Biochimica et Biophysica Acta, 292 (1973) 117–129

BBA 46427

KINETICS AND QUANTUM YIELD OF PHOTOCONVERSION OF PROTOCHLOROPHYLL(IDE) TO CHLOROPHYLL(IDE) *a*

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(Received July 11th, 1972)

SUMMARY

The kinetics of photoconversion of protochlorophyll(ide) to chlorophyll(ide) *a* were investigated in dark-grown barley leaves and in a preparation of protochlorophyll holochrome subunits. In the subunits the conversion obeyed first-order kinetics. This indicates that the excitation of protochlorophyll(ide), energy loss through deexcitation, and the reduction of excited protochlorophyll(ide) are all reactions that follow first-order kinetics with respect to protochlorophyll(ide) in protochlorophyll holochrome subunits.

In contrast, photoconversion in leaves obeyed neither first- nor second-order kinetics. This prompted the postulation of an additional route within macromolecular units of protochlorophyll holochrome, whereby energy is lost from excited protochlorophyll(ide) by a reaction that is not first order. Such a process might be energy transfer from excited protochlorophyll(ide) to newly-formed chlorophyll(ide) *a*.

A dynamic model describing photoconversion in macromolecular units was derived. The model is consistent with the observed progress of photoconversion in barley leaves and in protochlorophyll holochrome subunits from barley.

Determinations of the quantum yield of photoconversion in protochlorophyll holochrome subunits gave values of 0.4–0.5 molecules·quantum^{-1}. Estimates of the initial quantum yield of the photoconversion process in leaves fell into the same range. The dynamic model allows predictions on the progressively decreasing quantum yield as the photoconversion proceeds in macromolecular units.

INTRODUCTION

Seedlings of dark-grown angiosperms accumulate photoconvertible and some non-photoconvertible protochlorophyll(ide)'s. Upon illumination the photoconvertible portion of this pigment, is reduced to chlorophyll(ide) *a*. Protochlorophyll(ide)* is bonded, in a particular way that is necessary for its photoconvertibility, in protochlorophyll holochrome[1] which has been inferred to be a protochlorophyll(ide)–protein complex[2].

* Definitions: protochlorophyll(ide), as used generally in this paper, connotes ground-state protochlorophyll(ide), *i.e.* photoconvertible to chlorophyll(ide) *a*; total protochlorophyll(ide) refers to mixtures of photoconvertible and non-photoconvertible protochlorophyll(ide); protochlorophyll(ide)*, excited protochlorophyll(ide); chlorophyll(ide) *a**, excited chlorophyll(ide) *a*.

A number of studies on the kinetics of the photoconversion of protochlorophyll(ide) to chlorophyll(ide) *a* in etiolated leaves have been done[2-5]. Smith and Benitez[2] and Virgin[3] found that the photoconversion follows the second-order law, indicating to them that the reaction is bimolecular with respect to protochlorophyll(ide). Sironval *et al.*[4] observed that certain changes in the fluorescence properties of etiolated bean leaves could be related to first-order reaction kinetics, when driven by photons of wavelength 647 nm or longer, but not 630 nm or shorter. Thorne and Boardman[5], using 630, 640 and 647 nm light, found that photoconversion simulated second-order reaction kinetics up to about 75 % of maximal photoconversion in all cases.

Kinetic studies on the photoconversion of protochlorophyll(ide) in protochlorophyll holochrome isolated from etiolated bean leaves have also been done[6-9]. Boardman[8] found second-order reaction behaviour, as reported earlier[6,7] and postulated that the apparent second-order reaction was comprised of two first-order reactions, through which protochlorophyll(ide) conversion proceeds at two different rates. He suggested further that the reduction of the protochlorophyll(ide) molecule is an intramolecular event due to a restricted collision process between protochlorophyll(ide)* and hydrogen of the electron-donating portion of the apoprotein[8].

Kahn *et al.*[10], using partially photoconverted bean leaves and bean protochlorophyll holochrome, observed energy transfer at −196 °C from protochlorophyll(ide)* to chlorophyll(ide) *a in vivo* and *in vitro*. They concluded that protochlorophyll holochrome may consist of a number of protein subunits, each bearing one protochlorophyll(ide) molecule, and organized in a way that allows energy transfer among the pigment molecules. On the basis of these observations, Thorne and Boardman[5] reinvestigated the kinetics of the photoconversion of protochlorophyll(ide) to chlorophyll(ide) *a* in etiolated bean leaves and invoked an influence of energy transfer from protochlorophyll(ide)* to chlorophyll(ide) *a* to explain the observed kinetics of the photoconversion.

