On the Light-Stimulated Coupling between Rhodopsin and Its Disk Membrane Environment†

Rainer Uhl,*‡ Klaus Peter Hofmann, and Werner Kreutz

ABSTRACT: Disks from bovine ROS undergo a rapid shrinkage when flash illuminated with green light (Uhl, R., et al. (1977) *Biochim. Biophys. Acta 469,* 113-122). This can be monitored as a light scattering transient, referred to as the P signal. In this paper the P signal is studied at various temperatures and pH. The temperature dependence of the kinetics reveals that "P" consists of two sequential reaction steps. Both appear to occur within the receptor molecule rhodopsin. The actually observed event, the shrinkage of the disk, is therefore not rate limiting under the tested conditions. Both steps of "P" take place while there is only one spectroscopically detectable reaction of the rhodopsin molecule, the metarhodopsin I-metarhodopsin II transition. This implies that there are intermediates of the rhodopsin photolytic cycle which are not evident as spectroscopically separate species. The amplitude of "P", i.e., the extent of the disk shrinkage, is independent of the state of the equilibrium between the two photoproducts absorbing at 478 and 380 nm respectively and called MI and MII. A scheme is suggested in which the irreversible decay of MI (478) triggers the disk shrinkage (and maybe transduction), and in which there is an equilibrium between MII (380) and a proposed isochromic photoproduct MI′ (478).

The visual transduction process can be thought of as a sequence of "communication processes" on various levels of molecular organization in the receptor cell: (1) the interaction of light with the chromophore and, in turn, with the protein moiety of the pigment molecule rhodopsin; (2) the interaction of rhodopsin with its environment, the disk membrane; (3) the communication between disk and plasma membrane, probably due to a diffusable transmitter. The first "communication step" has been studied extensively, using spectroscopic techniques such as absorption spectroscopy (for a review, see Wald, 1968), flash photolysis (for a review, see Abrahamson & Wiesenfeld, 1972), and resonance Raman (see Lewis, 1976). All of these methods make use of the pigment nature of rhodopsin.

The final stages of "communication step" 3 have also been studied very carefully by means of intracellular and extracellular recordings (see Penn & Hagins, 1972; Yau et al., 1977; Kleinschmidt & Dowling, 1975). However, very little is known about the way rhodopsin communicates with its environment. The main reason for this is that the above mentioned techniques only allow the monitoring of events that either occur prior to the coupling between rhodopsin and the disk membrane (rhodopsin photolysis) or much later (rise of the receptor potential).

From an analysis of the complex kinetics of the rise of the receptor potential in both rods and cones it has been concluded that at least 4-10 first-order kinetic processes are involved in the coupling between rhodopsin and plasma membrane (Penn & Hagins, 1972; Baylor et al., 1974). One or more of these processes may actually reflect the coupling between rhodopsin and its subcellular environment. The complexity of the kinetics of the receptor potential, however, does not permit the resolution of this coupling. Moreover, none of the known reaction steps at the rhodopsin photolytic cycle possesses a rate similar to the ones calculated to fit the kinetic patterns of the light response; i.e., no trace of any of the known rhodopsin reactions can be found in the kinetics of the receptor potential. This leaves a gap between the processes (on the chronological scale and at the morphological level) that can be readily monitored by the above techniques.

This paper is an attempt to collect information on processes occurring within this gap. For this we have used a system which, on one hand, is far more physiologically intact than, for instance, rhodopsin in micelles or in reconstituted membranes, and, on the other hand, considerably less complex than the receptor cells in the intact retina. We have used isolated rod outer segments from the retinae of dark adapted cattle eyes. In this system a rapid light induced light scattering transient "P" can be observed which reflects a rapid disk shrinkage, i.e., a process where rhodopsin has already communicated with its environment (Uhl et al., 1977).

In this paper we shall compare the kinetics of this disk shrinkage with the kinetics of the MI/II[1] transition[2] at various temperatures and values of pH. From this comparison a model will be derived that can account for the trigger process of the above structural event.

We shall then speculate that the trigger process of the disk shrinkage can also be the trigger process of transduction and that the two kinetic processes that become obvious from "P" could close the above mentioned gap. This speculation seems legitimate on the basis of the following characteristics of the disk shrinkage phenomenon (Uhl et al., 1977):

(1) The extent of the disk shrinkage shows the same action spectrum as rhodopsin bleaching.

(2) Its kinetics (half-time of 15-20 ms at room temperature, 6-8 ms at physiological temperature) is fast enough for a process possibly involved in transduction.

(3) The fact that "P" reflects an event involving the entire disk implies that "communication processes" beyond the

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‡ Present address: Department of Chemistry of the University of Guelph, Guelph, Ontario, Canada, N1G 2W1.

