Low-Temperature Photoreactions of Halorhodopsin. 2. Description of the Photocycle and Its Intermediates†

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ABSTRACT: Photostationary states of halorhodopsin (HR, a retinal protein in the halobacterial membrane) and their further thermal conversions were investigated at 140–230 K by absorption spectroscopy in the visible. The difference spectra confirm several steps of the *all-trans*-HR photocycle, in the presence of chloride, proposed earlier on the basis of room temperature flash spectroscopy. Thus, at 140 K, the spectra reveal the $HR_{600} \rightarrow HR_{520}$ reaction, and at 170–230 K the $HR_{640} \rightarrow HR_{578}$ and the $HR_{520} \rightarrow HR_{578}$ reactions can be seen. No evidence for the expected $HR_{520} \rightleftarrows HR_{640}$ process was found, however. From the difference spectra at various temperatures, exact absorption spectra of HR_{600} and HR_{520} were calculated, and an estimate of the HR_{640} spectrum in a mixture also containing HR_{520} was obtained. The low-temperature absorption maxima of HR_{578} and its photointermediates relate to the room temperature maxima differently from what is expected from the spectra of the corresponding intermediates in the bacteriorhodopsin photocycle.

In the preceding paper (Zimányi et al., 1989), we reported on the photoreactions of the retinal protein halorhodopsin (HR)[1] at 80 K. At that temperature, the light-induced difference spectra of the pigment were dominated by the appearance of narrow spectral bands, which we interpreted as originating from the redistribution of conformational substates of the chromoprotein. A small amount of the bathoproduct, HR_{600}, was also formed, which decayed only above 110 K, consistent with the model in which this intermediate generates the other photocycle intermediates (Ogurusu et al., 1981; Taylor et al., 1983). At these temperatures, little useful information on the HR photocycle could be gained. Studies of HR_{600} and the other HR photocycle intermediates were feasible only at 140 K and above, because the amount of HR_{600} in the photostationary state was here greatly increased, and only in this temperature range was interference from changes in the vibrational spectrum absent.

With the exception of a few low-temperature spectra of some HR photointermediates (Weber & Bogomolni, 1981; Ogurusu et al., 1981, 1982), virtually all that is known about the HR photocycle is from room temperature flash spectroscopy. This approach yielded a model for the cycle in the presence of chloride (Oesterhelt et al., 1985; Lanyi & Vodyanoy, 1986; Tittor et al., 1987) which consists of the following sequence of reactions:

$$HR_{578} \xrightarrow{h\nu} HR_{600} \rightarrow HR_{520} \rightleftarrows HR_{640} \rightarrow HR_{565} \rightleftarrows HR_{578}$$

The $HR_{520} \rightleftarrows HR_{640}$ and the $HR_{565} \rightleftarrows HR_{578}$ reactions were proposed to be chloride dependent equilibria (Oesterhelt et al., 1985). The $HR_{640} \rightarrow HR_{565} \rightleftarrows HR_{578}$ steps were inferred from the fact that a chloride-dependent equilibrium between spectral forms HR_{565} and HR_{578} is measurable without illumination (Weber & Bogomolni, 1981; Ogurusu et al., 1982; Schobert et al., 1986), and it seemed likely that the chloride released during the decay of HR_{520} is regained via HR_{565} to complete the cycle. In the absence of chloride, the following reaction sequence was proposed (Lanyi & Vodyanoy, 1986; Tittor et al., 1987):

$$HR_{565} \xrightarrow{h\nu} HR_{600} \rightarrow HR_{640} \rightarrow HR_{565}$$

The identity of HR_{640} in the chloride-dependent cycle with HR_{640} in the absence of chloride has been assumed on the basis of the similarity of their decay kinetics (Lanyi & Vodyanoy, 1986) and the coincidence of their absorption maxima (Tittor et al., 1987), but this is not altogether proven as yet.

