Eur Biophys J (1986) 13:195–201

European Biophysics Journal

Photoenergetics of octopus rhodopsin

Convergent evolution of biological photon counters?

A. Cooper[1]*, S. F. Dixon[1], and M. Tsuda[2]

[1] Department of Chemistry, Glasgow University, Glasgow G12 8QQ, Scotland
[2] Department of Physics, Sapporo Medical College, Sapporo 060, Japan

Received August 1, 1985/Accepted in revised form October 14, 1985

Abstract. The enthalpy changes associated with each of the major steps in the photoconversion of octopus rhodopsin have been measured by direct photocalorimetry. Formation of the primary photoproduct (bathorhodopsin) involves energy uptake of about 130 kJ/mol, corresponding to storage of over 50% of the exciting photon energy, and is comparable to the energy storage previously observed in bovine rhodopsin. Subsequent intermediates involve the step-wise dissipation of this energy to give the physiological end-product (acid metarhodopsin) at a level only slightly above the parent rhodopsin. No significant differences in energetics are observed between rhodopsin in microvilli membrane suspensions or detergent dispersions. Use of different buffer systems in the calorimetric experiments shows that conversion of rhodopsin to acid metarhodopsin involves no light-induced protonation change, whereas alkali metarhodopsin photoproduction occurs with the release of one proton per molecule and an additional enthalpy increase of about 50 kJ/mol. Van't Hoff analysis of the effect of temperature on the reversible metarhodopsin equilibrium gives an enthalpy for the acid → alkali transition consistent with this calorimetric result, and the proton release is confirmed by direct observation of light-induced pH changes. Acid-base titration of metarhodopsin yields an apparent pK of 9.5 for this transition, though the pH profile deviates slightly from ideal titration behaviour. We suggest that a high energy primary photoproduct is an obligatory feature of efficient biological photodetectors, as opposed to photon energy transducers, and that the similarity at this stage between cephalopod and vertebrate rhodopsins represents either convergent evolution at the molecular level or strong conservation of a crucial functional characteristic.

* To whom offprint requests should be sent

Key words: Rhodopsin, vision, energetics, calorimetry

Introduction

Previous measurements of the light-induced energy changes in vertebrate visual pigment rhodopsin have highlighted various features of the primary photoreception process (Cooper and Converse 1976; Cooper 1979a, b, 1981, 1982). Particularly interesting was the unexpectedly high energy of the first photoproduct (bathorhodopsin) of bovine rhodopsin, corresponding to an energy storage of some 145 kJ/mol (35 kcal/mol), or more than 60% of the exciting photon energy (Cooper 1979b). This initially surprising result has since been confirmed by other techniques (Boucher and Leblanc 1985; R. R. Birge, personal communication), and reconciled theoretically (Birge and Hubbard 1980; Warshel and Barboy 1982), but contrasts sharply with the relatively low primary energy storage in the superficially similar, light-energy transducing system, bacteriorhodopsin (Birge and Cooper 1983). With hindsight, however, such high energy storage can be seen to convey distinct advantages to any photon detection system by providing a fast and efficient light-activated process whilst, at the same time, suppressing the thermally activated noise (Cooper 1979b, 1981). Consequently, it is of interest to see whether this, and other features of the bleaching energetics, are common to the primary photoreceptor macromolecules of other unrelated, or distantly related species. We report here on direct measurements of light-induced processes in octopus rhodopsin under various conditions, together with related observations on proton ionization and temperature effects.

The basic chemistry and photochemistry of visual pigments have been reviewed by Knowles and Dartnall (1977) and Fein and Szuts (1982). Cephalopod

rhodopsins, like their vertebrate counterparts, are membrane-bound proteins containing the 11-*cis* retinal chromophore covalently linked via a Schiff base to a lysine side chain. During the photoreaction, and probably very early on, this polyene chromophore isomerizes to an all-trans configuration, and the process involves a series of spectrally distinct intermediates which can be stabilized at low temperatures (Tsuda et al. 1980) or detected by rapid kinetic techniques (Tsuda 1979). In the case of octopus rhodopsin (absorbance max. 472 nm) irradiation with visible light initiates rapid formation of a red-shifted intermediate (bathorhodopsin, ca. 540 nm). This is stable in the dark at liquid nitrogen temperature but, on warming above −170 °C, the spectrum shifts to lumirhodopsin (ca. 485 nm). Further warming above −30 °C produces acid metarhodopsin (514 nm), which is the final photoproduct under physiological conditions. This last step may involve another intermediate, mesorhodopsin, spectrally similar to lumirhodopsin and appearing above about −100 °C (Tsuda 1979; Kusumi et al. 1983). Acid metarhodopsin is reversibly transformed to alkali metarhodopsin (376 nm) at high pH. These later stages of the photoprocess are markedly different from the vertebrate system where, at the analogous metarhodopsin stage, the blue-shifted pigment (meta II) is produced by lowering the pH, and where, in addition in a series of subsequent steps, spontaneous hydrolysis of the Schiff base and release of the retinal moiety occurs. No such hydrolysis occurs in the cephalopod systems, and the stable photoproduct (acid meta) readily reverts to the original rhodopsin on irradiation at the appropriate wavelength. This photoreversal probably contributes to physiological pigment regeneration in vivo (Hamdorf 1979).

