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On the Phytochrome Phototransformation Kinetics in Mustard Seedlings

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Summary. The in vivo phototransformation kinetics of mustard hook and cotyledon phytochrome exhibit a deviation from a single first order curve, quite similar to that for pumpkin hooks as reported in a previous paper (Boisard, Marmé and Schäfer, 1971). The $P_{fr} \rightarrow P_r$ kinetics can be characterized by the ratios $\varepsilon^{I}_{fr,\lambda} \cdot P^{I}_{fr}/\varepsilon^{II}_{fr,\lambda} \cdot P^{II}_{fr}$ and $\tau^{I}_{\frac{1}{2}}/\tau^{II}_{\frac{1}{2}}$ where P^{I}_{fr} and P^{II}_{fr} are two populations of phytochrome molecules which convert to P_r with a first order half-life of $\tau^{I}_{\frac{1}{2}}$ and $\tau^{II}_{\frac{1}{2}}$. These ratios depend on the length of time of etiolation. The ratio $\varepsilon^{I}_{fr,\lambda} \cdot P^{I}_{fr}/\varepsilon^{II}_{fr,\lambda} \cdot P^{II}_{fr}$ is independent of the amount of total P_{fr} present at the beginning of the $P_{fr} \rightarrow P_r$ phototransformation after a non-saturating dose of red light. The half-lives of the two populations, however, depend on the concentration of total P_{fr} initially present. $P_{fr} \rightarrow P_r$ phototransformation kinetics with different light intensities show that reciprocity holds.

Introduction

In a previous paper (Boisard, Marmé and Schäfer, 1971) it was shown that the phototransformation kinetics of phytochrome in pumpkin hooks deviate from first order curves. The time dependence of the $P_{fr} \rightarrow P_r$ light reaction could be described by assuming two populations of phytochrome molecules which are converted with two different rate constants. This treatment could also be an approximation of a single population converting with a single, but time-dependent, rate constant. This could be a consequence of an interaction of phytochrome molecules, which may be located in a well-ordered structure. In the present paper the phototransformations of phytochrome have been investigated in hooks and cotyledons of the mustard seedling.

Material and Methods

Plant Material. Mustard seedlings (*Sinapis alba*, L., 1969) were grown in the dark at 25° C on chromatography paper following a standard procedure (Mohr, 1966). The hooks and cotyledons were harvested in the cold using a dim green safelight, after different periods of etiolation.

Spectrophotometric Measurements of the $P_{fr} \rightarrow P_r$ Phototransformation kinetics were performed with a modified Ratiospect only (Marmé, 1969a, b). Parallel measurements on pumpkin hooks using a Ratiospect, a Cary 14 and a new spectrophotometer constructed by G. Larcher, Rouen (Larcher, 1971), demonstrated that the results as obtained with the Ratiospect can be reproduced with the other instruments (Boisard, Marmé and Schäfer, 1971). The measuring wavelengths were 728 nm (or 756 nm) and 805 nm. The intensity of the measuring beam was high enough to saturate the photoconversion within 20–25 min. The measuring temperature in all experiments was 0° C. At this temperature the decay of P_{fr} is negligible.

The $P_r \rightarrow P_{fr}$ *Phototransformation Kinetics* were performed with a Leitz Prado (for irradiation: $\lambda = 656$ nm) and the Ratiospect (for measurement of the newly formed P_{fr}; measuring wavelengths $\lambda_1 = 728$ nm, $\lambda_2 = 805$ nm).