A procedure[11] utilizing saponin, a mixture of detergents, has been developed for the isolation of photoactive subunits of protochlorophyll holochrome from etiolated barley leaves. Spectrofluorimetry on partially photoconverted samples of such protochlorophyll holochrome subunits cooled with liquid nitrogen gave no indication of energy transfer from protochlorophyll(ide)* to chlorophyll(ide) *a* which was consistent with a single protochlorophyll(ide) molecule per active unit[11]. As in bean, energy transfer at −196 °C has been observed in the protochlorophyll holochrome in barley leaves[5]. A study by Nielsen and Kahn (*cf.* ref. 11) indicated that the photoconversion of barley protochlorophyll holochrome subunits displayed first-order reaction kinetics.

We have reinvestigated in detail the kinetics of photoconversion of protochlorophyll(ide) in barley leaves and in preparations of protochlorophyll holochrome subunits from barley. The findings provide strong evidence that the basic process of protochlorophyll(ide) photoconversion is first-order and allow us to put forth a dynamic model describing the kinetics of the overall photoconversion process *in vivo* and *in vitro*.

The quantum yield of photoconversion of protochlorophyll(ide) in bean protochlorophyll holochrome has been calculated as 0.60 (refs 6 and 7), utilizing second-order kinetics to determine the initial rate. More recently higher quantum yields

have been calculated, indicating the conversion of two protochlorophyll(ide) molecules per absorbed photon[9]. We have redetermined the quantum yield of the overall process, employing protochlorophyll holochrome subunits and first-order kinetics. Also, the dynamic model has enabled us to estimate quantum yields of the photoconversion *in vivo*.

MATERIALS AND METHODS

Plant material and preparation of protochlorophyll holochrome subunits

Seedlings of barley (*Hordeum vulgare* L., cultivar Svalöfs Bonus) were grown and harvested as described earlier[11]. During the growth period and subsequent preparative procedures, leaves received no light except from a dim safe-light[12]. Protochlorophyll holochrome subunits were extracted as previously[11], except that the last centrifugation was omitted. Saponin Weiss Rein, art. 7685, Erg. B. 6, E. Merck AG, Darmstadt, Germany was used. Appropriate amounts of saponin, tricine buffer and glycerol were added to the protochlorophyll holochrome subunit preparation to give a solution with $A = 0.048$ due to protochlorophyll(ide) at 645 nm (Fig. 1) and 0.5 % (w/v) saponin, 43 % (v/v) glycerol and 0.1 M tricine adjusted to pH 8.5 with NaOH.

Photoconversion of protochloropyll(ide) to chlorophyll(ide) a in vivo and in vitro

The light sources used for the photoconversion of protochlorophyll(ide) were: *in vivo*, an Osram halogen ellipsoid reflector lamp (15 V, 150 W); and *in vitro*, a xenon lamp (Osram XBO 450 W/P) supplied with stabilized direct current.

The light from both sources was filtered through a 640-nm Depil interference filter (No. 272442) and a red glass cut-off filter (RG 1), both from Jenaer Glaswerk, Schott und Gen., Mainz, Germany. A 12.5-cm layer of water was introduced into the light path during the photoconversion of the protochlorophyll holochrome subunit preparation, but was omitted during the irradiation of the leaf pieces.

Energy measurements were made with a Schwartz thermopile (Hilger and Watts Ltd, London, type and serial number FT 17.1/64366). Precise agreement with the manufacturer's calibration of the thermopile was obtained using the method of Björn[13] and by comparison with an independently calibrated Kipp and Zonen thermopile.

Leaf pieces (2-g samples) in Expt 1 were irradiated at 25–27 °C for periods of 5–150 s with light of the intensity $4.5 \cdot 10^2$ ergs$\cdot$cm$^{-2}\cdot$s^{-1} ($3.9 \cdot 10^2$ ergs$\cdot$cm$^{-2}\cdot$s^{-1}, if infrared radiation had been removed by the interposition of a water filter). The same setup was used for the irradiations in Expt 2. However, during Expt 2, no energy measurements were made. The difference observed between the progress of photoconversion in the two experiments (see later) is thought to have arisen through a decrease in the quantum flux during the period intervening between Expt 1 and Expt 2. A 13 % decrease was measured some time after Expt 2 was concluded and is consistent with the results obtained in the two experiments. Therefore, we assign to Expt 2 the light intensity $3.4 \cdot 10^2$ ergs$\cdot$cm$^{-2}\cdot$s^{-1}, with the infrared radiation subtracted. Samples of 1.8 ml of the preparation of protochlorophyll holochrome subunits in 1.0-cm cuvettes were irradiated at 20 °C for periods of 5–300 s with light of the intensity $6.4 \cdot 10^2$ ergs$\cdot$cm$^{-2}\cdot$ s^{-1}. Maximal photoconversion was obtained by a 2-min irradiation with a tungsten filament lamp.

