Laser Flash Photolysis of 124-Kilodalton Oat Phytochrome in H_2O and D_2O Solutions: Formation and Decay of the I_{700} Intermediates

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Received June 19, 1986; Revised Manuscript Received November 5, 1986

ABSTRACT: The transient absorbance difference (ΔA) after laser flash photolysis (λ^{exc} = 650 nm) of the red-absorbing form (P_r) of 124-kDa oat phytochrome in H_2O and D_2O buffers was monitored at 696 nm in the temperature range 265–297 K. When extrapolating back to zero time, the value thus obtained for ΔA was unaffected by temperature and the H_2O/D_2O change. Furthermore, the flash energy dependence was similar in both buffers. These observations are consistent with the hypothesis that no proton transfer takes place in the primary photoreaction initiating the transformation of P_r to the far-red-absorbing form of phytochrome, in agreement with the preceding paper on a study of fluorescence lifetimes and quantum yields in the same buffer systems. The first-order decay rate constants of the I_{700}^i transients, formed as the primary photoproducts of P_r, are 20% lower in D_2O. This is compatible with a *solvent* H/D isotope effect but rather too small for an isotope effect on a proton transfer at room temperature in the transformation of I_{700}^i to subsequent products.

The primary products of the photochemical process transforming red- into far-red-absorbing phytochrome ($P_r \rightarrow P_{fr}$)[1] are a set of (at least) two intermediates, I_{700}^i, which are formed with a quantum yield of ≥0.5 and have already been spectroscopically and kinetically characterized [for the partially degraded 60- and 114/118-kDa phytochromes, see Braslavsky et al. (1980), Cordonnier et al. (1981), Inoue et al. (1982), Linschitz and Kasche (1967), Linschitz et al. (1966), Pratt and Butler (1970), Pratt et al. (1982), and Shimazaki et al. (1980); for "native" 124-kDa phytochrome, see Eilfeld and Rüdiger (1985), Furuya (1983), Inoue and Furuya (1985), Heihoff et al. (1987), Pratt et al. (1984), and Ruzsicska et al. (1985)]. They have an absorption maximum at 696 nm and convert thermally into the next set of intermediates, I_{bl}^i (Kendrick & de Kok, 1983), with lifetimes of about 3 and 30 μs at room temperature.

Much has been speculated about the molecular aspects of the $P_r \rightarrow P_{fr}$ transformation. $E \rightarrow Z$ isomerization of C=C double bonds, proton shifts, and conformational changes of chromophore and protein are being considered to play a role, but neither has the photochemical process been unequivocally identified nor is the chemical nature of the subsequent ground-state reactions known.

In an investigation of a possible proton transfer in the primary photoreaction of P_r, the fluorescence of P_r in H_2O and D_2O buffers has been studied by picosecond time-resolved detection and reported in the preceding paper (Brock et al., 1987). The results established that the change of solvent influences neither excited-state lifetimes (ca. 45 and 180 ps) nor fluorescence quantum yields (ca. 3 × 10^{-3}) of the two shorter lived functional (photoreversible) components (>90% and ca. 8%, respectively) of P_r. This is in contrast to conclusions by Moon et al. (1985) in a study with stationary fluorescence, which does not discriminate between components whose individual contributions to the total emission are vastly different. Although a proton-transfer process in the photoreaction was considered unlikely on the basis of the work by Brock et al. (1986), there remained the possibility—albeit also judged improbable—of a fortuitous compensation of excited-state P_r reaction and radiationless deactivation.

We now report on an investigation of the influence of D_2O (i) on the formation of the I_{700}^i's in the flash photolysis with visible detection of 124-kDa P_r and (ii) on the kinetics of the subsequent thermal decay of I_{700}^i to I_{bl}^i. The aim of our study was thus to complement the information on the initial photochemical step of the $P_r \rightarrow P_{fr}$ transformation and to provide a similar insight into the subsequent first set of ground-state reactions.

