R.A. Mathies, R.W. Schoenlein, L.A. Peteanu, Q. Wang and C.V. Shank

Femtobiology: Mechanism and Dynamics of the First Step in Vision

Abstract

The kinetics of the primary event in vision have been resolved by using femtosecond optical measurement techniques. The 11-*cis* retinal chromophore in rhodopsin is excited with a 35-fs pump pulse at 500 nm and the transient absorption changes are monitored with 10-fs probe pulses. Within 200 fs, increased absorption is observed between 540 and 620 nm demonstrating that the first step in vision, the 11-*cis* → all-*trans* isomerization of the rhodopsin chromophore, is complete in only 200 fs. The short time scale for this process suggests that the surface crossing from the 11-*cis* excited state to the all-*trans* photoproduct ground state involves nonstationary or coherent vibrational states and that the torsional *velocity* of the excited-state wavepacket in the 90° twisted transition state region is a critical parameter for determining the quantum yield of this important reaction. This new paradigm for visual photochemistry may be relevant for a variety of photochemical and photobiological processes.

Introduction

Visual excitation and photosynthesis are the most important photochemical reactions in biology. This paper will focus on the primary photoisomerization that initiates vision. These studies of femtobiology are important not only because they have elucidated the dynamics of the first step in vision, but also because they have uncovered a new paradigm for the mechanism of photochemical reactions. We begin by describing the protein rhodopsin that initiates visual excitation. This is followed by the presentation of our femtosecond optical studies on the photoexcitation of rhodopsin (Schoenlein et al., 1991b; Peteanu et al., 1993). The importance of nonstationary vibrational states or vibrational coherence in the photochemical isomerization reaction will be emphasized.

The Visual Protein Rhodopsin

Rhodopsin ($\lambda_{max} \cong 500$ nm) is an intrinsic membrane protein found in retinal rod cells. Light absorption by its bound 11-*cis* retinal prosthetic group initiates

a photochemical *cis* → *trans* isomerization that results in the excitation of the retinal rod cell (Wald, 1968). The initial red-absorbing (~ 550 nm) photochemical product is thought to contain a distorted all-*trans* chromophore (Eyring et al., 1980; Yoshizawa & Wald, 1963). Previous transient absorption experiments have shown that the red-absorbing photoproduct is formed in less than 6 ps (Busch et al., 1972). Time-resolved resonance Raman as well as Raman intensity analyses have argued that there is a very fast femtosecond photochemical isomerization (Hayward et al., 1981; Loppnow & Mathies, 1988). However, until the work presented here, the time scale for the first step in vision has remained unresolved.

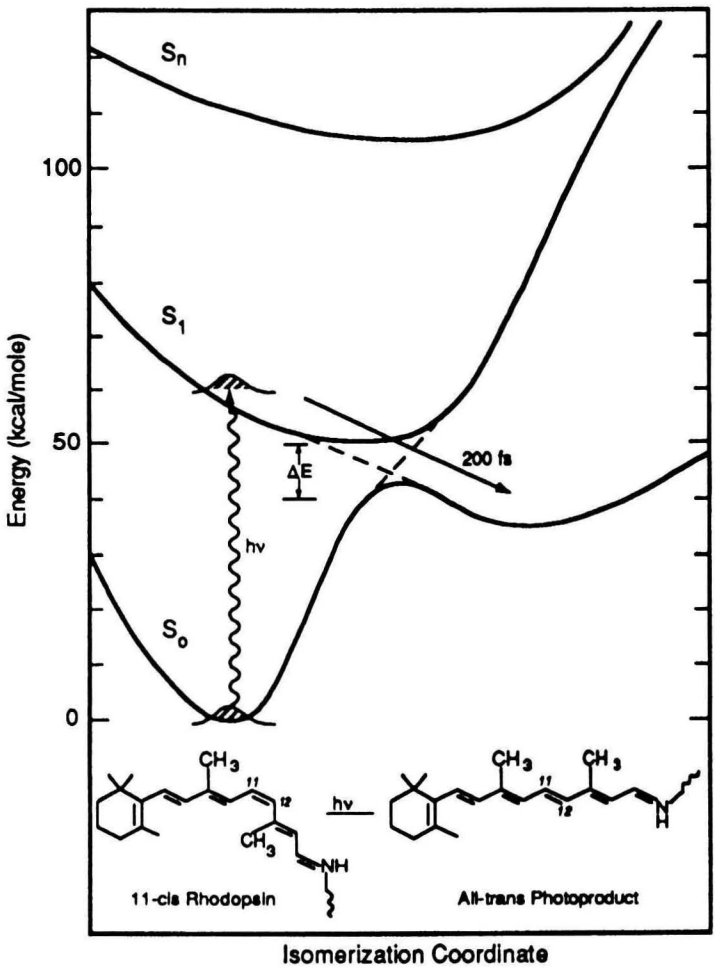

Fig. 1. Schematic ground and excited state potential surfaces for the 11-*cis* → all-*trans* isomerization in rhodopsin. The reaction path of the photoisomerization is indicated by the nonadiabatic potential surfaces (broken lines).