Materials and methods

Dark-adapted microvilli membranes, isolated in Sapporo from freshly obtained eyes of the edible octopus (species: *Paroctopus defleini*, local name: Mizudako) by the method of Tsuda (1979), were frozen and shipped to Glasgow over solid carbon dioxide where they were stored at −70 °C until use. Samples for photocalorimetry were prepared by sedimentation of the membranes at 38,000 g, 5 °C, washing and re-suspending in the appropriate aqueous buffer or detergent mixture. All manipulations were in the dark or under dim red light, unless otherwise specified, and samples for low-temperature studies also contained glycerol in proportion 2 : 1, by volume. Detergent-extracted rhodopsin solutions were clarified by further centrifugation after extraction. Buffer concentrations were normally 0.1 *M*, with 0.3 *M* NaCl added to maintain ionic strength. Buffers used include: borate, phosphate, *Tris*, ethylene diamine, imidazole and n-butylamine, pH 7.0 or 9.5 − 10.2, as appropriate.

The sucrose lauryl ester detergent, L-1690, was a kind gift of the Ryoto Co. Ltd. (Tokyo). All other reagents were of analytical, or equivalent grade, and glass-distilled water was used throughout.

Energy measurements were performed in the purpose-built photocalorimeter described previously (Cooper 1979 b, 1982). Briefly, this consists of a sensitive isothermal microcalorimeter, functional from liquid nitrogen to ambient temperatures, equipped with fibre-optics light guides for sample irradiation by monochromatic light from a stabilized Xe-arc source. The configuration is such that all light entering the sample vessel is absorbed either by the sample or by the container walls, so that the calorimeter measures the energy of the incident light plus any light-induced energy changes. The procedure adopted here was to irradiate the sample repeatedly with identical 2−4 min bursts of light (usually 460 nm) and to integrate the total energy in each pulse, until a photostationary state in the sample was reached. Thus the later pulses give the total irradiation energy which, by subtraction, allows estimation of the photochemically related energy changes in the earlier irradiation phases. In this way each sample, after complete photoreaction, acts as its own reference or control. This procedure differs slightly from previous work (Cooper 1982) where total irradiation energies were measured separately or compensated by irradiation of an inert reference. The present method was found to be more satisfactory in this instance and eliminates possible uncertainties due to mismatch of sample and reference cells and fibre optics. The major source of calorimetric error is baseline drift due to slow thermal fluctuations, and the effect was minimised by careful least-squares fitting of pre- and post-irradiation baselines to a quadratic function. Calorimetric experiments showing excessive baseline curvature or displacement were rejected. After each experiment the sample was withdrawn from the calorimeter, warmed to room temperature, and the total extent of photoreaction (as metarhodopsin) was estimated spectrophotometrically (Pye-Unicam SP-1800). Molar extinction coefficients at the appropriate wavelengths were taken from Tsuda et al. (1982). For measurements of spectral changes in membrane suspensions, the sample cuvette was placed at the secondary (scattering) cell position adjacent to the face of the photomultiplier, and the reference cell was oriented with the ground-glass faces in the light path to compensate for scattering. Photoconversion

to the metarhodopsin stage was studied at 5° or 20 °C by immersing the calorimeter in a precision circulating water bath.

Low-temperature data for formation of the earlier photoproducts (batho- and lumirhodopsin, − 195° and − 115 °C, respectively) were obtained by suspension of the calorimetric unit over liquid nitrogen in a stainless-steel dewar vessel, fitted with an automatic cryogenic level controller (Edwards). Some measurements were also attempted at about − 65 °C as the calorimeter warmed up in this system, in order to trap the meso-intermediate. Photoreversal in the calorimeter from batho- or acid metarhodopsins was studied by first irradiating the sample at 460 nm to generate the appropriate photostationary state, followed by periodic irradiation/energy integration at 550 nm (batho-) or 540 nm (meta-), as described above.

The effects of pH and temperature on the octopus metarhodopsin equilibrium were studied in a Pye-Unicam SP-1800 spectrophotometer fitted with a thermostatted cell compartment. Sample temperatures were measured directly in the cuvette with a calibrated thermocouple thermometer (Comark).

