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The Reduction of Protochlorophyllide into Chlorophyllide

VI. Calculation of the Size of the Transfer Unit and the Initial Quantum Yield of the Reduction *in vivo**

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Abstract

A simple and general model of the prolamellar body membrane is applied to kinetic data. The author calculates in this way the size *in vivo* of the transfer unit, and the quantum yield *in vivo* of the reduction of protochlorophyllide into chlorophyllide. It is shown that: (*1*) at ordinary temperature and in the leaf, the transfer unit must comprise about 18 pigment molecules, the size depending on the temperature; (*2*) at the start of the impact of photons on the leaf, the calculated quantum yield of the reduction is of the order of 1, or 2, depending on the value chosen for the molar coefficient of absorption of protochlorophyllide *in vivo*.

We introduce 4 propositions constituting a possible model of the prolamellar membrane and we apply the propositions to the empirical law [expression (*4*) below] proposed by SIRONVAL and KUYPER (1972) to relate two variables measurable when an etiolated (bean) leaf is illuminated for less than one second. These variables are (*1*) the proportion of pigment reduced in the leaf, and (*2*) the degree of the phototransformation of the emission spectrum of the leaf, estimated at liquid nitrogen temperature. We derive from the law the size of the transfer units found straight after illumination, and, relating this size to other data, we deduce the value of the initial quantum yield of the reduction of the pigment molecules.

Model of prolamellar membrane

(*1*) The membrane of the prolamellar body, before illumination, is an assembly of N' sub-units (protomers) each constituted by a polypeptide chain associated to u molecules of the pigment protochlorophyllide. The number u is constant.
(*2*) The u molecules of pigment in each sub-unit are reduced simultaneously, so the terms "reduced" and "non reduced" refer to each sub-unit as well as to each pigment molecule. Similarly, their spectra are also transformed simultaneously.

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376 (*3*) After an infinite fluence, *i.e.* an infinite duration of the flow of photons ($I \times t_\infty = p_\infty$), $N < N'$ sub-units of the prolamellar body are reduced. These sub-units are called "reducible".

(*4*) After an infinite fluence, the N reduced sub-units – and only they – are also transformed spectrally. In particular their emission spectrum at −196 °C is transformed ($E_{657} \rightarrow E_{688}$). In this specific sense, they are called "spectrally transformable".

On the basis of propositions (*1*) to (*4*), we may distinguish in the following way two variable proportions, i_p and c_p, which measure the action of the photons:

(*a*) i_p is the proportion of spectrally transformed sub-units among the N spectrally transformable sub-units in the membrane of the prolamellar body after fluence p, *i.e.*:

$$i_p = \frac{N_p}{N} \tag{1}$$

where N_p is the number of sub-units whose emission spectrum at −196 °C is transformed after p. i_p is also the proportion of molecules, among the spectrally transformable pigment molecules, whose emission spectrum at −196 °C is modified after p.

(*b*) After p, a number N_p^r of sub-units is reduced in the prolamellar body. The proportion c_p of sub-units reduced among the N reducible sub-units is written:

$$c_p = \frac{N_p^r}{N} \tag{2}$$

c_p is also the proportion of pigment molecules reduced among the reducible pigment molecules.

For an exposure of infinite duration to the flow of photons, the N spectrally transformable and reducible subunits are all spectrally transformed and reduced, so that:

$$\frac{N_{p\infty}^r}{N} = \frac{N_{p\infty}}{N} = 1 \tag{3}$$

Quantum yield of the spectral transformation of the sub-units; size of the transfer unit

Experimentally, it is found that the following is true after any fluence p:

$$(A - i_p)\, c_p = K i_p \tag{4}$$

where the constants A and K depend on the temperature (SIRONVAL and KUYPER 1972). This applies to each individual prolamellar body in the leaf. Assuming that law (*4*) expresses correctly the relation between i_p and c_p, we see that for an infinite

fluence $A - 1 = K$. The experiments also show that A is greater than 1. When ex- 377
panded in series and incorporating Eqs. (1) and (2), Eq. (4) is written:

$$\frac{N_p^r}{N} = \frac{KN_p}{AN}\left[1 + \frac{1}{A}\left(\frac{N_p}{N}\right) + \frac{1}{A^2}\left(\frac{N_p}{N}\right)^2 + \ldots\right] \tag{5}$$