[1] Abbreviations used: ROS, rod outer segments; MI/II, metarhodopsin I-metarhodopsin II reaction; "P", P signal.

[2] What we shall call MI/II transition in this paper is always the spectroscopically observable event, initiated by light and measured as an increase in absorbance at 380 nm or decrease at 478 nm. The actual underlying molecular events will be dealt with in the discussion.

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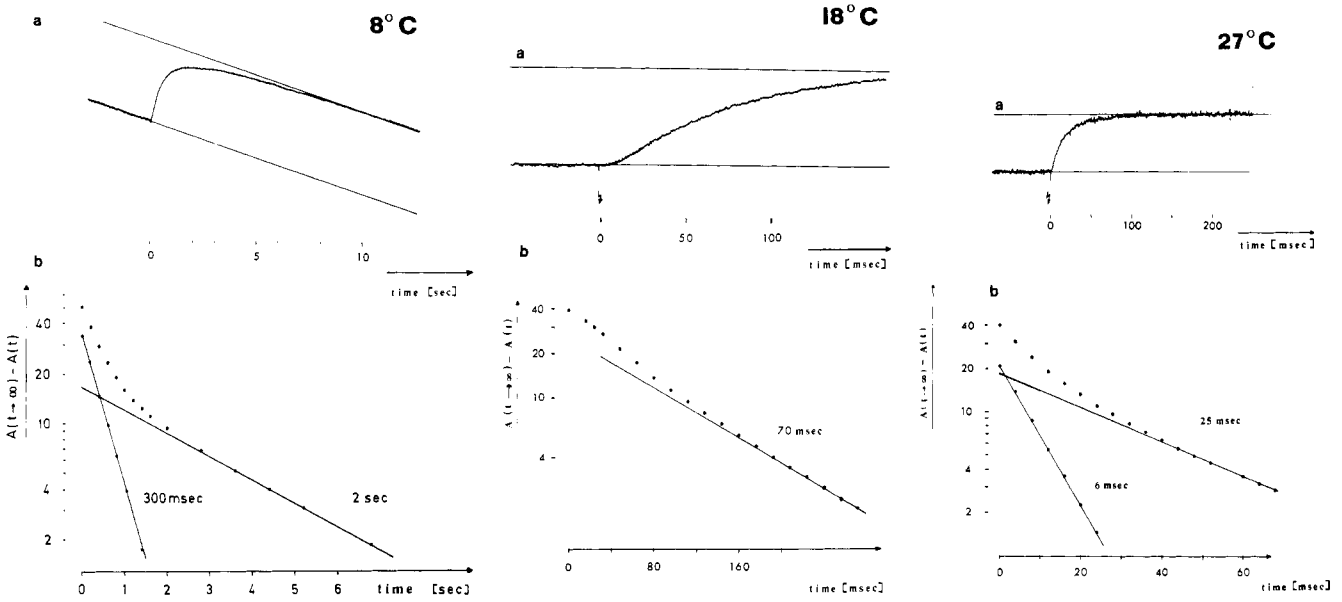

FIGURE 1: (a) Time course of P-signals obtained at 8, 18, and 27 °C. pH = 7, $\theta = 0 \pm 2°$, $\lambda = 750$ nm, rhodopsin concentration in the cuvette: 500 nM. Suspending medium: 140 mM KCl and 10 mM piperazine-1,4-diethanesulfonic acid (Pipes). If not stated otherwise, all measurements were performed under the same conditions. The bleaching rate was 2.5% per flash. (b) Kinetic analysis of the time course of the P signals in a.

rhodopsin interaction with its microenvironment are monitored.

(4) The fact that "P" cannot be regenerated by 11-*cis*-retinal, whereas the pigment rhodopsin can be in our system, clearly means that there are further requirements in the disk membrane, aside from the intactness of the pigment molecule, for the disk shrinkage to occur.

(5) The extent of the disk shrinkage as a function of the amount of rhodopsin bleached (Hofmann et al., 1976) clearly shows an adaptive behavior, similar to the phenomenon known as dark adaptation.[3] It leads to the assumption of interactive rhodopsin pools in the disk membrane.

Experimental Section

The kinetic light scattering device is identical with the one described in previous papers (Hofmann et al., 1976; Uhl et al., 1977). All measurements referred to in this communication were performed at 0° scattering angle, i.e., in transmission.