Absorption spectra for the HR photocycle intermediates have been calculated from time-resolved, room temperature flash-induced difference spectra (Tittor et al., 1987). These confirmed quite well earlier estimates for the rough positions of the absorption maxima for all the HR species.

In the present study of the low-temperature spectroscopy of HR between 140 and 200 K, evidence was obtained for several, but not all, of the steps proposed for the photocycle. Absorption spectra for the intermediates were also possible to calculate, and these were not quite consistent with what was expected on the basis of what is known about the temperature dependency of the spectra of BR species. To denote the HR intermediates in this study, we use the designations now current for the HR species, e.g., HR_{520} for the 520-nm intermediate, even when the absorption maximum we determined was not quite at this wavelength, in keeping with the fact that the absorption maxima of some retinal proteins will shift significantly at low temperatures [for example, see Becher et al. (1978)].

Materials and Methods

The preparation of HR, the low-temperature spectroscopy, and experimental conditions were all essentially as described in the preceding paper (Zimányi et al., 1989).

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[1] Abbreviations: HR, halorhodopsin; BR, bacteriorhodopsin; Tris, tris(hydroxymethyl)aminomethane; octyl glucoside, *n*-octyl β-D-glucopyranoside.

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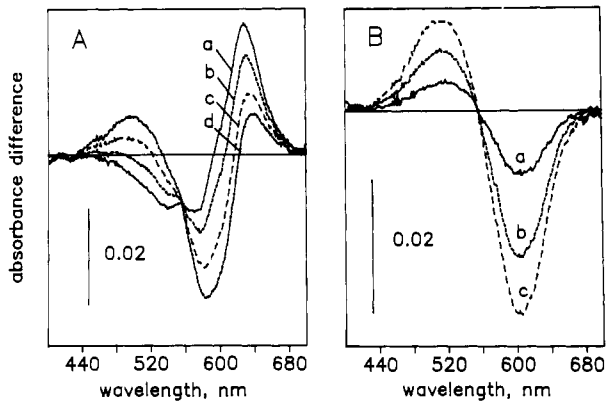

FIGURE 1: (A) Difference spectra of HR at 140 K after illumination and subsequent thermal reactions. Each spectrum is shown vs the preillumination spectrum. Curve a, after 3-min blue illumination; curve b, after 1 h at 140 K; curve c, after 6 h at 140 K; curve d, after 10 min at 170 K. Difference spectra showing the thermal reaction of HR in (A). Curve a was subtracted from curves b, c, and d of (A), yielding curves a, b, and c. The HR was solubilized in 0.5% octyl glucoside, 0.5% cholate, 4 M NaCl, and 25 mM Tris·HCl, pH 7.2, and mixed with 2 parts (w/w) of glycerol.

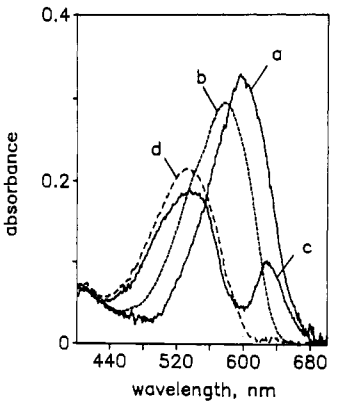

FIGURE 2: Estimated absolute spectra of the intermediates which account for the difference spectra in Figure 1A. Curve a, the spectrum of HR_{600}, calculated by subtracting 0.1 times the absorption spectrum of HR_{520} (from Figure 5B, curve d, and normalizing by −0.1) from Figure 1B, curve c. Curve b, the measured absorption spectrum of HR at 140 K, after subtracting light scattering. Curve c, the spectrum of the mixture which accounts for Figure 1A, curve d, consisting of HR_{520} and HR_{640}. It was calculated from that spectrum by adding 0.135 times the spectrum of HR_{578}, and normalizing by 0.135. Curve d, the spectrum of HR_{520} (from Figure 5B, curve d), shown here scaled for comparison with curve c. The amplitude of the spectrum of HR_{600} represents its extinction relative to that of HR_{578}, but the spectrum of the mixture of HR_{520} and HR_{640} contains the individual spectra of these intermediates times their fractional concentration.

