{ij}/\sum k_{ix}$.

The experimentally measurable slope of F_1 versus F_2 is proportional to the **rate constant** of spill over k_{21}. The terms for the probabilities p_{ij}, the rate constants k_{ij} and the lifetime τ_i of the excited pigment complex are determined by the conformation of a photosynthetic system. Hence, they are referred to as conformation terms.

5.4 The biophysical significance of the plot F_1 versus F_2

The absorption terms (J_1, J_2, α) conformation terms (p_{21}, k_{21}, τ_1, τ_2) and emission terms (F_1, F_2, $F_{1(\alpha)}$, $F_{1(\beta)}$) describe the energy fluxes flowing through a photosynthetic apparatus. All these terms can be linked together to the biophysical equation of a bipartite model as follows: (Bear in mind the definitions: $\alpha=J_1/(J_1+J_2)$; $p_{21}=k_{21}\cdot\tau_2$; $F_1=F_{1(\alpha)}+F_{1(\beta)}$

$$\frac{\alpha}{(1-\alpha)} = \frac{J_1}{J_2} = p_{21} \cdot F_{1(\alpha)} / F_{1(\beta)(o)}$$

Energy distribution term	*Absorption term*	*Conformation term*	*Terms for fluorescence of PS I*

This equation carries the same information as equation 1 below. Furthermore, it shows that it is impossible to calculate the value of α with one set of data of $F_{1(\alpha)}$ and $F_{1(\beta)}$. The terms α and p_{21} are unknown. A second independent signal is needed to solve the above equation for α. This second information can be found in excitation or fluorescence lifetime measurements. Both experiments lead to the same conclusion.

6. THE DETERMINATION OF ABSORPTION ENERGY DISTRIBUTION

The above equation for energy distribution in a bipartite system can be re-arranged and presented as :

Equation 1 (ref.9)

$$\alpha = \frac{F_{1(\alpha)}}{F_{1(\alpha)} + \frac{1}{p_{21}} \cdot F_{1(\beta)(o)}}$$

Replacing $F_{1(\beta)(o)} = F_{2(o)} \cdot \frac{p_{21}}{p_{2F}} \cdot p_{1F}$

leads to

$$\alpha = \frac{F_{1(\alpha)}}{F_{1(\alpha)} + \frac{p_{1F}}{p_{2F}} \cdot F_{2(o)}}$$

Replacing $p_{1F} = k_{1F} \cdot \tau_{1(o)}$ and
$p_{2F} = k_{2F} \cdot \tau_{2(o)}$ and
$k_{1F} = k_{2F}$

leads to

$$\alpha = \frac{F_{1(\alpha)}}{F_{1(\alpha)} + \frac{\tau_{1(o)}}{\tau_{2(o)}} \cdot F_{2(o)}}$$

Assuming: $F_{2(o)}/\tau_{2(O)} = F_{2(M)}/\tau_{2(M)}$ and $\tau_{1(o)} = \tau_{1(M)}$ leads to

Equation 2 (ref.10)

$$\alpha = \frac{F_{1(\alpha)}}{F_{1(\alpha)} + \frac{\tau_{1(M)}}{\tau_{2(M)}} \cdot F_{2(M)}}$$

This equation depends on the ratio of the experimental values $F_{1(\alpha)}/F_{2(M)}$. Therefore, it should be corrected to the shape of the total emission spectrum of F_1 and F_2.

The first equation (by Strasser and Butler, ref. 9) has been used in a combination of excitation and fluorescence data. The second equation (used by Wong and Govindjee and Merkelo, ref. 10) combines fluorescence lifetime measurements with fluorescence data. The first equation can be applied to a bipartite system but the combination of excitation and fluorescence data is technically very difficult to do. The second equation contains several assumptions. The signals for $F_{1(\alpha)}$ and $F_{2(M)}$ should be corrected to denote signals of the same relative area of the whole $F_{1(\alpha)}$ and $F_{2(M)}$ emission spectra. This correction is not necessary in equation 1 since it is cancelled by the ratio $F_{1(\alpha)}/F_{1(\beta)}$. Nevertheless, both equations and measuring techniques supply reasonable values for α and for the spill over probability p_{21}. It has to be emphasized here, that none of the equations consider either energy coupling between LHC and core antenna of PSII or grouping. However, as soon as the sample is placed in a high salt condition in the presence of DCMU, the fluorescence induction curve of chloroplasts is typically sigmoid at room temperature indicating that grouping occurs. The danger of all these equations is that, if the samples differ in their grouping or/and coupling constellations but have identical spill over constellations, then all these changes will appear

