RHODOPSIN PHOTOENERGETICS: LUMIRHODOPSIN AND THE COMPLETE ENERGY PROFILE

Alan COOPER
Chemistry Department, Glasgow University, Glasgow, G12 8QQ, Scotland

Received 5 December 1980

1. Introduction

Various intermediate stages in the photolysis of visual pigments have been recognised, but neither their stereochemical form nor their relation to the actual process of visual excitation have been resolved (review [1]). The relative energies of many of the intermediates in the bovine rhodopsin bleaching sequence have been determined in recent years by direct photocalorimetric methods [2–4]. Here I report the measurement of the enthalpy change associated with the light-induced formation of lumirhodopsin in bovine photoreceptor membranes at −75°C. This completes the sequence of calorimetrically accessible intermediates, and allows construction of an energy profile for the entire process which establishes firm thermodynamic constraints for proposed molecular mechanisms.

2. Materials and methods

Bovine rod outer segment membranes (ROS) were prepared by a standard sucrose density gradient technique [5] from the retinas of fresh, dark-adapted cattle eyes, obtained locally. Samples for photocalorimetry were prepared by suspension of ROS in a mixture of glycerol (2 vol.) and 0.1 M sodium phosphate buffer (1 vol.), pH 7.2 at room temperature. Rhodopsin concentrations and the extent of bleaching during calorimetry were determined from room temperature absorbance spectra of aliquots of the ROS suspension dispersed in 2% (w/v) CTAB (cetyltrimethylammonium bromide, Sigma Chem. Co.), 0.1 M phosphate buffer, pH 7.2, containing 10 mM hydroxylamine hydrochloride, assuming a molar extinction coefficient of 41 000 at 500 nm. Unless otherwise stated, all operations were performed in the dark or under dim red light.

Instrumental details of the low-temperature photocalorimeter have appeared in [3,6]. For these experiments the entire calorimetric unit was surrounded by a copper circulation coil and suspended in a Dewar vessel filled with methanol. The system was further insulated with expanded polystyrene foam and mounted in a temperature-controlled (20 ± 1°C) darkroom. The calorimeter was cooled to −75°C by circulation of methanol from a low-temperature bath, modified for proportional temperature control with a Pt resistance probe and precision electronic controller. Sample illumination was via glass fibre optics from a grating monochromator and a stabilised 200 W xenon arc lamp. Electrical calibration gave an instrumental sensitivity of 0.071 $\mu V/\mu W$ under these conditions. The amplified signal, integrated over 60 s intervals, was recorded digitally as a function of time throughout an experiment.

In a typical experiment the photocalorimeter was loaded with 2.0 ml ROS suspension ($E_{500} \simeq 1$) together with a reference sample of the same material totally bleached at room temperature prior to loading. After thermal equilibration at −75°C for several hours (usually overnight) the sample was irradiated briefly at 480 or 520 nm and the differential thermal response recorded. The sample was then removed, warmed to room temperature, and an aliquot taken for spectral determination of the amount of photoreaction in comparison with starting material. Control experiments included trials with bleached material in both calorimeter cells.

3. Results and discussion

Lumirhodopsin is the kinetically stable photoproduct of bovine rhodopsin at −75°C and the reaction only proceeds to subsequent stages on warming

 Elsevier/North-Holland Biomedical Press

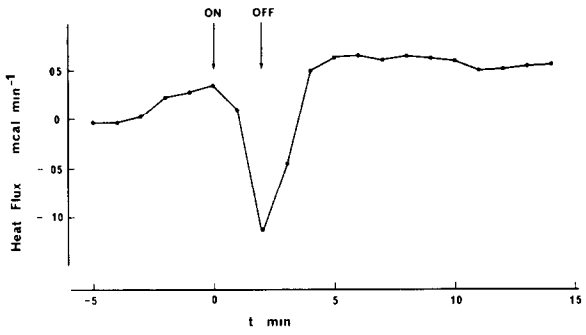

Fig.1. Calorimetric data showing differential energy uptake by bovine ROS at −75°C during a 2 min irradiation at 480 nm. In this experiment 7.3 nmol rhodopsin were bleached and the integrated heat energy uptake was ~1.8 × 10^{-4} cal.