RESULTS

Low-Temperature Photoreactions of Blue-Adapted Halorhodopsin. Illuminations of HR_{578} were carried out at 140 K after adaptation of the pigment with either blue or red light at 273 K. The first set of experiments were intended to explore the photoreactions of *all-trans*-HR, by using blue light adapted HR, which under these conditions contained 84% of the all-trans and 16% of the 13-cis pigment (from retinal extraction and HPLC fractionation of the isomers). Figure 1A curve a shows the difference spectrum for the photostationary mixture produced with blue illumination, similar in shape to that obtained by illuminating the sample at 80 K with blue light [cf. Zimányi et al. (1989)] and then warming it to 140 K. Several hours of incubation in the dark at 140 K (curves b and c) caused the accumulation of a blue-shifted form, with a single isosbestic point at 555 nm, and red-shifted product(s) were depleted during this time. The transition was completed when the sample was transiently (10 min) warmed to 170 K (curve d), and more prolonged heating (up to 2 h) at 170 K resulted in little further spectral changes. However, a portion of the red-shifted product remained (curve d). The kinetics of the thermal transition following the illumination are best demonstrated by subtracting the initial spectrum, i.e., curve a, from each of the spectra in Figure 1A (shown in Figure 1B). Here we see the transition of a red-absorbing spectroscopic species into a blue-absorbing one, as indicated by a maximum at 515 nm and a minimum at 604 nm. The similarity of the three difference spectra in Figure 1B and the existence of an isosbestic point (at 555 nm) strongly indicate that only two intermediates are involved in this transition. However, the transition began and ended with a mixture of intermediates, since both the initial spectrum and the final one in Figure 1A (curves a and d, respectively) are more complex than expected for a difference spectrum of a single intermediate *minus* HR_{578}. This can be seen also when one attempts to add a properly weighted spectrum of the parent HR species to any of these difference spectra in Figure 1A, in order to calculate the absolute spectra of the intermediates.

In the following, we attempt to reconstruct the spectra of the HR intermediates produced by illumination and subsequent thermal treatment. For BR, this reconstruction is relatively simple, because the spectra of BR and its principal intermediate, M_{412}, overlap very little, and hence the amount of photoconversion is easily estimated. For HR, such an intermediate is not produced, and the spectral reconstruction is more difficult. In general, we estimate the spectra of the intermediates according to established requirements (Lozier, 1982; Nagle et al., 1982): (1) the absorption of the putative intermediates should be nonnegative at all wavelengths, (2) the wavelength dependence of the absorption should be smooth, and (3) each intermediate should have a single principal broad absorption maximum. In addition, we consider the shape and bandwidth of existing intermediate spectra in the literature for both BR (Lozier & Niederberger, 1977; Becher et al., 1978) and HR (Tittor et al., 1987). The manipulations of the spectra are reasonable, since the percent photoconversions, which are used for the estimations, affect the calculated spectra simultaneously in all of the aspects listed above, and are thereby restricted to narrow ranges of values.

If it is assumed, on the basis of the HR photocycle discussed above, that the transition in Figure 1B represents the $HR_{600} \rightarrow HR_{520}$ reaction, the spectrum of HR_{600} can be calculated by subtracting an appropriately scaled spectrum of HR_{520} (obtained at higher temperature, cf. below) from the difference spectra. Curve a in Figure 2 shows the result, which is similar to the room temperature spectrum for HR_{600} (Tittor et al., 1987), both in its position and in its amplitude relative to the spectrum of HR_{578} (Figure 2, curve b).

