ENERGETICS OF RHODOPSIN AND ISORHODOPSIN

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1. Introduction

Bleaching of rhodopsin in vertebrate photoreceptor cells is an endothermic photochemical reaction in which photon energy is used specifically to drive the pigment glycoprotein system (rhodopsin) to a state of higher thermodynamic free energy (opsin + all-*trans* retinal) [1,2]. Neither the mechanism of visual excitation nor the chemistry of the photoreaction are clearly understood. Considerable theoretical effort is being directed towards the mechanics of the reaction, particular emphasis being placed on the possible topologies of the molecular energy surface in an attempt to explain the specific retinal isomerization process and the spectral properties of rhodopsin and its photoproducts [3–8]. Such studies are currently hampered by a lack of detailed information on the chemistry and structure of the retinal attachment site and on the energetics of the primary excitation events.

Native rhodopsin contains one molecule of 11-*cis* retinal (vitamin A aldehyde), probably bound as a protonated Schiff base to a specific lysine residue of the protein opsin which, together with other protein–chromophore interactions, results in a photosensitive pigment absorbing maximally in the visible region of the spectrum. Early experiments [9] showed that the 9-*cis* isomer of retinal could also form a photopigment (isorhodopsin) on reaction with opsin, and more recent work indicates that several other geometrical isomers [10,11], as well as chemically modified retinals (reviewed in [12]), can also be accommodated in the opsin binding site. This lack of absolute specificity implies either that the retinal binding site of opsin is rather open, with only a few molecular contacts being crucial for binding, or that the protein and/or retinal are sufficiently flexible that small conformational changes can take place to optimise interaction energies. Conformational changes would involve the uptake, or release, of molecular strain energy which should be reflected in the magnitude of the overall binding energies. As part of a program to determine experimentally the energetics of the complete rhodopsin photoreaction, I report here on a comparison of the enthalpies of total bleaching of rhodopsin and isorhodopsin in rod outer segment membranes using a direct calorimetric technique [2]. Significant differences in heat effects are observed which show that the ground-state enthalphy of isorhodopsin is higher than that of rhodopsin by about 5 kcal.mol^{-1}.

2. Experimental

Bovine rod outer segments (ROS) were prepared from fresh cattle retinas by the method in [13]. Ox eyes were obtained within 2–3 h of death from the local meat market and retinas dissected under dim red light. ROS preparation was performed in the dark, for rhodopsin, or under normal room lighting for opsin. Isorhodopsin ROS were subsequently regenerated from opsin-containing ROS suspensions by addition, in the dark, of stoichiometric amounts of 9-*cis* retinal (Sigma Chemical Co.) dissolved in a small volume of ethanol. Rhodopsin and isorhodopsin ROS suspensions were stored in the dark at −20°C under argon until use.

The technique of photocalorimetry has been

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detailed in [2]. Basically the instrument consists of a sensitive isothermal microcalorimeter with provision for monochromatic irradiation of the sample, thus allowing direct determination of the energetics of photochemical reaction under relatively low light intensities. The instrument is calibrated both electrically and chemically, using the potassium ferrioxalate photoredox system [2,14]. The present experiments utilised a new version of the photocalorimeter designed specifically for use at subzero temperatures, but set at 3°C in this instance. Suspensions of rhodopsin- or isorhodopsin-containing ROS, 2 ml sample volume, were irradiated in the photocalorimeter at 546 nm for 2–4 min. Pigment optical densities, at λ_{max}, were normally 1.5–3.0 and typically 10–20 nmol pigment was bleached during any one experiment. Suspensions were made up in 0.1 M Na-acetate buffer (pH 5.4) containing 50 mM hydroxylamine (as the hydrochloride) and 30% sucrose (w/w) to prevent sedimentation of the rods during the experiment. Pigment concentrations and extent of photoreaction were determined spectrophotometrically on aliquots of the ROS dissolved in 2% CTAB (cetyl trimethyl ammonium bromide), 0.2 M phosphate buffer (pH 6.7); using $\epsilon = 41\,000$ ($\lambda_{max} = 500$ nm) for rhodopsin and $\epsilon = 43\,000$ ($\lambda_{max} = 486$ nm) for isorhodopsin.