ROS fragments were prepared according to the following procedure. Cattle eyes were excised immediately after the death of the animal and stored in a light-tight container at room temperature for 30–60 min. Then the eyes were dissected under dim red light and the retinae placed in a physiological saline solution (120 mM NaCl, 3.5 mM KCl, 15 mM Na_2HPO_4/NaH_2PO_4 (pH 7), 5 mM glucose, 0.2 mM $CaCl_2$ and $MgCl_2$). A crude ROS suspension was obtained by gently grinding the retinae in the saline using mortar and pestle. This suspension was filtered through a 30-μm nylon mesh and layered on a discrete sucrose density gradient. A discrete gradient was chosen in order to not disturb the structural integrity of the ROS. A continuous sucrose density gradient usually yields ROS fragments with rather inhomogeneous disk to disk lattice distance, whereas our preparation procedure yields ROS with relatively uniform disk stacking as can be shown by electron microscopy (Uhl, 1976) and X-ray diffraction (Stange, 1977). Before freezing in liquid nitrogen the ROS suspension is washed in physiological saline once or twice and then, in order to perforate the plasma membrane, forced twice very rapidly through a thin Eppendorf pipet tip which was stuffed with glass wool. Even after this procedure and subsequent freeze-thawing a rather homogeneous disk to disk distance is seen in the electron microscope and only very few disks seem to have broken and formed smaller vesicles. This is consistent with the findings of Norisuye et al. (1976) that bovine disks, prepared after a similar procedure, stay mostly intact and exhibit a strikingly homogeneous size distribution.

Whereas the amplitude of the P signal was subject to minor variations from preparation to preparation, the kinetics at a given temperature and pH were found to be highly reproducible over a period of 2 years. Fresh preparations without freeze-thawing and even fresher ones without density gradient gave the same kinetic data as the preparations used for most of the measurements described in this paper. Furthermore, addition of 0.5 mM CaEDTA and argonating all the used solutions, a procedure which was reported to prevent oxidative damage to rhodopsin and ROS (Stone et al., 1978), had no influence on the observed kinetics. From this we have concluded that the described kinetics are genuine and not caused by artefactual inhomogeneities of the ROS system.

The simultaneous measurements of "P" and MI/II were performed by recording P signals with the first flashes at 750 nm and switching then, when "P" is exhausted, to a wavelength of 380 nm where MI/II is monitored. Thus it is accomplished that MI/II is not "distorted" by any light-scattering changes due to "P". However, both signals are obtained from the same ROS sample under identical measuring conditions. Since each flash bleached only 2.5% of the rhodopsin, the amplitude of "P" could not have possibly saturated.

Results

Arrhenius Plot of "P". The time courses of P signals at three different temperatures are shown in Figure 1, a. Two main features are obvious.

(1) At 18 °C "P" exhibits a sigmoid shape with a horizontal tangent at the time $t = 0$. Note that at 8 °C and at 27 °C no such behavior is observed. A sigmoid time course like the one at 18 °C is typically found for consecutive reactions in the

[3] Recently Wey & Cone (1978) have reported a very similar light scattering transient from fresh, dark adapted frog ROS which exhibits an almost identical adaptation behavior.

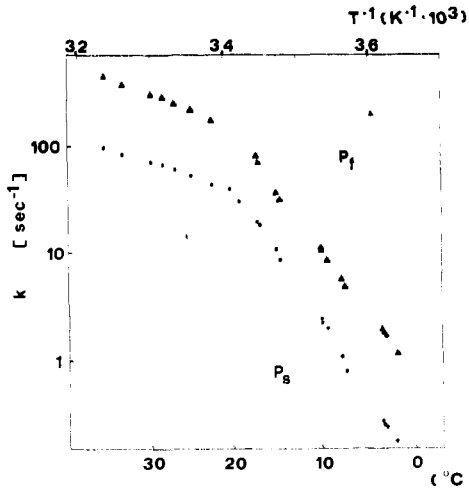

FIGURE 2: Arrhenius plot for the fast (k_f) and the slow (k_s) kinetic component of the P signal.

temperature range where both reaction steps have about the same rate.

(2) A kinetic analysis (Figure 1, b) of the three signals in Figure 1, a, shows that in each case the kinetics can be separated into two first-order processes. However, in the case of the P signal at 18 °C, this separation is somewhat obscured because both processes exhibit a sigmoid time course.

A separation into two and only two first-order processes can be accomplished for P signals in the temperature range between 2 and 37 °C. The rate constants k_f (fast component) and k_s (slow component) so obtained are depicted as an Arrhenius plot in Figure 2. For both, k_f and k_s, the plot shows two intersecting straight lines. The intersection occurs exactly in the temperature range where "P" exhibits a time course typical of consecutive reactions. Thus a more or less marked sigmoid shape for the time course of "P" is found between 12 and 23 °C. This strongly supports the previous assumption that at low and high temperatures respectively "P" reflects two different rate-limiting steps with markedly different activation energies. At 18 °C these reactions are approximately equally fast which explains the observed sigmoid time course. We have named the low-temperature rate-limiting step "P_1" and the reaction step which is rate limiting at higher temperature "P_2". Of course, for both "P_1" and "P_2" there is a fast ("P_{1f}" and "P_{2f}") and a slow component ("P_{1s}" and "P_{2s}"). The activation energies for these four processes, calculated from the slope of the Arrhenius plot, are as follows:

