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Kinetics and Saturation of Light-Induced Near-Infrared Scattering Changes in Isolated Bovine Rod Outer Segments

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Abstract. The axial and radial shrinkage of bovine rod outer segments, monitored by near-infrared scattering changes (P-signal), is investigated in dependence on the intensity of the activating flash. Suspensions of axially oriented and randomly oriented rod outer segments were measured. In the latter case, axial and radial effects are superimposed to another.
The following results are obtained:
1. The axial signal (P_a, $\tau \approx 10$ ms) and the radial signal (P_r, $\tau = 40-100$ ms), simultaneously measured on axially oriented rod outer segments, are similarly saturated with a half-saturation at a rhodopsin turnover of 3.5%.
2. For the saturation of the signal amplitude, measured on randomly oriented rod outer segments, a good fit is obtained by:

$$P(\varrho) \sim 1 - e^{-\beta\varrho} ,$$

ϱ: relative rhodopsin turnover by the flash;
β is found in the range $23 \leq \beta \leq 27$ in all measurements.

3. The kinetics of the signal, also measured on the isotropic sample, depends on the rhodopsin turnover, the apparent time constant becoming faster with increasing turnover. The distortion of the signal cannot be fitted by a sum of exponentials with a fixed set of time constants.

The signals from the isotropic sample are fitted by a phenomenological model. It introduces three first order processes concatenated in series; the first step is assumed as a rhodopsin transition inducing the two further processes.

The distortion of the signals with increasing ϱ is then described assuming a ϱ-dependent quenching of this induction, according to the measured amplitude saturation. The time constants remain thereby unchanged.

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The fit yields the values ln $2/k$ = 4, 11, and 45 ms with mean square deviations of 20%.

Key words: Rhodopsin – Light-scattering – Flash-photometry – Disc membrane – Rod outer segments

Introduction

Among the various flash-induced transients in the near-infrared scattering of bovine rod outer segments and isolated discs, a certain group of signals can be characterized by their common saturation at a rhodopsin bleaching of about 10%.

Recent investigations show that these signals can be attributed to different physical indicator processes.

The fast increase (10–50 ms) of the scattered light, observed in the undissolved disc stack, previously termed by us as "P-signal" (Hofmann et al. 1976), was interpreted as a shrinkage effect (Uhl et al. 1977).

By orienting the rod outer segments, we were able to distinguish a fast axial (termed P_a, 10 ms) and a slower radial (termed P_r, 40–100 ms) shrinkage of the disc stack (Hofmann et al. 1981). Signals from the isotropic sample must therefore contain both signals in linear superposition.

Starting with this result, the experimental data on the saturation and distortion of the signals with increasing flash intensity will be used to complement our previous consecutive reaction formulation (Uhl et al. 1978).

Materials and Methods

All measurements were done in isotonic saline containing 130 mM KCl, 0.5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5 mM (Ethylenedinitrilo)-tetra acetic acid (EDTA), 1 mM Dithiothreitol (DTT), 10 mM Piperazine – 1,4 – diethane sulfonic acid (PIPES).

The preparation was performed on a discontinuous sucrose gradient after filtering the rod outer segments (isolated by shaking the retinae in the above saline) through a nylon mesh (more details see Hofmann et al. 1981).

The measurement on axially oriented rod outer segments was performed using a fresh sample, the measurements on isotropic samples using suspensions frozen in liquid nitrogen and thawed immediately prior to the measurement.

The rod outer segments were oriented in a 12 kG magnetic field. The apparative details are given in two preceding papers (Hofmann and Emeis 1981; Hofmann et al. 1981).

An essential point for this study is the homogeneity of the flash excitation, two flash bulbs and two reflecting mirrors were therefore used.

Results

1. Time Course

Figure 1 shows a number of P-signals for various values of the relative rhodopsin turnover ϱ. The turnover is determined by the decrease of the light-scattering signal N, in a series of flashes, as described in a previous paper (Hofmann and Emeis 1981). All signals show a characteristic s-shaped time course, the lag after the flash becoming shorter and the consecutive slope steeper with increasing values of ϱ.