The red-shifted product which remains after warming to 170 K (Figure 1A, curve d) is kinetically clearly different from HR_{600}. This intermediate decays, however, after warming to 200 K. The difference spectrum, between after and before the warming, shown in Figure 3 curve a, contains a maximum at 578 nm and a minimum at 636 nm; i.e., it is very unlike the HR_{520} *minus* HR_{600} difference spectra in Figure 1B. The lack of increase of absorption below 500 nm indicates that this intermediate does not produce HR_{520} upon decay, but most likely HR_{578}. However, a difference spectrum constructed from spectra for HR_{600} and HR_{578} in Figure 2 (Figure 3, curve b) is also unlike the obtained spectrum. The position of the depletion band suggests instead that the intermediate absorbs to the red from HR_{600}. This is confirmed by adding a properly

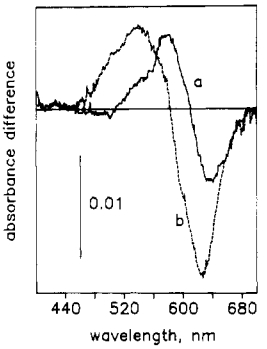

FIGURE 3: Difference spectrum of warming HR to 200 K after illuminating and allowing thermal decay at 170 K. A sample similar to that in Figure 1A, curve d, was warmed to 200 K for 25 min and recooled to 170 K. Curve a, difference spectrum between after and before warming to 200 K. Curve b, an HR_{578} *minus* HR_{600} difference spectrum reconstructed from absolute spectra in Figure 2.

scaled spectrum of HR_{578} to curve d in Figure 1A, which yielded the absolute spectrum of the mixture of intermediates remaining after warming the sample in Figure 1 to 170 K. The criterion of the best scaling factor for obtaining the latter spectrum was that HR_{520}, the other component of the mixture, should have a spectrum which resembles an authentic spectrum for this intermediate (calculated from a spectrum after illumination with deep red light at 170 K, where HR_{520} is virtually the only intermediate present; cf. below). The results of these manipulations are shown in Figure 2, curve c, where the red-shifted intermediate is seen to absorb at 632 nm, identifying it as HR_{640}.

After red, instead of blue, illumination at 140 K, we obtained a difference spectrum essentially identical with curve c in Figure 1A, except that the amplitude was smaller. This suggests that, although the amounts and the ratio of the intermediates produced may vary, the photostationary state formed at 140 K contains the same intermediates, independently of the color of light.

When the blue-illuminated sample was warmed from 140 K to 200 K, and HR_{640} decayed as described above, a difference spectrum characteristic of almost exclusively the HR_{520} *minus* HR_{578} was measured (Figure 4A, curve a). This difference spectrum is characterized by a maximum at 504 nm and a minimum at 591 nm. Further heating to 230 K and recooling to 200 K resulted in the partial reconversion of HR_{520} to HR_{578} (curve b). By adding 9% of the absorption spectrum of HR_{578} to curve a in Figure 4A, and properly normalizing the resulting spectrum, we obtained an estimation of the spectrum of HR_{520}, at 200 K (Figure 4B, curve b). A small contribution from HR_{640} could not be eliminated, indicating that this species coexists with HR_{520} at 200 K (cf. below). Another way to obtain the spectrum of HR_{520} at 200 K was by illumination with deep red light at this temperature, and adding a scaled HR_{578} spectrum to the difference spectrum as for Figure 4B curve b. The resulting spectrum, curve c, agrees well with the other estimate, curve b, except that the signal/noise ratio is better since more photoconversion is obtained under these conditions (27%).

