Biophys. Struct. Mechanism 2, 321–331 (1977)

Biophysics of Structure and Mechanism

Indication of the Metarhodopsin I–II Transition by Absorption-changes of Eriochromblack T

Hinderk M. Emrich* and Roland Reich

Max-Volmer-Institut für Physikalische Chemie und Molekularbiologie, Technische Universität Berlin

Abstract. The dye eriochromblack T (erio T), added to an aqueous suspension of bovine retinal outer segments solubilized by digitonin, shows a light-induced absorption-increase at $\lambda = 645$ nm. Erio T is shown to directly interact with micellar metarhodopsin I and metarhodopsin II. The absorption-changes of erio T can be regarded as an indication of the transition from the metarhodopsin I conformation (with associated Ca^{2+}) to the metarhodopsin II conformation (with associated H^+).

Key words: Photoreception – Rhodopsin-photolysis – Conformation-change – Solvatochromism.

1. Introduction

The conversion of metarhodopsin I (M_I) to metarhodopsin II (M_{II}) (meta I–II transition) is generally assumed to represent the primary event which triggers visual excitation [23]. This conversion is spectroscopically indicated by a strong difference in the absorption spectra of M_I ($\lambda_{max} = 480$ nm) and M_{II} ($\lambda_{max} = 380$ nm) [18, 23]. Moreover, there are several hints that the meta I–II transition is not localized to the chromophore, but that it involves some further important displacements of other atoms of the chromoprotein molecule:

1. The meta I–II transition is preceeded by several consecutive reactions but is just fast enough to allow for participation in visual excitation [3]. (The following decomposition to retinal and opsin is too slow for this.)

2. The azomethin group of retinal becomes accessible for aqueous reducing agents by this conversion [1, 2].

3. The meta I–II transition is accompanied by an H^+-uptake [5–7] and a release of Ca^{2+} [8, 11, 16].

* Present address: Max-Planck-Institut für Psychiatrie, D-8000 München 40, Kraepelinstr. 10, Federal Republic of Germany

4. The meta I–II equilibrium can be shifted to the M_I-conformation by external pressure, indicating a volume increase of the suspension during meta I–II transition [9]. However, an increase of volume generally implies a decrease of the averaged attraction forces between the atoms in the system (e.g. breaking of some hydrogen bonds or salt linkages [8, 16]).

5. The analysis of the H^+-uptake yields some hints on a conformative coupling of two rhodopsin molecules [7, 16].

However, from measurements of the circular dichroism of M_I and M_{II} it must be concluded that at least a significant portion of the dissymmetric local environment of retinal remains unchanged during meta I–II transition [19, 22]. (Only in the presence of strong detergents – other than digitonin – a marked loss in helical conformation is seen to accompany the meta I–II transition [19, 20].) Therefore an artificial indicator might be a useful tool in further investigations of this conversion.

Electrochromic absorption-changes of dye molecules incorporated in biological membranes are used at present to measure electric field-changes across these membranes [4, 5, 12–15, 25]. Changes of *local* electric fields in the micro-environment of adsorbed dye molecules can be caused also by conformation-changes in lipids. Thus, electrochromic (or "solvatochromic") absorption-changes and fluorescence-changes are induced, which can be used as an indicator of the underlying conformation-change. The change of the micro-environment of the dye molecule can be realized either by a transfer of the dye molecule from the water phase into the lipid phase (as proposed by Träuble [21] for the dyes 1-anilinonaphthalene-8-sulfonate, ANS, and bromthymolblue, BTB), or simply by a change of the orientation of the dye molecule *within* the lipid phase (as proposed by Walz [24] for chlorophyll *a*). A further possibility would be given by the exchange of ions in the micro-environment of the adsorbed dye molecule. This appears to be realized in the present experiments on the influence of meta I–II transition of the absorption of erio T.

2. Methods

Bovine rod outer segments (ROS) were isolated on a sucrose density gradient [6, 7]. The measuring equipment was a rapid registrating repetitive flash photometer after Witt [25]. The flash (Osram XIE 200) had an intensity of about 6 Ws and a half-life of 2×10^{-5} s. The photomultiplier (EMI 9558 AQ) was DC-coupled to an average-computer (Enhancetron 1024, ND). The electrical bandwidth was 1.4 kHz. The measuring light, monochromized by a Bausch and Lomb monochromator (optical bandwidth: $\pm$ 3 nm), was adjusted to $\lambda = 385$ nm (rise of M_{II}) or to $\lambda = 645$ nm (absorption-change of erio T). Filter combination: 385 nm: Balzers Filtraflex 155/115 + Schott OG 4 + Schott GG 10 colour-glasses at the flash-source; Schott DAL 385 interference-filter at the photocathode; 645 nm: Balzers Filtraflex-K3 at the flash-source; Schott DAL 645 interference-filter at the photocathode. In each experiment 35 single measurements were averaged. Length of cuvette: 50 mm; volume: 15 ml. In each experiment 0.2 ml ROS-suspension (4.2×10^{-5} M rhodopsin-concentration) + 0.2 ml digitonin (2%) were added to 14.6 ml electrolyte/buffer solution, indicated in the legends. The temperature was adjusted to 26.0° C. The erio T absorption spectra were measured using a Shimazu photometer.