$$E_{A(\text{"}P_{1f}\text{"})} = 42 \text{ kcal/mol}$$

$$E_{A(\text{"}P_{1s}\text{"})} = 50 \text{ kcal/mol}$$

$$E_{A(\text{"}P_{2f}\text{"})} = 12.5 \text{ kcal/mol}$$

$$E_{A(\text{"}P_{2s}\text{"})} = 10 \text{ kcal/mol}$$

The activation energies for "P_{2f}" and "P_{2s}" can be considered typical of ordinary molecular processes. However, the values found for "P_{1f}" and "P_{1s}" are considerably larger than those encountered in reactions involving small molecules. They are exclusively found for reactions within complex macromolecules where forming the activated state requires breakage of various bonds. The most likely candidate for such a complex molecule that undergoes major conformational changes immediately upon illumination appears to be rhodopsin. Therefore, it is quite likely that the processes with the high activation parameters, i.e., "P_{1f}" and "P_{1s}", are located within the protein moiety of the receptor molecule.

Comparison of the Kinetics of "P" and MI/II. A detailed kinetic study in our laboratory (Hoffman et al., in preparation) provided plausible evidence for the existence of two different, isochromic forms of rhodopsin. A very recent publication of Stewart et al. (1977) comes to identical results. The two forms of rhodopsin are in temperature and pH-dependent equilibrium and are photolyzed via two parallel pathways with slightly different rate constants. This is manifested, for instance, in the kinetics of the lumirhodopsin decay, which can be separated into two first-order processes, the rate constants of which are different by about a factor of 4–6. This is very similar to the behavior of the P-signal kinetics.[4] In our interpretation it is assumed that the fast component of the disk shrinkage is somehow connected to the fast pathway, the slow component of "P" to the slow pathway of rhodopsin photolysis. However, since the existence of a fast and slow component is presumably related to the two forms of rhodopsin, the appearance of two kinetic components in the case of "P_1" and "P_2" suggests that they both reflect events that take place in the rhodopsin molecule itself. For "P_1" this is already evident from its high activation energy.

Further support for the interpretation that there are two different rhodopsin photolysis pathways stems from the kinetics of the MI/II process. Neither Hagins in the living rabbit eye (1957), nor Junge in the perfused retina of the frog (personal communication), nor Stewart et al. in sonicated ROS or solubilized rhodopsin micelles (1976, 1977) find simple first-order kinetics for this process. Likewise, in our study dealing with isolated bovine ROS fragments, MI/II always was found to exhibit more complex kinetics: at most temperatures and pH values we studied, it appears as though it could be separated into two first-order processes. Under some conditions, however, the kinetics rather resembled a combination of three or four first-order processes. This more complicated behavior makes a straightforward comparison of the fast or slow components of "P" and MI/II impossible. Therefore, in this paper, a simpler approach on the basis of the half-life times of both MI/II and "P" was undertaken. It shall finally lead to a model which not only is in agreement with the presented data but furthermore provides an explanation for the more complex kinetic behaviour of MI/II compared to the P signal.

Figure 3 compares the Arrhenius plot of "P" and MI/II on the basis of simple half-life times: "P" again shows two intersecting straight lines. The deviation from those lines near their intersection is explained by the fact that at temperatures where both consecutive reaction steps have the same or a similar rate constant, the sigmoid time course provides a value for the half-life time, which is considerably higher than one would expect from the value of either rate constant. The Arrhenius plot of MI/II exhibits the following interesting features.

(1) The slope of the MI/II Arrhenius plot is greater in magnitude than that of "P_2", but less than that of "P_1".

(2) "P" is, at all measured temperatures, slower than MI/II.

(3) For an equilibrium reaction—which MI/II is considered to be (Matthews et al., 1963)—one would expect a slope of the Arrhenius plot that increases with increasing temperature. This is not observed. Therefore it is quite likely that MI/II is not a simple equilibrium process.

The pH dependence of the kinetics of both "P_1" and MI/II is shown in Figure 4. Note that only "P_1" is influenced by pH;

[4] Similar, though slightly slower, parallel first-order kinetics can also be found in a new light-scattering transient "A", which can be obtained from bovine ROS in the presence of Mg^{2+} and ATP (Uhl et al., 1978).