The fate of HR_{640} relative to HR_{520} could be best determined from difference spectra after blue light illumination at 170 K and subsequent incubation, because at this temperature it decays slowly while HR_{600} is very rapidly converted to HR_{520}. Figure 5A shows difference spectra obtained directly after such illumination (curve a, which resembles the spectrum after illumination at 140 K and warming to 170 K in Figure 1A, curve d), after 1 h at 170 K (curve b), and after warming to

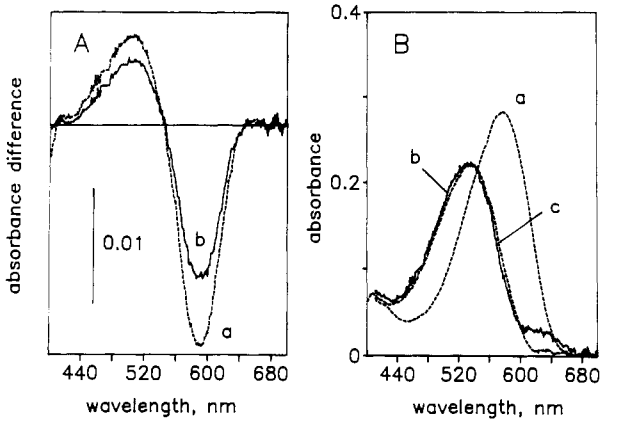

FIGURE 4: (A) Difference spectra of HR at 200 K. After blue illumination at 140 K, as in Figure 1A, the sample was warmed to 200 K, and a spectrum was taken (curve a). Then, the sample was warmed to 230 K, kept there for 10 min, and recooled to 200 K and another spectrum taken (curve b). The difference spectra are shown vs the preillumination spectrum. (B) Absolute spectra for HR_{520} from difference spectra at 200 K. Curve a, a spectrum for HR_{578} determined at 200 K, with light scattering subtracted. Curve b, 0.09 times the spectrum of HR_{578} was added to curve a in (A) and then normalized by 0.09 to yield the estimated spectrum of the intermediate(s) involved in the 200 K difference spectrum. A minor contribution from HR_{640} is evident. Curve c, The HR sample was illuminated with deep red light at 200 K, and 0.27 times the spectrum of HR_{578} was added to the difference spectrum and normalized by 0.27. The amplitude of these spectra represents the extinction of HR_{520} relative to that of HR_{578}, since the former was virtually the only intermediate present. The same sample as in Figure 1.

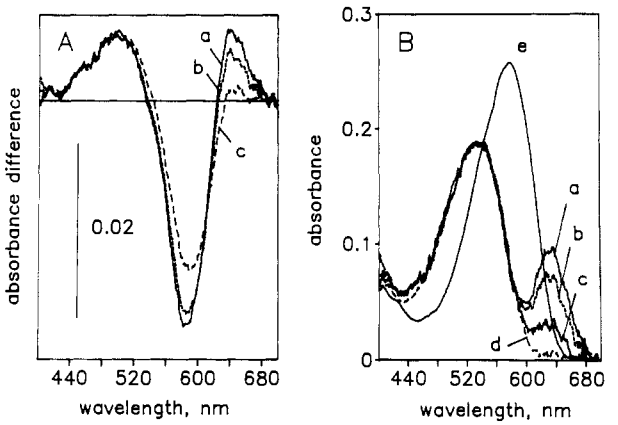

FIGURE 5: (A) Difference spectra for HR at 170 K. Curve a, spectrum after blue light illumination at 170 K. Curve b, spectrum after a subsequent 2-h incubation at 170 K. Curve c, spectrum after subsequent warming to 200 K for 25 min and recooling to 170 K. Difference spectra are shown vs the preillumination spectrum. (B) Absolute spectra of the mixture of intermediates produced thermally after blue illumination. Curves a, b, and c, absolute spectra from the difference spectra in (A), curves a, b, and c, respectively, by adding 0.13, 0.127, and 0.102 times the spectrum of HR_{578}, respectively, and scaling to fit the reference spectrum of HR_{520}. Curve d, absolute spectrum of HR_{520}, obtained by deep red illumination at 170 K and adding 0.22 times the spectrum of HR_{578} to the difference spectrum and normalizing. Curve e, the measured spectrum of HR_{578} at 170 K, with scattering subtracted. The amplitudes of the contributions of HR_{640} in curves a, b, and c represent the relative extinctions times their fractional concentrations.