Results

Phototransformation Kinetics $P_{fr} \rightarrow P_r$ *as a Function of the Length of Time of Etiolation.* The *in vivo* phototransformation kinetics of phytochrome $P_{fr} \rightarrow P_r$ were measured in mustard cotyledons and hooks, the $P_r \rightarrow P_{fr}$ phototransformation kinetics only in cotyledons. The curves are essentially of the same shape as those obtained from pumpkin hooks, i.e. they can be interpreted by assuming two populations of P_{fr}, (P_r) which convert with different rate constants to P_r, (P_{fr}) (Figs. 1 and 2). If one varies the time of etiolation between sowing and the photometric assay from 36 h to 120 h the $P_{fr} \rightarrow P_r$ phototransformation kinetics differ quantitatively from one another; 36 h after sowing the ratio of the two populations ($\varepsilon^{\mathrm{I}}_{fr,\lambda} \cdot P^{\mathrm{I}}_{fr} / \varepsilon^{\mathrm{II}}_{fr,\lambda} \cdot P^{\mathrm{II}}_{fr}$) is about 80/20. P^{I}_{fr} is the slow, and P^{II}_{fr} is the fast population; $\varepsilon^{\mathrm{I}}_{fr,\lambda}$, $\varepsilon^{\mathrm{II}}_{fr,\lambda}$ are the wavelength-dependent extinction coefficients of P^{I}_{fr} and P^{II}_{fr}. This value decreases to 50/50 at 72 h and increases again to about 65/35 at 120 h after sowing (Fig. 3). The ratio of the half-lives $\tau^{\mathrm{I}}_{\frac{1}{2}} / \tau^{\mathrm{II}}_{\frac{1}{2}}$ decreases from about 8.5 at 36 h to about 6.5 at 48 h and remains nearly constant until 120 h (Fig. 4). For comparison the ratios of the half-lives of pumpkin (Cucurbita pepo) and gourd (Cucurbita maxima) are plotted in Fig. 4.

The corresponding measurements of the phototransformation kinetics in mustard hooks reflect quite different behaviour. These experiments were started 48 h after sowing. At shorter periods of etiolation the total amount of phytochrome was too low to measure photoconversion kinetics. From 48 h to 60 h the ratio of the two populations ($\varepsilon^{\mathrm{I}}_{fr,\lambda} \cdot P^{\mathrm{I}}_{fr} / \varepsilon^{\mathrm{II}}_{fr,\lambda} \cdot P^{\mathrm{II}}_{fr}$) was about 55/45. During the next 12 h it increased to 65/35 and remained nearly constant up to 120 h (Fig. 5). The time-dependence of the ratios of the half-lives differs from those for cotyledons (Fig. 6). Using a 756 nm driving and measuring beam it could be shown for 48 h dark-grown mustard cotyledons that the ratios $\varepsilon^{\mathrm{I}}_{fr,\lambda} \cdot P^{\mathrm{I}}_{fr} / \varepsilon^{\mathrm{II}}_{fr,\lambda} \cdot P^{\mathrm{II}}_{fr}$ and $\tau^{\mathrm{I}}_{\frac{1}{2}} / \tau^{\mathrm{II}}_{\frac{1}{2}}$ did not depend on the wavelength of the driving beam.

Phototransformation Kinetics $P_{fr} \rightarrow P_r$ *after Various Degrees of Saturation of the* $P_r \rightarrow P_{fr}$ *Light Reaction.* In 48 h dark-grown mustard cotyledons one obtains 70 ± 3% for the slow converting P^{I}_{fr} and 30 ± 3% for the fast converting P^{II}_{fr} (Fig. 3). The $P_r \rightarrow P_{fr}$ photoconversion yields 59 ± 3% for

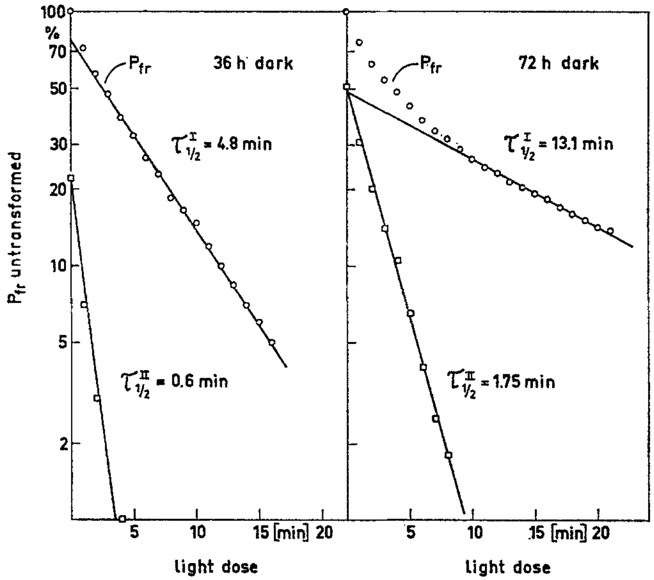

Fig. 1. *In vivo* kinetics of the phytochrome phototransformation ($P_{fr}\to P_r$) with 36 and 72 hours dark-grown mustard cotyledons. (○) represents the time course of total P_{fr}. (□) represents the calculated fast population