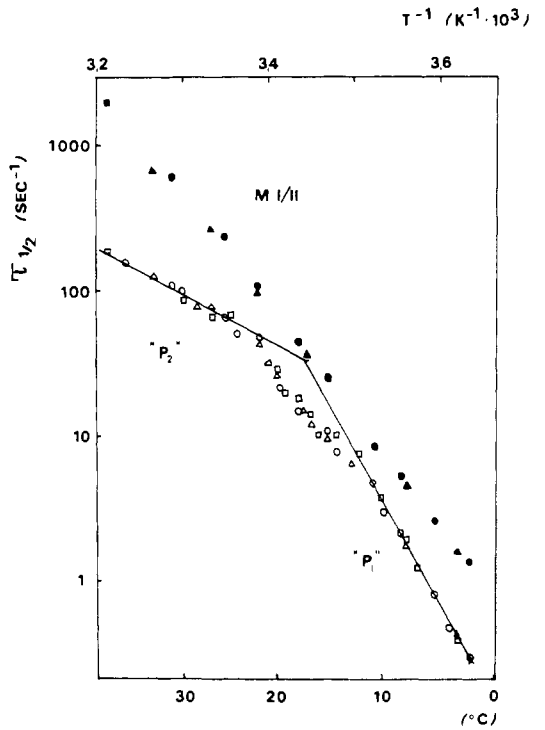

FIGURE 3: Comparison of the Arrhenius plot of both "P" and MI/II, using the reciprocal of the respective reaction half-life times. MI/II kinetics were monitored as increase in optical density at 380 nm. Where the same symbols are used for "P" and MI/II, it is indicated that these data were obtained from the same suspension under identical conditions.

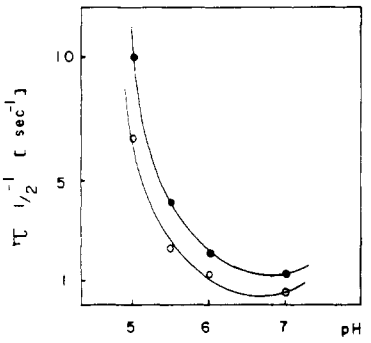

FIGURE 4: Comparison of the half-life times of both "P_1" and MI/II as a function of pH (simultaneous measurement). "P_2" exhibits no pH dependence of its kinetics. (●) MI/II; (○) "P_1".

"P_2" does not exhibit any pH dependence at all. There is a strong similarity between "P_1" and MI/II regarding their considerable acceleration at lower pH. Again, however, under all conditions "P_1" is slower than MI/II. The similar behavior of the kinetics of "P_1" and MI/II suggests that the two processes are somehow linked; the difference in their rate constants, however, excludes the possibility that both reflect the same event.

The Extent of the Disk Shrinkage and the State of the MI/II Equilibrium. The MI/II reaction is considered an equilibrium process. This was concluded from the existence of an equilibrium between two spectroscopically different species, absorbing at 478 and 380 nm, respectively. The state of this equilibrium, called MI (478)/MII (380) equilibrium, was shown to markedly depend on temperature, pH, and polarity of the reaction medium (Matthews et al., 1963). To see what influence the state of this equilibrium has on the extent of the disk shrinkage, the amplitude of MI/II and the amplitude of "P" were compared at different values of temperature

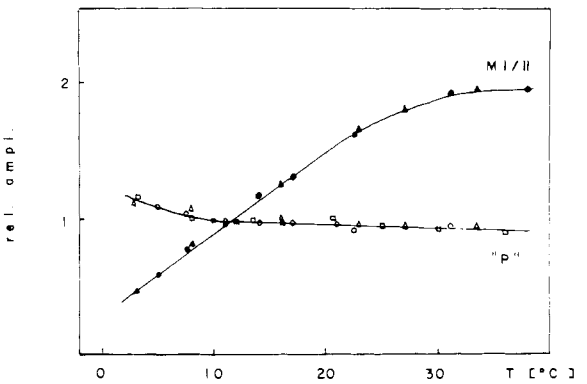

FIGURE 5: Comparison of the amplitude of "P" and MI/II as a function of temperature (simultaneous measurement).

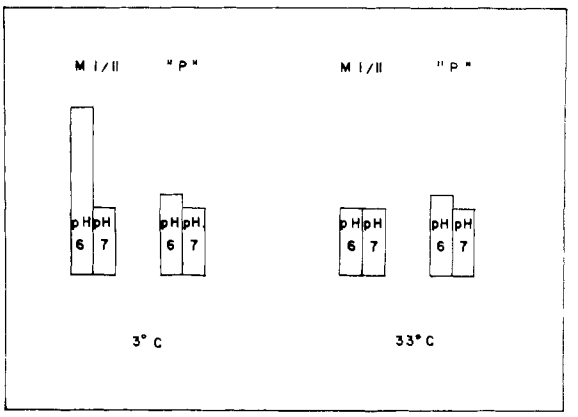

FIGURE 6: Comparison of the amplitude of "P_1" and MI/II and of "P_2" and MI/II at two different values of pH (simultaneous measurement).

