Selectivity coefficients were calculated from the data. The average value of S_1 ^3H (for animals fed separate food sources) was 0.2 ($\pm$0.1) while the average S_2 ^3H value (for animals fed mixed food) was 0.5 ($\pm$0.1).

Lampert[7], using an exposure time of 15 min and mixed food only, showed that young daphnids are remarkably selective in their feeding habits. Table 2 shows that feeding rate is highest during the shortest exposure times and lower during longer exposure times. In all cases, however, no selectivity was found.

There are two possible reasons for this difference: (1) *Daphnia* may digest bacteria faster than algae, or (2) the accumulation, in long-term experiments, of bacterial material as opposed to algal material may be due to the shorter retention time of ^{14}C-labelled substances (algal source), which might be used for intensive metabolic activities such as respiration. The results presented here and derived from long-term experiments with *Ceriodaphnia*[6] and *Daphnia*[7] suggest the following processes. Initially ^{14}C-labelled algal materials are ingested in larger amounts during normal feeding. Later, the rapid digestion of bacterial materials results in the accumulation of ^3H-labelled substances. I conclude that the rate of ingestion is influenced by the size of the particles present in natural or artificial bodies of water. This is important in ecosystems containing suspended particles of a diversity of sizes.

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Chromophore mobility in bacteriorhodopsin

THE sole protein found in the purple membrane of *Halobacterium halobium* contains retinal covalently bound by means of a protonated Schiff base linkage to lysine[1,2]. The only established function of this protein is to act as a light-driven proton pump producing a transmembrane proton gradient, which is coupled to ATP synthesis in the living organism[2,3]. In so far as the retinal prosthetic group resembles that found in the visual pigment of vertebrates, rhodopsin, the name bacteriorhodopsin has been widely adopted for this protein[3]. Considerable work has been done on the biochemistry and photochemistry of the purple membrane, both for its intrinsic interest as a unique photosynthetic membrane and with respect to the identification of possible mechanistic relationships between its photochemical cycle and that of the visual process. We report here a study of the later stages of the photochemical cycle by flash photometry with plane polarised light. Our results indicate conformational change in the bacteriorhodopsin molecule at the site of the visible chromophore.

There has been speculation as to whether rotational motion of the visual pigment is an essential link in the signal chain in the visual process[4]. It has been demonstrated that rhodopsin molecules undergo rapid Brownian rotation within the membrane[4,5] and the theory has been proposed (but criticised on energetic grounds[6]) that rotation about an axis parallel to the plane of the rod disks ('tumbling') could enable rhodopsin to act as a transmembrane ion carrier[4]. In contrast, bacteriorhodopsin exists in a crystalline form in the purple membrane[7]. Consequently, Brownian diffusion of individual molecules would be expected to be severely restricted, and this has been confirmed[8,9]. But during a study[10] of the temperature dependence of the photochemical cycle in aqueous suspensions we were surprised that the measured rotational relaxation times gave an activation energy for the medium viscosity (see below) which differed significantly from that characteristic of water. If the protein chromophore is rigidly bound within a crystal lattice which has dimensions comparable with those of the membrane, then rotational relaxation would be exclusively a consequence of the tumbling of the individual fragments, constrained only by the viscosity of the water in which they were suspended. This consideration induced us to carry out a more extensive study of rotational mobility.

The bacteriorhodopsin photochemical cycle contains a sequence of phototransients of which one, $M(410)$, has been shown to have the retinal Schiff base unprotonated[11]. The decay of $M(410)$ is linked kinetically to the reappearance of the ground state $BR(570)$, with a lifetime at room temperature in the millisecond region[12]. Rotational mobility was investigated by measurement of the time dependence of the decay of linear dichroism of the absorption band of $M(410)$ at 410 nm. By means of a conventional flash photometer[9], purple membrane samples were excited with μs light flashes filtered successively through a 500-nm cutoff filter and a linear polariser with its optical axis vertical (laboratory *a* axis, Fig. 1). Light transmission was then viewed in the *c* direction with a beam