in the calculations as changes in spill over properties **a priori.** So far, the **overestimation** of spill over can be avoided only when a theory is elaborated and when new simultaneous measuring techniques for trapping, coupling, grouping and spill over are developed. An analysis of the shape of the fluorescence induction curve can provide us with the necessary information about grouping. In a forthcoming paper, a new concept will be presented which allows measurement and calculation of a synergetic model including **trapping** of PSI and PSII, **spill over** from PSII to PSI, **grouping** between photosynthetic units, as well as absorption and dissipation fluxes of both photosystems. The data obtained from this concept show that when old experiments from the literature are analysed, the biggest conformational changes are always due mainly to changes in the grouping and slightly due to changes in the spill over constellation. The lack of mathematical freedom in the equations used by Butler led to the interpretation that every change in the energy distribution of the sample is a change in the spill over constellation (ref.15).

W.L. Butler proposed a method of calculating the absorption energy distribution α and the probability of spill over p_{21} by two sets of the four experimental values $F_{1(o)}$, $F_{1(M)}$, $F_{2(o)}$, $F_{2(M)}$ measured at low temperature. (Therefore, 8 independent experimental values are obtained). Many authors however, encountered some difficulties using this method. Many assumptions have to be made to solve the equations correctly but unfortunately, these assumptions are not in accordance with nature. The method uses two samples under different conditions (e.g. O mM Mg^{2+} and 10 mM Mg^{2+}) to solve the equations. If three samples under specific conditions were to be tested (e.g. O mM Mg^{2+}, 1 mM Mg^{2+} and 10 mM Mg^{2+}) then we could get three possible combinations each of which consists of a pair of two different samples (e.g. sample O and 1, sample O and 10, sample 1 and 10 mM Mg^{2+}).

Therefore, <u>two values</u> of α and p_{21} for each sample could be obtained. These two values should be <u>identical</u> if the behaviour of the sample corresponds to the statements assumed.
(see table 1 and 2)

The two sample or eight-point-method of calculating energy distribution by W.L. Butler is stated in the appendix of this paper without comments. Each author has to decide for himself whether or not he wants to base his data on this method.

Mono-, bi-, tri- and polypartite models are trials to quantify the complexity of a photosynthetic apparatus. Every model is far from nature but

it serves as a stimulus to correlate biological experimental measurements to their biophysical meaning. All these models provide the basis for the analysis of the photosynthetic apparatus in terms of non-equilibrium thermodynamics. This line of investigation may tell us some day (in biological terms like structure stability, adaptation ability, trend of development etc.) why variables such as trapping, coupling, spill over and grouping tend to optimize the overall state of a photosynthetic system in its natural environment.

7. APPENDIX: W.L. Butler's method of calculating the energy distribution term α and the probability p_{21} for energy transfer from PSII to PSI (ref. 11)

7.1 The experiment

Determination of the initial and maximal fluorescence at low temperature measured at an emission wavelength of PSI and simultaneously at an emission wavelength of PSII. The signals are: $F_{1(o)}$, $F_{1(M)}$, $F_{2(o)}$, $F_{2(M)}$. Two samples are measured under different conditions (e.g. high salt (+) and low salt (-) conditions).