(Figure 5) and pH (Figure 6). The results clearly show that there is no such influence of the state of the MI/II equilibrium on the amplitude of "P". Thus the reactions which maintain this equilibrium are effectively ruled out as the trigger process of "P" and neither reaction partner can be considered a trigger.

Discussion

A Model for the MI/II Transition. The light-induced light scattering signal "P", reflecting a rapid shrinkage of the ROS disks or rod sacs, consists of at least two consecutive reaction steps "P_1" and "P_2" as manifested from the Arrhenius plot. Both processes take place in the rhodopsin molecule itself.[5] This was concluded from the rhodopsin typical double exponential kinetics of "P_1" and "P_2", from the high activation energy of "P_1" and from the similar pH dependence of the kinetics of both "P_1" and MI/II. The latter finding also strongly suggests that "P_1" is the process to occur before "P_2" which, unlike "P_1" and MI/II is not affected by pH.

Both "P_1" and "P_2" occur *after* the rise of MI (completed in the μs range) and *before* the decay of MII, which is a rather slow reaction in the minute range. This clearly means that there must exist photolytic states of the rhodopsin molecule that do not appear as separate spectroscopic species, since *two* reac-

[5] Although both "P_1" and "P_2" occur within the rhodopsin molecule, the actually observed event, the shrinkage of the entire disk, takes place outside the receptor molecule. One has to conclude that the shrinkage itself, under the tested conditions, is considerably faster than the two intramolecular processes "P_1" and "P_2". The structural response of the disk membrane is therefore not rate limiting and does not show up in the kinetics of "P".

tions, "P_1" and "P_2", occur in the pigment molecule while there is only *one* spectroscopically visible transition, namely, MI/II. Thus a scheme for MI/II becomes possible that explains why the state of the equilibrium between MI (478) and MII (380) has no influence on the extent of the disk shrinkage (and may be on transduction). The reaction contains the MI/II equilibrium as a postponed reaction, preceded by an irreversible trigger process. In this way two pathways are possible:

$$\text{MI (478)} \xrightarrow{k_1} \text{MII (380)} \underset{k_3}{\overset{k_2}{\rightleftharpoons}} \text{MI' (478)} \quad (1)$$

$$\text{MI (478)} \xrightarrow{k_1} \text{MI' (478)} \underset{k_3}{\overset{k_2}{\rightleftharpoons}} \text{MII (380)} \quad (2)$$

In both cases it is assumed that the decay of MI with the rate constant k_1 is the trigger process for the disk shrinkage and can be monitored as "P_1". Assuming now that k_1 is affected by pH, it becomes clear why pH changes have a similar effect on "P_1" and MI/II, but not an identical one: The kinetics of MI/II do not only depend on k_1, but also on k_2 and k_3. Likewise the postponed equilibrium provides the explanation why the state of this equilibrium is of no bearing on the extent of the disk shrinkage.

Which of the two proposed schemes, 1 or 2, is correct, can be decided from the kinetic data given in Figures 3 and 4: it can easily be seen that, if 2 is correct, the trigger process (manifested as "P_1" with the rate constant k_1) would always be faster than the apparent rise of MII. The opposite is observed, however. To see why this finding is not contradictory to reaction 1 is a little more complicated: for reaction 1 the mathematical expression for the kinetics of MI/II obeys the following (eq 3):

$$[\text{MII}_{(t\to\infty)}] - [\text{MII}_{(t)}] = \frac{1}{k_2 + k_3 - k_1} \times \left(\frac{k_1 k_2}{k_2 + k_3} e^{-(k_2+k_3)t} + (k_3 - k_1)e^{-k_1 t}\right) \quad (3)$$

In the special case where k_3 equals k_1 or where both are similar, eq 3 has the simpler form (eq 4):

$$[\text{MII}_{(t\to\infty)}] - [\text{MII}_{(t)}] = \frac{k_1}{k_1 + k_2} e^{-(k_1+k_2)t} \quad (4)$$

The rate constant for MI/II therefore is $(k_1 + k_2)$ which is greater than k_1 alone, the rate constant of the trigger process "P_1". This implies that MI/III is faster than "P_1".

Since the same scheme should apply to both rhodopsin pathways, in this special case where k_1 and k_3 are equal or similar, the kinetics of MI/II should consist of two first order processes.

Where $k_1 \neq k_3$, however, each of the two rhodopsin pathways should exhibit a biphasic behavior for MI/II, thus providing a total of four kinetic components for this process. The fact that under certain conditions such a kinetic behavior is found in our preparation is taken as support for the proposed scheme. At the present time, however, since a systematic analysis of the MI/II kinetics under this aspect has not yet been undertaken, we consider the scheme as likely but not yet proven. Further support for it stems from the data below on MI/II, as found in the literature.