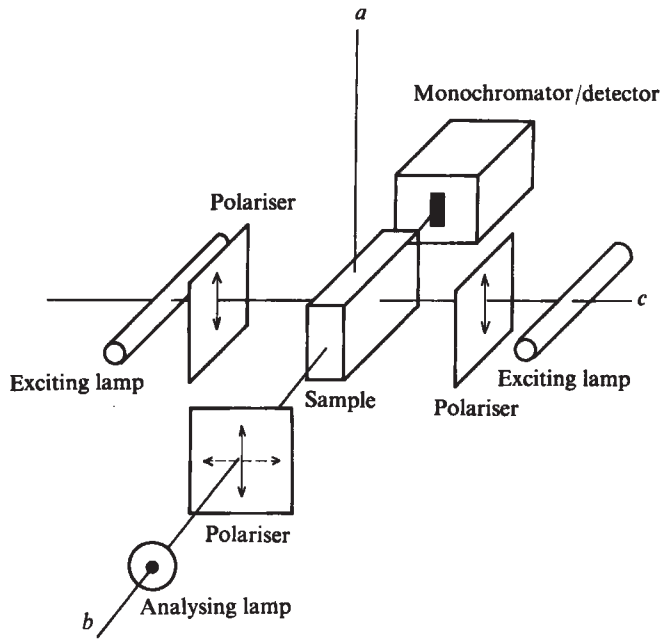

Fig. 1 Optical layout of flash photometer for measurement of relaxation of linear dichroism in phototransient absorption.

polarised alternately parallel and perpendicular to the exciting light. The time dependence for absorption of the two polarised analysing beams is given by[13]

$$A(t)_{\parallel} = 5/3 + \alpha\beta\exp(\theta t) \quad (1)$$

$$A(t)_{\perp} = 5/3 - \tfrac{1}{2}\alpha\beta\exp(\theta t) \quad (2)$$

where θ is the rotational rate constant, proportional to temperature and inversely proportional to medium viscosity and shape and size of the rotating species ($kT/V(r)\eta$), β is a function of the optical transition moments of the chromophore absorbing the exciting light and the species being viewed ($M(410)$), and α is a randomisation factor ($\alpha < 1$) which encompasses instrumental artifacts in addition to unidentified (non-Brownian) causes of depolarisation[13]. Total time-dependent absorption is then given by equations (1) and (2) multiplied by a weighting

factor which reflects the decay of the population of the absorbing species being viewed. By defining a function $R(t) = (A(t)_{=} - A(t)_{\perp})/(A(t)_{\parallel} + 2A(t)_{\perp})$ which is analogous to anisotropy commonly used for fluorescence polarisation[14], a purely exponential expression for dichroic decay is obtained.

$$R(t) = 3/10\alpha\beta \exp(\theta t) \quad (3)$$

This is independent of the decay kinetics of the phototransient.

As reported previously[9], little decay in dichroism can be detected at room temperature in aqueous suspensions of purple membrane during the lifetime of $M(410)$. Transient lifetimes, however, increase markedly with decreasing temperature ($Q_{10} \approx 3$) and adequate first-order plots were obtained (s.d. $< 10\%$) consistent with equation (3). The results are presented in Fig. 2(A) in the form of an Arrhenius plot of the viscosity dependent ratio T/θ. The best straight line yields an energy of activation for microviscosity, E, of 7.4 ± 0.3 (s.d.) kcalorie mol^{-1}. This is significantly higher than that for water[15], and shows the presence of rotational freedom in addition to that of fragment tumbling. Purple membrane, when treated with the protein crosslinking reagent glutaraldehyde, continues to undergo reversible photochemical cycling. The lifetimes are slightly increased, facilitating a more extended Arrhenius plot. This (see Fig. 2(B)) yields $E = 4.4 \pm 0.04$ (s.d.) kcalorie mol^{-1}, consistent with published values for water[15]. The size calculated[16] for the membrane fragments (diameter $= 0.5$ μm) assuming them to simulate oblate ellipsoids is in excellent agreement with published values (diameter $= 0.5$ μm)[1], affording a test for the validity of the theory.