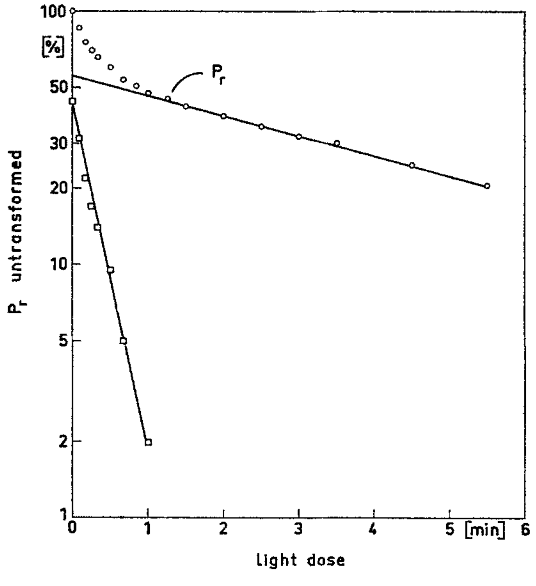

Fig. 2. *In vivo* kinetics of the phytochrome phototransformation ($P_r\to P_{fr}$) with 48 hours dark-grown mustard cotyledons. (○) represents the time course of total P_r. (□) represents the calculated fast population

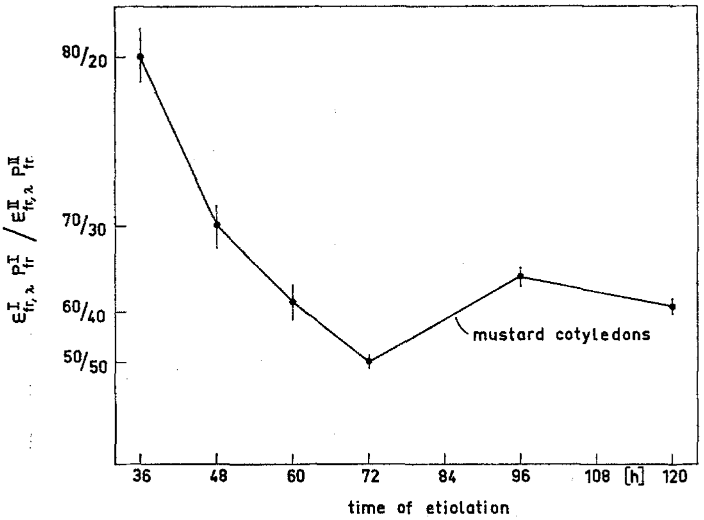

Fig. 3. Mustard cotyledons: dependence of the ratio $\varepsilon^{I}_{fr,\lambda} \cdot P^{I}_{fr}/\varepsilon^{II}_{fr,\lambda} \cdot P^{II}_{fr}$ on the length of time of etiolation. Each point represents the mean of at least 8 experiments with brackets equal to twice the standard error. The total P_{fr} at any time was normalized to 100%

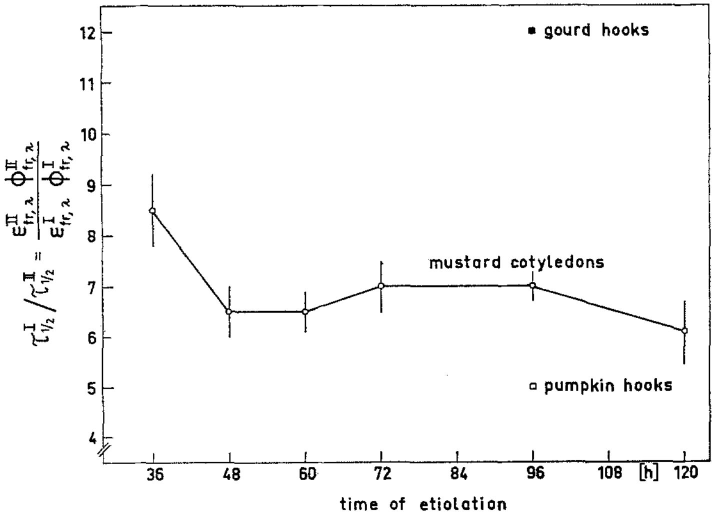

Fig. 4. Mustard cotyledons: dependence of the ratio $\tau^{I}_{\frac{1}{2}}/\tau^{II}_{\frac{1}{2}} = \varepsilon^{II}_{fr,\lambda} \cdot \phi^{II}_{fr,\lambda}/\varepsilon^{I}_{fr,\lambda} \cdot \phi^{I}_{fr,\lambda}$ on the length of time of etiolation. For comparison, the ratios of the two half-lives of P_{fr} in pumpkin and gourd hooks after 96 hours of etiolation are plotted in the same figure

