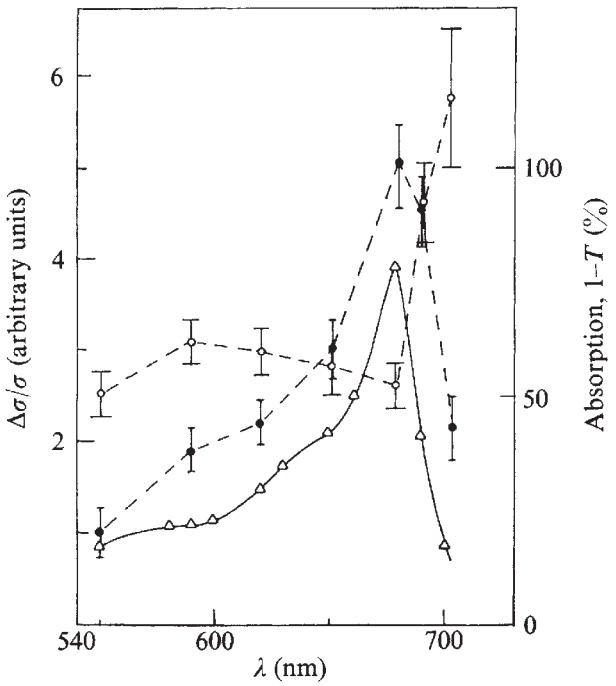

Fig. 2. Action spectrum and relative quantum yield for the microwave photoconductivity in a spinach chloroplast film. △, Absorption spectrum of the sample; ●, action spectrum, ○, relative quantum yield. The points are normalised to constant incident light intensity at 550 nm.

increase was also observed in the photosynthetic bacteria.

Superposition of pulsed light on steady background illumination, is a standard procedure in semiconductor physics to study trapping processes[10]. Such procedures were used in our experiments; they provided evidence for the trapping of carriers of both signs, and facilitated a rough determination of the trap concentration of $\sim 10^{13}$ cm^{-3}. The number of reaction centres, estimated from the chlorophyll content of the sample, was also $\sim 10^{13}$ cm^{-3}. Arrhennius plots of the temperature dependency of the photoconductivity signals and their decay times, gave activation enthalpies of 0.3 eV.

Light-induced changes in the real part of the dielectric constant, $\Delta\varepsilon'$, of $\sim 10^{-5}$, were observed. Changes in the dielectric constant can be related to changes in volume polarisability, α, using the Clausius–Mossotti relationship[10]. From this value of $\Delta\varepsilon$, we calculated a value of $\Delta\alpha$ of 10^{-20} cm^3. Assuming that α approximates the volume over which the charges are separated, $\alpha^{1/3} = 3\times10^{-7}$ cm, represents a separation length. This value is comparable with the presumed size of the pigment–protein complex of the reaction centre[19,20].

In the bacterial chromatophores the light-induced ESR signals exhibit decay half-life regimes of 30–50 ms, and of several seconds; the positive carriers exhibit very similar kinetic components. The reaction centreless mutant, *PM-8*, exhibits neither Faraday rotation, nor photo-induced ESR signals. Titration of the chromatophores with ferricyanide to maximum dark ESR signal intensity, concurrently eliminates the slow components of the photoconductivity signals. Taken together, these observations point to a common origin for the ESR and positive carrier photoconductivity signals.

The positive carrier component is thus interpreted as originating in the same species that produced the ESR signal I, the dimeric chlorophyll cation radical[20,21], and we suggest that this species behaves as a mobile hole (over a volume of 30° Å^3), when observed at microwave frequencies. The negative carrier component of the photoconductivity (few ms range) is tentatively interpreted as arising from electrons thermally released from the primary acceptors (the reverse process), and its decay as resulting from charge transfer to secondry acceptors; because of their larger energy gap, they would not contribute significantly to the population of the conducting state. Rigorous interpretation of the observed activation enthalpies is seriously hindered by the fact that they may represent a combination of activations for both carrier types, and it is not possible to separate their contributions from the available data.