Light-induced pH changes in un-buffered octopus rhodopsin membrane samples were observed using a combination glass electrode and Radiometer pH meter, connected to a sensitive linear recorder. The samples were stirred in a transparent, thermostatted vessel and flushed with nitrogen or argon gas throughout the experiment. Monochromatic light from a stabilized 200 W Hg-Xe arc source fitted with a calibrated variable interference filter (Oriel Scientific Co.) was used for irradiation, and the system was calibrated using standardised HCl or NaOH solutions.

Results

By judicious choice of calorimetric temperature and sample pH we have been able to halt the photolytic sequence of octopus rhodopsin at each of the major intermediate stages and measure the nett enthalpy change in each case. All photoreactions in the forward direction, starting from rhodopsin, are clearly endothermic as exemplified in Fig. 1 A, C i.e. the initial irradiation steps give a lower integrated calorimetric energy response compared to later irradiations of the photostationary state. (In most experiments the photoreaction was essentially complete within the first two irradiation periods.) In contrast, photoreversal to the parent rhodopsin is exothermic (Fig. 1 B, D). The relative enthalpies of the different intermediates under various conditions are given in Table 1, and compared to the equivalent bovine intermediates in Fig. 2. By far the greatest energy uptake occurs in the very first step of the photoreaction (Table 1), and corresponds to storage of just over 50% of the exciting photon energy at the rhodopsin absorbance maximum. This stored energy can be released by photoreversal at longer wavelengths (Fig. 1). Photoproduction of subsequent

Table 1. Calorimetric enthalpies of formation of octopus rhodopsin photoproducts

Rhodopsin →	Temperature [°C]	ΔH (S.D.) [kJ/mol]	Conditions
Bathorhodopsin	−195	130.5 (8.4)	a
Lumirhodopsin	−115	53.3 (5.2)	a
Mesorhodopsin	−65	~18 (~10)	a
Acid meta.	5	17.4 (7.8)	b
	5–20	19.6 (7.0)	c
Alkali meta.	5–20	68.9 (4.1)	d

[a] 10% detergent, 0.1 *M* phosphate, pH 7; 2 : 1 glycerol. 460 nm irradiation
[b] 10% detergent, 0.1 *M* buffers, pH 7, 0.3 *M* NaCl. 460 nm irradiation
[c] Membrane suspension, 0.1 *M* buffers, pH 7, 0.3 *M* NaCl. 460 nm irradiation
[d] Membrane suspension, 0.1 *M* buffers, pH 9.5–10.2, 0.3 *M* NaCl. Corrected for buffer ionization. 540 nm irradiation

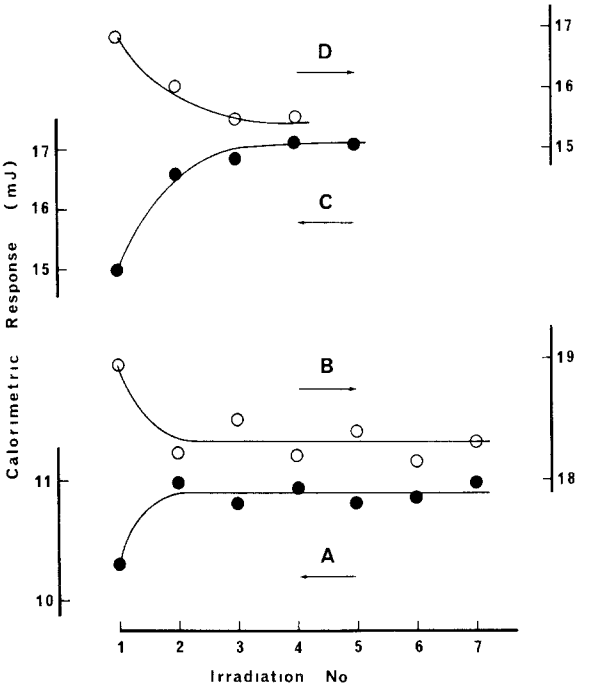

Fig. 1 A–D. Examples of photocalorimetric data showing energy uptake on bleaching and release on photoreversal. Each point is the integrated heat energy detected during successive, identical 2 minute irradiations of an appropriate sample. **A** Rhodopsin → Acid metarhodopsin (at 20 °C, 460 nm); **B** Acid meta → Rhodopsin (5 °C, 540 nm); **C** Rhodopsin → Bathorhodopsin (− 195 °C, 460 nm); **D** Batho → Rhodopsin (− 195 °C, 540 nm). Note the different energy scales indicated by the arrows. The amounts of rhodopsin photoreacting in these examples were in the range 10–30 nmoles. The lines are intended solely to guide the eye