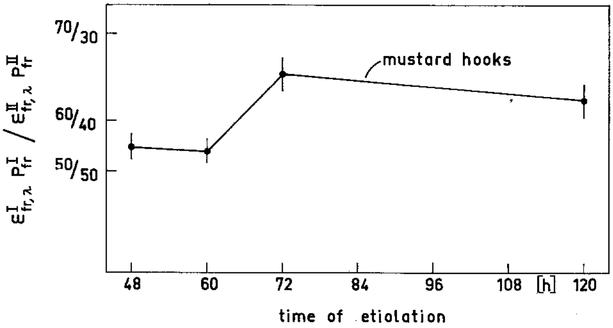

Fig. 5. Mustard hooks: dependence of the ratio $\varepsilon^{I}_{fr,\lambda} \cdot P^{I}_{fr}/\varepsilon^{II}_{fr,\lambda} \cdot P^{II}_{fr}$ on the length of time of etiolation. Each point represents the mean of at least 8 experiments with brackets equal to twice the standard error. The total P_{fr} at any time was normalized to 100%

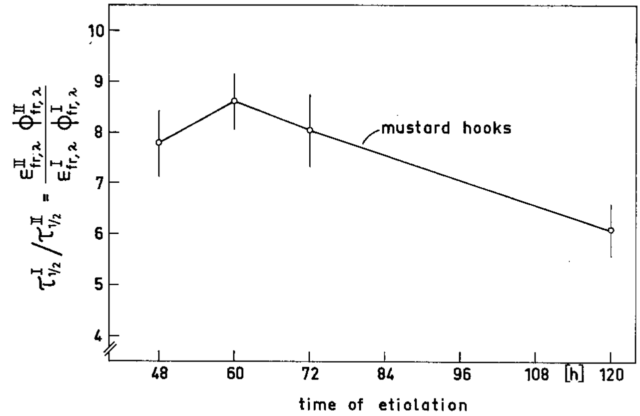

Fig. 6. Mustard hooks: dependence of the ratio $\tau^{I}_{\frac{1}{2}}/\tau^{II}_{\frac{1}{2}} = \varepsilon^{II}_{fr,\lambda} \cdot \phi^{II}_{fr,\lambda}/\varepsilon^{I}_{fr,\lambda} \cdot \phi^{I}_{fr,\lambda}$ on the length of time of etiolation

P^{I}_{r} and 41 ± 3% for P^{II}_{r} (mean of 8 experiments). One of the kinetic curves is plotted in Fig. 2. These data suggest that the phototransformation part of the models (Fig. 7) presented for pumpkin phytochrome (Boisard, Marmé and Schäfer, 1971) need to be modified, since one would expect the same ratios of the two populations for the phototransformations in both directions. To test this, the following experiment was carried out with 48 h dark-grown mustard cotyledons. The phototransformation, $P_r \rightarrow P_{fr}$, was begun after a saturating dose of far-red light and the photoreaction ceased at a point when almost all of the fast converting P^{II}_{r} had been transformed into P_{fr} (but only a small amount of the slow converting

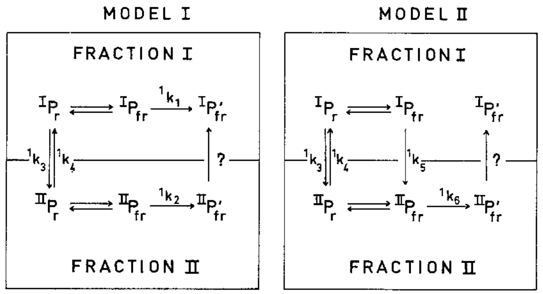

Fig. 7. Diagram of the two models of pumpkin phytochrome (from Boisard, Marmé and Schäfer, 1971: Fig. 5)