Pigment determinations

Leaf segments were ground with a mortar and a pestle, using sand, a small amount of $CaCO_3$, and an appropriate amount and concentration of acetone to give a ratio acetone–water of 4:1 (v/v) in the extract. The acetone extractions were initiated in a cold room, and in the case of illuminated leaves, within 1 min after the conclusion of the irradiation.

Samples of the protochlorophyll holochrome subunits, taken before the final dilution with glycerol, were diluted 1:4 with acetone before or after being exposed to light to convert all photoconvertible protochlorophyll(ide) to chlorophyll(ide) *a*.

Absorption spectra of the 80% acetone extracts of the leaves and the protochlorophyll holochrome subunit preparation were recorded after a clarifying centrifugation.

Total protochlorophyll(ide) as well as chlorophyll(ide) *a* were quantitated using the equations of Anderson and Boardman[14] after substituting millimolar extinction coefficients ($l \cdot mmole^{-1}$) for the specific absorption coefficients ($l \cdot g^{-1}$) of the pigments. The occurrence in etiolated leaves of protochlorophyllide) as well as its phytol ester, protochlorophyll, presents a problem in the conversion of specific absorption coefficients to molar extinction coefficients. Boardman[15] and Houssier and Sauer[16] have considered the problem and arrived at differing conclusions. We have used the molecular weights of the phytolated pigments to convert the specific absorption coefficients[14] to millimolar extinction coefficients (Table I). The latter coefficients lead to the determination of constant amounts, on a molar basis, of total protochlorophyll(ide) *plus* chlorophyll(ide) *a* in samples of leaves or protochlorophyll holochrome subunits, respectively, with widely differing degrees of photoconversion ranging from zero to maximal (Nielsen, O.F. and Kahn, A., unpublished).

TABLE I

MILLIMOLAR EXTINCTION COEFFICIENTS OF PROTOCHLOROPHYLL(IDE) AND CHLOROPHYLL(IDE) *a* IN 80% ACETONE

The wavelengths at which the millimolar extinction coefficients are given, 627 and 666 nm, correspond to the absorption maxima of protochlorophyll(ide) and chlorophyll(ide) *a*, respectively, observed in this work. The specific absorption coefficients[14] from which the millimolar extinction coefficients were derived were given at 626 and 663 nm, respectively. These small discrepancies in the wavelengths are neglected.

Wavelength (nm)	*Extinction coefficient* ($l \cdot mmole^{-1}$)	
	Protochloro-phyll(ide)	*Chloro-phyll(ide) a*
627	31.1	13.3
666	0.2	73.3

As an alternative procedure to pigment quantitation in acetone extracts, the fraction of photoconvertible protochlorophyll(ide) converted to chlorophyll(ide *a in vitro* was obtained directly from absorption spectra of the protochlorophyll holochrome subunit preparations (*cf.* Fig. 1) by a method previously used by Boardman[8], assuming linearity between the absorbance and the concentration of chlorophyll (ide) *a*.

Absorption spectra were recorded with a Cary 17 spectrophotometer equipped with a scattered transmission accessory and an EMI 9659 QB multiplier phototube.

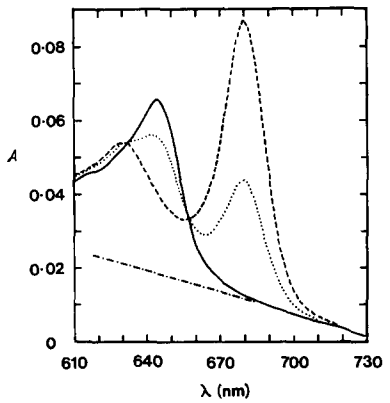

Fig. 1. Absorption spectra of barley protochlorophyll holochrome subunit preparation (1 cm light path): ———, before irradiation; ·····, after exposure to 640 nm light for 50 s; – – – –, after maximal photoconversion; –·—·–, line used to correct for scatter when obtaining the initial absorption at 640 nm by protochlorophyll(ide).

RESULTS

Photoconversion of protochlorophyll(ide) in etiolated barley leaves

The fraction of protochlorophyll(ide) remaining in segments of leaves after various doses of light was determined. The results were not consistent with first-order (Fig. 2A) or second-order (Fig. 3A) kinetics for the photoconversion process, since the points depart from linearity in both test plots.