3. Results

A preparation of rod outer segments solubilized by digitonin shows practically no light-induced absorption-changes at $\lambda = 645$ nm. However, after addition of 3×10^{-5} M erio T, a strong absorption-increase is observed (Fig. 1). For this signal, the solubilisation of the rod outer segments by digitonin is a necessary condition; without digitonin, the signal is only very small.

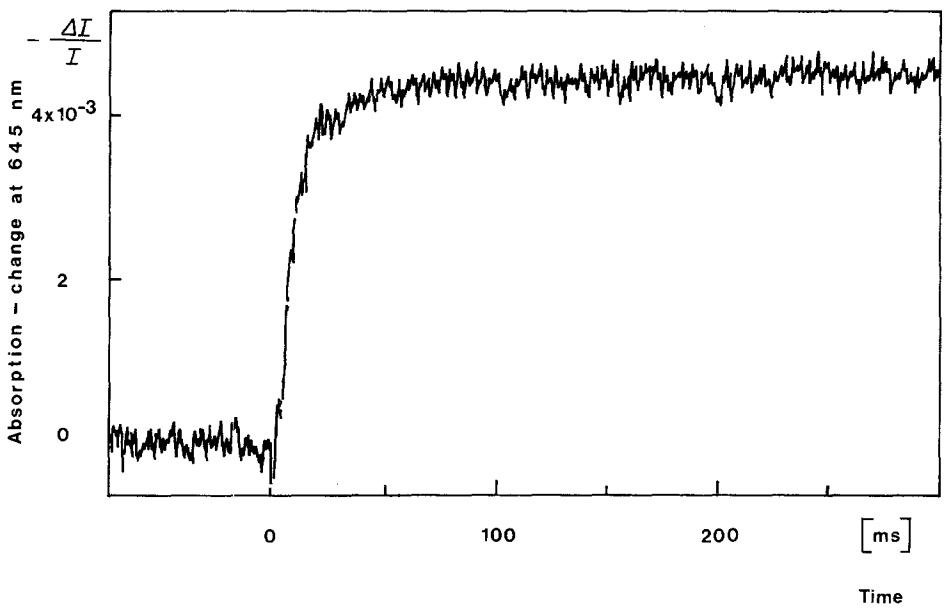

Fig. 1. Flash-induced absorption-increase at $\lambda = 645$ nm. Erio T-concentration: 3×10^{-5} M; phosphate buffer concentration: 13×10^{-5} M; pH = 5.8; temperature: 26.0° C

The time course of this absorption-change shows a characteristic deviation from a simple first-order process, as has frequently been observed also in the meta I–II transition and the accompanying proton-uptake [7]. The kinetic analysis was performed in the following way: In a semilogarithmic plot of deviation of the absorbance from its equilibrium value as a function of time t, the slow component was separated by extrapolating the straight line obtained at long times to $t = 0$. The mean rate constant of the fast component was obtained by substracting the amplitude of the slow component from the total change and plotting the difference once more in a logarithmic scale as a function of t.

In Figure 2, the rate constants of the slow and of the fast component of the absorption-change of erio T at 645 nm are compared with the corresponding rate constants of the absorption-increase of M_{II} at 385 nm and of the proton-uptake ΔH^+ (from [7])[1]. The rate constants of erio T agree with those of ΔH^+, whereas the kinetics of the ΔM_{II}-signal are slightly faster.