2. Saturation

In former investigations, we applied the dose in a series of flashes and observed the successive decrease of the signal amplitude (Hofmann et al. 1976). In order

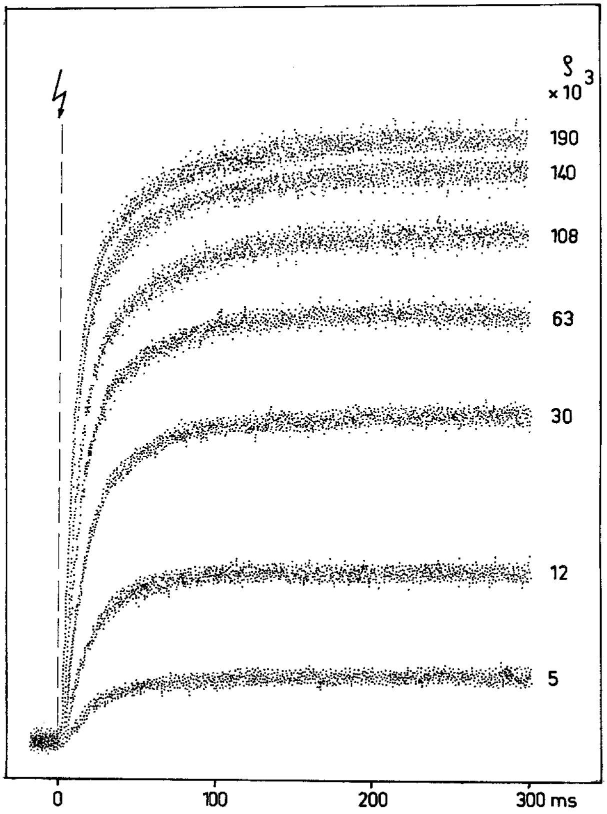


Fig. 1. P-signals for various flash intensities. Parameter of the signals is the relative rhodopsin turnover induced by the flash applied at $t = 0$. Measuring parameters: $c_{\text{rhod}} = 3 \cdot 10^{-6}$ M, scattering angle: $2° \leq \theta \leq 6°$, pH $= 6.0$, $T = 22°$ C. It is seen that the signals become faster with increasing turnover

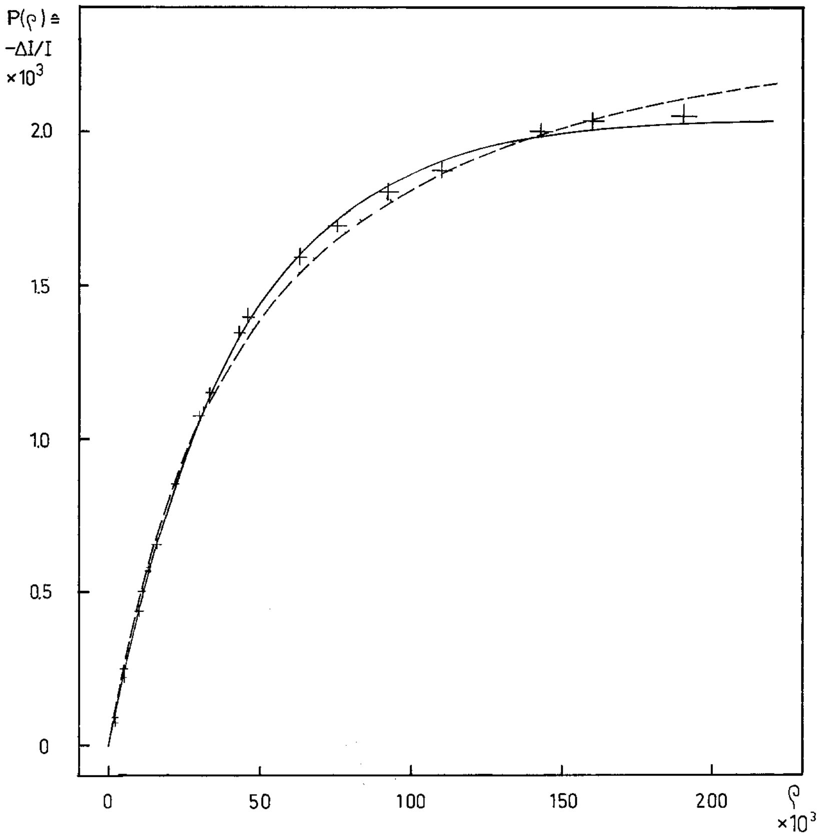


Fig. 2. Amplitude of the P-signal as a function of the relative rhodopsin turnover ϱ induced by the flash. In contrast to Fig. 3, this measurement was performed using isotropic samples. Every measuring point corresponds to a new sample from the same preparation. The fit for an exponential [solid line, formula (1)] and a hyperbolic function [dotted line, formula (2)] is drawn in. Data are from the same measurement as in Fig. 1