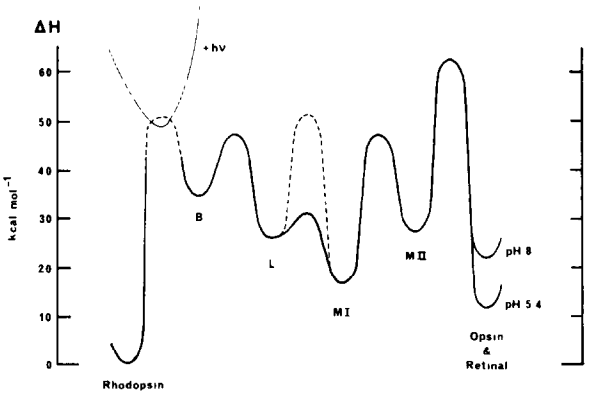

Fig.2. Energy profile for the bleaching of rhodopsin in cattle ROS. Enthalpies of metastable intermediates are determined by direct calorimetry. Activation barriers for the thermal processes from bathorhodopsin (B) onwards are from kinetic data. In the case of the lumirhodopsin (L) to metarhodopsin I (MI) transition the solid line is for ROS near physiological temperature, and the dashed line for the same system in the −40 to −50°C range [7]. The ground-state barrier between rhodopsin and B must approach close to, or even overlap with the electronically excited energy levels (upper curve) in this region [3]. (1 kcal = 4.184 kJ = 4.335 × 10^{-2} eV.)

The activation parameters, taken from [7–10] are as follows (kcal/mol):

B → L, 12.5; L → MI, 4.5 (+3 to +18°C), 25.2 (−40 to −50°C); MI → MII, ≃ 30; MII → Opsin + Retinal, 35

Calorimetric enthalpies ([2,3] and present work) (kcal/mol):

B, 34.7(±2.2); L, 26.3(±4.2); MI, 16.8(±1.4); MII, 27.4(±1.3); Opsin + Retinal, pH 8.0, 22.1(±2.0), pH 5.4, 11.7(±2.0)

to higher temperatures [1]. Light-induced formation of lumirhodopsin in the photocalorimeter is consistently endothermic (fig.1), though photoreversal to isorhodopsin during extensive bleaches makes the effect difficult to quantitate. This is a particular problem here because of the proximity of the absorbance maxima of lumirhodopsin (497 nm) and the parent rhodopsin (500 nm), but the effect could be minimised by working with relatively dilute ROS suspensions and limited bleaching (5–10 nmol). Under these conditions the enthalpy increase during lumirhodopsin formation was found to be 26.3 (±4.2) kcal/mol (mean and standard deviation of 5 determinations, 20.0–31.1 kcal/mol), thereby placing this intermediate roughly midway in energy between its immediate neighbours, batho- and metarhodopsin I. No dependence on irradiating wavelength was detected, within the relatively large experimental uncertainty limits.

By combining this datum with the enthalpies of other intermediates determined [2,3] and with kinetic activation energy barriers observed in bovine rhodopsin under similar conditions [7–10], it is now possible to draw a potential energy diagram covering the entire sequence of major events in the bleaching of rhodopsin (fig.2). This cannot, by itself, define the molecular mechanism of the process, but it does for the first time provide stringent thermochemical limits within which models may be developed. Some caution may be required, however, since of necessity the enthalpies of the various photoproducts have been measured at different temperatures, and differences in heat capacity [11] between the different states might change the details of the picture somewhat when extrapolated to physiological temperatures, as indicated by the temperature-dependent activation parameters for lumirhodopsin decay [7]. Nevertheless, the overall energetics are consistent with a general mechanism involving rapid and efficient retinal photoisomerization as a first step [12,13], whilst at the same time unwanted thermal activation is suppressed by the large ground-state barrier [3]. Stored energy is subsequently released by conformational relaxation in stages to the metarhodopsin I state [7], following which the retinal–opsin linkage is probably hydrolysed [2,14] prior to release of the chromophore from the protein. These latter stages are undoubtedly more complex than pictured here, involving transient intermediates which cannot be resolved by calorimetry.

Acknowledgements

Experimental aspects of this work were aided by a grant from the UK Science Research Council. I thank Margaret Nutley for expert technical assistance.

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