EXPERIMENTAL PROCEDURES

Chemicals. Ethylene glycol-d_6 and D_2O were Merck Uvasol (deuterium grade, minimum 99% and 99.75%, respectively). For other chemicals, the isolation and characterization of phytochrome, the preparation of the sample in H_2O and D_2O solutions, and their handling, see Brock et al. (1987).

The concentrations of the air-saturated P_r solutions were in the range 0.75–2.5 μM, calculated from the measured absorbance and an absorption coefficient at 667 nm of 1.20 × 10^5 M^{-1} cm^{-1} (Kelly & Lagarias, 1985). The values therefore refer to chromophore concentrations. No loss in absorbance was noticed even after some 100 laser flashes each followed by photoreversion with far-red irradiation. For concentrations ≤1.2 μM, no turbidity was noticed in H_2O buffer solutions from room temperature down to 265 K. Only H_2O samples of higher concentrations turned turbid upon being cooled but completely cleared again upon being returned to room temperature. While dilute D_2O samples (0.75 μM) could be kept free from turbidity at room temperature, it was impossible to completely avoid this effect at lower temperatures. The otherwise negligible turbidity of the more concentrated D_2O samples increased upon being cooled and only incompletely disappeared again on being warmed up to room temperature.

Flash Photolysis. The laser flash photolysis apparatus with absorption detection has been described previously (Braslavsky et al., 1980; Neumann et al., 1984). The samples were excited at 650 nm (frequency-doubled Nd-YAG pumped DCM dye

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[1] Abbreviations: I_{700}^i, (set of) primary photoproducts in the $P_r \rightarrow P_{fr}$ transformation; I_{bl}^i, (set of) intermediates formed in a ground-state reaction from I_{700}^i; P_r and P_{fr}, red- and far-red-absorbing forms of phytochrome, respectively.

0006-2960/87/0426-1418$01.50/0

Table I: Average Values for the Two First-Order Decay Rate Constants ($1/\tau_i$) of the I^i_{700}'s at Different Temperatures in H_2O and D_2O Solutions and Corresponding Arrhenius Parameters (E_a, A), $E_0 > 25$ mJ[a]

	$1/\tau_1 \times 10^{-5}$ (s^{-1}, ±25%)		$1/\tau_2 \times 10^{-4}$ (s^{-1}, ±10%)	
T (K)	H_2O	D_2O	H_2O	D_2O
297	3.0 (14)	2.6 (9)	2.82 (14)	2.57 (9)
290	1.4 (7)	1.1 (5)	1.51 (7)	1.25 (5)
284	1.5 (4)	0.86 (4)	1.05 (4)	0.85 (4)
277	0.57 (9)	0.52 (6)	0.533 (9)	0.458 (6)
269	0.35 (10)	0.21 (3)	0.339 (10)	0.262 (3)
265	0.23 (6)	0.18 (2)	0.221 (6)	0.187 (2)

E_a (kJ·mol^{-1})				log A (s^{-1})			
for $1/\tau_1$		for $1/\tau_2$		for $1/\tau_1$		for $1/\tau_2$	
H_2O	D_2O	H_2O	D_2O	H_2O	D_2O	H_2O	D_2O
50 ± 2 (56)	55 ± 4 (29)	50 ± 1 (56)	54 ± 1 (29)	14.1 ± 0.4	15.1 ± 0.7	13.3 ± 0.2	13.9 ± 0.3
54 ± 1[b]		58 ± 1[b]		14.9 ± 0.2[b]		14.6 ± 0.1[b]	

[a] Number of experiments used for the calculation of the average is shown in parentheses. The determinations were performed with sets of nine H_2O and five D_2O samples, repeating some of the measurements at randomly varied temperatures. Errors are standard deviations. [b] Data from Ruzsicska et al. (1985).

laser) with 15-ns flashes of an energy between 3 and 35 mJ. The flash light impinged on the frosted side of a quartz cuvette. The cuvettes used were either square (1 cm), thermostated with a Peltier element, or rectangular with a 4-mm excitation path and a 1-cm analysis path and equipped with a jacket for external liquid cooling. A pulsed Xe lamp served as the analysis light source. The beam passed through a 695-nm cut-off filter in order to minimize photoconversion of the sample. Excitation and analysis beams were at right angles. After flashing, the transmitted analysis light was selected by a monochromator at 696 nm and detected with a Hamamatsu R928 photomultiplier. The energy of each flash was measured by a pyroelectrical energy meter (Rj-7100 and RjP-735, Laser Precision Corp.) and adjusted to the desired value with neutral density filters. The signal was fed into a Biomation 8100 transient recorder (10-ns sampling time), and successive shots were averaged by a PDP11/04-VAX 780 computer system.