Support for the Proposed Scheme from Results of Other Authors. (a) Stewart et al. (1976) described the MI/II kinetics to consist of four easily to separate kinetic components when measured in digitonin micelles. We assume that in digitonin the rate constant differences between k_1, on the one hand, and k_3, on the other hand, are more pronounced and that therefore all four rates, k_{1_f}, k_{1_s} as well as $(k_2 + k_3)_f$ and $(k_2 + k_3)_s$ appear unmasked.

(b) As already mentioned, either state of the equilibrium between MI' and MII or kinetics of their transition are affected by pH (Matthews et al., 1963; Sengbusch & Stieve, 1971; Hoffman, 1977): lower pH accelerates MI/II and shifts the equilibrium towards MII. Below pH 6, however, there is still a considerable increase in rate constant for MI/II (factor of 10–15 between pH 6 and pH 4.5), whereas the state of the equilibrium remains constant, i.e., is identical for both pH. For a reaction scheme

$$\text{MI (478)} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{MII (380)} \quad (5)$$

one would expect that each acceleration would also affect the ratio of k_1/k_2 and therefore the equilibrium. Particularly at low temperatures where the equilibrium favors MI (478) this effect should be rather pronounced, but is not observed. In our model there is no such problem arising, if one assumes that two proton binding sites of the pigment molecule are involved in MI/II. One acts as a catalytic site, affecting k_1, the other one determines whether the pigment molecule is in the unprotonated (MI') or protonated form (MII). Both binding sites have different p*K*s, thus causing the different pH dependence of kinetics and equilibrium.

(c) In the same way Matthews et al.'s (1963) finding is understood, namely, that glycerol affects the state of the MI/II equilibrium, but not the MI/II kinetics. In terms of the proposed scheme this means that only k_2 and k_3 are affected by glycerol, but not k_1.

(d) Likewise the apparent incongruity of reaction enthalpies and entropies on one side and the respective activation parameters of MI/II on the other side, first stated by Ostroy et al. (1966), can be dissipated in the following manner. Kinetic data of MI/II deal with three constants k_1, k_2, and k_3, whereas the thermodynamic data concerning the state of equilibrium only involve k_2 and k_3. This is to say that the kinetically monitored part of the rhodopsin photolytic cycle, called MI/II, is not identical with the process that establishes the equilibrium after the light-induced cascade of dark reactions in the rhodopsin has already taken place.[6]

Some Speculations on the Possible Meaning of MI and MI' for the Transduction Mechanism. The MI/II reaction has been generally considered the trigger process for transduction (Wald, 1968). The main evidence for this is that MI/II is the last of the light-stimulated dark reactions that is fast enough to be the trigger (the following MII decay, with its kinetics in the minute range, is far too slow), but the first one that involves the rhodopsin environment: MI/II is accompanied by a proton uptake (Falk & Fatt, 1966; McConnell et al., 1968; Emrich, 1971) and a rapid change in birefringence (Liebmann et al., 1974) and it requires the presence of water.

If the trigger process MI/II were a simple equilibrium reaction, the following problem would arise: in the visual process the bleaching of *one* rhodopsin should lead to *one* chain of transduction events, terminating in *one* transient hyperpolarization of the plasma membrane. However, the rate of the MI/II reaction under physiological conditions (ca. 1000 s^{-1}, which in the case of a simple equilibrium reaction would cor-

[6] Further support for this comes from recent data of Hoffmann (1977). Our model predicts that a low pH k_1 is markedly larger than $k_2 + k_3$, and therefore the time course of MI/II should show a fast rise (with a rate constant of k_1) and a subsequent partial decay of the MII amplitude (with the rate $k_2 + k_3$). Exactly this is observed by Hoffmann at pH 4.

respond to $k_1 + k_2$) and the equilibrium constant of 10 (corresponding to k_1/k_2) imply that the MI/II reaction is driven back and forth with a rate of about 90 s^{-1}. Therefore the rhodopsin environment has to be able to distinguish between the first time this happens (when it "communicates" that the pigment has seen light) and all the following times.

In our model, the actual trigger process is irreversible. The decay of MI contains the trigger information, but not the decay of MI'. However, the molecular difference between MI and MI', the difference that enables one to trigger further events and prevents the other one from doing it, remains to be elucidated. It is possible that the intermediate MI, which is able to trigger the disk shrinkage, still possesses enough of the stored light energy (Rosenfeld et al., 1976) to trigger subsequent processes, whereas MI' no longer contains "useful" energy to a sufficient extent.