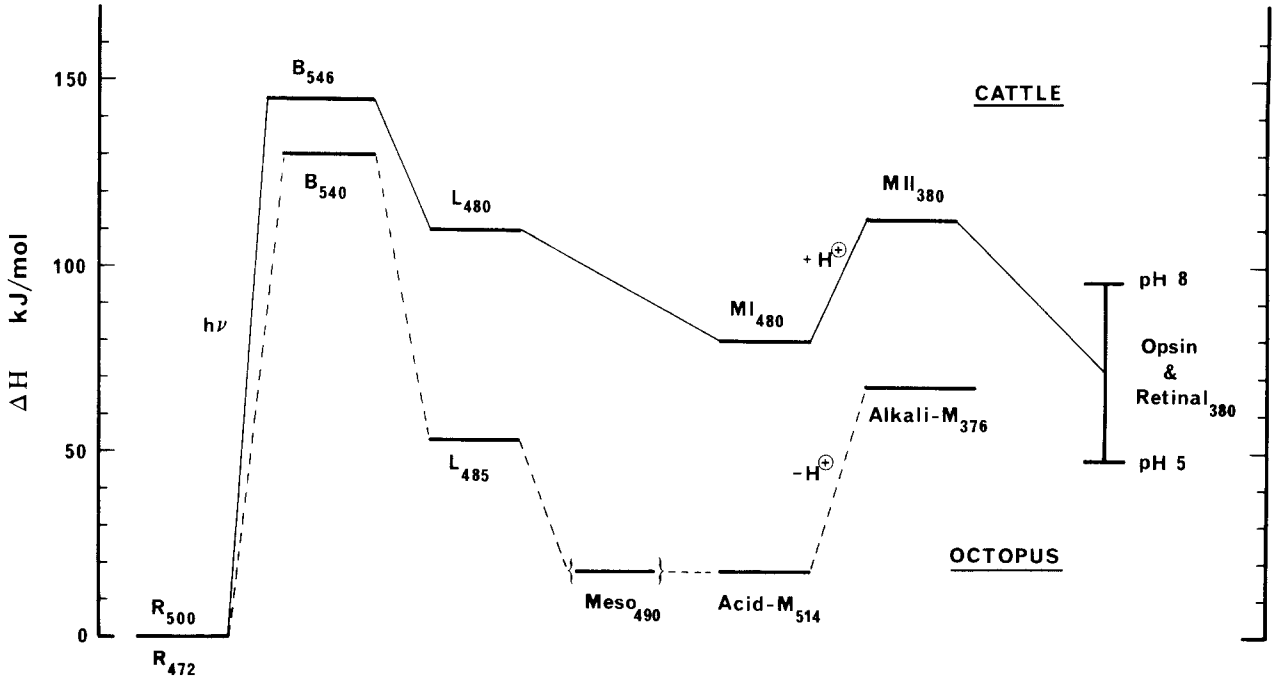

Fig. 2. Comparison of the relative enthalpy levels of the various octopus rhodopsin photoproducts with the equivalent bovine intermediates (from Cooper 1981). Subscripts denote the approximate absorbance maxima (nm) of each species. (R = rhodopsin, B = batho-, L = lumi-, M = meta-)

intermediates involves significantly smaller enthalpy changes (Table 1, Fig. 2). Accurate measurements of the meso-intermediate at about −65 °C were thwarted by poor temperature stability of the photocalorimeter in this region, but tentative indications are that the enthalpy increase is indistinguishable from that of acid metarhodopsin (i.e. about + 18 kJ/mol). Photoreversal of acid metarhodopsin to the parent rhodopsin by irradiation at 540 nm is exothermic, and of magnitude compatible with true reversibility (Fig. 1). No evidence for formation of the 9-*cis* pigment, isorhodopsin, during calorimetric irradiations was found and, in any case, this would not be expected to markedly affect the energetic measurements (Cooper 1979a). All energies quoted are averages of a series of experiments performed with both microvilli membranes and detergent solubilized rhodopsin (L-1690, 10% w/v) under various buffer conditions, and no significant variations were observed. In particular, the enthalpies of acid metarhodopsin formation were independent of the particular buffer species employed (Fig. 3), indicating that no change in the state of protonation of the system is involved in this step (Cooper and Converse 1976). In contrast, formation of alkali metarhodopsin at pH > 9 depended markedly on the heat of ionization of the buffer species (Fig. 3). This arises because, in a well-buffered system, any hydrogen ions displaced in the process are taken up by the buffer species with consequent secondary heat