Phytochrome was transformed back to the P_r form after each flash with saturating far-red light from a slide projector provided with a 2-mm RG 9 filter (Schott, Mainz). Thus, repeated cycling guaranteed that D was exhaustively exchanged for H in the D_2O runs and any D/H effect was maximized.

RESULTS

Flash photolyses of 124-kDa P_r were performed in the temperature range 261–297 K at concentrations from 0.75 to 2.5 μM. In order to explore a possible kinetic H/D isotope effect, the I^i_{700} decays measured in the D_2O and H_2O buffers were compared. The evolution of the absorbance difference (ΔA) with time was fitted to a sum of two exponentials and a constant term, according to eq 1, for a period of up to 2 ms

$$\Delta A = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) + a_3 \qquad (1)$$

after the laser flash. This expression contains five adjustable constants, of which three (a_1, a_2, and a_3) are amplitudes, and two ($1/\tau_1$ and $1/\tau_2$) are first-order rate constants. Equation 1 has already proved to be the most adequate to fit the decay curves of I^i_{700} in a broad range of wavelengths stretching from 410 to 715 nm (Ruzsicska et al., 1985). Second-order kinetics can be ruled out on the basis of the invariance of the lifetimes when the laser energy was varied by 1 order of magnitude and the P_r concentration by a factor of 4. The values for τ_1 range from 3 to 50 μs and for τ_2 from 35 to 500 μs in the temperature range of 261–297 K. By use of the fitted curve, the initial absorbance difference (ΔA_{init}) after the laser flash was calculated. Typical values are $a_1 \simeq a_2/4$ and $a_3 \simeq 0.20\Delta A_{init}$.

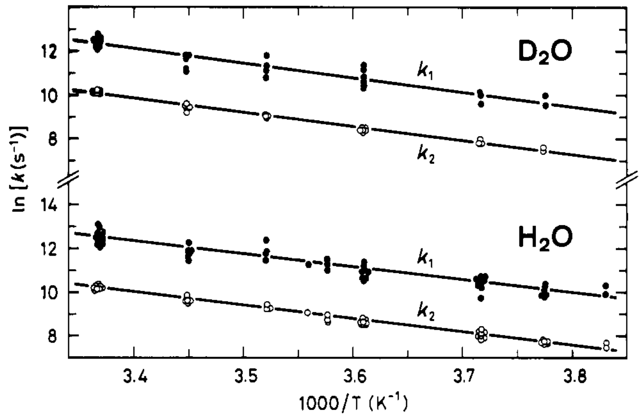

FIGURE 1: Arrhenius plot for the first-order decay rate constants of I^i_{700}. Corresponding parameters are listed in Table I.

Table I gives rate constants at the different temperatures and the Arrhenius parameters. The Arrhenius plots for both decay lifetimes in the H_2O and D_2O media are shown in Figure 1. The lifetimes in D_2O typically are about 20% higher than in H_2O. This difference is significant since it has systematically been found in samples from several P_r isolations and in measurements performed many months apart.

Our previously measured rate constants in H_2O (Ruzsicska et al., 1985) are within the error range of the present data (see Table I) at all temperatures, and the error ranges are similar in both studies. An apparent discrepancy between the Arrhenius parameters derived from the two sets of rate constants is due to the greater accuracy of the present data, which have been obtained from more measurements performed over a wider temperature range.