7.2 Empirical definitions

1) $F_{i(M)}-F_{i(o)} = F_{i(v)}$ i can be substituted for either 1 or-2

2) $(F^{-}_{1(v)}/F^{+}_{1(v)}).(F^{-}_{2(v)}/F^{+}_{2(v)}) = R$

3) $F_{1(\alpha)} = F_{1(o)}-F_{2(o)}\cdot F_{1(v)}/F_{2(v)} = F_{1(o)}-F_{1(\beta)(o)}$

7.3 Definitions of a bipartite model

1) Absorption energy distribution $\alpha = J_1/(J_1+J_2)$
2) Energy transfer probability from PSII to PSI

$$p_{21} = \frac{\text{Energy flux from PS II to PS I}}{\text{Total excitation rate of PS II}} = \frac{E_{21}}{E_2}$$

3) Intercept in the plot $F_{1(t)}$ versus $F_{2(t)}$ is $F_{1(\alpha)} = J_1 \cdot p_{1F}$
4) Slope of the plot $F_{1(t)}$ versus $F_{2(t)} = p_{1F}\cdot k_{21}/k_{2F}$

Table 1

Data from ref:		(11)		(14)	
mM Mg^{2+}	0	5	0	1	10
$F_{1(o)}$	76	60	80	73	55
$F_{1(M)}$	97	83	105.5	97.5	75.5
$F_{2(o)}$	28	32	34.5	39.5	43
$F_{2(M)}$	67	114	95	130	156.5
	.119	.066	****	.050	.034
p_{21}			.231	.161	****
(according to Butler)			.125	****	.058
	.324	.271	****	.226	.171
α			.510	.486	****
(according to Butler)			.361	****	.260

All calculations for α and p_{21} of the same sample should be identical.

Table 2

	pH 6.2			pH 8.8			pH 7.0		
	SD	Na^+	Mg^{2+}	SD	Na^+	Mg^{2+}	SD	Na^+	Mg^{2+}
$F_{1(o)}$	237.2	277.0	216.8	232.6	204.9	174.4	266	347	219
$F_{1(M)}$	300.8	339.2	257.8	265.9	225.0	186.3	323	426	276
$F_{2(0)}$	22.3	29.5	36.6	19.9	24.0	47.7	59	56	59
$F_{2(M)}$	65.1	62.1	100.0	47.7	36.4	63.6	100	100	133
	-.368	-.527	****	.151	.193	****	.049	.062	****
p_{21}	****	.184	.071	****	.557	.368	****	.040	.018
	.510	****	.312	.364	****	.263	0	****	0
	1.790	1.937	****	.569	.452	****	.099	.133	****
α	****	.419	.366	****	.704	.588	****	.090	.063
	.759	****	.718	.761	****	.506	0	****	0
p_{21}	.14	.24	.09	.13	.23	.18	.25	.27	.13
α	.46	.48	.43	.54	.49	.42	.36	.40	.33

calculations of Butler (12)

of Wong, Govindjee, Merkelo (10)

7.4 Necessary assumptions to make

1) Total light absorption flux for all samples is constant
$J_1^+ + J_2^+ = J_1^- + J_2^-$ therefore $\Delta J_2 = -\Delta J_1$!!

2) The probability of fluorescence emission of PSI is constant
$$p_{1F}^+ = p_{1F}^-$$

3) The sum of the rate constants of fluorescence emission, heat dissipation and photochemistry of PSII is constant.
$K^+ = k_{2F}^+ + k_{2D}^+ + k_{2b}^+ = k_{2F}^- + k_{2D}^- + k_{2b}^- = K^-$

4) The rate constants of fluorescence emission of PSII are constant:
$k_{2F}^+ = k_{2F}^-$ and $\Delta k_{2b} = -\Delta k_{2D}$!!

