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NATURE OF THE PRINCIPAL PHOTOINTERMEDIATE OF HALORHODOPSIN

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Two alternative hypotheses have been presented as to the nature of the principal halorhodopsin photointermediate: a) it is a form whose its absorption band is shifted from the 575 nm position to 500 or 520 nm, and b) it is a form whose absorption band is shifted to only about 565 nm, but with an altered band shape so it exhibits a fortuitous difference peak near 500 nm. Such a shift with a maximum near 500 nm is also obtained in the dark when chloride is removed from the sample, suggesting the hypothesis that the spectral changes reflect the transient detachment of chloride from a binding site (Ogurusu et al, J.Biochem. Tokyo 95, 1073-1082, 1984). Comparison of the quantum yields of flash-induced absorption changes in halorhodopsin and bacteriorhodopsin strongly suggests, however, that hypothesis b) is untenable.

Halorhodopsin, a retinal pigment similar to bacteriorhodopsin (1,2), is a light-driven transport system for chloride ions (3). It is interesting, therefore, that halides have been found to exert strong effects on the chromophore of this pigment and its photochemical reactions (4-6). Most significantly, in the presence of chloride the flash-induced absorption changes proceed in such a way as to give a difference spectrum with a minimum near 600 nm (corresponding to the depletion of the original chromophore) and a maximum near 500 nm (5-7), but in the absence of chloride the maximum is near 660 (6). At intermediate chloride concentrations both kinds of spectral changes are observed (6), and the apparent affinity constant for chloride agree with the K_m for the transport. In addition, the kinetics of the decay for both kinds of photoreactions is affected by halides. Corrections for the overlap between the absorption band of the pigment produced by the flash illumination and the 580 nm absorption band of halorhodopsin gave 520 nm as the probable absorption maximum for the chloride-dependent photointermediate (6).

Recently Ogurusu et al (8) showed that addition of chloride to purified halorhodopsin in the dark produced a small red-shift

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in the visible absorption band and complex changes in the shape of the bands, so as to give a difference spectrum with a maximum near 600 nm and a minimum near 500 nm. This spectrum resembled to a remarkable extent the mirror-image of the flash-induced difference spectrum in the presence of chloride, and the authors (8) proposed tentatively that the flash-induced absorption changes might originate from the transient loss of chloride from a binding site. The hypothesis thus provides a ready explanation for the flash-induced spectroscopic changes, in terms which are relevant to the chloride transport. A consequence of this hypothesis is that the maximum observed near 500 nm in the flash experiments does not correspond to any real photointermediate with an absorption maximum near 500 nm, but can be attributed rather to a fortuitous spectroscopic effect.

Since the two interpretations predict very different magnitudes of absorption changes for the same extent of photoconversion, we report here on a comparison between the amplitudes of flash-induced absorption changes in bacteriorhodopsin (with earlier determined quantum efficiency for photocycling) and halorhodopsin. The results do not support the hypothesis suggested by the results of Ogurusu et al (8).

MATERIALS AND METHODS

Bacteriorhodopsin and halorhodopsin were purified by previously described methods (9,10). The samples were extensively dialyzed against 1 M sodium nitrate containing 0.05 M phosphate, pH 6.0 ("nitrate buffer"). Absorption spectra were measured with a Gilford Model 2600 spectrophotometer. Flash-induced absorption changes were measured with a cross-beam flash spectrometer described earlier (6). Different flash intensities were obtained with the aid of neutral density filters. The measuring beam was set at 580 nm, the flash was through a 610 nm long-pass filter. The flash energy was 4 kV, signal averaging was with 512 repetitions.

RESULTS

Fig. 1 shows absorption spectra for two light-adapted samples of halorhodopsin in the nitrate buffer, one of which received enough 4 M NaCl to give a final concentration of 0.36 M chloride and the other the same volume of nitrate buffer. Chloride-dependent changes are noticeable in the spectra, and are even more readily seen in the difference spectrum between the two samples: there is a larger increase of absorption near 600 nm and a smaller decrease near 500 nm. These changes correspond closely to the results reported by Ogurusu et al (8) under essentially