to avoid recovery effects (Hofmann and Schnetkamp, unpublished) arising from the successive illumination, we apply in this study the dose in one single flash.

Randomly Oriented Rod Outer Segments. In Fig. 2, the amplitude of the P-signal is plotted versus the relative rhodopsin turnover induced by the flash. Any point represents the measurement with a fresh aliquot from one and the same preparation, identical with that used for the measurement shown in Fig. 1.

Several functions can be tested in order to describe the curve in Fig. 2. If one attempts to model the simple curve by only two parameters, the fit by an exponential (1) or by a hyperbolic function (2) is suggested:

$$P(\varrho) \sim 1 - e^{-\beta\varrho}, \tag{1}$$

$$P(\varrho) \sim \frac{\varrho}{\varrho + \sigma}. \tag{2}$$

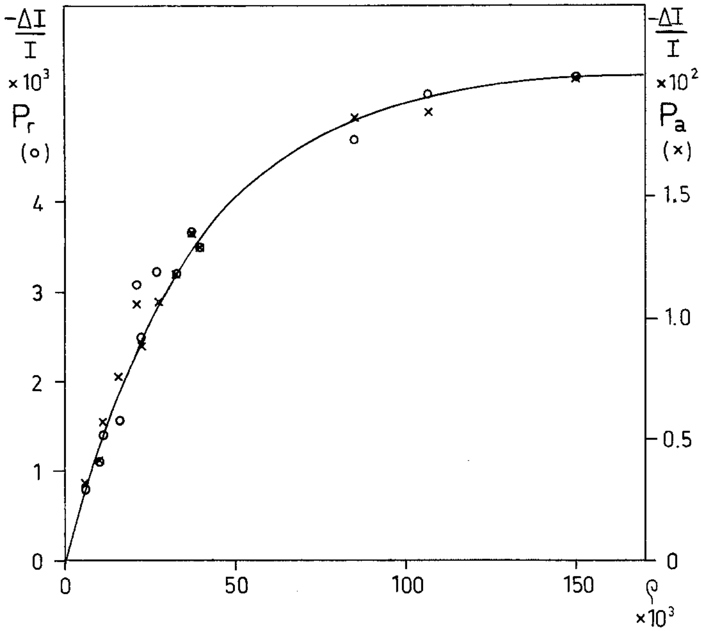


Fig. 3. Amplitudes of the axial and radial components of the *P*-signal as a function of the relative rhodopsin turnover ϱ induced by the flash. (×) axial component, (O) radial component. Scattering angle $15° \leq \theta \leq 25°$; $c_{\text{rhod}} = 8 \cdot 10^{-7}$ M, pH = 6.0, $T = 22°$ C

Both functions are drawn into Fig. 2 by solid and dotted lines respectively. It is seen that the curvature of the measured function is better described by (1) than by (2): for the function (2), the mean square deviation is four times higher than that of (1) and a systematic deviation occurs in addition.

For the different preparations, the exponential function yielded in all cases a good fit to the measured saturation with only two parameters. More parameters must be introduced when using the hyperbolic function or a cooperative function, as given by Changeux et al. (1967), in order to describe the steep curvature of the saturation.

Axially Oriented Rod Outer Segments. In axially oriented rod outer segments, a radial and an axial component is separately observed. In the isotropic sample, one expects to find these components in a linear superposition. The model curves, which will be calculated for the much better measurable signals from the isotropic sample, have therefore to contain two additive components.

In Fig. 3, a simultaneous measurement of the axial and the radial signal as a function of the rhodopsin turnover is shown. It is seen, that a quite similar saturation is found for both signals within the higher error of this measurement.