200 K and recooling to 170 K (curve c, which resembles the spectrum after illumination at 140 K and warming to 200 K in Figure 4A curve a). These spectra are complex but confirm that the decay of HR_{640} is accompanied mainly by an increase in the absorption band of HR_{578}; i.e., this form returns thermally to HR_{578} rather than to HR_{520}. Adding the HR_{578} absorption spectrum to curves a–c with appropriate scaling

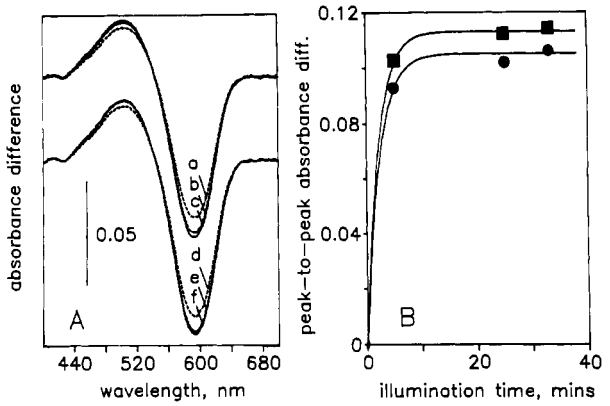

FIGURE 6: (A) Difference spectra of red-adapted HR (curves a, b, and c) and blue-adapted HR (curves d, e, and f) at 200 K. The adaptation was at 270 K, after which the temperature was lowered to 200 K and the HR was illuminated for 5 min (curves a and d), 25 min (curves b and e), and 35 min (curves c and f) with red light. The difference spectra are given vs the preillumination spectrum at 200 K. Similar sample as in Figure 1. (B) Peak-to-peak absorption difference for red- and blue-adapted HR from Figure 5A vs the illumination period. (■) Blue-adapted sample; (●) red-adapted sample. The solid lines are fitted curves of the form: constant$[1 - \exp(-kt)]$.

factors generated absorption spectra for the mixtures of the intermediates (Figure 5B, curves a–c). These consist of HR_{520} and decreasing relative amounts of HR_{640}. As in Figure 2, in selecting the scaling factor we chose ones which produced spectra for HR_{520} most like an authentic spectrum for this intermediate (obtained at 170 K with deep red light and included as curve d in Figure 5B).

To summarize, illumination of blue-adapted HR at 140 K appears to produce varying amounts of two, kinetically different, red-shifted intermediates, HR_{600} and HR_{640}, as well as HR_{520}. It would be expected from the photocycle model that HR_{600} should produce first HR_{520} and then HR_{640} by thermal decay, but we always find that a mixture of the latter two is produced, and the composition shifts toward relatively more HR_{520} and less HR_{640} at 170 K or higher temperatures. An explanation would be that the rate of the $HR_{520} \rightarrow HR_{640}$ step has a lower temperature coefficient than that of the $HR_{640} \rightarrow HR_{578}$ step, resulting in smaller steady-state amounts of HR_{640} at higher temperatures, during the return to HR_{578}.