7.5 Correlation of data of the two samples

1) The ratio of the intercept of plot $F_{1(t)}$ versus $F_{2(t)}$
$$F_{1(\infty)}^+ / F_{1(\infty)}^- = J_1^+ \cdot p_{1F} / J_{1F}^- \cdot p_{1F} = J_1^+ / J_1^- = \alpha^+ / \alpha^-$$

2) The ratio of the slope of plot $F_{1(t)}$ versus $F_{2(t)}$
$$R = \frac{p_{21}^-}{p_{21}^+} \cdot \frac{p_{2F}^+}{p_{2F}^-} \cdot \frac{p_{1F}^-}{p_{1F}^+} = \frac{k_{21}^-}{k_{21}^+}$$

3) The ratio of the energy transfer probability
$$p_{21}^- / p_{21}^+ = R \cdot \Sigma k_{2x}^+ / \Sigma k_{2x}^- \quad \text{this ratio can be written as}$$
$$= R \cdot \frac{\Sigma k_{2x}^+ / k_{21}^+}{(k_{21}^- + k_{2F}^- + k_{2D}^- + k_{2b}^- + (k_{21}^+ - k_{21}^+))/k_{21}^+}$$
this equation is identical with
$$= R \cdot \frac{1 / p_{21}^+}{R + \frac{1}{p_{21}^+} - 1}$$

7.6 The four necessary equations for α^+, α^-, p_{21}^+, p_{21}^- are:

$$1)\quad p_{21}^- = R \cdot p_{21}^+ / (1 + (R - 1) \cdot p_{21}^+)$$

$$2)\quad \alpha^+ / \alpha^- = F_{1(\alpha)}^+ / F_{1(\alpha)}^-$$

$$3)\quad \alpha^+ = F_{1(\alpha)}^+ / (F_{1(\alpha)}^+ + F_{1(\beta)(o)}^+ / p_{21}^+)$$

$$4)\quad \alpha^- = F_{1(\alpha)}^- / (F_{1(\alpha)}^- + F_{1(\beta)(o)}^- / p_{21}^-)$$

7.7 The solution for the system of equations

The four equations can be used to solve for α^+, α^-, p_{21}^+, p_{21}^-. The probability of energy transfer from PSII to PSI and the absorption energy distribution term α can be calculated from the experimental data as follows:

$$p_{21}^+ = 1 / \left(1 - \frac{F_{2(v)}^+ \cdot (F_{1(o)}^+ - F_{1(o)}^-)}{F_{1(v)}^+ \cdot (F_{2(o)}^+ - F_{2(o)}^-)}\right)$$

$$\alpha = 1 / \left(1 - \frac{1 / p_{21}}{1 - F_{1(o)} \cdot F_{2(v)} / F_{1(v)} \cdot F_{2(o)}}\right)$$

The following table (see table 1) shows the calculations on energy distribution α and spill over probability p_{21} (from the literature) based on the experimental data obtained by W.L. Butler (11,14) using the two sample method. This method has an interesting theoretical approach, however, it lacks experimental consistency (a warning to those who intend to use the method).

A comparison of calculations on α (energy distribution) and p_{21} (spill-over probability) based on the data obtained by Butler (two sample method) and the data obtained by Wong, Govindjee (fluorescence emission and lifetime method) is shown in table 2. SD (sucrose buffer), Na^+ (buffer with Na^+), Mg^{2+} (Buffer with Na^{2+} and Mg^{2+}).

Table 1 does not show any consistency at all since many completely different random values of p_{21} (spill over probability) and α (incident absorption

energy distribution) are obtained from one and the same sample.

Table 2 shows that the method of Wong, Govindjee, Merkelo offers reasonable and generally acceptable values of p_{21} and α. The inconsistency found in Butler's two sample method is attributed to very strict and unbiological assumptions made by him e.g. $\Delta J_1 = -\Delta J_2$ or $\Delta k_{2b} = -\Delta k_{2D}$

They suggest that every absorption change in PSII should be compensated by an absorption change in PSI or/and every change in the rate constant of photochemistry should be parallel to an opposite change in the rate constant of heat dissipation. However, nature seems to vary these terms independently of one another. Both reported methods do not give any attention to grouping which is reflected in the lateral movements of protein complexes as revealed by electron microscopy. Furthermore, it is also reflected in the sigmoid shape of the fluorescence induction curve at room temperature in the presence of DCMU.

The message is: More experimental signals rather than initial/maximal fluorescence intensities and lifetime measurements are needed to describe energy distribution in a model. The model should include the four distinctly different types of energy transfer fluxes namely: trapping, spill-over, grouping and coupling.

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