3. Results and discussion

Under normal conditions the complete photolysis reaction in ROS is rather too slow for calorimetric studies and also somewhat ill-defined, since all-*trans* retinal released on photolysis may go on to form non-specific Schiff base complexes with free amino groups in the various protein and lipid components of the ROS membrane. The addition of hydroxylamine at pH 5.4 gives a much cleaner reaction, reacting rapidly at the *meta*-rhodopsin II stage to give opsin plus free retinal oxime [16,17]. The enthalpy of this reaction, coupled with the heat of retinal oxime formation measured separately, readily gives the enthalpy for the overall photolysis process [2].

The experiments in [2], confirmed here with the new version of the photocalorimeter, have shown that bleaching of rhodopsin ROS in the presence of hydroxylamine at pH 5.4, 3°C, is essentially isothermal:

$$\text{Rhodopsin} + \text{Hydroxylamine} \xrightarrow{h\nu} \text{Opsin} + \text{Retinaloxime (all-}\textit{trans}\text{)}$$

$$\Delta H = +0.3\ (\pm 1.1)\ \text{kcal.mol}^{-1} \qquad (1)$$

A series of experiments under identical conditions, but using isorhodopsin ROS, gave consistently exothermic heats of reaction:

$$\text{Isorhodopsin} + \text{Hydroxylamine} \xrightarrow{h\nu} \text{Opsin} + \text{Retinaloxime (all-}\textit{trans}\text{)}$$

$$\Delta H = -4.9\ (\pm 1.2)\ \text{kcal.mol}^{-1} \qquad (2)$$

These enthalpies may be combined with the known heat of retinal oxime formation [2] to give the enthalpy changes for complete bleaching in both cases. The more interesting quantity, however, is the relative energy difference between the two pigments. Since the products of reactions (1) and (2) are identical [10,15], this may be obtained directly by subtraction:

$$\text{Rhodopsin} \longrightarrow \text{Isorhodopsin} \qquad (\text{pH } 5.4, 3^{\circ}\text{C})$$

$$\Delta H = +5.2\ (\pm 2.3)\ \text{kcal.mol}^{-1}$$

Thus, ignoring any small volume changes, the ground-state energy of isorhodopsin is higher than that of rhodopsin by ~5 kcal.mol^{-1} (0.22 eV), implying that isomerization of the retinal chromophore from the 11-*cis* to the 9-*cis* configuration, when constrained in the binding site of opsin, is an endothermic process. This is in contrast to theoretical estimates which predict that isorhodopsin is the more stable of the two pigments [6]. Comparison with the energy difference between unconstrained isomers would be of interest here, but this has not yet been determined. However, by analogy with the difference between 11-*cis* and all-*trans* retinals measured in solution [18], this energy is likely to be small.

Possibly at least part of the energy difference between rhodopsin and isorhodopsin arises from

distortion of the 9-*cis* conformation in order to fit the opsin binding site, which has been optimized by evolutionary pressures to take the 11-*cis* retinal conformation most readily. This is interesting in view of a class of theories regarding the origin of the colour of rhodopsin. It has been argued that part of the bathochromic shift on binding of retinal to opsin might be due to torsional strain induced in the chromophore [20]. This is clearly not supported by the present observations which indicate that the chromophore is likely to be more strained in isorhodopsin than rhodopsin, whereas the isorhodopsin absorbance spectrum is blue-shifted by ~15 nm with respect to rhodopsin.

It is worth noting here that rates of pigment formation with 9-*cis*, and other retinal isomers, are much slower than for the 11-*cis* isomer ([10,11] and unpublished observations) and that the resulting pigments are much less thermally stable [11,19]. This suggests that for other visual pigment analogues as well, the ground-state energy is significantly higher than native rhodopsin.

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