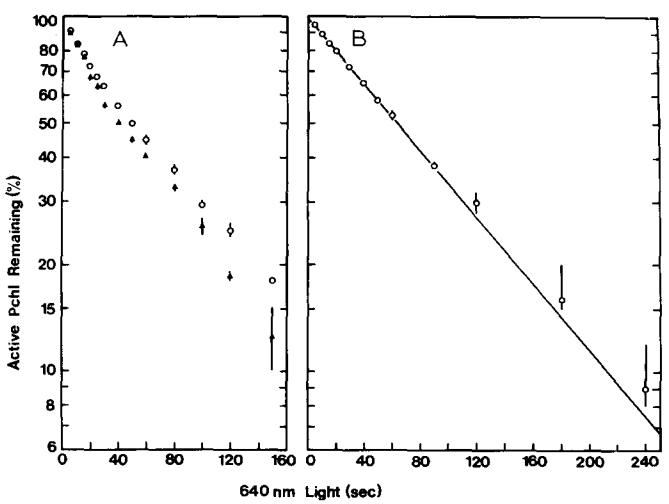

Fig. 2. Semi-logarithmic plots of the progress of photoconversion of protochlorophyll(ide): test for first-order kinetics. The vertical lines through the points give the ranges. (A) In etiolated leaves. All points represent the means of two replicates. ▲, Expt 1, light intensity $3.9 \cdot 10^2$ ergs·cm^{-2}·s^{-1}; ○, Expt 2, light intensity $3.4 \cdot 10^2$ ergs·cm^{-2}·s^{-1}. (B) In a preparation of protochlorophyll holochrome subunits. All points represent the means of three replicates. Light intensity $6.4 \cdot 10^2$ ergs·cm^{-2}·s^{-1}.

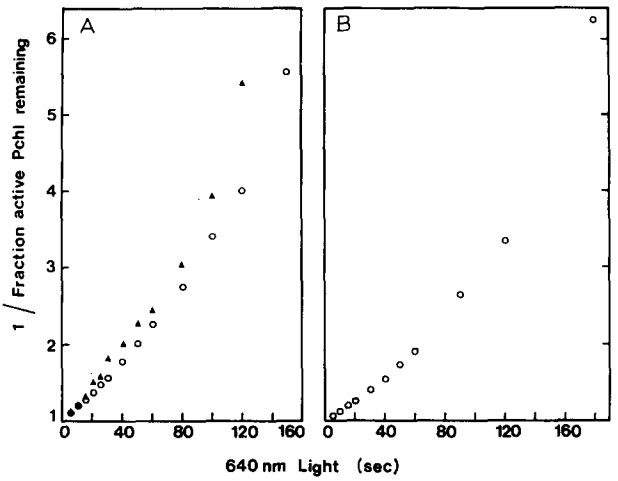

Fig. 3. Progress of photoconversion of protochlorophyll(ide): test for second-order kinetics. (A) In etiolated leaves as in Fig. 1A. (B) In the preparation of protochlorophyll holochrome subunits as in Fig. 1B.

Photoconversion of protochlorophyll(ide) in a preparation of protochlorophyll holochrome subunits

In contrast to the results with leaves, when the fraction of protochlorophyll(ide) remaining in aliquots of a solution of protochlorophyll holochrome subunits was plotted logarithmically *versus* irradiation time (Fig. 2B) a relationship consistent with first-order kinetics was obtained. The small deviations of the points from linearity at low fractions of protochlorophyll(ide) remaining are not considered significant. In each of these cases, at least one of the three experimental observations departs from the line by less than 1 % of the protochlorophyll(ide) present initially. As can be expected from the consistency of the data with first-order reaction kinetics, the same data show inconsistency with second-order kinetics (Fig. 3B) except at small fractional photoconversions. The close approach to linearity with energy dose for the reciprocal as well as the logarithm of the fraction of protochlorophyll(ide) remaining, when the fraction of photoconversion is small has a trivial explanation. If one plots fractions between 1.0 and 0.7 on a logarithmic ordinate *versus* their respective reciprocals on an arithmetric abcissa, with both axes to the same scales used in the ordinates of Figs 1 and 2, only small departures of the points from linearity appear.

A dynamic model describing the photoconversion of protochlorophyll(ide) to chlorophyll(ide) a

We take as the starting point the formulation of Boardman[8]:

$$\text{Protochlorophyll(ide)} + h\nu \rightarrow \text{protochlorophyll(ide)}^* \qquad (1)$$

$$\text{Protochlorophyll(ide)}^* + 2\,\text{H} \rightarrow \text{chlorophyll(ide)}\ a \qquad (2)$$

where H is hydrogen from an unknown reductant. Reaction 1 is purely photochemical and therefore can be assigned a first-order rate constant, k_1, which depends

on the light intensity, wavelength, and the absorption characteristics of the reactant. Thus, k_1 describes the rate of absorption of light by protochlorophyll(ide). Reaction 2 is thermochemical[2,17], and despite its superficial resemblance to a second-order reaction, we consider it first order with respect to protochlorophyll(ide)* and assign the rate constant k_2. These postulates are consistent with the observed first-order kinetics of photoconversion in preparations of protochlorophyll holochrome subunits and the intramolecular, restricted collision process between protochlorophyll(ide)* and the reductant as proposed earlier[8].