---

[1] The symbol ΔH^+ denotes the amount of protons per volume of the suspension, which are adsorbed by metarhodopsin after one flash. ΔH^+ is not identical with the decrease of the proton concentration C_{H+}, since the solution is slightly buffered. ΔM_{II} is the amount of M_{II} per volume that is formed after one flash

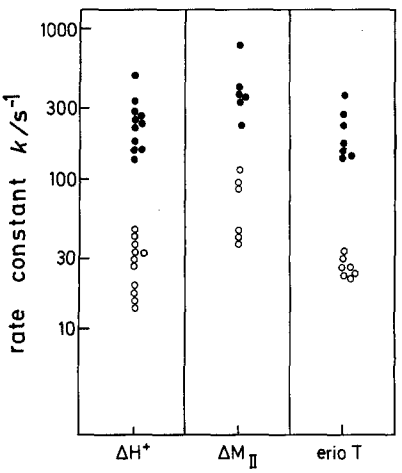

Fig. 2. Rate-constants of the light-induced proton uptake (ΔH^+), of the absorption-increase at λ = 385 nm (ΔM_{II}), and of the absorption-increase at λ = 645 nm (erio T) in a rhodopsin-digitonin-erio T suspension, pH = 5.8, concentrations cf. Figure 1. Each point represents a single measurement. In these measurements different ROS-preparations have been used, leading to the high degree of scattering of the kinetic results. ΔH^+ is calculated from the pH-change of the slightly buffered solution as measured by the calibrated absorption-change of bromcresolpurple at λ = 596 nm [7]. O slow components; ● fast components

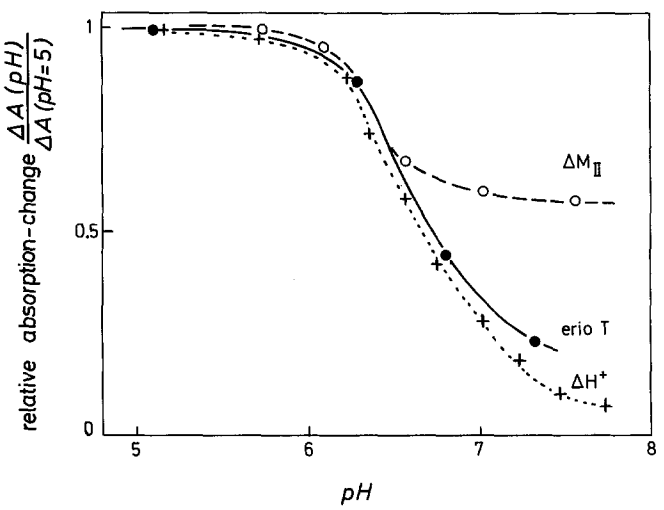

Fig. 3. pH-dependence of the amplitudes of the relative light-induced absorption-changes at λ = 645 nm (erio T, ●) and at λ = 385 nm (ΔM_{II}, O), and of the proton uptake (ΔH^+, +). All relative signals are referred to the corresponding values at pH = 5

As a further test whether the erio T-signal really indicates the meta I–II transition, its dependence on pH and on Ca^{2+}-concentration can be compared with the dependence of the ΔM_{II}-signal on these parameters.

In Figure 3, the pH-dependence of the relative absorption-change of erio T (solid line) is compared with that of ΔM_{II} (dashed line) and of the proton uptake ΔH^+ (dotted line). Again, the curves of erio T and of ΔH^+ agree well: The amplitude is high in the acid and low in the alkaline region with an inflection point at pH $\approx$ 6.6.

The curve of ΔM_{II} qualitatively shows a similar behaviour. The quantitative differences are interpreted in the discussion.

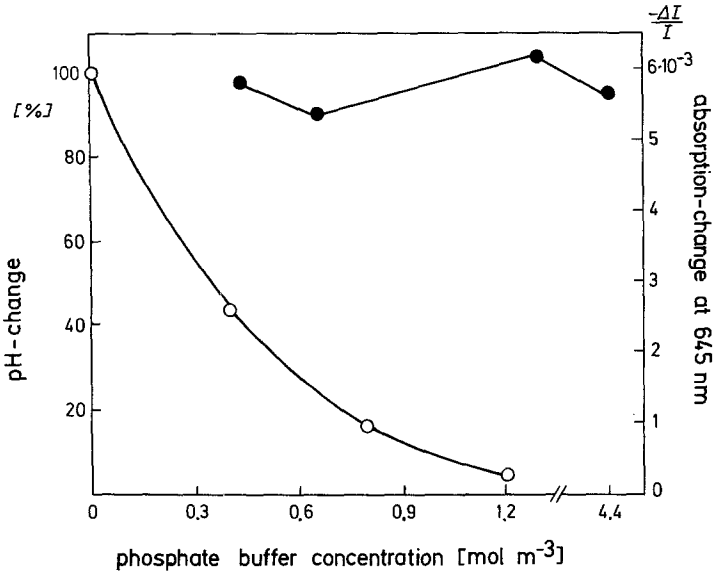

Fig. 4. Relative pH-change (as measured by the absorption-change of bromcresolpurple at λ = 596 nm) and absorption-change of erio T at λ = 645 nm in a rhodopsin-digitonin suspension after a flash, as function of phosphate buffer concentration; pH = 5.8