The time course of many photochemical and photobiological reactions can be studied by using compressed femtosecond (fs) optical pulses (Shank, 1986). Such pulses in the red were previously used to observe the isomerization of the retinal chromophore in bacteriorhodopsin, a related pigment that functions as a light-driven proton pump (Mathies et al., 1988). Recent advances in the generation of femtosecond pulses in the blue-green region of the spectrum now make it possible to study the primary photo-processes in vision as well (Schoenlein et al., 1991a). The work presented here reveals that the primary *cis-trans* isomerization in vision occurs in only 200 fs and is one of the fastest photochemical reactions ever studied.

Femtosecond Spectroscopy of Rhodopsin

The femtosecond laser system consists of a colliding-pulse, mode-locked dye laser with a 400 Hz excimer-pumped dye amplifier system (Schoenlein et al., 1991a). This laser system produces 35 fs pump pulses at 500 nm and ~ 10 fs probe pulses (centered either at 620 nm or at 500 nm). The bandwidth of the compressed probe pulses permits the resolution of spectral dynamics of the rhodopsin molecule following the narrow band (~ 15 nm) pump. The probe pulse is then dispersed and detected *after* passing through the sample, providing simultaneously both high spectral and time resolution. Rhodopsin from 400 cattle retinas was purified and solubilized in detergent solution at a concentration of 15 OD/cm at 500 nm. The 3 ml sample was flowed through a 300 μm pathlength wireguided drip jet. In all measurements the maximum signal ($\Delta T/T$) is $<2\%$ and linearity in both the pump and probe powers has been verified.

Fig. 1 presents the structures of the chromophore in 11-*cis* rhodopsin and in its all-*trans* photoproduct along with schematic potential surfaces for the torsional isomerization. In the traditional picture of photochemical processes, vertical excitation would be followed by vibrational dephasing and relaxation, surface crossing to the ground state (and back to the reactant since the quantum yield is not unity) and photoproduct cooling. The rate of photoproduct production can be followed by probing at ~ 570 nm. Fig. 2 presents transient absorption spectra of the primary photochemistry and Fig. 3 presents the corresponding single wavelength kinetic traces. At early times (~ 33 fs) there is a dramatic increase of absorption at 500 nm. This is due to absorption to a higher-lying excited state from the pump-induced nuclear wavepacket produced near the Franck-Condon region on S_1. By 200 fs, this signal has completely disappeared, revealing the full bleach of the rhodopsin ground state absorption at 500 nm. The prompt dissipation of the excited-state absorption demonstrates that rhodopsin exhibits very rapid excited-state torsional nuclear dynamics. The photoproduct absorption in the 550-570 nm region increases rapidly and is fully formed in only 200 fs. After this time the photoproduct absorption band relaxes by shifting slightly to the blue but does not further increase in area. These data show that the all-*trans* photoproduct is formed in only 200 fs and that little to

