THE APPLICATION OF PRESSURE RELAXATION TO THE STUDY OF THE EQUILIBRIUM BETWEEN METARHODOPSIN I AND II FROM BOVINE RETINAS

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1. Introduction

In the sequence of reactions from rhodopsin to the end products of photo-bleaching, opsin and all-*trans* retinal, the interconversion of metarhodopsin I to metarhodopsin II (MR I ⇌ MR II) is the first step which is on the right time scale to be directly involved in triggering a physiological response. It is also widely accepted that the very marked temperature and pressure dependence of this reaction indicates that it involves considerable structural rearrangement [1,2]. In different hypotheses for the transduction of information from rhodopsin to the plasma membrane of the rod cell, after absorption of a photon, the protein opsin is supposed to be involved either in the opening of an ion channel for release of calcium [3] or in the activation of a chain of enzyme reactions [4]. In either case it is of major interest to characterize the transition to find out whether the equilibration MR I ⇌ MR II involves a single step and whether the structure of the protein or of the lipid environment is principally involved.

Clearly the answers to the above questions will come from a long term investigation of rhodopsin from different sources with a range of techniques. In the investigations reported here we have applied the pressure relaxation technique [5] to enable us to study the kinetics of the equilibration MR I ⇌ MR II and to compare the results obtained with those derived from flash spectroscopy on the same material.

The results presented here illustrate a number of points. It is of considerable interest that the pressure relaxation technique can be applied to the study of rate processes in a highly organised system – the rod outer segments (ROS). We have shown that the transformation MR I ⇌ MR II in ROS can be described by a single exponential and that this kinetic homogeneity is unaffected by temperature and pressure.

2. Method and materials

Retinas were obtained from bovine eyes which had been stored in the dark at 0°C for approximately 12 h between slaughter and dissection. Rod outer segments were prepared by tumbling the retinas in 45% sucrose, 0.16 M phosphate pH 7 at 4°C for 2 h. They were then isolated by the technique of Smith, Fager and Litman [6], as were the disks. The preparations were used within 3 days, being stored at 4°C.

The kinetic investigations of the reactions which occur after photoflashes were carried out in a dual beam flash spectrophotometer designed by Dr O. T. G. Jones of this laboratory. A 2 kV snaked xenon flash tube with a Kodak Wratten 16 gelatine filter was used for initiation of the photochemical reactions and the course of resulting changes in optical extinction were observed with light from a quartz iodine lamp and an Applied Photophysics monochromator. The photomultiplier (EMI 9824A) was protected from the flash by a Corning 9863 glass filter. The temperature in the thermostated cell was continuously monitored with a thermocouple probe of a digital thermometer. The solutions were stirred between each flash which bleached about 2% of the rhodopsin in the sample. An example is shown in fig.2a.

Pressure relaxation kinetics of the reaction MR I ⇌ MR II were studied by the method described by Davis and Gutfreund [5]. All kinetic data were stored first in a Datalab (DL 905) transient recorder and transferred to cassette tapes via an Intel 8080 controlled cassette recorder. The data were processed from the

tapes in a PDP 8/e digital computer. A non-linear least square routine was used to fit exponentials and the theoretical exponential, calculated from the derived rate constant, was drawn on top of the raw data in the figures presented in this paper.

Determination of the concentration of MR I and MR II were carried out by the method described by Lamola, Yamane and Zipp [2] using a Shimadzu MPS-50L spectrophotometer. The samples used to study the relaxations of the equilibrium between the two intermediates were bleached in the pressure cell at 1°C until a photostationary state was reached. At this temperature this 'pseudo equilibrium' is quite stable since the reactions of MR II to form MR III or hydrolyse to opsin and retinaldehyde, are very slow.