A Phenomenological Model. In a previous study (Uhl et al. 1978), we already attempted to describe the time course seen in the *P*-signal as a consecutive reaction. We introduced two linear consecutive processes P_1 and P_2. The metarhodopsin reaction was discussed as the first of these processes.

We will investigate the role of metarhodopsin II in a separate study, based on simultaneous absorption- and scattering measurements (Emeis and Hofmann to be published).

For the present work, we only assume an inducing rhodopsin product M. It will be seen below that a risetime of 4 ms for this product fits well to the data.

On account of the new finding of two scattering events superimposed in the P-signal, we have to introduce two further processes.

It cannot be decided unambiguously from the data, in which of the consecutive processes ϱ-dependent functions have to be introduced and whether the processes are connected parallel or in series to the inductor. The experimentally found similar saturation (Fig. 3) favours, however, the assumption of a series. Otherwise, one would be forced to assume that both independent saturations were equal by chance.

Therefore, the model assumes that the faster process P_a contains the saturation and that the slower process P_r is directly consecutive to P_a without further nonlinear characteristics.

This means in a symbolic representation:

$$P \equiv R \xrightarrow{f(k_1,t)} M \xrightarrow{g(k_2,\varrho,t)} X_a \xrightarrow{h(k_3,t)} X_r \tag{3}$$

X_a and X_r are hypothetical states of the system. Their production can be directly observed in the axial and radial signals P_a and P_r. The transition from rhodopsin (R) to the the M-state is related to the process previously termed P_1, the concatenated reactions $M \rightarrow X_a$ and $X_a \rightarrow X_r$ replace P_2. M, X_a, and X_r are connected by the functions f, g, and h. The first and the third function are solely determined by the time constants k_1 and k_3, the second step must contain at least two quantities, k_2 and ϱ.

With first order time functions and the saturation given by (1), one obtains:

$$f(t) = 1 - e^{-k_1 t}, \tag{4}$$

$$g(\varrho, t) = 1 - \exp\,[\beta\varrho(1 - e^{-k_2 t})]\,, \tag{5}$$

$$h(t) = 1 - e^{-k_3 t}. \tag{6}$$

This parametrisation is certainly not unique and tries only the most simple assumption.

M, X_a and X_r are now concatenated by convolutions:

$$P(t) = A_a\left\{\frac{d}{dt}f(t) * g(\varrho, t)\right\} + A_r\left\{\frac{d}{dt}\left[\frac{d}{dt}f(t) * g(\varrho, t)\right] * h(t)\right\}. \tag{7}$$

Table 1. Fit parameters for P-signals in the range $0.005 \leq \varrho \leq 0.19$, $0 \leq t \leq 300$ ms. Symbols as in formula (7). The quantity in the last column is a measure for the mean relative deviation of the fitted function from the average of the data points [χ^2: sum of mean square deviations (M.S.D.) NP: number of data points]. It is further seen from the table that the mean square deviation of the whole set of parameters is located within ± 20 % of the average (lowest line). This means that all signals are fitted by sole variation of ϱ

ϱ	$\ln 2/k_1$	$\ln 2/k_2$	$\ln 2/k_3$	A_s/A_f	$\frac{\sqrt{\chi^2} \cdot 10^3}{(A_f + A_s) \cdot \mathrm{NP}}$
0.005	5	10.5	59	0.39	2.0
0.013	3	11.5	54	0.33	1.5
0.029	4	9.3	34	0.37	2.3
0.0425	4	9.0	34	0.30	1.6
0.063	3	12.0	34	0.32	3.3
0.076	3	10.0	42	0.34	1.6
0.11	4	12.0	45	0.31	2.3
0.16	3	15.0	41	0.31	2.3
0.19	4	13.0	38	0.34	2.5
Average (M.S.D.)	3.7 (0.7)	11.4 (1.8)	42.3 (8.5)	0.33 (0.03)	