Effect of Adaptation with Blue and Red Light on the HR Photoreactions. In the following, photostationary states of blue and red light adapted HR (Smith et al., 1984; Lanyi, 1986) were compared after illuminations at 200 K, in attempting to separate the photoreactions of the *all-trans*- and *13-cis*-retinal-containing pigments. Figure 6A compares the effect of prolonged red illumination on red- and blue-adapted HR at 200 K, respectively. In both cases, more than 30 min was required to reach the photostationary state, which contained HR_{520} as virtually the only intermediate. The amount of photoconversion by red light was as high as 35–40% under these conditions, but somewhat different in the two samples. Figure 6B shows the peak-to-peak absorption changes as functions of illumination time. The amount of HR_{520} formed was consistently somewhat higher in blue-adapted HR, by an amount which correlates well with the percentage of *all-trans*-retinal in the two samples, as determined by HPLC following retinal extraction: 84% and 76%, respectively. Therefore, we conclude that the HR_{520} formed in the low-temperature photostationary states originates from the *all-trans*-retinal-containing HR. The same conclusion was reached earlier from flash-induced absorbance changes (Lanyi, 1986). No spectra of the photoreaction of *13-cis*-HR could be measured in these experiments.

Discussion

We have studied the low-temperature photoreactions of HR by absorption spectroscopy in order to describe the sequence of events in the photocycle and to characterize the spectra of the intermediates. As generally agreed, such investigations give information complementary to those obtainable from room temperature flash kinetic experiments and provide the basis for other low-temperature measurements such as vibrational spectroscopy. However, a complication of low-temperature spectroscopy is that after illumination photostationary states are formed, which are often mixtures of more than two species with overlapping absorption bands. Usually, the amounts and absolute spectra of the participating spectral forms cannot be determined unambiguously unless overlap between the spectra is absent. We found that a combination of obtaining photostationary-state spectra and then following further changes of these spectra at either the same or a higher temperature overcame these difficulties, since a time or temperature interval could be always found where (nearly) only one form was transformed to another.

The results we obtained in this way confirm, by and large, the earlier suggested photocycle scheme (Oesterhelt et al., 1985; Lanyi & Vodyanoy, 1986; Tittor et al., 1987) and provide absorption spectra for the photointermediates of the *all-trans*-HR photocycle. We describe the difference spectra for the transitions $HR_{600} \rightarrow HR_{520}$ at 140 K (Figure 1B), $HR_{520} \rightarrow HR_{578}$ at 200–230 K (Figure 4A), and $HR_{640} \rightarrow HR_{578}$ at 170–200 K (Figures 3 and 5A). However, even though flash spectroscopy suggested that HR_{520} decays via HR_{640} (Oesterhelt et al., 1985; Lanyi & Vodyanoy, 1986; Tittor et al., 1987), the low-temperature spectra do not provide evidence for this. Attempts to show the $HR_{520} \rightarrow HR_{640}$ transition were made after deep red light illumination, where HR_{520} was produced in large excess, but no increase in absorption due to HR_{640} occurred with time at 170 or 200 K (not shown). Attempts to show the opposite reaction also failed: after blue light illumination, where HR_{640} was produced in large amounts, no increase in HR_{520} was seen (Figure 5). These negative results do not necessarily contradict the proposed equilibration of these forms, since the data are consistent with the possibility that in the temperature range studied the $HR_{520} \rightarrow HR_{640}$ reaction is slower than the $HR_{640} \rightarrow HR_{578}$ reaction (i.e., the rate of the latter increases more rapidly with temperature), and, therefore, the concentration of HR_{640} can only decrease as it approaches its steady-state value during thermal conversion. Since we detected no thermal $HR_{520} \rightarrow HR_{640}$ reaction, the question must be raised, however, as to how HR_{640} is produced (e.g., at 140 K with blue light in Figure 1A). There are three possibilities: (1) HR_{640} is produced rapidly by thermal pathway from HR_{520} already during the illumination. This would mean that Figure 1A curve a contains HR_{640} as well as HR_{600}, but seems unlikely, since the lifetime of HR_{520} is long at 140 K. (2) HR_{640} is produced by thermal decay of HR_{520} after the illumination, as the concentration of HR_{520} increases. In this case, the difference spectra in Figure 1B refer to the entire $HR_{600} \rightarrow HR_{520} \rightleftarrows HR_{640}$ reaction. This also seems unlikely, because the slow approach to the steady-state concentration of HR_{640} during the return to HR_{578} at 170 K (Figure 5) seems to rule out rapid equilibration between HR_{520} and HR_{640} at 140 K. (3) HR_{640} is produced by a photoreaction of HR_{520} during the illumination. The latter seems to be the most likely possibility, particularly in view of the fact that the yield of HR_{640} is higher, and that of HR_{520} is lower, after blue than red illumination.