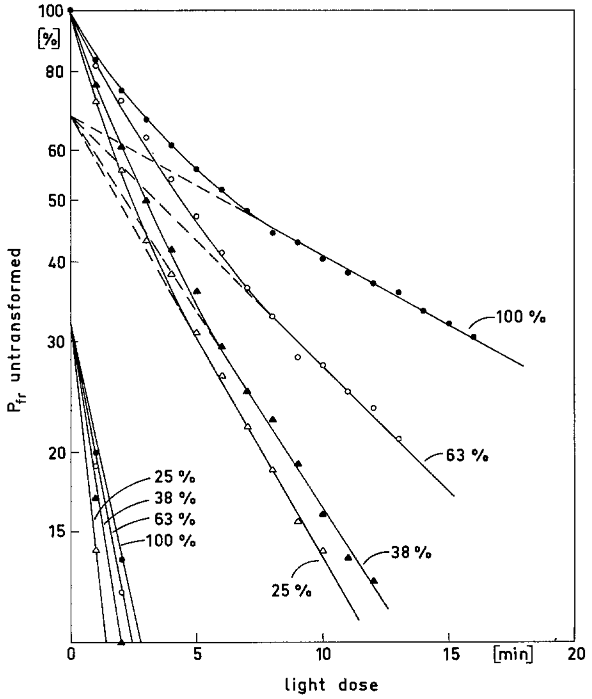

Fig. 8. $P_{fr} \rightarrow P_r$ phototransformation kinetics with 48 hours dark-grown mustard cotyledons after a saturating [(●), 100%] and various non-saturating doses of red light [(○), 63%; (▲), 38%; (△), 25%]. The amount of P_{fr} at the beginning of the phototransformation is normalized to 100%

P_r^{I}). The phototransformation kinetics of the newly formed P_{fr} to P_r were measured immediately. From the pumpkin model a strong increase of the fast converting P_{fr} population compared to the slow transforming one was

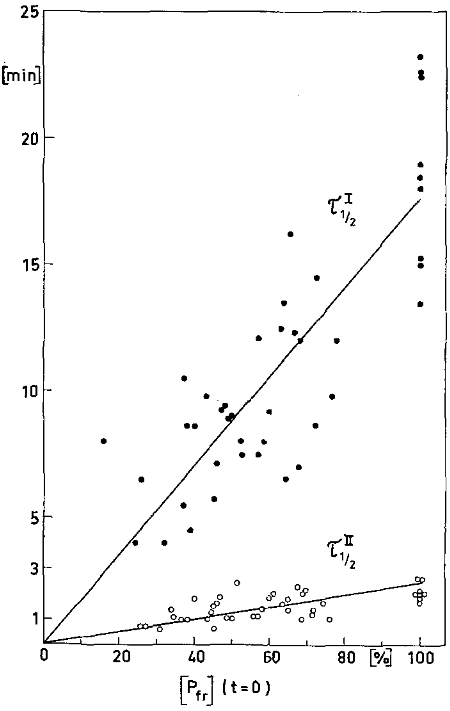

Fig. 9. $\tau_{\frac{1}{2}}^{I}$ (●) and $\tau_{\frac{1}{2}}^{II}$ (○) as a function of the amount of total P_{fr} present at the beginning of the phototransformation kinetics

expected. However, Fig. 8 shows that the ratio $\varepsilon_{fr,\lambda}^{I} \cdot P_{fr}^{I}/\varepsilon_{fr,\lambda}^{II} \cdot P_{fr}^{II}$ did not change. This experiment was repeated for various doses of red light, i.e. for different amounts of newly formed P_{fr}. The results are summarized in Fig. 8, which shows a set of phototransformation curves of the same sample. In all cases the ratios are the same, while the half-lives of the slow and the fast populations increased with increasing concentrations of total P_{fr} at the beginning of the photoconversion $P_{fr} \rightarrow P_{r}$. Fig. 9 shows the dependence of the half-lives $\tau_{\frac{1}{2}}^{I}$ and $\tau_{\frac{1}{2}}^{II}$ on the amount of P_{fr} initially present. $\tau_{\frac{1}{2}}^{I}$ (the half-life of the slow converting population) shows a pronounced dependence on the content of P_{fr}, whereas the increase of $\tau_{\frac{1}{2}}^{II}$ with increasing concentration of P_{fr} was only slight. In Fig. 10 the half-lives of each set of phototransformations obtained from one sample were normalized to the corresponding half-lives after a saturating dose of red light, i.e. to the highest content of P_{fr} ($=100\%$). Similar results were obtained for 96 h dark-grown pumpkin hooks.