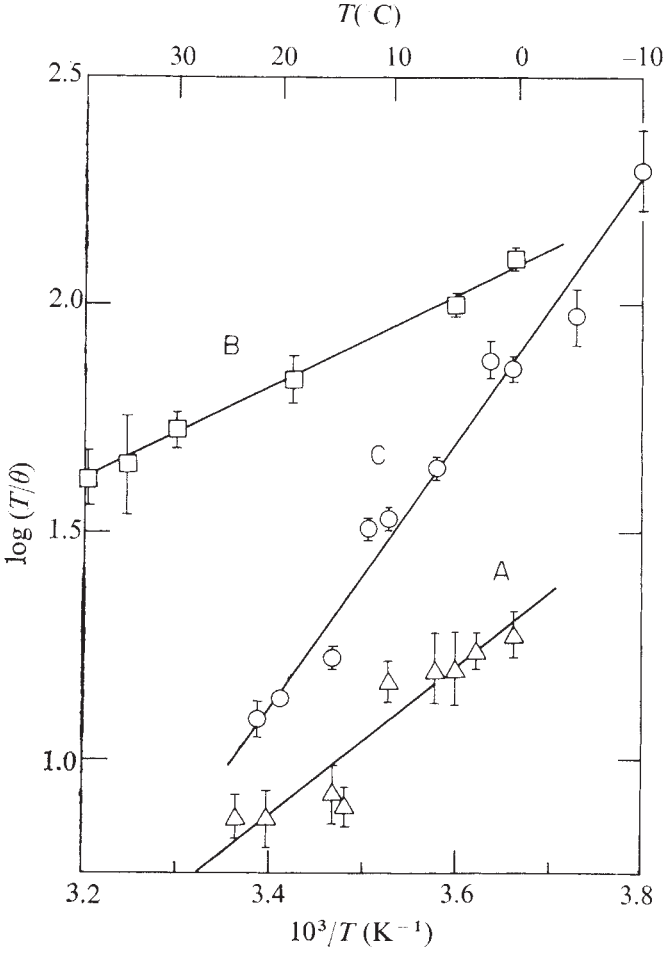

Fig. 2 Arrhenius plots of the viscosity-proportional function T/θ. Bars represent standard deviation in the measurement of θ. △, Membrane fragments in water; □, membrane fragments pretreated with glutaraldehyde and suspended in water; ○, membrane fragments in a 20% polyacrylamide gel.

Both untreated and glutaraldehyde-fixed purple membranes were incorporated into a 20% polyacrylamide gel by *in situ* polymerisation of acrylamide monomer added to aqueous purple membrane suspensions. At this concentration the membrane fragments may be expected to be completely immobilised. The photochemistry seems unchanged, with phototransient yields and lifetimes in the polymer indistinguishable from those in pure water. For the glutaraldehyde-fixed sample no dichroic relaxation was detectable within the whole temperature range (detection limits at 20 and 0 °C, respectively, $\theta = 10^{-1}$ and 10^{-2} s^{-1}). But for the unfixed polymer first-order dichroic decay was observed, yielding $E = 13 \pm 0.05$ (s.d.) kcalorie mol^{-1} (Fig. 2(C)).

The results presented here show that the chromophore of bacteriorhodopsin is not totally immobilised within the purple membrane. The significant rotational rate constants found, 20 s^{-1} at room temperature, are too high to be attributable to rotation of individual protein molecules *in toto* within the crystal lattice, and must indicate internal conformational change. It is tempting to speculate, based on the close coincidence of E for rotational mobility and that found for the formation of $M(410)$ (12 kcalorie mol^{-1})[12], that the deprotonation step which produces this intermediate is controlled by intramolecular movement involving the retinylidene group (which simulates Brownian motion). The establishment of internal mobility within bacteriorhodopsin suggests that it might be profitable to look for an analogous process, in contrast to the criticised tumbling mechanism, in the transmission of the visual signal in rhodopsin subsequent to the primary *cis–trans* isomerisation.

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Three-chain merokeratin from wool may be a fragment of the microfibril component macromolecule

WOOL fibres consist mainly of long cortical cells filled with microfibrils, 7–8 nm diameter, embedded in a matrix. The microfibrils are composed of proteins with a lower sulphur content and a higher α-helix content than wool; the matrix proteins have a higher sulphur content than wool and no α helix[1,2]. When reduced and carboxymethylated low-sulphur proteins from wool (SCMKA) are separated by starch gel electrophoresis, there are two