The ΔA_{init} values listed in Table II show no temperature or solvent dependence for the more dilute samples. At the higher concentration, both ΔA_{init} and the turbidity concomitantly increased upon cooling. The fact that the ΔA_{init} values were consistently higher in D_2O may be an artifact due to the turbidity. The data of this concentration series have therefore not been evaluated any further.

Finally, the dependence of ΔA_{init} on the laser fluence was measured in both media (since the irradiated cross section was kept constant, the fluence was always proportional to the measured energy). The results were adjusted to an empirical equation of the form (Lachish et al., 1976)

$$\Delta A_{init} = C_1[1 - \exp(-C_2E_0)] \qquad (2)$$

in which C_1 and C_2 are adjustable constants and E_0 is the laser

Table II: Initial Absorbance Differences, ΔA_{init}, of the I^{i}_{700} Transients at 696 nm for Two Phytochrome Concentrations in H_2O and D_2O at Different Temperatures, after Laser Excitation of 124-kDa Pr, $\lambda^{\text{exc}} = 650$ nm and $E_0 > 25$ mJ[a]

	$\Delta A_{\text{init}} \times 10^2$ (±0.05)			
	$A_{667} = 0.30$		$A_{667} = 0.09$	
T (K)	H_2O*	D_2O*	H_2O**	D_2O**
297	2.72	2.93	0.65	0.67
290	2.88	3.16	0.73	0.69
284	3.20	3.39		
277	3.29	3.64	0.64	0.68
269			0.64	0.64

[a] Errors are standard deviations. (*) and (**) are designations indicating columns with values of matched sample pairs measured under identical geometrical conditions, i.e., the same setting of excitation and analysis beams.

Table III: Laser Flash Energy Dependence of Initial I^{i}_{700} Absorbance at 696 nm for H_2O and D_2O Media at 297 K: Best Fit Constants to Equation 2 of the Values Shown in Figure 2[a]

medium	$C_1 \times 10^2$	C_2 (mJ^{-1})	A_{650}[b]
H_2O*	1.08 ± 0.04	0.16 ± 0.02	0.043
D_2O*	1.12 ± 0.04	0.11 ± 0.01	0.043
H_2O**	1.90 ± 0.04	0.20 ± 0.01	0.048
D_2O**	2.02 ± 0.04	0.22 ± 0.01	0.048

[a] Errors are standard deviations. (*) and (**) are designations indicating matched sample pairs [see (*) and (**) explanation in Table II footnote]. [b] Absorbance along a laser path of 4 mm.

flash energy (in mJ). The results for two matched samples are shown in Figure 2. The results of Table III indicate no dependence of C_1 or C_2 on solvent. On the other hand, the energy dependence of the transient absorption amplitude in Figure 2 suggests that the decay measurements (see Tables I and II) must have been performed in the energy saturation region ($E_0 > 25$ mJ).

DISCUSSION

The measurements carried out in this work involve two different consecutive processes: the primary photochemical step of the P_r transformation, to which the values of ΔA_{init} are linked, and the decay of the I^{i}_{700} intermediates to the I^{i}_{bl}'s, for which the rate constants were determined.

The ΔA_{init} value is in principle a measure of the efficiency of the primary photoreaction channel. Since the lifetime of excited P_r is very short [45 ps: Brock et al. (1987)], photochromic interconversion I^{i}_{700}'s $\rightleftarrows \text{P}_\text{r}$ is established already during the 15-ns duration of the excitation flash [Holzwarth et al., 1984; Jabben et al., 1984; cf. also Pratt and Butler (1970) and Pratt et al. (1984)], which complicates the interpretation of the ΔA_{init} values.

The most general mechanism for our system, which accounts for the build up of the I^{i}_{700}'s during the flash and discards triplet-state formation (Land, 1979; Jabben et al., 1984), is shown in Chart I.

The thermal reactions of the I^{i}_{700}'s are not considered because they occur on a much longer timescale ($\text{I}^{i}_{700} \rightarrow \text{I}^{i}_{\text{bl}}$ occurs in the microsecond range; cf. Table I).