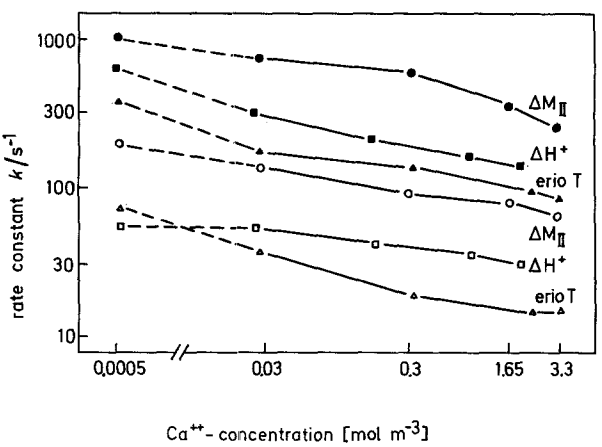

Fig. 5. Rate constants of the light-induced absorption-changes of erio T, ΔM_{II}, and of ΔH^+, as a function of Ca^{2+}-concentration. Open symbols: slow components; filled symbols: fast components. The Ca^{2+}-concentration of 5×10^{-7} M was adjusted using EDTA as Ca^{2+}-chelating agent

From the agreement between the signals of erio T and of ΔH^+ one might conclude that erio T plays the same role as a simple pH-indicator in the aqueous solution. However, this possibility is excluded by the experiments shown in Figure 4: If the pH-change in the aqueous phase is suppressed by increasing the buffer concentration, the erio T-signals remain constant.

Finally, one has to test the dependence on Ca^{2+}-concentration. Recently it has been demonstrated that the kinetics of the meta I–II reaction and of the H^+-uptake are slowed down by Ca^{2+} [8]. The same is true for the absorption-change of erio T: The rate constants of the slow and the fast components of ΔM_{II}, of ΔH^+, and of the erio T-signal show the same dependence on Ca^{2+}-concentration (Fig. 5).

Furthermore it has been shown that the amplitude of ΔM_{II} is decreased by Ca^{2+}, indicating a shift of the meta I–II equilibrium to M_I [8, 16].

Such a shift can only be performed by some molecular attraction forces between Ca^{2+} and metarhodopsin, i.e. some adsorption or complex formation. Let the symbols C_I and C_{II} denote the overall concentrations of M_I and M_{II}, respectively, including all possible complexes of these two conformations with Ca^{2+} and H^+. If (in an experiment of thought) C_I and C_{II} are fixed at their former equilibrium ratio during the addition of Ca^{2+} to the solution, a shift of the equilibrium from M_{II} towards M_I means that the chemical potential of M_I becomes smaller than that of M_{II} (so that the reaction "wants to go" towards the new equilibrium), i.e. the M_I-Ca^{2+} complex must be more stable than a possible M_{II}-Ca^{2+} complex (if the latter exists at all). Therefore it must be thermodynamically expected that, with a suitable concentration of Ca^{2+}, the meta I–II transition is accompanied by a release of Ca^{2+} into the solution. This is in agreement with observations of Nöll [11].

In Figure 6 the dependence on Ca^{2+}-concentration of the erio T-signal (solid line) is compared with that of the ΔM_{II}-signal (dashed line).

An increase of the Ca^{2+}-concentration from 10^{-3} M to 10^{-1} M causes a decrease of the erio T-signal which is similar to that of ΔM_{II}. However, on *decreasing* the

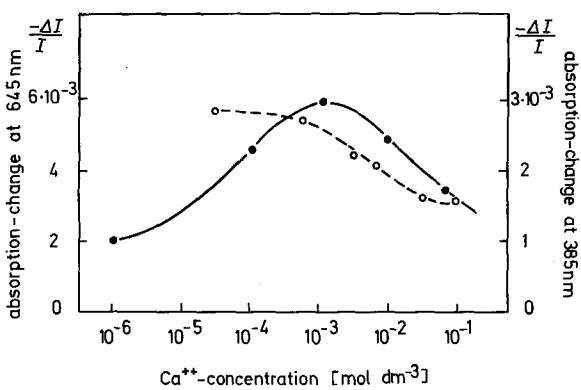

Fig. 6. Absorption-changes at λ = 645 nm, due to erio T (solid line) and at λ = 385 nm, due to the formation of M_{II}, (dashed line), as function of Ca^{2+}-concentration at constant pH = 5.8