Next, we must consider the possibilities for the deexcitation of protochlorophyll(ide)* which do not lead to its reduction but return protochlorophyll(ide)* to the ground-state. Accordingly we write:

$$\text{Protochlorophyll(ide)}^* \rightarrow \text{protochlorophyll(ide)} + \text{lost energy} \qquad (3)$$

describing the known self-fluorescence of protochlorophyll(ide)* in bean protochlorophyll holochrome at room temperature[10] and other internal transitions restoring the ground state. We assign the rate constant k_3 to express the sum of the rate constants of these first-order reactions.

We must now account for the deviation from first-order kinetics observed for the photoconversion process in leaves and in bean protochlorophyll holochrome. In these cases, as distinct from protochlorophyll holochrome subunits, there must be at least one reaction that is not first order and that competes effectively with the reactions leading to the loss of protochlorophyll(ide)* as described above. Such a reaction might be energy transfer from protochlorophyll(ide)* molecules to molecules of newly-formed chlorophyll(ide) *a* within the same holochrome macromolecule[5]. The term protochlorophyll holochrome macromolecule is used to describe a unit containing multiple protochlorophyll(ide) molecules or, after partial conversion, protochlorophyll(ide) and chlorophyll(ide) *a* molecules with energy transfer capability. At −196 °C such energy transfer takes place in leaves[10,5] and in bean protochlorophyll holochrome macromolecule preparations[10]. Thorne and Boardman[5] assumed the energy transfer to take place also at room temperature. We write the assumption:

$$\text{Protochlorophyll(ide)}^* + \text{chlorophyll(ide) } a \rightarrow \text{protochlorophyll(ide)} + \text{chlorophyll(ide) } a^* \qquad (4)$$

This deexcitation of protochlorophyll(ide)* is assigned a constant, k_4, and k_4CP^* is the instantaneous rate at which protochlorophyll(ide)* falls back to protochlorophyll(ide) by Reaction 4. C and P^* are, respectively, the mean number of chlorophyll(ide) *a* and protochlorophyll(ide)* molecules per holochrome macromolecule. The formulation is not intended to imply a second-order reaction with collision necessary between protochlorophyll(ide)* and chlorophyll(ide) *a*.

Reactions 1 through 4 can be summarized and combined as

$$\text{Protochlorophyll(ide)} \underset{\text{Reactions 3+4}}{\overset{\text{Reaction 1}}{\underset{k_3, k_4C}{\overset{k_1}{\rightleftharpoons}}}} \text{protochlorophyll(ide)}^* \xrightarrow[k_1]{\text{Reaction 2}} \text{chlorophyll(ide) } a \qquad (5)$$

While there is a superficial resemblance to the treatment developed by Thorne and Boardman[5], the model departs from theirs in the basic derivation and in some further details.

The kinetic statements below stem from the foregoing postulations and provide the dynamic model:

$$-\frac{dP}{dt} = k_1P - k_3P^* - k_4CP^* \tag{6}$$

$$\frac{dC}{dt} = k_2P^* \tag{7}$$

where P is the mean number of protochlorophyll(ide) molecules per holochrome macromolecular unit.

Since the extent of energy transfer is independent of the absolute concentration of pigments in dilute solutions containing units with multiple chromophores, concentrations are expressed here as pigment molecules per holochrome macromolecular unit. Thus, the statement of the model formally is independent of the concentration of pigment in the total system.

At the light intensities used, P^* at any instant is negligible in comparison with P *plus* C, so we can write:

$$C = P_0 - P \tag{8}$$

where P_0 is the initial number of protochlorophyll(ide) molecules per unit, and therefore:

$$-\frac{dP}{dt} = \frac{dC}{dt} \tag{9}$$

The simultaneous solution of the differential Eqns 6, 7 and 9 leads to:

$$-\frac{dP}{dt} = k_1P + \frac{k_4C}{k_2}\cdot\frac{dP}{dt} + \frac{k_3}{k_2}\cdot\frac{dP}{dt} \tag{10}$$

and the substitution of (P_0-P) for C (*cf.* Eqn 8) and $x\cdot P_0$ for P, where x is P/P_0, the fraction of protochlorophyll(ide) remaining at time t, lead to:

$$dt = \frac{k_4P_0}{k_1k_2}\,dx - \left(\frac{k_4P_0}{k_1k_2} + \frac{k_2+k_3}{k_1k_2}\right)\frac{1}{x}\,dx \tag{11}$$

and in integrated form:

$$t = \frac{k_4P_0}{k_1k_2}(x-1) - \left(\frac{k_4P_0}{k_1k_2} + \frac{k_2+k_3}{k_1k_2}\right)\ln x \tag{12}$$