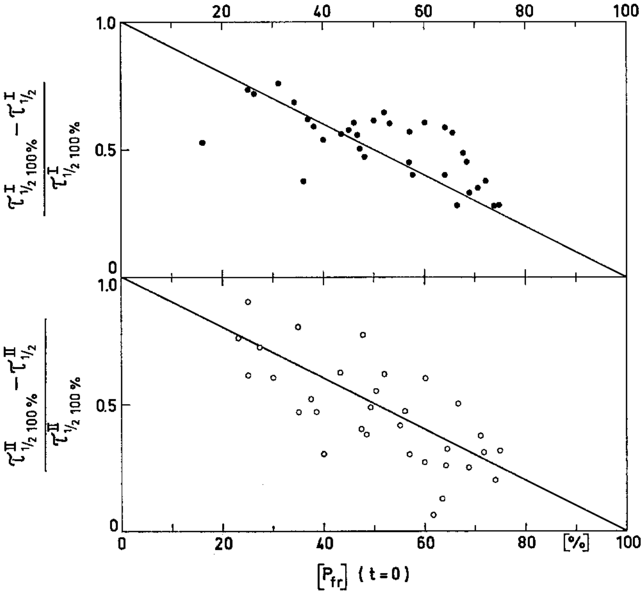

Fig. 10. $\frac{\tau^{I}_{\frac{1}{2}100\%} - \tau^{I}_{\frac{1}{2}}}{\tau^{I}_{\frac{1}{2}100\%}}$ (●) and $\frac{\tau^{II}_{\frac{1}{2}100\%} - \tau^{II}_{\frac{1}{2}}}{\tau^{II}_{\frac{1}{2}100\%}}$ (○) as a function of the amount of total P_{fr} present at the beginning of the phototransformation kinetics

Table. *Test of reciprocity of the $P_{fr} \rightarrow P_r$ light reaction with two different light doses*

Light dose	I	t	P_{fr} transformed (%)	
			experiment I	experiment II
a) $I_1 \times t_a$	I_1	t_a	42	44
	$4.2\ I_1$	$1/4.2 \cdot t_a$	44	43.5
	$29\ I_1$	$1/29 \cdot t_a$	42	42
b) $I_1 \times t_b = 3\ (I_1 \times t_a)$	I_1	t_b	64	64.5
	$4.2\ I_1$	$1/4.2 \cdot t_b$	64.5	63.5
	$29\ I_1$	$1/29 \cdot t_b$	63.5	63.5

Law of Reciprocity. To test reciprocity, experiments with different intensities of photoconverting light were performed. Fig. 11 shows the results for two intensities, I_1 and I_2, with $I_1 = 4.4 \cdot I_2$. The kinetics obtained show no significant differences.

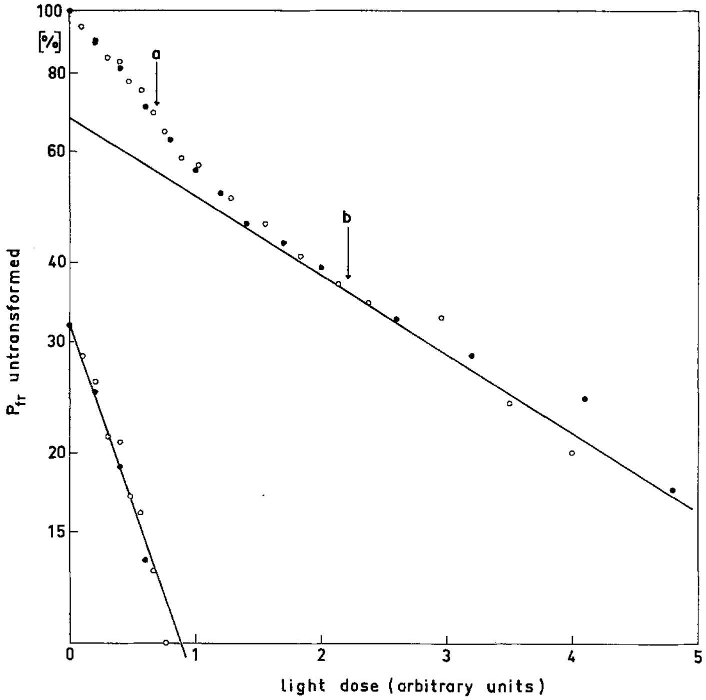

Fig. 11. Two $P_{fr} \rightarrow P_r$ phototransformation kinetics of 48 hours dark-grown mustard seedlings, performed with different intensities of illumination [(○), I_1; (●) 4.4 · I_1] and plotted as P_{fr} untransformed as a function of light dose

To confirm reciprocity over a larger intensity interval, the converted P_{fr} was measured for two light doses only (arrows a and b in Fig. 11). Each light dose was obtained with three different intensities: $I_1 = 4.2\, I_2 = 29.0\, I_3$. The Table demonstrates that no deviation from reciprocity was detected.