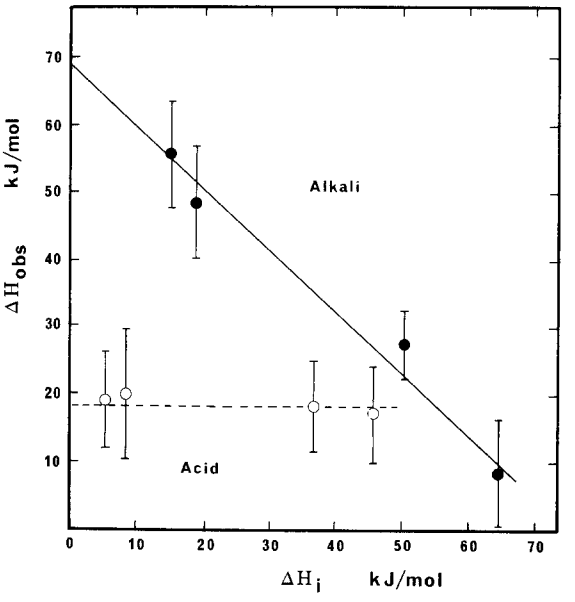

Fig. 3. Effect of differing buffer ionization heats (ΔH_i) on the observed enthalpies of acid or alkali metarhodopsin formation. Buffers used include: borate, phosphate, *Tris*, ethylene diamine, imidazole and n-butylamine, all at 0.1 M concentration, pH 7.0 or 9.5–10.2, as appropriate. The error bars indicate standard deviations of a series of determinations in each buffer system

effects, and the calorimeter detects the sum of these heat effects. A plot of the observed apparent enthalpies of alkali metarhodopsin formation versus heat of buffer ionization, taken from the tabulation

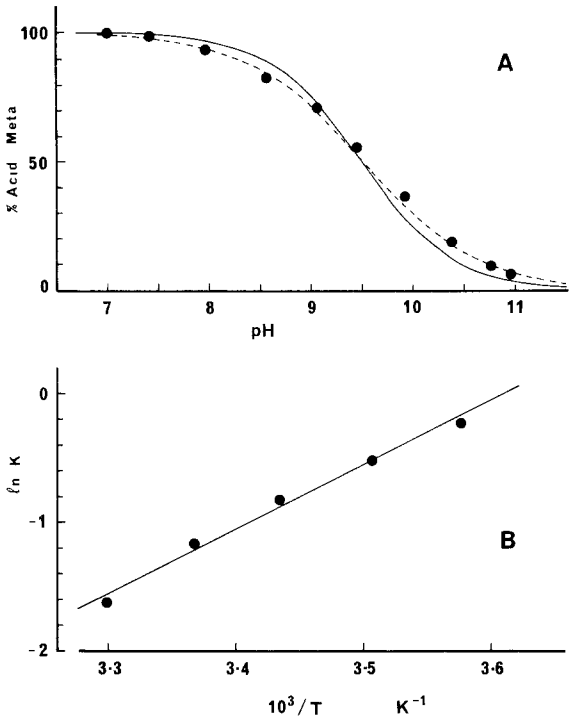

Fig. 4. **A** pH titration of octopus acid/alkaline metarhodopsin dispersed in 2% (w/v) L-1690, 80 m*M* *Tris* or borate buffer as appropriate. The solid line gives the theoretical behaviour for simple titration of a single acidic group with pK 9.5, whereas the dashed line is the best fit to the data with nominal protonation stoichiometry of 0.79. **B** Van't Hoff plot of the temperature dependence of the octopus metarhodopsin equilibrium. K = [acid meta]/[alkali meta]

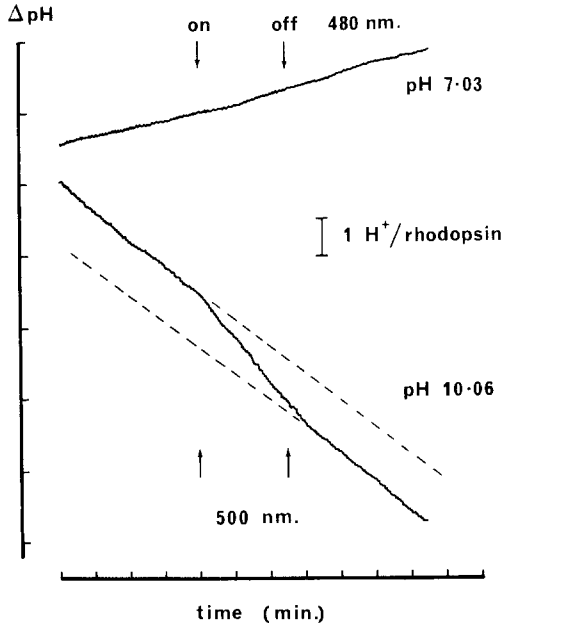

Fig. 5. Tracings of light-induced pH effects on un-buffered suspensions of octopus microvilli membranes in 0.4 *M* NaCl at 6 °C. The dashed lines are linear extrapolations of pre- and post-illumination drift

by Christensen et al. (1976), is linear (Fig. 3) with gradient 0.91 (+/− 0.09). This is consistent with the release of one proton per rhodopsin molecule during photoconversion at high pH. Extrapolation to zero buffer ionization heat gives + 68 (+/− 4) kJ/mol for alkali metarhodopsin formation in the absence of buffer effects.