This equation, transformed to:

$$-\frac{t}{x-1} = -\frac{k_4P_0}{k_1k_2} + 2.303\left(\frac{k_4P_0}{k_1k_2} + \frac{k_2+k_3}{k_1k_2}\right)\frac{\log x}{x-1} \tag{13}$$

describes, in a system of coordinates with $\log x/(x-1)$ as the abscissa and $-t/(x-1)$

as the ordinate, a straight line with the slope 2.303 $[(k_4P_0/k_1k_2 + (k_2 + k_3)/k_1k_2]$ and the intercept $-k_4P_0/k_1k_2$.

Accordingly, the statement (13) allows tests of the model. Experimental observations on the progress of photoconversion *in vivo* and *in vitro*, obtained as the fraction of the initial concentration of protochlorophyll(ide) remaining (equal to x) at time t, can be plotted in the appropriate system of coordinates and examined. The model predicts that the experimental points will in both cases fall along a straight line, intercepting the ordinate at zero when deexcitation of protochlorophyll(ide)* occurs solely by first-order processes ($k_4 = 0$), and at a value other than zero, when $k_4 \neq 0$.

The data on the progress of protochlorophyll(ide) conversion to chlorophyll(ide) *a* in barley leaves, given previously in Figs 2A and 3A, are replotted in Fig. 4. The lines were determined by the method of least squares and intercept the ordinate at −60 s (Expt 1) and at −70 s (Expt 2). The significance of the departures of the points from the lines can be assessed from the changes necessary in the corresponding values of x to bring the points onto the lines. In Expt 1, only one deviant point requires a change in x by as much as 0.03 to place it on the line; the others require changes of 0.02 or less. The points from Expt 2 show only rare and very small deviations from linearity. Thus, there is good agreement in both experiments between the predictions from the dynamic model and the experimental observations on photoconversion *in vivo*.

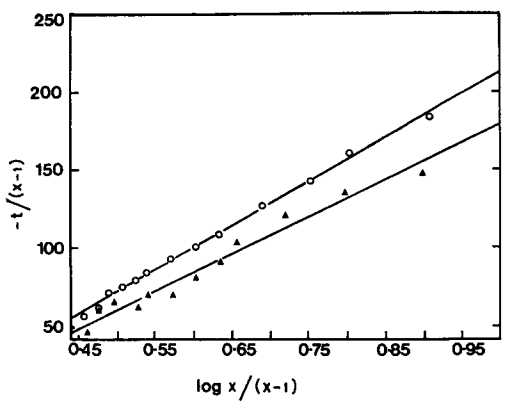

Fig. 4. Progress of photoconversion of protochlorophyll(ide) in barley leaves: test of the dynamic model. t is irradiation time in seconds, and x is the fraction of photoconvertible protochlorophyll(ide) remaining at time t. ▲, Expt 1; ○, Expt 2.

The model can also be evaluated with regard to the progress of photoconversion in a preparation of protochlorophyll holochrome subunits. Setting $k_4 = 0$ in Eqn 12 eliminates the non-first-order terms and leads to:

$$t = \frac{k_2 + k_3}{k_1 k_2} \ln x \qquad (14)$$

The evidence that this overall process follows first-order kinetics, in agreement with Eqn 14, has already been given in Fig. 2B.

Quantum yield of protochlorophyll(ide) photoconversion

The first-order rate constant, k, for the photoconversion of protochloro-

phyll(ide) to chlorophyll(ide) *a* in the preparation of protochlorophyll holochrome subunits was $1.09 \cdot 10^{-2}$ s^{-1} (*cf.* Fig. 2B). We express the total protochlorophyll(ide) concentration in a 1.0-cm cuvette, obtained as molecules·ml^{-1}, in molecules·cm^{-2} of surface area exposed to the actinic light beam (line 1, Table II). Multiplication of the total initial concentration of protochlorophyll(ide) by the fraction of the total protochlorophyll(ide) that was photoconvertible (line 2) gave the initial concentration of photoconvertible protochlorophyll(ide), $[\text{protochlorophyll(ide)}]_0$. The initial rate of photoconversion (line 3), $-\mathrm{d}[\text{protochlorophyll(ide)}]_0/\mathrm{d}t$ in molecules·cm^{-2}·s^{-1} was then calculated from the first-order rate expression:

$$-\frac{\mathrm{d}[\text{protochlorophyll(ide)}]}{\mathrm{d}t} = k \cdot [\text{protochlorophyll(ide)}]_0$$