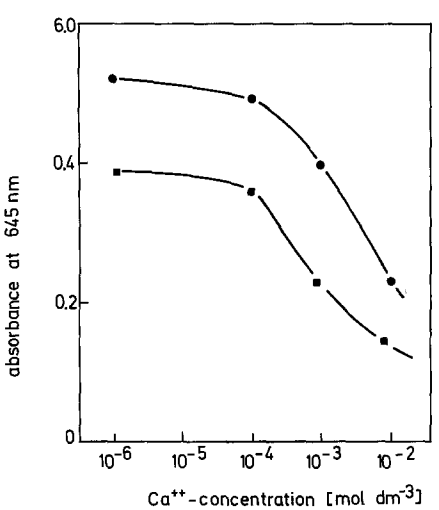

Fig. 7. Absorbance at λ = 645 nm of an aqueous solution of erio T, $C = 5 \times 10^{-5}$ M, as a function of Ca^{2+}-concentration. Upper curve: pH = 6.3; lower curve: pH = 5.5

Ca^{2+}-concentration to 10^{-4} and to 10^{-6} M, the erio T-signal is diminished *again,* whereas the ΔM_{II}-signal still increases. Obviously, a further influence of Ca^{2+} on erio T appears to be superimposed.

In aqueous solution, Ca^{2+} forms a weak complex with erio T [17]. The influence of Ca^{2+}-concentration on the absorbance of erio T at $\lambda = 645$ nm in an aqueous

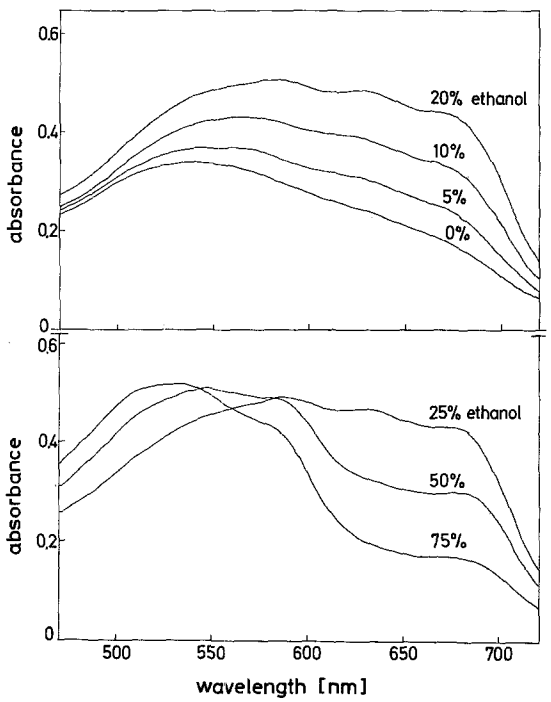

Fig. 8. Absorption spectrum of erio T, $C = 5 \times 10^{-5}$ M, at a constant Ca^{2+}-concentration $= 10^{-3}$ M, in ethanol-water mixtures of different percentages. pH = 5.5

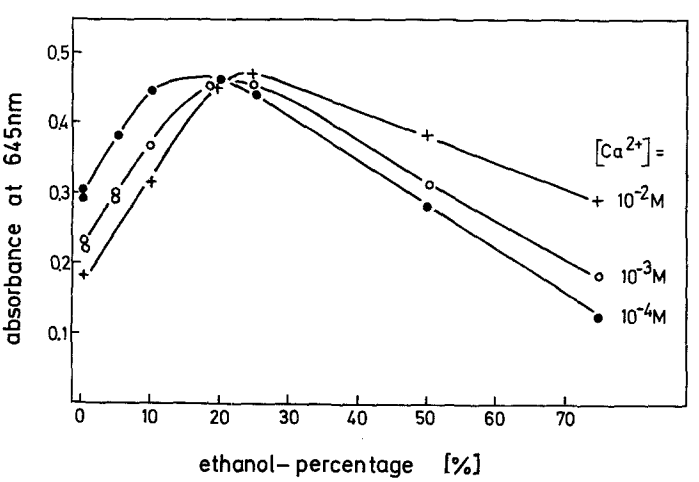

Fig. 9. Absorbance of erio T at $\lambda = 645$ nm as a function of ethanol percentage in the ethanol-water mixture, at different Ca^{2+}-concentrations. $C_{\text{erio T}} = 5 \times 10^{-5}$ M = constants. pH = 5.5

solution (without rhodopsin and digitonin) is shown in Figure 7. The dye changes from the free form to the occupied form in the concentration range between 10^{-4} M and 10^{-2} M Ca^{2+}. (At $\lambda = 645$ nm, the absorbance of the occupied form is smaller than that of the free form.)