The reversible acid/alkali metarhodopsin equilibrium is both temperature and pH dependent – high pH and high temperature both favouring alkali metarhodopsin. The pH profile of the reaction, observed at 20 °C on totally photoconverted octopus rhodopsin dispersed in detergent buffer, is sigmoidal with apparent pK 9.5 (Fig. 4A), and shows some deviation from ideal titration behaviour (see discussion). A Van't Hoff plot of the effect of temperature on this equilibrium, in 0.1 *M* borate buffer, is linear (Fig. 4B) and gives a value of + 56.5 (+/− 3.5) kJ/mol for the acid → alkali metarhodopsin reaction, after correction for the small temperature dependence of the buffer pH, consistent with the calorimetric enthalpy difference of about + 50 kJ/mol (Table 1).

Direct observations of pH changes during illumination (Fig. 5) show no change at low pH's, but a reproducible pH drop accompanying alkali metarhodopsin formation at high pH, despite the appreciable drift evident in these un-buffered samples. Subsequent irradiations, after total photoconversion of the rhodopsin, showed no such effects. The average proton release, from a series of determinations, is 1.08 (+/− 0.16) protons/rhodopsin under these conditions.

Discussion

Cephalopods are molluscs, very remote from vertebrates in evolutionary terms. But despite this wide phylogenetic separation, their visual systems display striking convergence at the anatomical level (Packard 1966, 1972; Wells 1983) which may extend to the biochemical level, bearing in mind the broad similarities in the chemistry and photolytic sequences of cephalopod and vertebrate rhodopsins (Knowles and Dartnall 1977). Photoproducts of cephalopod rhodopsins are, in addition, capable of activating bovine photoreceptor phosphodiesterase and GTP-binding protein (Ebrey et al. 1980; Saibil and Michel-Villaz 1984). This basic similarity is now seen to extend also to the molecular energetics of the primary photoprocess (Fig. 2). In particular, the relatively high energy of octopus bathorhodopsin parallels our previous observations on the similar process in bovine rhodopsin (Cooper 1979b), and suggests that photon energy storage in the primary photoreaction is a common, if not obligatory feature

of all rhodopsins. One can easily see why this might be the case. In visual photoreceptors, as in all photon detectors, a primary requirement is optimisation of signal-to-noise ratio. If, as is generally believed, the very first step in photoreception involves rapid cis-trans photoisomerization of the retinyl moiety, via the singlet excited state, to give the meta-stable bathorhodopsin intermediate, then any thermal (dark) isomerization would give rise to spurious signals and thermal noise (Ashmore and Falk 1977). Now, for free retinal in organic solvents the relative energies of the cis-trans isomers are quite similar, and the kinetic activation barriers are sufficiently small that thermal isomerizations are rapid at physiological temperatures (Hubbard et al. 1965) and would lead to severe background noise problems in the retina. In typical laboratory devices, such as photomultiplier tubes, one would normally attempt to suppress this 'dark current' by cooling to low temperatures. But this course of action is clearly not convenient in a biological situation, and it would seem that in the course of evolution an alternative strategy has emerged: that is, to constrain the chromophore in a specific protein matrix which, among other things, makes isomerization an energetically expensive process with high activation barriers (Cooper 1979b; Birge 1981) and, thereby, much less prone to spurious thermal excitation. Whether this represents true convergent evolution at the molecular mechanics level, or conservation of a vital functional characteristic already present in common ancestral photoreceptor proteins, remains open to speculation. But the recently described homologies in amino acid sequence between vertebrate and insect rhodopsins indicate that these proteins have a very ancient pedigree (O'Tousa et al. 1985; Zuker et al. 1985).