3. Results and discussion

Figure 1 shows, in the form of an Arrhenius plot, the temperature dependence of the interconversion, after a photoflash, of MR I to MR II observed by transmission at 380 nm in intact ROS disk preparations. It is of interest to note that the energy of activation is remarkably similar for this reaction in the two forms, although the rate constants are significantly different. Successive flashes, each resulting in about 2% bleaching of rhodopsin, were carried out on each sample at each temperature. No significant changes in rate constants were observed as the extent of bleaching increased. As will be seen below, the rate of interconversion MR I $\rightleftharpoons$ MR II is the same when all the bleached rhodopsin is distributed between these two forms.

The activation parameters for the two types of preparations studied are given in table 1. It must be pointed out that the apparent overall linearity of the Arrhenius plot does not exclude changes in slope at the low temperature end of the graph. The wide range of data reported in the literature [7] makes it essential that accurate data should be produced under identical conditions when different preparations and methods

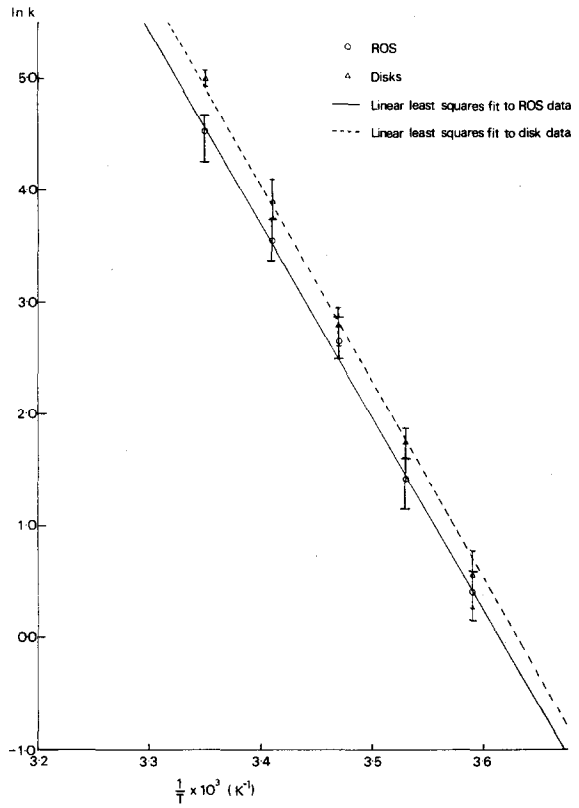

Fig.1. Arrhenius plots of the rate of formation of metarhodopsin II, observed at 380 nm, in ROS and disks in a medium of 0.16 M phosphate pH 7.0.

are compared. The remarkably large energy of activation of this process must indicate a very major structural rearrangement and an important step in the response to bleaching.

At temperatures between 0°C and about 6°C, fully bleached rhodopsin in disks or ROS will remain in a state of equilibrium between MR I and MR II for considerable periods of time. This equilibrium is dependent on pH, temperature and pressure [1,2] in the following way:

Table 1
Calculations of activation parameters 310.15 K (37°C)

Preparation	$\Delta E^{\dagger}$/kJ . mol^{-1}	$\Delta H^{\dagger}$/kJ . mol^{-1}	$\Delta S^{\dagger}$/J . mol^{-1} . K^{-1}	$\Delta G^{\dagger}$/kJ . mol^{-1}
Disk membranes	149.0	146.4	293.0	54.8
Rod outer segments	143.5	141.0	274.0	56.0