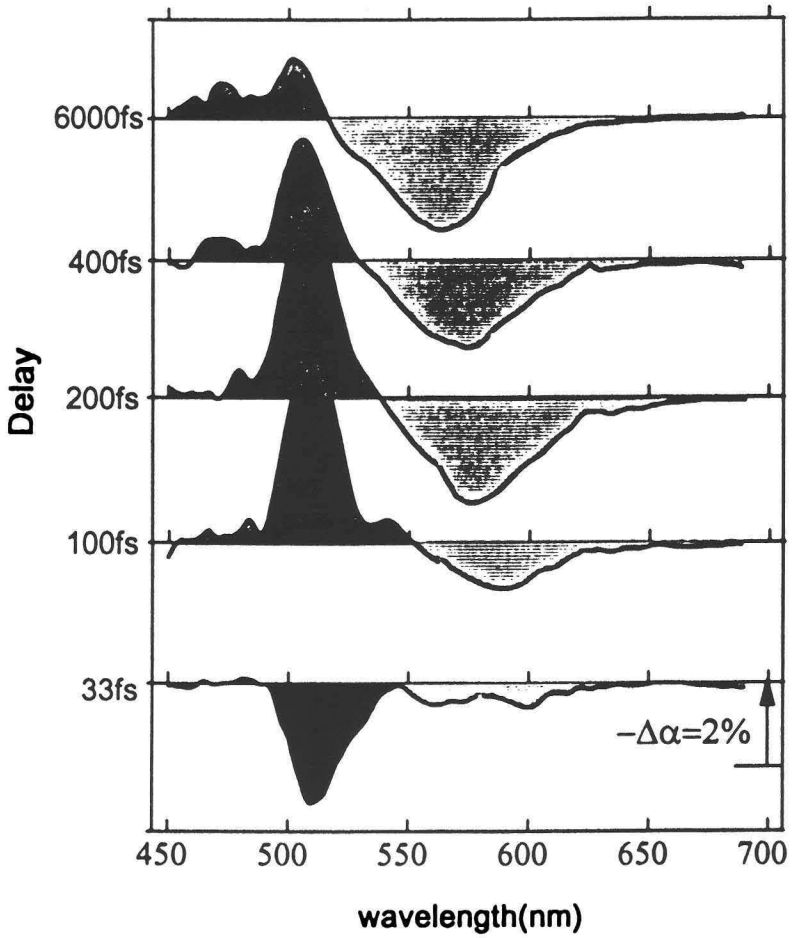

Fig. 2. Difference spectra of 11-*cis* rhodopsin (~ 10 fs probe) at various delays following a 35-fs pump pulse at 500 nm.

no photoproduct is formed after this time. The kinetic traces at 570 nm in Fig. 3 confirm this conclusion.

The time course of the absorption traces at 500 nm, within the rhodopsin bleach, is more complicated. The spectral traces in Fig. 2 suggest that the filling-in of the reactant bleach is slower than the photoproduct production. The kinetic traces in Fig. 3 at 500 nm quantify this observation. There is an initial absorption increase due to excited state absorption. Rapid wavepacket dynamics reveal the full bleach by ~ 150 fs. This bleach is filled in with a biphasic process having a fast (~ 200 fs) and a slow (~ 5 ps) time constant. The slow recovery that occurs after photoproduct formation is most likely due to cooling of hot reactant and photoproduct molecules and/or conformational relaxation on the ground state surface.

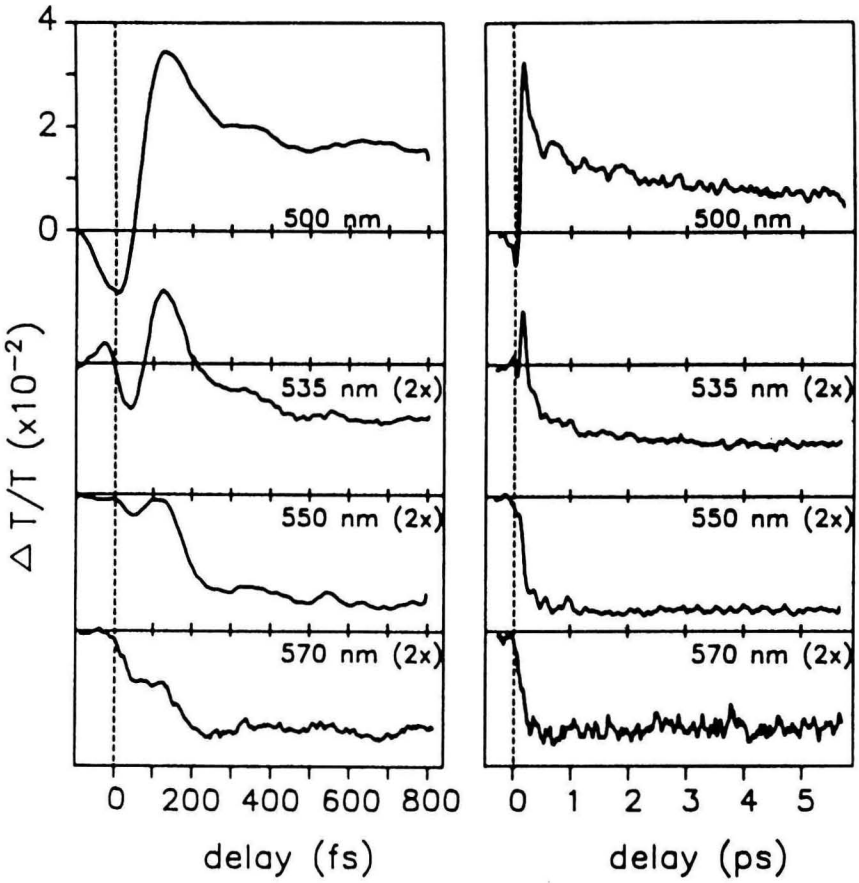

Fig. 3. Transient absorption measurements ($\sim$ 10 fs probe) of 11-*cis* rhodopsin at the indicated wavelengths following a 35-fs pump pulse at 500 nm.