Since absorbance at 650 nm was very low (see Table III), the rates of absorption were approximated in Chart I, where ϵ_e and ϵ'_e are the molar absorption coefficients of P_r and the I^{i}_{700}'s at the excitation wavelength and l is the length of the excitation beam. Assuming steady-state conditions for the excited species, the I^{i}_{700} concentration at the end of the flash, $[\text{I}^{i}_{700}]_{t=0}$, is given by eq 3 (for the derivation see Appendix).

$$[\text{I}^{i}_{700}]_{t=0} = C_3[1 - \exp(-C_2E_0)] \tag{3}$$

The result depends on the total energy of the laser flash and not on the pulse shape. A similar dependence of the signal to that given by eq 3 has been obtained by Lachish et al. (1976) for a two-level system. Our kinetic scheme, though more complicated, provides for the same type of dependence because a stationary state is established. Nevertheless, the constants (C_2 and C_3) have a different meaning here, as defined in eq 4 and 5. In these equations, $\phi_{\text{react}} = k_{\text{react}}/(k_{\text{react}} + k_\text{f}$

$$C_2 = \kappa l(\ln 10)(\phi'_{\text{react}}\epsilon'_\text{e} + \phi_{\text{react}}\epsilon_\text{e})/V \tag{4}$$

$$C_3 = \phi_{\text{react}}\epsilon_\text{e}[\text{P}_\text{r}]_{\text{tot}}/(\phi'_{\text{react}}\epsilon'_\text{e} + \phi_{\text{react}}\epsilon_\text{e}) \tag{5}$$

$+ k_\text{d})$. An equivalent expression defines ϕ'_{react} (i.e., ϕ_{react} and ϕ'_{react} are the quantum efficiencies of the reaction channels of P_r and the I^{i}_{700}'s, respectively). $[\text{P}_\text{r}]_{\text{tot}}$ is the total phytochrome concentration (=phytochrome concentration before the flash), κ is a constant converting moles of photons into energy, and V is the irradiated volume.

Since the absorption spectra of P_r and the I^{i}_{700}'s strongly overlap

$$\Delta A_{\text{init}} = (\epsilon'_\text{a} - \epsilon_\text{a})l_\text{a}[\text{I}^{i}_{700}]_{t=0} \tag{6}$$

where ϵ_a and ϵ'_a refer to the analyzing wavelength and l_a is the beam length in the analysis direction. When $[\text{I}^{i}_{700}]_{t=0}$ in eq 6 is replaced by the expression in eq 3, an equation analogous to eq 2 is obtained (with $C_1 = \Delta\epsilon_\text{a}l_\text{a}C_3$). Nevertheless, some additional considerations should be taken into account before the experimental data are interpreted. An incomplete overlap of the laser and analysis beams will cause uncertainties in the values of V and l_a (Bazin & Ebbesen, 1983), which precludes the calculation of absolute concentrations values. At low absorbances and with first-order rate constants only, the real

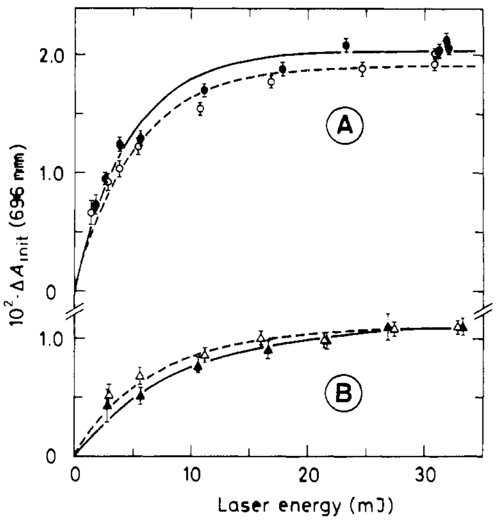

FIGURE 2: Absorbance difference at 696 nm, ΔA_{init}, after the laser flash ($\lambda^{\text{exc}} = 650$ nm) as a function of the flash energy and fitted curves according to eq 7, for two matched sample pairs (see footnotes b and c of Table II): (A) (●) in H_2O and (○) in D_2O; (B) (▲) in H_2O and (△) in D_2O. For fitted parameters see Table III. Errors are standard deviations.