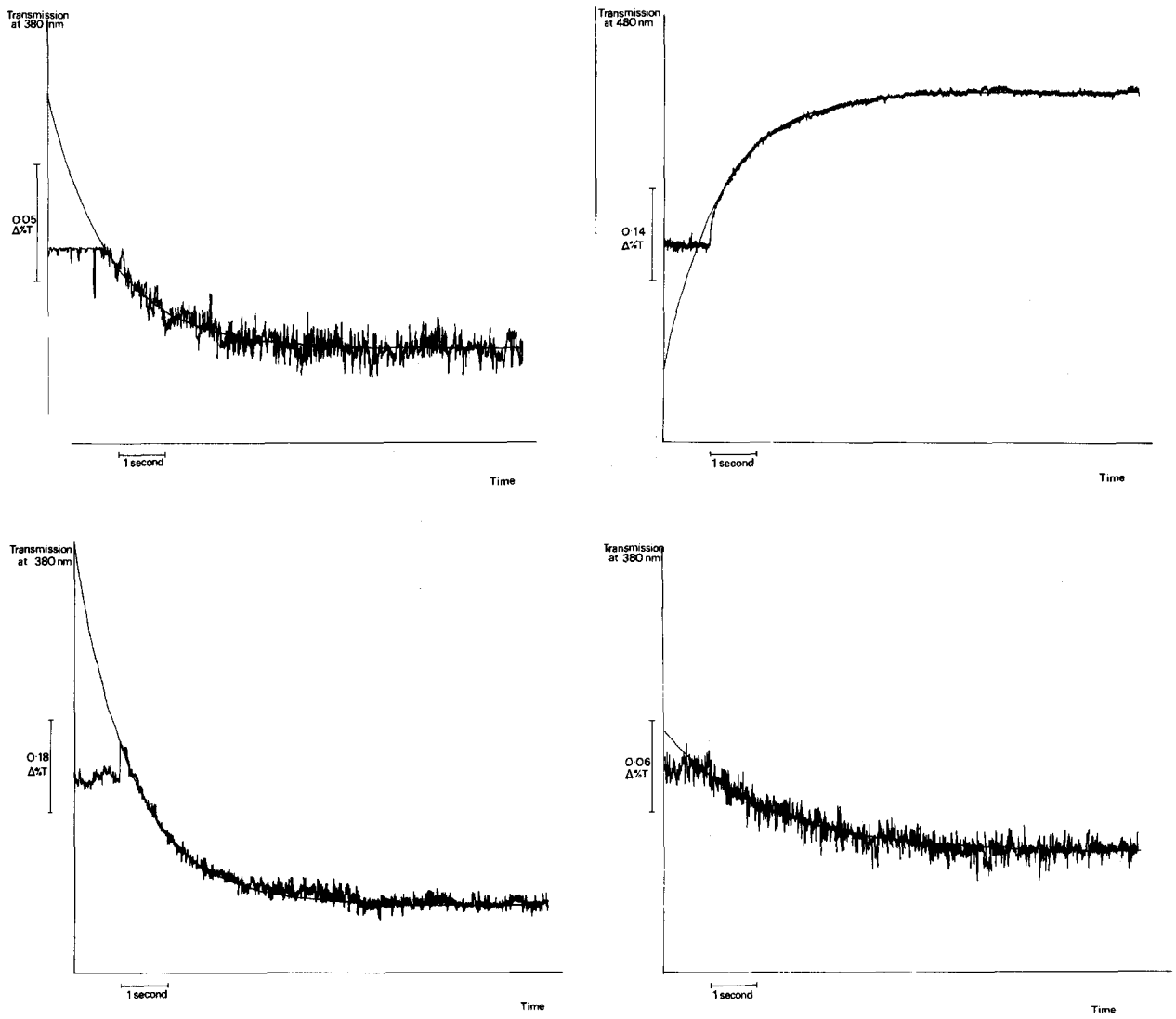

Fig.2. Examples of kinetic records of the interconversion metarhodopsin I ⇌ metarhodopsin II at 0°C as described in the results section: (a) disks in 0.16 M phosphate pH 7.0, after photoflash observed at 380 nm, (b) disks in 0.1 M imidazole pH 7.0 after pressure relaxation observed at 480 nm, (c) disks in 0.1 M imidazole pH 7.0 after pressure relation observed at 380 nm, (d) ROS in imidazole pH 7.0 after pressure relaxation observed at 380 nm. The solid lines are the least squares fit to the data.