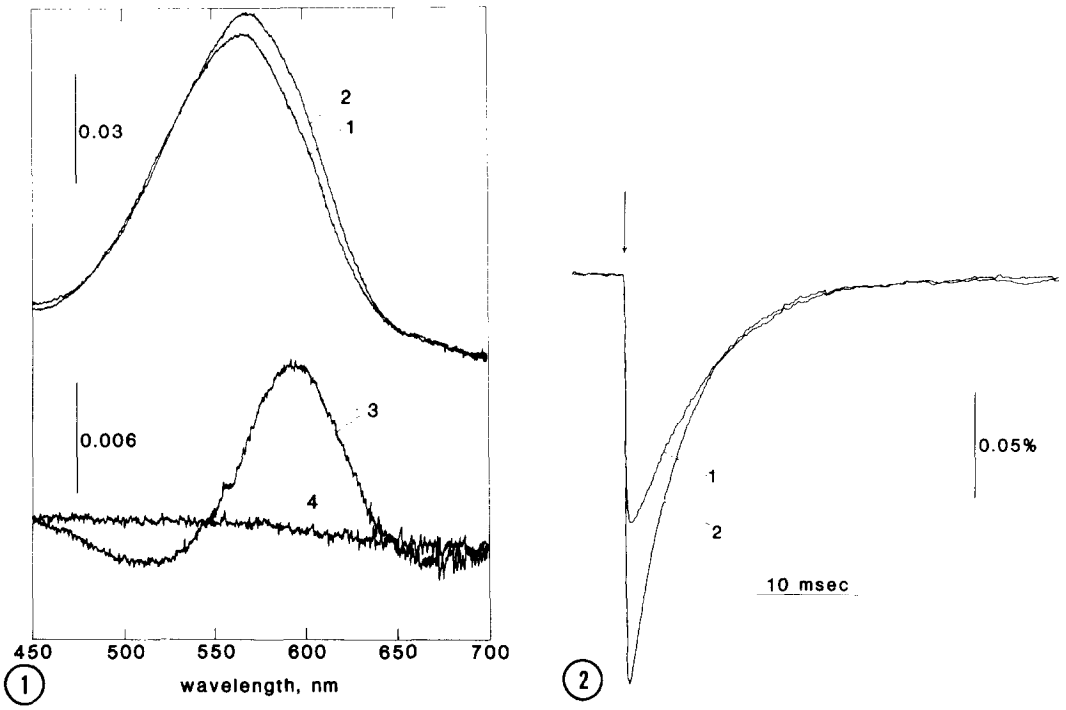

Fig. 1 Absorption spectra for halorhodopsin in the absence of chloride (trace 1) and in the presence of 0.36 M chloride (trace 2). Trace 3 is the difference between traces 1 and 2; trace 4 is a baseline. Vertical bars are absorption.

Fig. 2 Absorption changes at 580 nm following flash illumination for halorhodopsin (trace 1) and bacteriorhodopsin (trace 2). The time of the flash is indicated by an arrow. The sample of halorhodopsin used was from Fig. 1, and a matching sample of bacteriorhodopsin was prepared in the same buffer. The vertical bar refers to percent change in light intensity per flash.

the same conditions. They also reported that half-maximal changes at 600 nm were seen at 0.31 M chloride. Matched samples of light-adapted bacteriorhodopsin under the conditions in Fig. 1 did not exhibit these chloride-dependent changes (not shown).

The two samples (bacteriorhodopsin and halorhodopsin) with chloride added were analyzed for flash-induced absorption changes, and the traces are shown in Fig. 2. The rise-time of the absorption decrease is not resolved, but the decay back to the original chromophore is largely exponential for both pigments. The amplitudes of the maximal changes were obtained by extrapolation to the instant of the flash, and are plotted as functions of relative flash intensity in Fig. 3. It appears that the fractional change of absorbance for halorhodopsin is about 54% that in bacteriorhodopsin. Virtually the same ratio is obtained when the absorption change amplitudes are compared

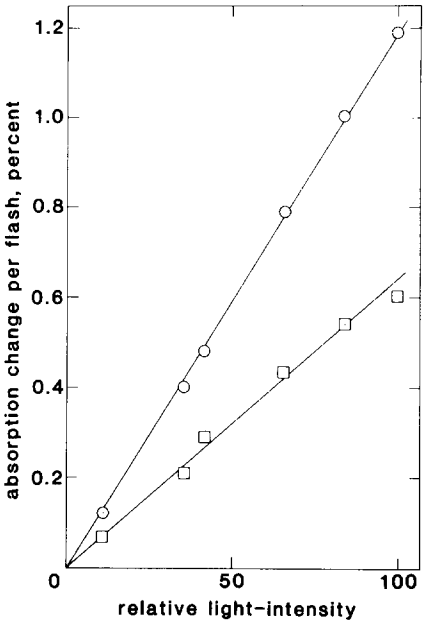

Fig. 3. Relationship of maximal flash-induced absorption change at 580 nm (given as percent decrease in absorption after flash) to relative light-intensity. Bacteriorhodopsin (⊙), halorhodopsin (⊡).

without extrapolation. No differences were observed between bacteriorhodopsin in the nitrate-chloride buffer and in buffer containing chloride alone (not shown).

