Dark-Processes Following Photoconversion of Butterfly Rhodopsins*

G. D. Bernard

Yale University, Department of Ophthalmology and Visual Science, P.O. Box 3333, New Haven, CT 06510, USA

Abstract. Photoconversion of rhodopsin to metarhodopsin by a short actinic flash creates photochemical changes in the absorbance spectrum of the butterfly rhabdom, which are measurable as changes in the reflectance spectrum of the intact eye. The difference spectrum relaxes in the dark, but changes considerably in shape when doing so. The positive peak caused by the accumulation of metarhodopsin relaxes to zero much faster than the negative peak caused by the loss of rhodopsin. The positive peak actually undershoots zero absorbance-difference before its final asymptotic approach to zero, whereas the negative peak approaches zero monotonically.

The entire temporal evolution of difference spectra can be quantitatively reproduced by only assuming different kinetics for the dark-processes of metarhodopsin's decay and of rhodopsin's recovery. A consequence of this analysis is that no long-lived, coloured intermediates can be detected in the rhabdom other than metarhodopsin.

Metarhodopsin's decay is well approximated by a first-order process, but has a time-constant that depends strongly on temperature. Examples are 71 min at 12.5 °C, 18 min at 23 °C, and 4 min at 26.5 °C.

Rhodopsin's recovery is kinetically complex. The rate of recovery shortly after a small photoconversion is somewhat slower than the rate for metarhodopsin's decay. At later times, or for a large photoconversion, rhodopsin's recovery is very much slower than metarhodopsin's decay.

Key words: Insect photoreceptors – Visual pigments – Unstable metarhodopsin – Dark regeneration – Bleaching

Introduction

One of the cornerstones of the photochemistry of insect rhodopsins is that their metarhodopsins are stable. A considerable amount of work on the microspectrophotometry of insect visual pigments indicates that the rhodopsins of insects

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do not bleach but are changed by light to a stable metarhodopsin. Many of the
techniques for photochemical measurement and analysis assume that the
concentrations of rhodopsin and metarhodopsin in a rhabdom do not change if
the eye is left in the dark. This assumption is adequately supported by a large
body of work on some invertebrate species (Goldsmith 1972; Hamdorf 1979;
Langer et al. 1982), but not for all species.

The concentrations of rhodopsin and metarhodopsin actually do change in
the dark in some situations. Dark-regeneration with half-times of less than
30 min has been reported in four diverse groups of invertebrates [octopus –
Schwemer (1969); fly – Stavenga et al. (1973); butterfly – Stavenga (1975b);
lobster – Bruno et al. (1977)]. The thoroughly dark-adapted rhabdom contains
no metarhodopsin in the lobster (Bruno et al. 1977) and crayfish (Cronin and
Goldsmith 1982). The dark-processes can depend strongly on temperature.
Bruno et al. (1977) showed that dark-regeneration of lobster rhodopsin has a
half-time of 55 min at 15°C, but only 25 min at 22°C. They also showed that the
dark-adapted lobster rhabdom contains no metarhodopsin, and that the
chronically illuminated rhabdom contains much less metarhodopsin at 25°C
than it does at 1°C.

Stavenga (1975a, b) studied the dark processes in a butterfly as its rhodopsin
R535 was dark-regenerated from metarhodopsin M480, by following the
relaxation process with dim monochromatic light of various wavelengths. The
relaxation was monotonic at 567 nm, but was biphasic at 506 nm. He suggested
two possibilities, a) M480 passes through a violet-absorbing intermediate as it is
regenerated to R535, or b) more than one kind of rhodopsin was photoconverted
by the yellow actinic light.

Bernard (1977) confirmed Stavenga's experimental results, but suggested
that the metarhodopsin was unstable, and was decaying at a faster rate than the
rate for dark-regeneration of rhodopsin, making it possible to bleach the
rhabdoms. I recently presented evidence to support this suggestion (Bernard
1983). The purpose of the present paper is to quantitatively characterize the
kinetics of both the decay of metarhodopsin and the regeneration of rhodopsin
in the dark, to show how butterfly rhabdomeres can indeed be bleached in the
living, intact animal, and to show that the kinetics of the dark-processes depend
strongly on temperature.

Methods

An optical specialization of the butterfly eye allows in vivo measurements of
photochemical difference spectra. In each ommatidium a tapetal reflector
located at the proximal end of rhabdom creates coloured eyeshine (Miller and
Bernard 1968; Ribi 1978; Miller 1979). By immobilizing an intact butterfly on
the stage of an incident light microspectrophotometer (MSP) and focussing on
the deep pseudopupil, it is possible to measure the light that has survived a
double-pass through the rhabdom. Discrimination against stray light is superb
(Franceschini and Kirschfeld 1971; Stavenga 1975a, b; Bernard 1977, 1979).
Thus, the optical density (absorbance) of the rhabdom is proportional to
one-half times the common logarithm of the measured reflectance.