$$\begin{array}{l} \text{MR I} \rightleftharpoons \text{MR II} \\ \qquad + \Delta T \rightarrow \\ \leftarrow + \Delta P \\ \qquad + \Delta H^{+} \rightarrow \end{array}$$

We have applied the pressure relaxation technique developed in this laboratory [5] to study the kinetics of the above equilibration. Imidazole buffer (pH 7) was used for these experiments since its pK is virtually insensitive to pressure. Figures 2b and c show the records of two observations of the relaxation of the equilibrium after pressure release to atmospheric on the same sample of disks. One observation being recorded as transmission at 380 nm to observe the formation of MR II, while the other was recorded at

480 nm to observe the disappearance of MR I. While the approach to equilibrium after a photoflash at 0°C (fig.2a) is clearly represented by a single exponential, the two relaxation records indicate a small rapid perturbation prior to the principal exponential decay. This rapid phase is likely to be due to light scattering effects. The fact that the rate profiles of the disappearance of MR I and the appearance of MR II are identical, if normalised in amplitude, is the best evidence that there is a single rate limiting process in the transition. Figure 2d shows the record of a similar experiment carried out on a sample of ROS.

The pressure relaxation technique permits repeated reproducible experiments on the same sample under identical conditions and provides evidence of reversibility. The dependence of the equilibrium on pressure from 500 to 2500 atmospheres was used to calculate a volume change of more than 60 ml . mol^{-1} for the formation of MR II from MR I [2]. We investigated the effects of pressure in the range of 30 to 150 atmospheres and calculated a volume change of 108 ml . mol^{-1} for the reaction in disks at 0°C from the data plotted in fig.3.

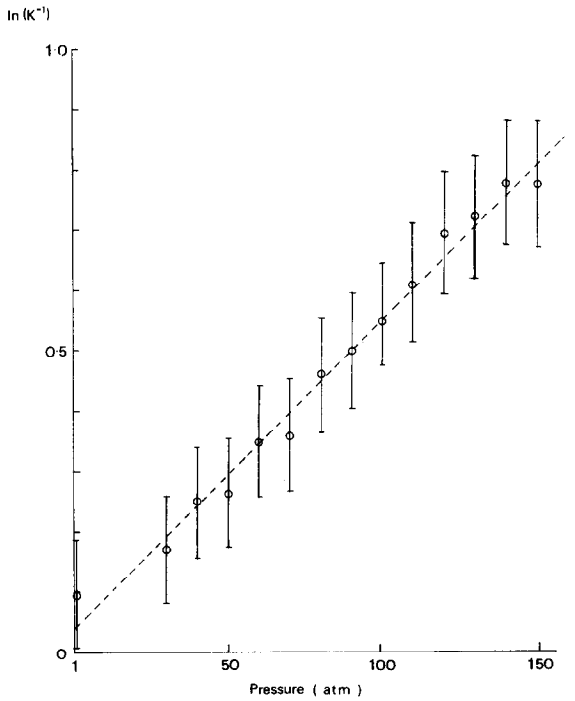

Fig.3. The effect of pressure on the equilibrium metarhodopsin I ⇌ metarhodopsin II in disks at 1°C in imidazole pH 7.0, observed as described in the method section.

The results presented in this paper should help to focus on some important characteristics of a step in the reaction of rhodopsin which is likely to play a key role in its physiological response to light absorption. It seems likely that further information about the function of the protein and its lipid environment will come from the application of varied and detailed kinetic analysis of this single step by the techniques outlined above. The application of equilibrium perturbation should be extended to the alteration of other physical parameters as well as to systems with controlled lipid replacement. Clearly the study of this reaction with rhodopsin preparations from other species living in different environments will be informative. Some explanation may come from the fact that frogs, for instance, have a rhodopsin system which is relatively temperature insensitive.

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