Chart I

process	rate
$\text{P}_\text{r} + h\nu \rightarrow \text{P}^*_\text{r}$	$I_0(\ln 10)\epsilon_\text{e}l[\text{P}_\text{r}]$
$\text{P}^*_\text{r} \rightarrow \text{P}_\text{r} + h\nu_\text{f}$	$k_\text{f}[\text{P}^*_\text{r}]$
$\text{P}^*_\text{r} \rightarrow \text{P}_\text{r} + \Delta$	$k_\text{d}[\text{P}^*_\text{r}]$
$\text{P}^*_\text{r} \rightarrow \text{I}^{i}_{700}$	$k_{\text{react}}[\text{P}^*_\text{r}]$
$\text{I}^{i}_{700} + h\nu \rightarrow \text{I}^{i*}_{700}$	$I_0(\ln 10)\epsilon'_\text{e}l[\text{I}^{i}_{700}]$
$\text{I}^{i*}_{700} \rightarrow \text{I}^{i}_{700} + h\nu_\text{f}$	$k'_\text{f}[\text{I}^{i*}_{700}]$
$\text{I}^{i*}_{700} \rightarrow \text{I}^{i}_{700} + \Delta'$	$k'_\text{d}[\text{I}^{i*}_{700}]$
$\text{I}^{i*}_{700} \rightarrow \text{P}_\text{r}$	$k'_{\text{react}}[\text{I}^{i*}_{700}]$

and the measured absorbances remain proportional, permitting the evaluation of relative changes for matched samples under identical geometrical conditions [see (*) and (**) explanation in Table II footnote]. Since C_1 and C_2 are determined by relatively numerous unknowns, it is easier to consider the quantity R defined in eq 7. R depends on the geometrical

$$R = C_1C_2/\kappa(\ln 10)A_{650} = \Delta\epsilon_a l_a \phi_{\mathrm{react}}/V \qquad (7)$$

arrangement of the excitation and analysis beams through V and l_a and on the medium through $\Delta\epsilon_a$ and ϕ_{react}. The ratio of the R's for matched samples (see above and footnote *a* in Table II) does not depend on V and l_a and gives the relative value of the product $\Delta\epsilon_a\phi_{\mathrm{react}}$. For the two pairs of matched samples in Table III, we obtain H/D ratios for $\Delta\epsilon_a\phi_{\mathrm{react}}$ of 1.3 ± 0.2 and 0.9 ± 0.1. No trend is seen in these values, which would indicate that a proton transfer is taking place in the primary process.

The situation is thus reminiscent of that encountered when searching in vain for an unequivocal H/D effect on the $\mathrm{P_r}$ fluorescence lifetime and quantum yield (Brock et al., 1987). Proton transfer was judged unlikely to participate at the deactivation of the $\mathrm{P_r}$ excited state, although it was recognized that accidentally compensating changes in k_d and k_{react} might render the sum of the two rate constants solvent independent and hence the fluorescence lifetime invariant in H_2O and D_2O. Similarly, it cannot be ruled out that the invariance of the product $\Delta\epsilon_a\phi_{\mathrm{react}}$ results from compensatory variations of the two factors. However, an exact compensation of all these quantities appears forbiddingly fortuitous. We rather prefer the alternative hypothesis that a proton shift is involved neither in the primary photoreaction of the $\mathrm{P_r} \rightarrow \mathrm{P_{fr}}$ transformation nor in any other deactivation mode of excited $\mathrm{P_r}$.