A New Paradigm for Visual Photochemistry

Our observation that the *cis-trans* isomerization of rhodopsin is complete in only 200 fs has important implications for the photochemistry of vision and for photochemistry in general. First, 200 fs is faster than typical vibrational dephasing and vibrational relaxation times (Fragnito et al., 1989) suggesting that the photochemistry occurs from a nonstationary vibrational state. The idea that the photochemistry occurs through a vibrationally coherent process is also consistent with the fact that 200 fs is approximately the half-period of a 50 cm^{-1} torsional vibration. This frequency is appropriate for modeling the excited-state torsional isomerization potential energy surface of rhodopsins (Pollard et al., 1990). We conclude that the isomerization occurs in one continuous coherent

torsional motion as indicated in Fig. 1. In contrast, the traditional picture of photochemistry assumes that vibrational relaxation and dephasing bring the system to the bottom of the excited-state potential energy surface. Surface crossing then occurs from these perhaps thermally excited but stationary vibrational states. Our observations indicate that this traditional picture is not correct for rhodopsin; the surface crossing that leads to photoproduct occurs through vibrationally coherent states.

How should one model the rapid photoisomerization in rhodopsin? Our results suggest that after excitation the photoproduct is formed in an essentially barrierless transition following the nonadiabatic potential surface indicated by the broken lines in Fig. 1. The nonadiabatic surface carries the wavepacket rapidly and directly from the *excited state* of the reactant to the *ground state* of the product. This is envisioned as a dynamic coupling where the time-dependent wavepacket tunnels through the avoided crossing between the excited-state and ground-state potential surfaces. Such crossings can be described as a Landau-Zener process where the probability of crossing from the excited state of the reactant to the ground state of the product is given by (Bagchi & Fleming, 1990): $P_{LZ} \propto \exp -\{\Delta E^2/(2h\,|\Delta F|\,v)\}$. Here ΔE is the minimum energy gap between the adiabatic (solid) surfaces (see Fig. 1), h is Planck's constant, v is the wavepacket torsional velocity in the transition state and ΔF is the difference in slope of the two dashed (nonadiabatic) potential surfaces. For reasonable estimates of these parameters ($\Delta E = 5$ kcal/mole, $v\,|\Delta F| = 100\ \mathrm{cm}^{-1}/\mathrm{fs}$) we calculate that P_{LZ} is very close to the experimental value of the isomerization quantum yield (2/3). This shows that the Landau-Zener tunneling mechanism is a competent 1-dimensional model for the isomerization process in vision and supports the idea that vibrational coherence plays an important role in determining the quantum yield of this reaction.

The idea that the isomerization process in rhodopsin involves a vibrationally coherent tunneling process leads to several important predictions. (1) In the first pass through the transition state only 2/3 of the molecules in the ensemble couple through the surface to form product so the other 1/3 must reflect off and presumably vibrationally dephase on the excited-state potential surface. This residual dephased excited-state population may then undergo an incoherent internal conversion. However, the photoproduct is predominantly made through the initial fast vibrationally coherent process. Vibrational coherence and the femtosecond nuclear dynamics are thus critical for the process of vision. If the isomerizing rhodopsin chromophore lost its torsional kinetic energy before reaching the transition state, the quantum yield and hence the exquisite sensitivity of vision would be dramatically reduced. (2) One would expect that if the initial nuclear dynamics are slower then the quantum yield would be reduced. Indeed, femtosecond experiments on isorhodopsin, whose quantum yield is reduced to 0.2 (Suzuki & Callender, 1981), show that the initial dynamics are significantly slower (Schoenlein et al., 1993). Finally, the dynamic crossing of a wavepacket to the ground-state potential surface would predict that oscillations may be seen in the ground state of the product as has been observed in the case of ozone

(Banin & Ruhman, 1993). Indeed, close inspection of the 500 nm and 570 nm traces in Fig. 3 reveals oscillations that are experimentally reproducible. The vibrational frequency of these oscillations ($\sim 100\,cm^{-1}$) is qualitatively consistent with low-frequency torsional modes of rhodopsins (Loppnow & Mathies, 1988) suggesting that rapid dynamic passage through the transition state may be producing impulsive torsional excitation of the photoproduct. Detailed analysis of these oscillations may provide a basis for more accurate modeling of the photochemical potential surfaces in rhodopsin. In conclusion, this work presents experimental evidence for a new paradigm for visual photochemistry that may also be relevant for a wide variety of efficient photobiological processes (Vos et al., 1993).

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