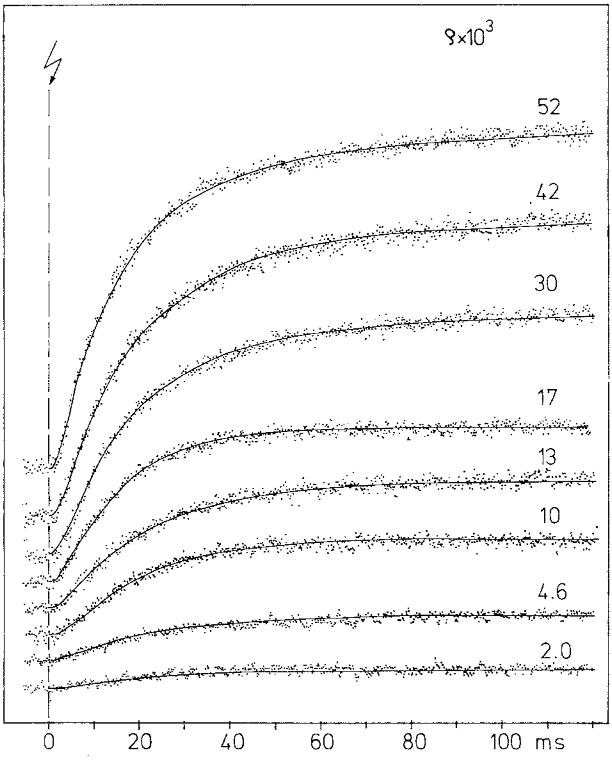
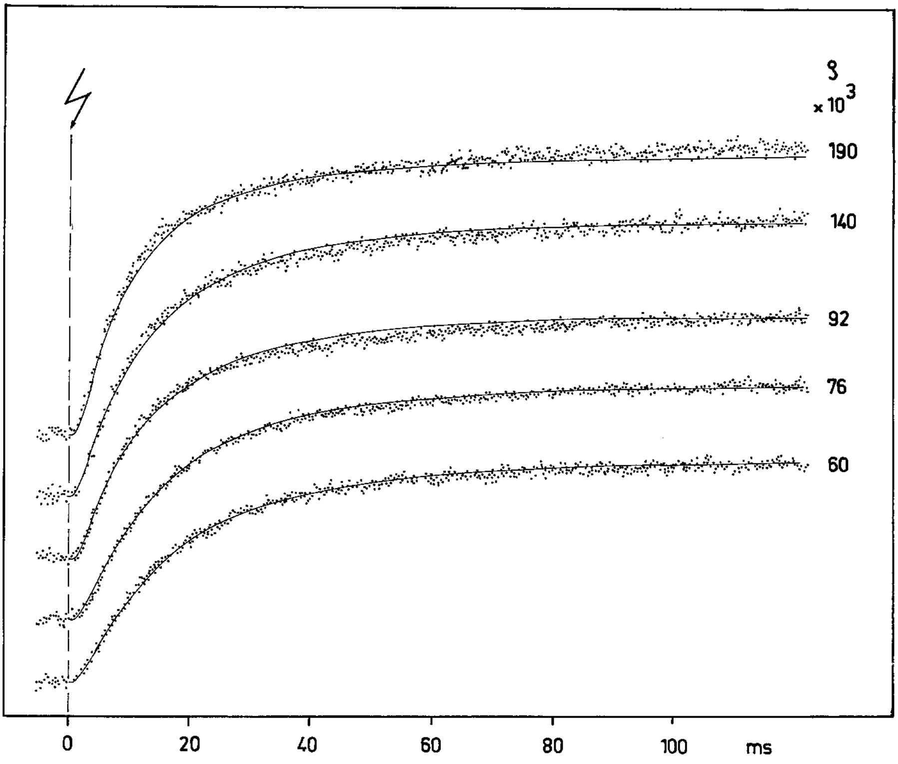


Fig. 4a and b. Time course of the P-signal for various flash intensities corresponding to a relative rhodopsin turnover of (**a**) $0.002 \leq \varrho \leq 0.052$, (**b**) $0.06 \leq \varrho \leq 0.19$. The fits according to (7) are plotted as solid lines. Measuring parameters as in Fig. 1. To visualize the behaviour at small times, only the range $t \leq 120$ ms is shown. The quality of the fit in the whole range $0 \leq t \leq 300$ ms and the values of the fit parameters are given in Table 1



This convolution expression means that the P-signal consists of two additive components, since two of the concatenated processes are directly observable in light-scattering. In the first term, f acts on g, while in the second term the convolution of f and g acts on h. The allowed simplifications of (7) are discussed in the appendix.