Williams (1970), from quite different measurements, also has suggested the existence of isochromic forms of rhodopsin intermediates. Like us he uses, rather than the spectroscopic property, the capability of the pigment molecule to act in a certain way as a criterion for different forms. What he calls MI and MI', however, cannot be identical with MI and MI appearing in our scheme. His two isochromic forms appear to belong to the different rhodopsin pathways.

Finally, we should like to come back to the problem that no trace of any of the known rhodopsin photolytic processes has been found in the kinetics of the rise of the receptor potential. In this paper we have provided evidence that a trace of MI/II can be seen in the kinetic process "P_1", and we have shown that "P_1" is followed by a process "P_2", which also most likely occurs within the pigment molecule. This process "P_2" finally leads to the shrinkage of an entire disk. If it could be shown now that the processes "P_1" and "P_2" that terminate in the disk shrinkage are not "cul de sac" but actual part of the transduction chain, the above gap could be considered closed since the kinetics of "P_2", the process that follows the MI decay, is slow enough (ca. 20 ms at room temperature) to be visible as a trace in the time course of the light response. Before this can be said, further studies will have to explore the molecular basis of the disk shrinkage phenomenon. But even if it should turn out that "P_1" and "P_2" are not *the* processes that mediate between rhodopsin and disk membrane in transduction, they will still be examples of *a* light-stimulated communication between the pigment and its environment.

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References

Abrahamson, E. W., & Wiesenfeld, J. R. (1972) in *Handbook of Sensory Physiology* (Datnall, H. J. A., Ed.) Vol. VII/1, pp 69–121, Springer-Verlag, New York, N.Y.

Baylor, D. A., Hodgkin, A. L., & Lamb, T. D. (1974) *J. Physiol. 242,* 685–791.

Emrich, H. M. (1971) *Z. Naturforsch. B 26,* 352–356.

Falk, G., & Fatt, P. J. (1966) *J. Physiol. 185,* 211–224.

Hagins, W. A. (1957) Ph.D. Thesis, University of Cambridge.

Hoffmann, W. (1977) Ph.D. Thesis, Freiburg, West Germany.

Hofmann, K. P., Uhl, R., Hoffman, W., & Kreutz, W. (1976) *Biophys. Struct. Mech. 2,* 61–77.

Kleinschmidt, J., & Dowling, J. E. (1975) *J. Gen. Physiol. 66,* 617–648.

Lewis, A. (1976) in *The SPEX Speaker,* Vol. XXI, No. 2, pp 1–8.

Liebmann, P. A., Jagger, W. S., Kaplan, M. W., & Bargoot, F. G. (1974) *Nature (London) 251,* 31–36.

Matthews, R. G., Hubbard, R., Brown, P. K., & Wald, G. (1963) *J. Gen. Physiol. 47,* 215–240.

McConnell, D. G., Rafferty, C. N., & Dilley, R. A. (1968) *J. Biol. Chem. 243,* 5820–5826.

Norisuye, T., Hoffmann, W. A., & Yu, H. (1976) *Biochemistry 15,* 5678–5682.

Ostroy, S. E., Erhardt, F., & Abrahamson, E. W. (1966), *Biochim. Biophys. Acta 112,* 265–277.

Penn, R. D., & Hagins, W. A. (1972) *Biophys. J. 12,* 1073–1094.

Rosenfeld, T., Honig, B., Ottolenghi, M., Hurley, J., & Ebrey, T. G. (1976) *Pure Appl. Chem. 49,* 341–351.

Sengbusch, G. V., & Stieve, H. (1971) *Z. Naturforsch. B 26,* 861–867.

Stange, S. (1977) Ph.D. Thesis, Freiburg.

Stewart, J. G., Baker, B. N., Plante, È. O., & Williams, T. P., (1976) *Arch. Biochem. Biophys. 172,* 246–251.

Stewart, J. G., Baker, B. N., & Williams, T. P. (1977) *Biophys. Struct. Mech. 3,* 19–29.

Stone, W. L., Farnsworth, C. C., & Dratz, F. A. (1978) *Biophys. J. 21,* 135a.

Uhl, R. (1976) Ph.D. Thesis, Freiburg.

Uhl, R., Hofmann, K. P., & Kreutz, W. (1977) *Biochim. Biophys. Acta 469,* 113–122.

Uhl, R., Borys, T., & Abrahamson, E. W. (1978) *Biophys. J. 21,* 136a.

Wald, G. (1968) *Nature (London) 219,* 800–807.

Wey, C. L., & Cone, R. A. (1978) *Biophys. J. 21,* 135a.

Williams, T. P. (1970) *Vision Res. 10,* 525–533.

Yau, I. W., Lamb, T. D., & Baylor, D. A. (1977) *Nature (London) 269,* 78–80.