The influence of polarity of the solvent on the absorption spectrum of erio T is shown in Figure 8. (The polarity is diminished by addition of ethanol.) In Figure 9 the absorbance of erio T at 645 nm is shown as a function of polarity of the solvent (ethanol-concentration) and of Ca^{2+}-concentration. At ethanol-concentrations below 20%, the absorption decreases with increasing Ca^{2+}-concentration (due to formation of the Ca^{2+}-erio T-complex, cf. Fig. 7); however, at ethanol-concentrations *above* 20%, this dependence is inverted.

4. Discussion

At $\lambda = 645$ nm the absorbance of the erio T-Ca^{2+}-complex is smaller than that of free erio T (Fig. 7). This statement should hold also for erio T which is adsorbed in the rhodopsin-digitonin micelles, if the polarity in the micro-environment of erio T corresponds to an ethanol-water-mixture of less than 20% (Fig. 9). Then the light-induced adsorption-increase of erio T in the rhodopsin-digitonin micelles can be explained by the dissociation of an erio T-Ca^{2+}-M_I-complex, due to the release of Ca^{2+} from the micelles which has been demonstrated to accompany meta I–II transition [8, 11, 16]. This absorption-increase of erio T has a maximum at a Ca^{2+}-concentration of 10^{-3} M (Fig. 6). If the concentration is smaller than 10^{-3} M, the amount of erio T-Ca^{2+}-M_I-complexes is correspondingly smaller, and thus also the absorption-increase due to the dissociation of Ca^{2+} from this complex during meta I–II transition is smaller (Fig. 6). However, if the Ca^{2+}-concentration increases above 10^{-3} M, the absorption-increase becomes smaller again, since meta I–II transition is suppressed more and more.

A change of the concentrations of erio T and of erio T-Ca^{2+}-complexes *in the aqueous phase* cannot be the reason for the absorption-changes observed in Figure 6, since the relative change of the concentration of free Ca^{2+}-ions in the aqueous phase due to the release of Ca^{2+} from the rhodopsin is too small, especially at $C_{Ca^{2+}} = 10^{-1}$ M. Moreover, according to Figure 7, the *sign* of the absorption-change would be wrong in this case.

We have still to discuss the reason for the difference between the curves of ΔM_{II} and ΔH^+ in Figure 3. According to the model proposed in [7], meta I–II transition consists of two steps: firstly the formation of an unfolded structure (M_{II}*) with exposure of a base from the hydrophobic core of the molecule to the aqueous phase, and secondly the protonation of this base (in the acid pH-range), leading to a stabilization of the unfolded M_{II}-conformation:

$$M_I \underset{K^*}{\rightleftharpoons} M_{II}^* \overset{+H^+}{\underset{K_B}{\rightleftharpoons}} M_{II}H^+ . \tag{1}$$

The first equilibrium is characterized by the constant

$$K^* = C_{M_{II}^*}/C_I , \tag{2}$$

where $C_{M_{II}}$ and C_I are the mean equilibrium concentrations [mol/l] of M_{II}* and M_I in the suspension. K* is only a function of the temperature, whereas the second equilibrium, characterized by the proton-association constant

$$K_B = \frac{C_{M_{II}H^+}}{C_{M_{II}^*} C_{H^+}}, \tag{3}$$

can be shifted to the right side of (1) by increasing the H^+-concentration.

The light-induced absorption-change at 385 nm is proportional to the amount ΔM_{II} which is formed after the flash, and this amount is a definite fraction of the amount of rhodopsin which was photolysed by the flash. This fraction is determined by the meta I–II equilibrium:

$$\Delta M_{II} \sim \frac{C_{II}}{C_I + C_{II}}. \tag{4}$$

The concentration C_{II} is given by the sum of the concentrations of the protonized and the unprotonized form, which have both the same absorption spectrum:

$$C_{II} = C_{M_{II}^*} + C_{M_{II}H^+}. \tag{5}$$

Inserting (2), (3) and (5) into (4), we get for ΔM_{II} in Figure 3:

$$\Delta M_{II} \sim \frac{1 + K_B C_{H^+}}{1/K^* + 1 + K_B C_{H^+}}. \tag{6}$$

For high values of C_{H^+} (low pH), the fraction in (6) approaches unity, i.e. the equilibrium in (1) is shifted completely to M_{II} (high amplitude of the ΔM_{II}-signal in Fig. 3). On the other hand, for low values of C_{H^+}, the fraction in (6) will approach zero only under the condition $K^* \ll 1$, which is fulfilled at 2° C. At 23° C, however, K^* has a value of ≈ 0.4 [7], which explains that still considerable amounts of M_{II} are formed even at pH = 8 (the amplitude of ΔM_{II} is smaller than in the acid pH-region, but *not* zero, cf. Fig. 3).