The derivative of Eq. (5) with respect to N_p tends towards K/A as N_p tends towards zero. Consequently, at the start of the spectral phototransformation, for a very small N_p, we have:

$$N^r_{p(\text{first photons})} = \frac{K}{A} N_{p(\text{first photons})}$$

We conclude that at the start of the impact of photons, the ratio of the number of spectrally transformed sub-units N_p to the number of reduced sub-units N_p^r is equal to A/K.

These results reflect the existence of transfer units as follows: when an initial sub-unit is reduced, the emission spectrum (at -196 °C) of A/K sub-units is transformed as a result of a transfer of energy to the reduced sub-unit. A/K thus expresses the size of the transfer unit in terms of the number of spectrally transformed sub-units per transfer unit at the start of illumination.

Let r' be the number of photons needed to reduce 1 sub-unit; r' is also the number of photons needed to transform A/K sub-units spectrally. The number of transformed sub-units per photon absorbed is therefore:

$$n' = \frac{A/K}{r'} \tag{6}$$

n' is the quantum yield of the spectral transformation of the sub-units, expressed in sub-units per photon absorbed and estimated at the start of the irradiation. If r' is constant during the phototransformation, n' is also constant since A and K are constant.

Number of pigment molecules per sub-unit and quantum yield of the reduction

The quantum yield n of the spectral phototransformation of the pigment molecules in the leaf can be measured by following the kinetics of this transformation (SIRONVAL *et al.* 1968a). In red monochromatic radiation, $\lambda = 647$ nm, the kinetics are close to the first order and the rate constant k is the product of n by the molecular coefficient of absorption $\varkappa_{647}^{vivo}$ of the pigment to be transformed *in vivo*, multiplied by the flux intensity I_0 of the incident radiation:

$$k = n\,\varkappa_{647}^{vivo}\,I_0 \tag{7}$$

378 In this expression, the value of $\varkappa_{647}^{vivo}$ is the Napierian logarithm of the ratio I_0/I for a molar concentration of pigment and a thickness of 1 cm, I being the radiant flux density leaving the absorbant medium. Since k is constant, n is constant during the phototransformation. On the other hand, since n is the number of spectrally transformed pigment molecules per photon absorbed, the number n' of spectrally transformed sub-units per photon is $n' = n/u$, u being the number of pigment molecules per sub-unit. Substituting value from Eq. (6) for n', we find:

$$u = \left(\frac{n}{A/K}\right) r' \tag{8}$$

This expression gives the size of a sub-unit in pigment molecules per sub-unit, estimated at the start of the impact of photons. The size of the constant $(n/(A/K))$ is expressed in molecules of pigment per photon. If r' is assumed constant during phototransformation, n' and u are also constant.

Table 1

Comparison of the values of n and A/K in the leaf at three temperatures.

$\frac{I}{T} \times 10^4$	n	$\frac{A}{K}$
38.8	24.2	12·3
33.8	17.6	8.6
32.3	16.8	8.3

[The values of A/K are calculated from Table 3 in SIRONVAL and KUYPER (1972). The values of n are calculated, using expression (7), from the mean values of the spectral transformation rate constant k measured in the leaf at the temperatures stated; the method is described in SIRONVAL *et. al.* (1968a). The leaf receives 647 nm monochromatic photons for a period shorter than one second. The irradiance is 35×10^{-11} einstein s^{-1} cm^{-2}. $\varkappa_{647}^{vivo}$ is calculated from the molar coefficient of absorption of protochlorophyll(ide) in ether at 623 nm according to KOSKI and SMITH (1948) by multiplying it by 2.3 (*i.e.* $\varkappa_{647}^{vivo} = \varepsilon_{623} \times 2.3$; $\varepsilon_{623} = 36\,000 \times 10^3$ cm^2 mol^{-1}; we thus have the expression: $k = n\,2.3\varepsilon_{623}\,I_0$ — see RABINOWITCH 1951 — Vol. II/1, p. 838; SIRONVAL *et al.* 1968a — Appendix I). — Beans of cv. Commodore were grown as described in the papers quoted. They were 20 d old.]