Discussion

Phototransformation Kinetics $P_{fr} \rightarrow P_r$ as a Function of the Length of Time of Etiolation. Assuming two time-independent rate constants k_1 and k_2 for the photoconversion, and two extinction coefficients $\varepsilon^{\mathrm{I}}_{fr,\lambda}$ and $\varepsilon^{\mathrm{II}}_{fr,\lambda}$ for the two populations, the time course of the total P_{fr} can be described by the equation:

$$\varepsilon_{fr,\lambda} \cdot P_{fr} = \varepsilon^{\mathrm{I}}_{fr,\lambda} \cdot P^{\mathrm{I}}_{fr} + \varepsilon^{\mathrm{II}}_{fr,\lambda} \cdot P^{\mathrm{II}}_{fr} = \varepsilon^{\mathrm{I}}_{fr,\lambda} \cdot [P^{\mathrm{I}}_{fr}]^{t=0} \cdot e^{-k_1 \cdot t} + \varepsilon^{\mathrm{II}}_{fr,\lambda} \cdot [P^{\mathrm{II}}_{fr}]^{t=0} \cdot e^{-k_2 \cdot t}$$

Figs. 3 and 5 show that the ratio of the two populations is a function of the length of time of etiolation:

$$\frac{\varepsilon^{\mathrm{I}}_{fr,\lambda} \cdot P^{\mathrm{I}}_{fr}}{\varepsilon^{\mathrm{II}}_{fr,\lambda} \cdot P^{\mathrm{II}}_{fr}} = f(t = \text{time of etiolation}).$$

From the 756 nm phototransformation kinetics of mustard cotyledons, it follows that the proportion $\varepsilon^{\mathrm{I}}_{fr,\lambda}/\varepsilon^{\mathrm{II}}_{fr,\lambda} \neq f(\lambda)$. $\varepsilon^{\mathrm{I}}_{fr,\lambda}$ and $\varepsilon^{\mathrm{II}}_{fr,\lambda}$, however, are wavelength-dependent. Without speculating on the function $f(t)$ itself, one may state that if

a) $\varepsilon^{\mathrm{I}}_{fr,\lambda}/\varepsilon^{\mathrm{II}}_{fr,\lambda} \neq f(t)$, then $P^{\mathrm{I}}_{fr}/P^{\mathrm{II}}_{fr}$ must be a function of time.

b) $\varepsilon^{\mathrm{I}}_{fr,\lambda}/\varepsilon^{\mathrm{II}}_{fr,\lambda} = f(t)$, then $P^{\mathrm{I}}_{fr}/P^{\mathrm{II}}_{fr}$ need not necessarily be a function of time.

The ratio of the half-lives $\tau^{\mathrm{I}}_{\frac{1}{2}}/\tau^{\mathrm{II}}_{\frac{1}{2}}$ from mustard cotyledons for $t > 48$ h (Fig. 4) is not a function of time and wavelength:

$$\tau^{\mathrm{I}}_{\frac{1}{2}}/\tau^{\mathrm{II}}_{\frac{1}{2}} = \frac{\varepsilon^{\mathrm{II}}_{fr,\lambda} \cdot \phi^{\mathrm{II}}_{fr,\lambda} \cdot N_\lambda}{\varepsilon^{\mathrm{I}}_{fr,\lambda} \cdot \phi^{\mathrm{I}}_{fr,\lambda} \cdot N_\lambda} \neq f(t, \lambda)_{t>48\,h}.$$

ϕ_λ is the quantum efficiency; N_λ is the intensity of the converting light. From this equation, it follows that if

a) $\varepsilon^{\mathrm{I}}_{fr,\lambda}/\varepsilon^{\mathrm{II}}_{fr,\lambda} = f(t)$, then $\phi^{\mathrm{I}}_{fr,\lambda}/\phi^{\mathrm{II}}_{fr,\lambda}$ must also depend on time.

b) $\varepsilon^{\mathrm{I}}_{fr,\lambda}/\varepsilon^{\mathrm{II}}_{fr,\lambda} \neq f(t)$, then $\phi^{\mathrm{I}}_{fr,\lambda}/\phi^{\mathrm{II}}_{fr,\lambda} \neq f(t)$.