The curve ΔH^+ (pH) in Figure 3 gives the amount of protons per volume which are taken up during meta I–II transition. This ΔH^+ is equal to the amount of $M_{II}H^+$ which is formed after one flash and which is determined by the meta I–II equilibrium:

$$\Delta H^+ \sim \frac{C_{M_{II}H^+}}{C_I + C_{II}}. \tag{7}$$

Inserting (2), (3), and (5) into (7), we get

$$\Delta H^+ \sim \frac{K_B C_{H^+}}{1/K^* + 1 + K_B C_{H^+}}. \tag{8}$$

For high values of C_{H^+} (low pH), i.e. for $K_B C_{H^+} \gg 1$, the fraction in (8) does not differ from that in (6). But for low values of C_{H^+} (high pH), the fraction in (8) approaches zero, in contrast to that in (6) (cf. Fig. 3).

The formula (8) yields a sigmoid curve for ΔH^+ as a function of the pH, which is similar to the titration curve of a simple pH-indicator in an aqueous solution. For comparison, if HX is the absorbing form and X^- the nonabsorbing form of a pH-indicator with a proton-association constant

$$K_B = \frac{C_{HX}}{C_{X^-}\ C_{H^+}}, \tag{9}$$

the absorbance A is proportional to the fraction of the absorbing form:

$$A \sim \frac{C_{HX}}{C_{HX} + C_{X^-}}. \tag{10}$$

Insertion of (9) yields

$$A \sim \frac{K_B\, C_{H^+}}{1 + K_B\, C_{H^+}} = \frac{K_B \cdot 10^{-pH}}{1 + K_B \cdot 10^{-pH}}. \tag{11}$$

The inflection point of this well known titration curve lies at $pH = \log K_B$. For high values of C_{H^+}, i.e. for $K_B\, C_{H^+} \gg 1/K^*$, the fraction in (8) does not differ from that in (11). Otherwise the value of ΔH^+ according to (8) is smaller, so the inflection point is shifted from $pH = \log K_B$ to lower pH values by the term $1/K^*$ in the denominator of (8) (cf. Fig. 3).

From the agreement between the curves of erio T and of ΔH^+ (the latter reflects the concentration-increase of $M_{II}H^+$) it can be concluded that the erio T-signal is caused by a *direct* molecular interaction of erio T with micellar $M_{II}H^+$. An *indirect* interaction by means of the change of C_{H^+} in the aqueous phase is excluded by the finding of Figure 4. This conclusion is confirmed by the observation that the erio T-signals are very small (nearly absent) if rod outer segments are *not* solubilized by addition of digitonin, although the ΔH^+-signals (using e.g. bromcresolpurple) are still present, only showing slower kinetics [6, 7].

The detergent digitonin appears to be necessary to hold the erio T in direct molecular contact with the metarhodopsin. So, in ROS suspensions, the concentration of metarhodopsin-erio T complexes is only very small, thus the same is true for the erio T-signals.

So the absorption-changes of erio T in rhodopsin-digitonin micelles can be regarded as a direct indication of the transition from the M_I-conformation (with associated Ca^{2+}) to the M_{II}-conformation (with associated H^+).

Finally the question arises if the absorption-changes of erio T may be called "solvatochromic" (i.e. caused by local electrochromism), or if they are more a kind of mesomeric effect, like the color-change of a pH-indicator induced by protonisation. However, this may be merely a matter of definition, since in the case of erio T there appears to exist a continuous shading between inductive and mesomeric effects (cf. Figs. 8, 9).

Thanks are due to the Deutsche Forschungsgemeinschaft for financial support (Em 18/1–4).