DISCUSSION

In the bacteriorhodopsin system quantum efficiency is defined as the number of pigment molecules photoconverted per photon absorbed (11-13). Photoconversion can be estimated from the absorption changes after the flash, but the decreased absorption by the original chromophore is partly compensated for by increased absorption by all those photointermediates which absorb at the wavelength of measurement. Thus, the fraction of pigment photoconverted is always somewhat greater than the fractional change in absorption near the absorption maximum. On a several-millisec time-scale for bacteriorhodopsin at 580 nm the error is not large, because it originates mainly from contributions from the intermediates N_{530} and O_{640} (14), which do not accumulate in large quantities. The principal photointermediate, M_{412}, should absorb only slightly at 580 nm. At room temperature in aqueous suspension the quantum efficiency of bacteriorhodopsin photocycling was determined to be about 0.3 (12-14) or between

0.6 and 0.8 (15). This number is below 1 because of the establishment of a photostationary state between the original chromophore and a batho intermediate on a psec time-scale (16,17). The same considerations apply to the relationship of absorption changes to photocycling for halorhodopsin if it is assumed that the photocycle includes the suggested P_{520} intermediate (hypothesis a). However, here the absorption changes at 580 nm will more seriously underestimate the extent of photocycling than for bacteriorhodopsin, because the intermediate accumulates in large quantities and must have significant absorption at this wavelength. Nevertheless, according to hypothesis a) the percent absorption changes for the two retinal pigments should be more-less comparable, and agree roughly with the percent photoconversion.

On the other hand, if the absorption changes are due to loss of chloride from the pigment, (hypothesis b), the same extent of photocycling will produce a much smaller absorption change in halorhodopsin than in bacteriorhodopsin. Thus, the absorption change at 580 nm upon addition of chloride in Fig. 1 is only 7.0% at 0.36 M chloride, and is estimated to be 12.6% at infinite chloride concentration. This latter change in absorption corresponds to the entire pigment content of the sample gaining or losing chloride, i.e. 100% photoconversion. Hence, this hypothesis predicts that the percent change in absorption will be only about 1/8th of the percentage photoconverted.

The data in Fig. 3 show that bacteriorhodopsin and halorhodopsin under identical conditions exhibit similar magnitudes of flash-induced absorption changes, within a factor of 2. If the quantum efficiency for bacteriorhodopsin is taken as 0.3 to 0.6 molecules per photon, the quantum efficiency for halorhodopsin is estimated from hypothesis a) to be 0.2 to 0.3 (depending also on the extent of underestimation for halorhodopsin). Hypothesis b) yields a quantum efficiency of 1.3 to 2.4. It is very likely that the quantum yields for the two pigments are in reality not very different from one another, because a bathointermediate is produced for halorhodopsin (5) as well. It seems very unlikely that the quantum efficiency of halorhodopsin can be greater than one. Recent results with actinometry suggest (Oesterhelt, personal communication), that the quantum efficiency of the halorhodopsin photocycle is about 0.35. Thus, the results seem to rule out hypothesis b), the model raised by Ogurusu et al (8).

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REFERENCES

1) Lanyi, J.K. and Weber, H.J. (1980) J.Biol.Chem. 255, 243-250.
2) Spudich, J.L. and Bogomolni, R.A. (1983) Biophys.J. 43, 243-246.
3) Schobert, B. and Lanyi, J.K. (1982) J.Biol.Chem. 257, 1030-10313.
4) Lanyi, J.K. and Schobert, B. (1982) Biochemistry 22, 2763-2769.
5) Weber, H.J. and Bogomolni, R.A. (1981) Photochem.Photobiol. 33, 601-608.
6) Schobert, B., Lanyi, J.K. and Cragoe, E.J., Jr. (1983) J.Biol. Chem. 258, 15158-15164.
7) Tsuda, M., Hazemoto, N., Kondo, M., Kamo, N., Kobatake, Y.and Terayama, Y. (1982) Biochem.Biophys.Res.Comm. 108, 970-976.
8) Ogurusu, T., Maeda, A. and Yoshizawa, T. (1984) J.Biochem. (Tokyo) 95, 1073-1082.
9) Oesterhelt, D. and Stoeckenius, W. (1974) Methods Enzym. 31, 667-678.
10) Sugiyama, Y. and Mukohata, Y. (1984) J.Biochem. (Tokyo), in press.
11) Goldschmidt, C.P., Kalisky, O., Rosenfeld, T. and Ottolenghi, M. (1977) Biophys.J. 17, 179-183.
12) Lozier, R.H. and Niederberger, W. (1977) Fed.Proc. 36, 1805-1809.
13) Becher, B. and Ebrey, T.G. (1977) Biophys.J. 17, 185-191.
14) Stoeckenius, W., Lozier, R.H. and Bogomolni, R.A. (1979) Biochim.Biophys. Acta 505, 215-278.
15) Oesterhelt, D. and Hess, B. (1974) Eur.J.Biochem. 37, 316-326.
16) Hurley, J.B. and Ebrey, T.G. (1978) Biophys.J. 22, 49-66.
17) Goldschmidt, C.R., Ottolenghi, M. and Korenstein, R. (1976) Biophys.J. 16, 839-843.