The conclusions to be drawn from the H/D response of the rate constants of the second reaction step, $\mathrm{I}^{i}_{700} \rightarrow \mathrm{I}^{i}_{\mathrm{bl}}$, are less secure. A solvent isotope effect decreases these rate constants by 20% on changing from H_2O to D_2O. This effect is much less than expected for a kinetic H/D effect on a proton-transfer process. The value of k_H/k_D strongly depends on the nature of the transition state, but it normally is not below 1.4 at room temperature (Bell, 1974; Kwart, 1982). The magnitude observed here rather is characteristic of a solvent isotope effect influencing reaction steps in which no proton shift is involved, provided that reactants and transition state differ in solvation status [see, for instance, Blandamer et al. (1984), Matta et al. (1985), and Swain and Bader (1960)]. We should keep in mind, however, that quite an extended temperature range is needed in order to unambiguously determine a primary kinetic isotope effect. Even in the case of a proton transfer, the rate constants for both isotopes coincide over a wide temperature range when tunneling takes place (Grellmann et al., 1982), and they become distinctly different only at lower temperatures. (Note that our solvent system freezes at 255 K. Low-temperature measurements therefore would have to be performed at higher concentrations of ethylene glycol or with similar solvent systems, which would rather drastically change the medium.) In view of this, we cannot exclude a proton transfer in the decay of the I^{i}_{700}'s, although the measured H/D effect is insufficient support.

CONCLUSIONS

There is no compelling indication that the primary photochemical step in the $\mathrm{P_r} \rightarrow \mathrm{P_{fr}}$ transformation be influenced by the change from H_2O to D_2O buffer media, which is consonant with a similar observation for the radiative deactivation of excited $\mathrm{P_r}$ (Brock et al., 1987). This is in favor of a configurational C=C double bond isomerization as the primary photoprocess [cf. Thümmler and Rüdiger (1983)], although the possibility, albeit unlikely, cannot be discarded that a tautomerization takes place instead, involving a proton that is not exchanged for deuteron in D_2O during the $\mathrm{P_r}$–$\mathrm{P_{fr}}$ cycle.

The situation concerning the subsequent ground-state reaction $\mathrm{I}^{i}_{700} \rightarrow \mathrm{I}^{i}_{\mathrm{bl}}$ is more ambiguous. The transformation is slowed down by the H_2O–D_2O change but insufficiently in the temperature range between 261 and 297 K to unequivocally distinguish between a tautomerization as the rate-determining step and a solvent H/D influence on a reaction *not* involving a proton transfer.

ACKNOWLEDGMENTS

We thank W. Schlamann for the phytochrome isolation and L. Lindgens GmbH and Co., Mülheim an der Ruhr, for providing the facilities to grow the seedlings.

APPENDIX

On the basis of the kinetic scheme proposed for the $\mathrm{P_r}$–I^{i}_{700} system established during the laser flash (Chart I), the steady-state concentrations of the excited species are

$$[\mathrm{P_r^*}] = I_0(\ln 10)\epsilon_e l[\mathrm{P_r}]/(k_f + k_d + k_{\mathrm{react}}) \qquad (8)$$

and

$$[\mathrm{I}^{i*}_{700}] = I_0(\ln 10)\epsilon'_e l[\mathrm{I}^{i}_{700}]/(k'_f + k'_d + k'_{\mathrm{react}}) \qquad (9)$$

The time derivative of $[\mathrm{I}^{i}_{700}]$ is

$$\mathrm{d}[\mathrm{I}^{i}_{700}]/\mathrm{d}t = k_{\mathrm{react}}[\mathrm{P_r^*}] + (k'_f + k'_d)[\mathrm{I}^{i*}_{700}] - I_0(\ln 10)\epsilon'_e l[\mathrm{I}^{i}_{700}] \qquad (10)$$

When the expressions for $[\mathrm{P_r^*}]$ and $[\mathrm{I}^{i*}_{700}]$ in eq 8 and 9 are introduced into eq 10 and the mass balance, $[\mathrm{P_r}]_{\mathrm{tot}} = [\mathrm{P_r}] + [\mathrm{I}^{i}_{700}]$, is taken into account, we obtain

$$\mathrm{d}[\mathrm{I}^{i}_{700}]/\mathrm{d}t = I_0(\ln 10)l\{\phi_{\mathrm{react}}\epsilon_e[\mathrm{P_r}]_{\mathrm{tot}} - (\phi'_{\mathrm{react}}\epsilon'_e + \phi_{\mathrm{react}}\epsilon_e)[\mathrm{I}^{i}_{700}]\} \qquad (11)$$

or, after rearranging

$$\mathrm{d}[\mathrm{I}^{i}_{700}]/(C_3 - [\mathrm{I}^{i}_{700}]) = C_2VI_0\,\mathrm{d}t/\kappa \qquad (12)$$