Table 1 compares the values of A/K to those of n at three temperatures. At a given temperature, n is constant during the phototransformation, as are A and K. The ratio A/K and n are greater than one when the measurements are made between +37 and −20 °C. $n \cong 2(A/K)$, regardless of temperature, so that:

$$u \cong 2r' \tag{9}$$

The equation (9) shows that if r' photons are needed to reduce one sub-unit, then the sub-unit in question contains about $2r'$ molecules of pigment. It is concluded that

at the start of irradiation, each photon absorbed reduces of the order of 2 pigment molecules. Referring to the model [proposition (*2*)], this amounts to saying that each sub-unit reduced at the start contains about two molecules of pigment (since the reduction of the pigment molecules in one sub-unit is assumed to be simultaneous, *i.e.* that it corresponds to a single absorption act), or in other words that $r' = 1$ at the start.

DISCUSSION

The reasoning may be summarized as follows. We calculate, from the spectral transformation rate constant k, the number of pigment molecules n whose emission spectrum at low temperature is modified as a result of the absorption of one photon; n is constant during phototransformation. We also calculate the number of pigment molecules whose emission spectrum is transformed per molecule reduced at the start of the photon impact; this is equal to A/K. The ratio of the values of n and A/K gives the number of pigment molecules reduced at the start per photon absorbed. We find that this number is 2. Proposition (*2*) of the model then implies that the number u of pigment molecules per sub-unit absorbing photons at the start of irradiation is 2.

The value 2 for the quantum yield of the reduction is considered as the most plausible by SCHULTZ (1970) on the basis of direct measurements on a protochlorophyllide-lipoprotein preparation (PCH suc), extrapolated to the origin. However, it would seem that the first molecules reduced in this type of preparation are not in a dimer state (MATHIS and SAUER 1972). It remains to be seen how far these data are valid in the leaf and in our experimental conditions.

The evaluation of yield by our method depends on the measurement of n and A/K. The value of A/K is calculated by extrapolation to the start of the photon impact, assuming that law (*4*) is correct and applicable from the start. Direct measurement at the start is lacking. The value of n comes from measurement of the rate constant k of the spectral transformation produced by 647 nm photons for which the transformation tends towards a first order. We use expression (7). The illuminance is measured with care, after calibration of the thermopile with a Standard of Spectral Irradiant (SIRONVAL *et al.* 1968a). We assume that the absorption coefficient $\varkappa_{647}^{vivo}$ is equal to the molar coefficient of extinction measured by KOSKI and SMITH (1948) at the red maximum of protochlorophyll(ide) in ether, multiplied by 2.3.

BROUERS (1972) finds that for the dimer of artificial protochlorophyllide formed in a dry polar solvent and absorbing around 650—655 nm (in benzene, CCl_4 and liquid paraffin) the molar coefficient at the red maximum is about twice that of the monomer. If the pigment of the *in vivo* lipoprotein-pigment complex behaves as the dimer of BROUERS, a value of $\varkappa_{647}^{vivo}$ about twice as high as the one adopted to calculated n must be used. In this case n will be lower than we have indicated in Table 1 and the quantum yield of the reduction derived from Eq. (8) will be close to 1.

In earlier papers we drew attention to the high value of n, *i.e.* the size of the transfer unit expressed as the number of protochlorophyllide molecules per unit ($n > 10$; SIRONVAL *et al.* 1968b; SIRONVAL and BROUERS 1970). THORNE (1971) also evaluates the size of the "groups" of protochlorophyllide molecules in which an energy transfer takes place in the leaf during spectral transformation. His reasoning is based essentially on a statistical model. He finds, in primary bean leaves aged 21 d, a group size of 17.5 protochlorophyllide molecules (at +20 °C). This quantity is slightly higher than the one we obtain for n by using KOSKI and SMITH's value for ε_{647}^{vivo} [extrapolation from the values of n at −15 °C and +23 °C (Table 1) gives: $n \cong 18$ at +20 °C].

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