It is interesting to speculate why such energy storage is not so dramatic in the photon energy transducing bacteriorhodopsin of *Halobacterium halobium* (Birge and Cooper 1983), which is a superficially similar system based on photoisomerization of protein-bound retinal (Stoeckenius 1980; Henderson 1977), but lacking any sequence homology with true rhodopsins (Ovchinnikov 1982; Ovchinnikov et al. 1982), and where efficient photon energy storage might have been thought to be of prime importance. In general terms the difference may lie in that, whereas thermal processes in a photon detector can totally mask the signal, similar processes in an energy transducer contribute nothing to thermodynamic energy balance at equilibrium. That is to say: at thermodynamic equilibrium any energy gained from thermal activation of the bacteriorhodopsin cycle in one direction is cancelled by similar thermal processes in the reverse direction. Only when the cycle is pumped preferentially in one direction by specific, non-equilibrium effects such as light-activated isomerization, does any nett energy gain accrue. Thus, there is no strong evolutionary pressure to suppress thermal processes, unlike photodetector systems where spurious signals due to thermal activation in one direction are not cancelled by the equivalent reverse process. In this context, Birge (1981) has argued alternatively that a low ground-state, thermal barrier to isomerization in bacteriorhodopsin is, indeed, required for rapid completion of the photocycle in this system. These two arguments are not necessarily mutually exclusive.

The molecular mechanism of energy storage in bathorhodopsins has not yet been established experimentally, but probably involves a combination of stereochemical strain and electrostatic forces arising from *cis-trans* isomerization of the retinal molecule in the constrained protein milieu (Birge and Hubbard 1980; Warshel and Barboy 1982). This strain is presumably then liberated as the system relaxes through lumi- and meso-intermediates to the metarhodopsin stage where, in the case of cephalopods, it stops.

All the experimental techniques employed here give a consistent picture of the later photointermediates. Formation of the physiological end product, acid metarhodopsin, occurs without change in protonation of the system. On the other hand, both calorimetry and direct pH observations (Figs. 3 and 5) indicate the release of one proton during alkali metarhodopsin formation. This suggests that acid → alkali metarhodopsin is a simple proton ionization process. pH titration data, however, do show slight deviations from ideal acid-base equilibrium (Fig. 4A), which can be parameterized in terms of a notional protonation stoichiometry of about 0.8. Similarly non-ideal titration behaviour, with apparent proton stoichiometries of between 0.5 and 0.7, has been observed in the vertebrate metarhodopsin system (Matthews et al. 1963; Abrahamson and Fager 1973; Parkes and Liebman 1984), and it is not clear whether this apparent fractional protonation is due to subtle ionic strength effects, competing ionizable groups on the protein, or the presence of un-resolved intermediates in the metarhodopsin transition. Bearing in mind the spectral changes involved in this transition, involving a blue-shift typical of deprotonated retinyl Schiff bases, the most likely site for this deprotonation step is the imine nitrogen of the chromophoric Schiff base, and this is confirmed by resonance Raman experiments (Kitagawa and Tsuda 1980). The analogous meta I → meta II transition of vertebrate systems also involves deprotonation of the Schiff base nitrogen

though, paradoxically, the overall reaction involves the uptake of about one proton. Whereas the retinal-opsin linkage in vertebrate metarhodopsins is susceptible to hydrolysis and to attack by hydrophillic reagents such as hydroxylamine, the octopus metarhodopsins are quite stable to hydrolysis and resistant to hydroxylamine. This suggests that whatever conformational changes are associated with the bleaching, they do not expose the Schiff base linkage to the solvent environment. The relatively high pK for the octopus metarhodopsin equilibrium is consistent with the imine group remaining buried in a non-polar environment at this stage. The relatively low energy of acid metarhodopsin, compared to the parent rhodopsin, indicates that relatively little strain is induced by *cis-trans* isomerization of the retinal chromophore in the protein matrix, or that different conformational states of roughly similar energy exist in order to accommodate either retinyl configuration. As a consequence there is little driving force for Schiff base hydrolysis, in contrast to the situation in the bovine sequence where the higher metarhodopsin energies will tend to lead in the direction of breakage of the imine linkage and expulsion of the all-*trans* chromophore.

Acknowledgements. It is a pleasure to thank Mr. N. Kawase of the Mitsubishi-Kasei Food Corporation (formerly Ryoto Co. Ltd.) for samples of L-1690, and the Asagi Co. (Yoich, Hokkaido) for the supply of live octopus eyeballs. S.F.D. is supported by an S.E.R.C. research studentship.

References

Abrahamson EW, Fager RS (1973) The chemistry of vertebrate and invertebrate visual photoreceptors. Curr Top Bioenerg 5:125–200

Ashmore JF, Falk G (1977) Dark noise in retinal bipolar cells and stability of rhodopsin in rods. Nature 270:69–71

Birge RR (1981) Photophysics of light transduction in rhodopsin and bacteriorhodopsin. Annu Rev Biophys Bioeng 10:315–354

Birge RR, Cooper TM (1983) Energy storage in the primary step of the photocycle of bacteriorhodopsin. Biophys J 42:61–69

Birge RR, Hubbard LM (1980) Molecular dynamics of cis-trans isomerization in rhodopsin. J Am Chem Soc 102: 2195–2205