Knowledge of the number of quanta absorbed per second by the photoconvertible protochlorophyll(ide) molecules present initially was the only further information required for the calculation of the quantum yield. From the absorbance at 640 nm (line 4) of the non-illuminated preparation of protochlorophyll holochrome subunits (*cf.* Fig. 1) after correction for scatter, the fraction of the light absorbed by the total protochlorophyll(ide) present initially was obtained (line 5). This value, multiplied by the fraction of protochlorophyll(ide) that was photoconvertible initially (line 2) and by the actinic light flux (line 6), gave the number of quanta·cm^{-2}·s^{-1} (= quanta·ml^{-1}·s^{-1}) absorbed initially by photoconvertible protochlorophyll(ide) (line 7). The procedure assumes identical absorption properties for convertible and nonconvertible protochlorophyll(ide) at 640 mm. The initial rate of conversion (line 3) divided by the initial absorption by photoconvertible protochlorophyll(ide) (line 7) gave the quantum yield (line 8) 0.43 molecules·$quantum^{-1}$. Work on another preparation of protochlorophyll holochrome subunits from barley gave a quantum yield of 0.48 (Nielsen, O. F. and Kahn, A., unpublished).

TABLE II

DETERMINATION OF THE INITIAL QUANTUM YIELDS OF PHOTOCONVERSION OF PROTOCHLOROPHYLL(IDE) TO CHLOROPHYLL(IDE) *a* IN ISOLATED BARLEY PROTOCHLOROPHYLL HOLOCHROME SUBUNITS AND IN DARK-GROWN BARLEY LEAVES

	Protochlorophyll holochrome subunits	*Leaves*	
		Expt 1	*Expt 2*
1. Initial total protochlorophyll(ide) concentration (molecules·cm^{-2})	$7.58 \cdot 10^{14}$	$3.90 \cdot 10^{14}$	$3.69 \cdot 10^{14}$
2. Photoconvertible fraction of protochlorophyll(ide)	0.75	0.86	0.83
3. Initial rate of conversion (molecules·cm^{-2}·s^{-1})	$6.20 \cdot 10^{12}$	$7.38 \cdot 10^{12}$	$5.82 \cdot 10^{12}$
4. Initial absorbance of total protochlorophyll(ide) at 640 nm	0.043	0.07	0.07
5. Initial fraction of 640 nm light absorbed	0.094	0.15	0.15
6. Actinic light flux (quanta·cm^{-2}·s^{-1})	$2.05 \cdot 10^{14}$	$1.24 \cdot 10^{14}$	$1.08 \cdot 10^{14}$
7. Initial absorption by photoconvertible protochlorophyll(ide) (quanta·cm^{-2}·s^{-1})	$1.45 \cdot 10^{13}$	$1.60 \cdot 10^{13}$	$1.35 \cdot 10^{13}$
8. Quantum yield (molecules·$quantum^{-1}$)	0.43	0.46	0.43*

* This value is 0.38, assuming the same light flux as in Expt 1 (see Materials and Methods)

The good agreement between the predictions from the model and the experimental observations on photoconversion *in vivo* allows estimates of the quantum yield of photoconversion of protochlorophyll(ide) in etiolated barley leaves. The calculations are based on three approximations:

(1) The protochlorophyll(ide) content of the 2-g leaf samples was evenly distributed over 45 cm^2, the area occupied when the leaves of a sample were arranged in contact and in a single layer.

(2) The absorbance measured on non-illuminated leaf samples at 640 nm, *minus* correction for scatter, gives the absorption due to total protochlorophyll(ide) at 640 nm *in vivo*, and this absorbance averages 0.07 (line 4, Table II).

(3) Convertible and non-convertible protochlorophyll(ide) have the same absorption characteristics at the excitation wavelength, 640 nm, as is assumed also for protochlorophyll(ide) in protochlorophyll holochrome subunits.

Transformation of Eqn 11 leads to

$$\frac{dx}{dt} = \frac{x}{\left(\frac{k_4 P_0}{k_1 k_2}\right) x - \left(\frac{k_4 P_0}{k_1 k_2} + \frac{k_2 + k_3}{k_1 k_2}\right)} \tag{15}$$

where the quantities in the parentheses can be determined using Eqn 13 and the plots in Fig. 4. The initial rates (line 3, Table II) were obtained by multiplying dx_0/dt with the initial concentration of total protochlorophyll(ide) (line 1) and with the fraction of that protochlorophyll(ide) which was photoconvertible (line 2). The initial absorption by photoconvertible protochlorophyll(ide) (line 7) and the quantum yields (line 8) were determined as for protochlorophyll holochrome subunits. The calculated quantum yields were 0.46 and 0.43 in Expt 1 and Expt 2, respectively. Thus, the initial quantum yields calculated for photoconversion of protochlorophyll(ide) organized into units in barley leaves and for protochlorophyll(ide) in a saponin-containing preparation of protochlorophyll holochrome subunits are approximately the same. As photoconversion proceeds, however, the respective quantum yields diverge, as will be discussed subsequently.