When eq 12 is integrated between the limits $[\mathrm{I}^{i}_{700}] = 0$ at the beginning of the laser flash and $[\mathrm{I}^{i}_{700}]_{t=0}$ at the end

$$\ln\{(C_3 - [\mathrm{I}^{i}_{700}]_{t=0})/C_3\} = -C_2E_0 \qquad (13)$$

which can be rearranged into the form of eq 3.

Registry No. D_2O, 7789-20-0.

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Study of 124-Kilodalton Oat Phytochrome Photoconversions in Vitro with Laser-Induced Optoacoustic Spectroscopy[†]

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Received June 19, 1986; Revised Manuscript Received November 5, 1986

ABSTRACT: The radiationless deactivation processes undergone by the excited red-absorbing form (P_r) of 124-kDa oat phytochrome were studied by laser-induced optoacoustic spectroscopy. The use of poly(vinylidene fluoride) foil as a broad-frequency-band piezoelectric detector permitted the time-resolved measurement of the pressure wave produced after the absorption of the 580-, 660-, and 695-nm laser flashes. The prompt heat dissipation is the same for 124- and 60-kDa P_r when determined for the same transit time of the acoustic wave across the laser beam radius. This supports the concept that the primary photochemical process is confined to the tetrapyrrole chromophore. At shortest possible observation time (which minimizes transient contributions) ≤50% of the absorbed light energy is released as heat, and the quantum yield of the phototransformation of P_r to the first set of intermediates (I^i_{700}) is ≥50%. These data correct earlier values obtained from the heat dissipation at longer observation times.

Previous studies of the fluorescence lifetimes of the P_r[1] form of phytochrome (Holzwarth et al., 1984; Wendler et al., 1984) and the kinetic behavior of the two primary photoproducts (the I^i_{700}'s), which arise in the transformation of P_r into the P_{fr} form [see the preceding paper by Aramendía et al. (1987) and references cited therein],[2] have been carried out with oat phytochrome preparations of various molecular sizes ("small" = 60 kDa, "large" = 114/118 kDa, and "native" = 124 kDa). The results indicated that the transformations upon irradiation of P_r, affording the I^i_{700}'s and the next set of intermediates (I^i_{bl}) (Kendrick & de Kok, 1983), are mainly restricted to the bilitriene chromophore and are not affected by the molecular weight of the protein (in the range ≥60 kDa).

The dissipation of the excitation energy is mainly governed by radiationless processes, since the fluorescence quantum yield of P_r is very low (for the 124-kDa form: $\phi_f = 2.9 \times 10^{-3}$; Holzwarth et al., 1984) and the quantum yield for the $P_r \rightarrow P_{fr}$ transformation has a maximum value of 0.15 [Kelly & Lagarias, 1985; see also Holzwarth et al. (1984)]. These

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[†] This work was supported by a fellowship award to K.H. from the Alfried Krupp von Bohlen und Halbach Stiftung.

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[1] Abbreviations: I^i_{700}, (set of) first intermediates formed from photoexcited P_r; I^i_{bl}, (set of) intermediates into which the I^i_{700}'s are transformed in a ground-state reaction; LIOAS, laser-induced optoacoustic spectroscopy; P_r and P_{fr}, red and far-red light absorbing forms of phytochrome, respectively; PVF_2, poly(vinylidene fluoride) (foil detector); PZT, Pb–Zr–Ti (ceramic detector); τ_a, acoustic transit time; τ'_a, observed acoustic transit time.

[2] The set of first intermediates can be composed of more than two I^i_{700}'s; e.g., Furuya (1983) has found that three such photoproducts are formed from pea P_r.

0006-2960/87/0426-1422$01.50/0