Boucher F, Leblanc RM (1985) Energy storage in the primary photoreaction of bovine rhodopsin. A photoacoustic study. Photochem Photobiol 41:459–465

Christensen JJ, Hansen LD, Izatt RM (1976) Handbook of proton ionization heats. John Wiley, New York

Cooper A, Converse CA (1976) Energetics of primary processes in visual excitation: photocalorimetry of rhodopsin in rod outer segment membranes. Biochemistry 15: 2970–2978

Cooper A (1979a) Energetics of rhodopsin and isorhodopsin. FEBS Lett 100:382–384

Cooper A (1979b) Energy uptake in the first step of visual excitation. Nature 282:531–533

Cooper A (1981) Rhodopsin photoenergetics: lumirhodopsin and the complete energy profile. FEBS Lett 123:324–326

Cooper A (1982) Calorimetric measurements of light-induced processes. Methods Enzymol 88:667–673

Ebrey T, Tsuda M, Sassenrath G, West JL, Waddell WH (1980) Light activation of bovine rod phosphodiesterase by non-physiological visual pigments. FEBS Lett 116: 217–219

Fein A, Szuts EZ (1982) Photoreceptors: their role in vision. Cambridge University Press, Cambridge, UK

Hamdorf K (1979) The physiology of invertebrate visual pigments. In: Autrum H (ed) Handbook of sensory physiology, vol VII/6A. Springer, Berlin Heidelberg New York, pp 145–224

Henderson R (1977) The purple membrane from *Halobacterium halobium.* Annu Rev Biophys Bioeng 6:87–109

Hubbard R, Bownds D, Yoshizawa T (1965) The chemistry of visual photoreception. Cold Spring Harbour Symp Quant Biol 30:301–315

Kitagawa T, Tsuda M (1980) Resonance Raman spectra of octopus acid and alkaline metarhodopsins. Biochim Biophys Acta 624:211–217

Knowles A, Dartnall HJA (1977) The eye, vol 2B. 2nd edn. Academic Press, New York

Kusumi A, Tsuda M, Akino T, Ohnishi S, Terayama Y (1983) Protein-phospholipid-cholesterol interaction in the photolysis of invertebrate rhodopsin. Biochemistry 22:1165–1170

Matthews RG, Hubbard R, Brown PK, Wald G (1963) Tautomeric forms of metarhodopsin. J Gen Physiol 47:215–240

O'Tousa JE, Baehr W, Martin RL, Hirsh J, Pak WL, Applebury ML (1985) The *Drosophila* ninaE gene encodes an opsin. Cell 40:839–850

Ovchinnikov YA (1982) Rhodopsin and bacteriorhodopsin: structure–function relationships. FEBS Lett 148:179–189

Ovchinnikov YA, Abdulaev NG, Feigina MY, Artamonov ID, Zolotarev AS, Miroshnikov AI, Martynov UI, Kustina MB, Kudelin AB, Bogachuk AS (1982) The complete amino acid sequence of visual rhodopsin. Bioorg Khim 8: 1011–1014

Packard A (1966) Operational convergence between cephalopods and fish: an exercise in functional anatomy. Arch Zool Ital 51:523–542

Packard A (1972) Cephalopods and fish: the limits of convergence. Biol Rev 47:241–307

Parkes JH, Liebman PA (1984) Temperature and pH dependence of the metarhodopsin I – metarhodopsin II kinetics and equilibria in bovine rod disk membrane suspensions. Biochemistry 23:5054–5061

Saibil HR, Michel-Villaz M (1984) Squid rhodopsin and GTP-binding protein crossreact with vertebrate photoreceptor enzymes. Proc Natl Acad Sci USA 81:5111–5115

Stoeckenius W (1980) Purple membrane of halobacteria: a new light-energy converter. Acc Chem Res 13:337–344

Tsuda M (1979) Transient spectra of intermediates in the photolytic sequence of octopus rhodopsin. Biochim Biophys Acta 545:537–546

Tsuda M, Tokunaga F, Ebrey T, Yue KT, Marque J, Eisenstein L (1980) Behaviour of octopus rhodopsin and its photoproducts at very low temperature. Nature 287:461–462

Tsuda M, Terayama Y, Takahashi M (1982) Absorption spectra and molar extinction coefficients of octopus rhodopsin and its photoproducts. Jinbun Shizen Kagaku Kiyo 23:37–41

Warshel A, Barboy N (1982) Energy storage and reaction pathways in the first step of the vision process. J Am Chem Soc 104:1469–1476

Wells MJ (1983) Caphalopods do it differently. New Sci 100: 332–338

Zuker CS, Cowman AF, Rubin GM (1985) Isolation and structure of a rhodopsin gene from *D. melanogaster.* Cell 40:851–858