(7) contains the experimentally determined parameters ϱ (from the determination of the turnover) and β [from the result (1)].

Concerning the relation of (7) to our previous linear analysis (Uhl et al. 1978), it can be shown that (7) is well approximated by a sum of exponentials for a turnover of $\varrho = 3\%$ which was applied in the previous study (see Appendix).

The Eq. (7) can be considered as an adequate description if it fits to all measured signals with a constant set of the parameters A_f, A_s, k_1 k_2, and k_3.

Furthermore, the values given by the modeling of the signals from the isotropic sample must be in accordance with the experimentally found signals on axially oriented samples.

As is seen from Fig. 4 and Table 1, all these parameters are really constant within ± 20%, when they are kept free and calculated by a Marquardt fit-program. The values of the mean square deviation are for all signals (Table 1) by a factor of ca. 5 lower than the experimental noise.

As expected, the fit yields time constants which are in good accordance to the apparent reaction times seen in the signals P_a and P_r.

It should be mentioned that the fit procedure is not sensitive to a commutation of the time constants k_1 and k_2. The present choice is only supported by the above mentioned similarity of the k_1-process to the metarhodopsin transition.

Discussion

The expression (7) was found to describe the results. It states that rhodopsin, with a delay of ca 4 ms, induces two postponed processes which become visible in the axial and radial near-infrared scattering signals. They can be described by first order reaction times of 12 ms and 45 ms.

To introduce the measured saturation (1) into the model, it was sufficient to assume that, with increasing rhodopsin turnover, the ability of rhodopsin decreases gradually to induce the second process (gradual quenching).

This result reflects the experimental finding that the axial shrinkage P_a and the radial shrinkage P_r are similarly saturated. This will be essential for the molecular interpretation since it makes it probable that the saturation is an intrinsic property of the molecular processes and not merely a property of the indicator "shrinkage". Otherwise, it would be difficult to understand that the saturation of the axial and radial indicator is the same.

The saturation was best described by the exponential relation (1), better than by a hyperbolic function (2).

This result alone is certainly not to be taken as decisive for the underlying mechanism. It is, however, expected to be valuable, when we have learned more about the membrane units which are actually responsible for the consecutive processes. Generally, two different processes would fit to an exponential law: a catalytic action of rhodopsin on other single membrane units and a more unspecific interaction (mutual quenching) of rhodopsin molecules in the M-state (Hofmann 1980).

Near-infrared transmission transients found by Kühn et al. (1981) are saturated in proportion to the amount of G-protein in the disc-membrane preparation; when the natural amount of G-protein is bound to the membrane, the saturation level is comparable to the values found in this study. This would suggest that the above mentioned formally derived catalysable units are identical to the G-protein. So far, however, we were not able to find an influence of the G-protein on the fast axial shrinkage. Preliminary experiments are discussed in a previous paper (Hofmann et al. 1981).

The exponent β in (1) is numerically equal to the exponent in the Rushton equation for the bleaching dependence of dark adaptation (Rushton 1965). Thus, the states X_a and X_r might be regarded as candidates for the hypothetical membrane-bound regulative step which was already hypothesized by Robinson (1975). The various aspects of this problem (e.g., concerning the rhodopsin photoproduct triggering dark adaptation; Pugh 1975; Ernst and Kemp 1979) will be discussed elsewhere.

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Appendix

1. Considerations About the Convolution (7)

In the consecutive reaction chain, C(t) is given by

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C\,, \tag{A1}$$

$$C(t) \sim 1 - \frac{1}{k_2 - k_1}\left(k_2\, e^{-k_1 t} - k_1\, e^{-k_2 t}\right). \tag{A2}$$