Within the time interval 36 h $< t <$ 48 h, when $\tau^{\mathrm{I}}_{\frac{1}{2}}/\tau^{\mathrm{II}}_{\frac{1}{2}}$ is a function of time, the correlation between ε and ϕ is more complex. The data from mustard hooks (Figs. 5 and 6) show that the phototransformation properties of mustard phytochrome can be quite different in different organs.

Phototransformation Kinetics $P_{fr} \rightarrow P_r$ after Various Degrees of Saturation of the $P_r \rightarrow P_{fr}$ Light Reaction. Fig. 8 shows the independence of the ratio $\varepsilon^{\mathrm{I}}_{fr,\lambda} \cdot P^{\mathrm{I}}_{fr}/\varepsilon^{\mathrm{II}}_{fr,\lambda} \cdot P^{\mathrm{II}}_{fr}$ from the concentration of P_{fr} initially present. To explain these data one must assume, instead of the two parallel light reactions (Fig. 7), a mechanism for a rapid redistribution of the newly formed P_{fr} to a constant proportion in each of the two P_{fr} pools. This suggestion can be illustrated by a hypothetical scheme as shown in Fig. 12. The red light converts P^{I}_r and P^{II}_r to a common intermediate P_1 which fills up the P^{I}_{fr} and P^{II}_{fr} pools to form a constant ratio in the dark. The extent of these reactions are functions of the length of time of etiolation (Fig. 3). The phototransformation half-lives of the two populations depend on the total P_{fr} present at the beginning of the $P_{fr} \rightarrow P_r$ photoconversion (Fig. 9). $\tau^{\mathrm{I}}_{\frac{1}{2}}$ and $\tau^{\mathrm{II}}_{\frac{1}{2}}$ seem to increase linearly (but with different slopes) with increasing total P_{fr}. No saturation of the increase could be detected. Fig. 10 shows in a more clearcut way the dependence of both half-lives on the amount of P_{fr}. If the linear extrapolation of the curves is correct, both

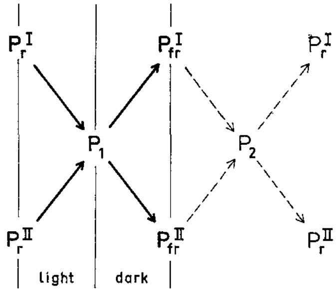

Fig. 12. Modified model of the phototransformation reaction in mustard and pumpkin seedlings. The photoreaction step of $P_r \rightarrow P_{fr}$ is based on the data given in Fig. 8, whereas the dotted arrows represent an analogue behaviour of the $P_{fr} \rightarrow P_r$ photoreaction (which has not yet been justified)

half-lives seem to become extremely small when total $P_{fr} \rightarrow 0\%$. When the amount of P_{fr} at the beginning of the $P_{fr} \rightarrow P_r$ phototransformation is low, it is much more probable that a P_{fr} molecule will be transformed by an absorbed far-red photon than when the total P_{fr} concentration is high; whereas during a phototransformation kinetics (Figs. 1, 2, 8) the amount of P_{fr} (P_r) seems to have no influence on the probability of phototransformation ($\varepsilon \cdot \phi$).

Law of Reciprocity. Fig. 11 and the Table show no deviation from the law of reciprocity. The ratio of the two phytochrome populations, as defined by a formal treatment, does not depend on the intensity of the photoconverting light.

We do not yet know which molecular properties of the phytochrome are reflected by the shape of the photoconversion kinetics. There may be two populations (in this case our treatment is not only a formal one), or there may exist some sort of cooperative mechanism which leads to a time-dependent rate constant. Experiments on decay, dark reversion and photostationary states in vivo have already shown that the light reaction $P_{fr} \rightarrow P_r$ is not the only process which depends on the length of time of etiolation (Marmé, Marchal and Schäfer, 1971; Schäfer, Marchal and Marmé, 1971). We also do not yet know the molecular mechanism of the dependence of the half-lives of the $P_{fr} \rightarrow P_r$ phototransformation on the concentration of P_{fr} at the beginning of the phototransformation. The data suggest either that the increasing P_{fr} concentration inhibits the $P_{fr} \rightarrow P_r$ light reaction, or that the increasing P_r concentration stimulates the $P_{fr} \rightarrow P_r$ photoconversion. On the other hand, the half-lives of the two populations do not change during a photoconversion kinetics. The phytochrome system, at least in mustard and pumpkin seedlings as detected by spectrophotometric methods in vivo, becomes increasingly complex.

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