DISCUSSION

In deriving the dynamic model, we have, like Thorne and Boardman[5], written a term with properties of a second-order reaction. The process is taken to represent energy transfer from protochlorophyll(ide)* molecules to newly-formed chlorophyll(ide) *a* molecules within a protochlorophyll holochrome macromolecule, leading to the regeneration of ground state protochlorophyll(ide). Energy transfer from protochlorophyll(ide)* to chlorophyll(ide) *a* has not been demonstrated at room temperature in living leaves or in preparations of protochlorophyll holochrome macromolecules. In fact, little, if any, energy transfer from protochlorophyll(ide)-637 to protochlorophyll(ide)-650 occurs at room temperature in protochlorophyll holochrome macromolecules extracted from bean leaves[10].

Smith and French[6] and Smith[7] determined for the photoconversion of protochlorophyll(ide) in bean protochlorophyll holochrome macromolecule preparations quantum yields ranging from 0.49 to 0.70, based on initial rates calculated from a

second-order expression. Smith's[7] reporting on his Expt a allows the reconstruction of his determination. The kinetics are qualitatively in excellent agreement with those observed on barley leaves; the dynamic model (Eqn 13) describes the progress of photoconversion, whereas first- and second-order kinetics do not. We have taken Smith's data and calculated the initial rate of photoconversion using our Eqn 15. Despite the difference in Smith's and our mode of calculating the initial rate, the result, and hence, the quantum yield (0.625 and 0.64, respectively) is the same.

The uncertainties in estimating the actinic light absorption by protochlorophyll(ide) are sufficiently large that we do not attribute significance to the differences between the calculated quantum yields for the photoconversion of protochlorophyll(ide) in Smith's protochlorophyll holochrome[7] and protochlorophyll(ide) in barley leaves and in protochlorophyll holochrome subunits.

Recently, Schultz[9], working with protochlorophyll holochrome from bean, calculated quantum yields high enough to support the idea of cooperative action between two protochlorophyll(ide) molecules, *i.e.* the absorption of one photon leads to the photoconversion of two protochlorophyll(ide) molecules. We have no explanation for these results.

The dynamic model lends itself to estimating the instantaneous quantum yields throughout the progress of photoconversion *in vivo*. The relative instantaneous rate of photoconversion, dx/dt, for any x can be determined from Eqn 15 as described above. The instantaneous rate of photoconversion equals $(dx/dt) \cdot [\text{protochlorophyll(ide)}]_0$. The instantaneous rate of quantum absorption is approximated by the product of the initial rate of photoconvertible protochlorophyll(ide)'s quantum absorption multiplied by x.

Progressive decreases in the quantum yield as photoconversion proceeded in Expt 2 have been calculated. With the assumption that the absorption of a single photon can lead to the reduction of one protochlorophyll(ide) molecule, the initial quantum yield implies that 57 % of the absorbed energy is lost to the photoconversion process by self-fluorescence and internal transitions of protochlorophyll(ide)*. The energy loss increases to 74 % when 50 % of the initial protochlorophyll(ide) remains, 78 % when 30 % remains, and 81 % when 5 % remains.

The increasing loss of energy as photoconversion proceeded is attributed to the organization of protochlorophyll(ide) in macromolecular units, and as already noted, possibly through increasing energy transfer from protochlorophyll(ide)* to chlorophyll(ide) *a*[5]. Within this framework, the results are compatible with energy transfer competing effectively at room temperature with self-fluorescence from protochlorophyll(ide)*, as was found[10] at −196 °C. This notion is open to quantitative test.

While the dynamic model can give additional information, for instance on relationships among the rate constants, we choose not to draw further conclusions until the model has been evaluated more thoroughly.

ACKNOWLEDGEMENTS

This study was supported by grants to Professor Diter von Wettstein from the National Institutes of Health, U.S. Public Health Service (GM 10819); the Carlsberg Foundation; and the Danish Natural Science Research Council.

We appreciate greatly the interest and significant aid of cand. scient. Morten Kielland-Brandt, who performed the mathematical treatment giving the precise statement of the model and allowing its test. The skillful technical assistance of Mrs Betty Netting and Miss Karen Birgit Pauli is gratefully acknowledged. We are grateful also to Professor Lars Olaf Björn and Dr Axel Madsen, who participated in the checks on the calibration of the thermopile used for the measurement of light energy in the quantum yield determinations.

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