The general behaviour, therefore, can be understood in terms of photoliberated charges migrating for short distances through a dielectric medium. The microwave Hall mobilities are incompatible with charge transport through conduction bands; rather, they are similar to those expected for tunnelling or hopping processes[10].

These observations and interpretations, which will be elaborated fully elsewhere, strongly support the model in which the primary charge separating events occur in the reaction centres in photosynthesis. The fact that such signals are not observed in the reaction-centreless mutant of *R. spheroides*, together with stringent controls, and other evidence not reported here, rules out an artefactual source of the signals. The action spectra for the microwave photoconductivity, resembling, as they do, those for photosynthesis, point to a close connection between the two processes. The number of traps, as well as the maximum number of free carriers, approximates the number of reaction centres, thus connecting the observations with the structure of the photosynthetic unit.

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Kinetic evidence for a conformational transition in rhodopsin

AFTER light absorption, rhodopsin decays through a series of spontaneous reactions[1,10], the kinetics of which have been the object of considerable study. In a variety of conditions the decay kinetics of bathorhodopsin (prelumirhodopsin), lumirhodopsin, and metarhodopsin I (meta I) have been found to be non-first order, but are consistent with a set of parallel, independent first-order reactions[2–10]. These kinetics have been assumed to result from the existence of multiple forms of each of these intermediates, and it has been proposed that rhodopsin itself exists in different forms or different environments in some conditions[7]. The kinetics of meta I → meta II in both dodecyltrimethylammonium bromide- and digitonin-solubilised phospholipid-free rhodopsin are also consistent with two forms of meta I which decay independently (J. G. S. and E. O. Plante, unpublished). We show here that the effect of temperature changes on these kinetics is consistent with the hypothesis that

rhodopsin exists in two forms in equilibrium resulting from the accessibility of two stable conformational states separated by a small difference in free energy.

We have studied the kinetics of rhodopsin decay reactions using flash photolysis of detergent-solubilised rhodopsin containing less than 0.2 mol phospholipid per mol rhodopsin. The kinetics of meta I → meta II were determined from absorbance measurements at 380 nm and of lumirhodopsin → meta I from absorbance measurements at about 415 nm, which is near the isosbestic point of meta I and meta II. In each case the absorbance against time function could be fitted by a sum of two exponential decay functions of the form $\Sigma A_i \exp(-t/\tau_i)$. We used a phospholid-free preparation so that we could exclude phospholipid heterogeneity of the digitonin micelle as a source of multiexponential kinetics.

We have assumed that these kinetics result from the independent decay of two forms of each of these species, and thus that the amplitudes of the two exponential terms are proportional to the initial concentrations of these two forms. Figure 1 shows that for meta I → meta II a change in temperature from 20 to 37 °C results in a change in the ratio of these two amplitudes, but not in their sum (Fig. 1*a* and *b*). Furthermore, this change is reversible (Fig. 1*c*). Such kinetics are caused by two components which are interconvertible, but only on a time scale which is long compared with the time constants for decay of meta I. Thus, these two components exist in equilibrium, but this equilibrium is established before the appearance of meta I.

We also found that the kinetics of decay of the earlier intermediate, lumirhodopsin, at 20 and 30 °C were consistent with double-exponential kinetics with approximately the same relative amplitudes as those determined from the meta I decay kinetics at these two temperatures. Presumably, the decay kinetics of bathorhodopsin, the earliest of the intermediates, would also be double exponential with the same ratio of amplitudes in these conditions, but we have as yet made no attempt to test this experimentally. Bathorhodopsin does, however, decay with double-exponential kinetics in rod outer segment (ROS) suspensions[6]. Thus, all of these intermediates display the same type of double-exponential decay kinetics. This suggests that rhodopsin itself exists in two forms before photolysis.