References

1. Akhtar, M., Blosse, P. T., Dewhurst, P. B.: The active site of the visual protein, rhodopsin. Chem. Comm. **13**, 631–632 (1967)
2. Bownds, D.: Site of attachment of retinal in rhodopsin. Nature (Lond.) **216**, 1178–1181 (1967)

3. Cone, R. A., Cobbs, W. H.: Rhodopsin cycle in the living eye of the rat. Nature (Lond.) **221,** 820–822 (1969)
4. Emrich, H. M., Junge, W., Witt, H. T.: Further evidence for an optical response of chloroplast bulk pigments to a light-induced electrical field in photosynthesis. Z. Naturforsch. **24b,** 1144–1146 (1969)
5. Emrich, H. M., Junge, W., Witt, H. T.: An artificial indicator for electric phenomena in biological membranes and interfaces. Naturwissenschaften **56,** 514–515 (1969)
6. Emrich, H. M.: Optical measurements of the rapid pH-change in the visual process during the metarhodopsin I–II reaction. Z. Naturforsch. **26b,** 352–356 (1971)
7. Emrich, H. M., Reich, R.: Über Primärreaktionen beim Sehvorgang. Thermodynamischer und kinetischer Einfluß des pH-Wertes auf die Metarhodopsin-I–II-Umwandlung. Protonenverbrauch als Auswirkung einer Konformationsänderung. Z. Naturforsch. **29c,** 577–591 (1974)
8. Emrich, H. M., Reich, R.: The effect of Ca^{2+} on the metarhodopsin I–II transition. I. Experiments. Pflügers Arch. **364,** 17–21 (1976)
9. Lamola, A. A., Yamane, T., Zipp, A.: The metarhodopsin I $\rightleftharpoons$ metarhodopsin II equilibrium: Effects of detergents and pressure. Exp. Eye Res. **18,** 19–27 (1974)
10. Matthews, R. G., Hubbard, R., Brown, P. K., Wald, G.: Tautomeric forms of metarhodopsin. J. gen. Physiol. **47,** 215–240 (1963)
11. Nöll, G. N.: Reaktionskinetische Untersuchungen an Rinder-Rhodopsin und Stäbchenaußensegmenten sowie 11-cis Retinal. Dissertation, Aachen 1974
12. Reich, R., Schmidt, S.: Über den Einfluß elektrischer Felder auf das Absorptionsspektrum von Farbstoffmolekülen in Lipidschichten. I. Theorie. Ber. Bunsenges. physik. Chem. **76,** 589–598 (1972)
13. Reich, R., Scheerer, R., Sewe, K.-U.: Electrochromic spectra of dyes in lipid layers. Ber. Bunsenges. physik. Chem. **80,** 245–246 (1976)
14. Reich, R., Scheerer, R.: Effect of electric fields on the absorption spectrum of dye molecules in lipid layers. IV. Electrochromism of oriented chlorophyll *b*. Ber. Bunsenges. physik. Chem. **80,** 542–547 (1976)
15. Reich, R., Scheerer, R., Sewe, K.-U., Witt, H. T.: Effect of electric fields on the absorption spectrum of dye molecules in lipid layers. V. Refined analysis of the field-indicating absorption-changes in photosynthetic membranes by comparison with electrochromic measurements in vitro. Biochim. biophys. Acta (Amst.) (in press) (1976)
16. Reich, R., Emrich, H. M.: The effect of Ca^{2+} on the metarhodopsin I–II transition. II. Model calculations and hypothesis on a molecular mechanism of visual excitation. Pflügers Arch. **364,** 23–28 (1976)
17. Schwarzenbach, G., Biedermann, W.: Die komplexometrische Titration der Erdalkalien und einiger anderer Metalle mit Eriochromschwarz T. Chimia **2,** 56–59 (1948)
18. v. Sengbusch, G., Stieve, H.: Measurements on bovine rod outer segments. Z. Naturforsch. **26b,** 488–489 (1971)
19. Shichi, H.: Circular dichroism of bovine rhodopsin. Photochem. Photobiol. **13,** 499–502 (1971)
20. Shichi, H., Lewis, M. S., Irreverre, F., Stone, A. L.: Biochemistry of visual pigments. I. Purification and properties of bovine rhodopsin. J. biol. Chem. **244,** 529–536 (1969)
21. Träuble, H.: Phasenumwandlungen in Lipiden. Mögliche Schaltprozesse in biologischen Membranen. Naturwissenschaften **58,** 277–284 (1971)
22. Waggoner, A. S., Stryer, L.: Induced optical activity of the metarhodopsins. Biochemistry **10,** 3250–3254 (1971)
23. Wald, G.: Die molekulare Basis des Sehvorgangs (Nobelvortrag). Angew. Chem. **80,** 857–867 (1968)
24. Walz, D.: Pigment containing lipid vesicles. III. Role of chlorophyll *a* as sensor for aggregational states of lecithin. J. Membrane Biol. (in press) (1976)
25. Witt, H. T.: Coupling of quanta, electrons, fields, ions, and phosphorylation in the functional membrane of photosynthesis. Quart. Rev. Biophys. **4,** 365–477 (1971)

Received May 26, 1976