This function can also be represented by

$$C(t) \sim \int_0^t - k_1\, e^{-k_1 t'}\, e^{-k_2 (t - t')}\, dt' \tag{A3}$$

which is just the definition of a convolution. In (7), the linear transition $B \rightarrow C$, is replaced by the ϱ-dependent function $g(\varrho\ , t)$. Since k_1, k_2, and k_3 are enough different, the rigorous expression (7) can be replaced by

$$\begin{aligned} P(\varrho, t) = {} & A_f\left[1 - e^{-\beta\varrho \cdot g'(k_1, k_2, t)}\right] \\ & + A_s\left[(1 - e^{-\beta\varrho}) \cdot h'(k_1, k_2, k_3, t)\right] \end{aligned} \tag{A4}$$

$$\text{with } g'(t) = C(t) \text{ in (A1)} \tag{A5}$$

$$\begin{aligned} \text{and } h'(t) \sim 1 & - \frac{k_2 k_3}{(k_2 - k_1)(k_3 - k_1)}\, e^{-k_1 t} - \frac{k_1 k_3}{(k_1 - k_2)(k_3 - k_2)}\, e^{-k_2 t} \\ & - \frac{k_1 k_2}{(k_1 - k_3)(k_2 - k_3)}\, e^{-k_3 t}. \end{aligned} \tag{A6}$$

The time constants obtained with (A4) differ by maximal 10% from those obtained with (7) (tested for $\varrho = 0.05$).

2. Approximation by a Sum of Exponentials

Consider the first term in (A4). Neglecting the s-characteristics, it can be replaced by

$$P(\varrho, t) \sim 1 - e^{-\beta\varrho(1 - e^{-k_2 t})}. \tag{A7}$$

Expansion of the exponential function yields:

$$P(\varrho,t) \sim 1 - \left(1 - \beta\varrho(1 - e^{-k_2 t}) + \frac{1}{2}\beta^2\varrho^2(1 - e^{-k_2 t})^2 - \ldots\right),$$

and for $\beta\varrho = 1$ ($\varrho \approx 3\%$) results:

$$P(\varrho,t) \sim 1 - \frac{8}{15}e^{-k_2 t} - \frac{6}{15}e^{-2k_2 t} - \frac{1}{15}e^{-4k_2 t}.$$

This solution deviates by ca 1% from (A7). One would obtain k_2, $2\,k_2$, $4\,k_2$, and k_3 as time constants for the P-signal.

References

Changeux J-P, Thiery J, Tung Y, Kittel C (1967) On the cooperativity of biological membranes. Proc Natl Acad Sci USA 57: 335–341

Ernst E, Kemp CM (1979) Reversal of photoreceptor bleaching and adaptation by microsecond flashes. Vision Res 19: 363–365

Hofmann KP, Uhl R, Hoffmann W, Kreutz W (1976) Measurements of fast light-induced light-scattering and -absorption changes in outer segments of vertebrate light sensitive rod cells. Biophys Struct Mech 2: 61–77

Hofmann KP (1980) Die lichtinduzierte Kontraktion der Disk-Membran und ihre Verbindung zur Signal-Transduktion. Habilitationsschrift, Universität Freiburg

Hofmann KP, Emeis D (1981) Comparative kinetic light-scattering and -absorption photometry. Biophys Struct Mech 8: 23–34

Hofmann KP, Schleicher A, Emeis D, Reichert J (1981) Light-induced axial and radial shrinkage effects and changes of the refractive index in isolated bovine rod outer segments and disc vesicles – physical analysis of near-infrared scattering changes. Biophys Struct Mech 8: 67–93

Kühn H (1978) Light-regulated binding of rhodopsin kinase and other proteins to cattle photoreceptor disc membranes. Biochemistry 17: 4389–4395

Kühn H, Bennett N, Michel-Villaz M, Chabre M (1981) Interactions between photoexcited rhodopsin and GTP-binding protein: kinetic and stoichiometric analysis from light-scattering changes. Proc Natl Acad Sci USA (in press)

Pugh EN Jr (1975) Rushton's paradox: rod dark adaptation after flash photolysis. J Physiol (Lond) 248: 413–432

Robinson GW (1975) Rhodopsin cooperativity in visual response. Vision Res 15: 35–48

Rushton WAH (1965) Bleached rhodopsin and visual adaptation. J Physiol (Lond) 181: 645–655

Uhl R, Hofmann KP, Kreutz W (1977) Measurement of fast light-induced disc-shrinkage within bovine rod outer segments by means of a light-scattering transient. Biochim Biophys Acta 469: 113–122

Uhl R, Hofmann KP, Kreutz W (1978) On the light-stimulated coupling between rhodopsin and its disk membrane environment. Biochemistry 17: 5347–5352

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