According to this interpretation, the ratio of the two amplitudes determined from the meta I → meta II kinetics is equal to the equilibrium constant for the transition between these two forms of rhodopsin. The values of the thermodynamic

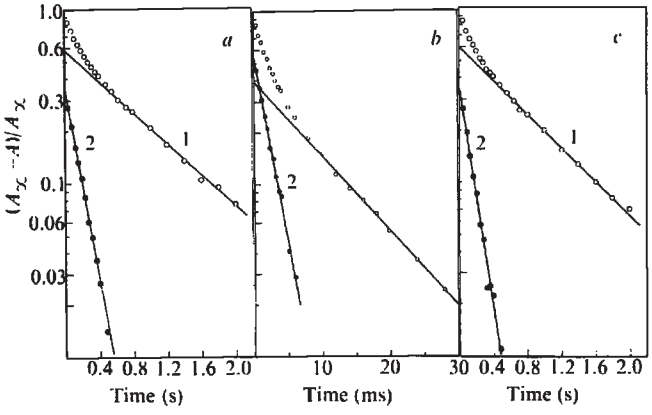

Fig. 1 Kinetics of change in A_{380} with phospholipid-free rhodopsin solubilised in 2% (w/v) digitonin in distilled water, pH ≃ 6. A_{380}, as a function of time, is relative to the preflash absorbance of the solution. Time constants and amplitudes were extracted from the data by linear least squares fitting and the 'peeling off' procedure. ○, $(A_\infty - A)/A_\infty$; ●, difference between these points and line 1. *a*, Sample photolysed at 20 °C; $A_1 + A_2 = 0.95$, $A_2/A_1 = 0.62$. *b*, Sample photolysed at 37 °C; $A_1 + A_2 = 0.96$, $A_2/A_1 = 1.4$. *c*, Sample held at 37 °C for 3 min, and photolysed at 20 °C; $A_1 + A_2 = 0.95$, $A_2/A_1 = 0.59$.

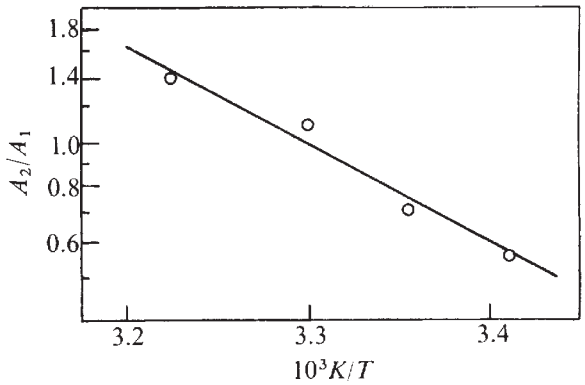

Fig. 2 Van't Hoff plot for the proposed reaction $R_1 = R_2$ over the range 20–37 °C. At each temperature, A_1 and A_2 were determined as described in Fig. 1. A straight line was fitted to the data points by linear least squares, giving $\Delta H^0 = 9.8$ kcalorie and $\Delta S^0 = 32$ e.u.

parameters for this hypothetical transition were calculated from the temperature dependence of the equilibrium constant in the range 20–37 °C (Fig. 2). For phospholipid-free rhodopsin solubilised in 2% (w/v) digitonin in distilled water, pH ≃ 6, $\Delta H^0 = 9.8$ kcalorie, $\Delta S^0 = 32$ e.u., and at 37 °C, $\Delta G^0 = -0.2$ kcalorie. These values are of approximately the same magnitude as those reported for transitions between some conformational substates of α-chymotrypsin[11,12]. Two components in these kinetics could also be due to the availability of two different environments in the digitonin micelle, but we have recently obtained similar results with ROS solubilised with cetyltrimethylammonium bromide, Emulphogene BC720, Amonyx-LO and with detergent-free suspensions of sonicated ROS. Thus, it seems likely that the heterogeneity inferred from these kinetics resides in the state of the protein itself.

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Erratum

In the article "Seismic evidence for local undulation of olivine–spinel phase boundary", by S. K. Dey-Sarkar and R. A. Wiggins (*Nature*, **257**, 572; 1975) the last two sentences of the first paragraph should read: While analysing seismograms between 14 and 18°, a group of anomalous data was observed localised in a specific region which could be explained only by a local undulation of the 410-km discontinuity. Here we present these observations and give a speculative explanation for the undulation.