Table I: Absorption Maxima for HR_{578} and Its Photocycle Intermediates at Various Low Temperatures[a]

species	absorption maximum (nm) at			
	80 K	140 K	170 K	200 K
HR_{578}	577[b]	577	578	577
HR_{600}		598		
HR_{520}		532	532	535
HR_{640}		630	632	

[a] The numbers were estimated from calculated absolute spectra, such as in Figures 2, 4B, and 5B. [b] From the preceding paper (Zimányi et al., 1989).

We have obtained fairly good estimates for the spectra of the HR photointermediates, as indicated by the fact that, for example, a single spectrum for HR_{520} was consistent with difference spectra at 140 K, 170 K, and 200 K, including the HR_{520} *minus* HR_{600} (Figure 1B) as well as the HR_{520} *minus* HR_{578} difference spectra (Figure 4A), with the extent of photoconversion the only adjustable variable. Table I shows the estimated absorption maxima for HR_{578} and its photointermediates at various temperatures. The results indicate that the temperature dependency of the absorption maxima of HR intermediates is different from those of the BR intermediates reported [for example, see Becher et al. (1978)]. It appears from Table I that the absorption band of HR_{578} shifts little upon cooling, unlike that of BR, which is red shifted from 568 nm at room temperature to 578 nm at 77 K (Becher et al., 1978). Likewise, we find that the absorption band of HR_{600} at 140 K is at 598 nm, equal within error to the 600 nm measured for this intermediate at room temperature (Tittor et al., 1987). In contrast, the bathoproduct of BR at 77 K shows considerable red shift relative to its absorption at room temperature (Becher et al., 1978). According to our data, the absorption band of HR_{520} is at 532–535 nm. Its position does not vary (within error) between 140 K and 200 K, similarly to the L_{550} intermediate of BR, which it resembles in other respects (Fodor et al., 1987). Hence, the discrepancy between the room temperature value of 520 nm for this intermediate (Tittor et al., 1987) and what we find in this study is surprising. Our preliminary results with transient spectroscopy at room temperature yielded 525 nm for the absorption maximum of HR_{520} (Zimányi et al., 1988). As expected, our extinction coefficients for HR_{600} and HR_{520} relative to that of HR_{578} (in Figures 2 and 4B) correspond, within a few percent, to those reported for room temperature by Tittor et al. (1987). The extinction coefficients of HR_{578} and HR_{600} at 80 K are 64 700 and 69 800 M^{-1} cm^{-1}, respectively, and those of HR_{578} and HR_{520} at 200 K are 53 900 and 42 000 M^{-1} cm^{-1}, respectively.

The red shifts in the visible spectra of BR and its intermediates at low temperature can be attributed to a reduced population of vibrational quantum levels which are superimposed on asymmetrical electronic potential wells (Warshel & Karplus, 1974). The insensitivity of the absorption maxima of HR and its photointermediates to cooling suggests that the potential well of the ground state is steeper in HR than in BR. A steep ground-state potential well for HR would have the consequence that this pigment be red shifted even at room temperature, unlike BR which shows a red shift only at low temperatures. This may be the origin of the 10-nm difference in the absorption maxima of BR and HR at room temperature, which disappears at 77 K (Becher et al., 1978; this study).

A deeper ground-state potential well would correspond to a more immobilized state of the chromophore in HR than in BR. The same conclusion about these chromophores was reached in the preceding paper (Zimányi et al., 1989), on grounds of the dependency of the quantum yields on temperature and the equilibration of conformational substates in the two pigments during illumination.

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