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The First Step in Vision: Femtosecond Isomerization of Rhodopsin


The kinetics of the primary event in vision have been resolved with the use of femtosecond optical measurement techniques. The 11-cis retinal prosthetic group of rhodopsin is excited with a 35-femtosecond pump pulse at 500 nanometers, and the transient changes in absorption are measured between 450 and 580 nanometers with a 10-femtosecond probe pulse. Within 200 femtoseconds, an increased absorption is observed between 540 and 580 nanometers, indicating the formation of photoprotein on this time scale. These measurements demonstrate that the first step in vision, the 11-cis→11-trans torsional isomerization of the rhodopsin chromophore, is essentially complete in only 200 femtoseconds.

Light detection by the visual system is one of nature’s most important information transduction processes. It consists of a series of chemical reactions that are initiated by the absorption of a photon and culminate in the stimulation of the optic nerve. It has long been known that the primary step in vision is the photoisomerization of the retinal chromophore in rhodopsin (1). However, understanding the dynamics of this isomerization remains a fundamental problem in photochemistry and biology. The time course of many photochemical reactions can be studied with compressed femtosecond optical pulses (2). Such pulses were previously used to observe the isomerization of the retinal chromophore in bacteriorhodopsin, a related pigment which functions as a light-driven proton pump (3). Recent advances in the generation of femtosecond pulses in the blue-green spectral region now make it possible to study a much wider range of ultrafast processes with a time resolution of 10 fs (4). We report here the room temperature investigation of the cis-trans isomerization in rhodopsin which reveals that the first step in vision occurs on a 200-fs time scale and is one of the fastest photochemical reactions ever studied.

Rhodopsin ($\lambda_{\text{max}} \sim 500$ nm) consists of the 11-cis retinal prosthetic group (Fig. 1) bound within the protein opsin. Yoshizawa and Wald (5) determined that the absorption of light results in the cis-to-trans isomerization of 11-cis retinal to form a red-absorbing photoprotein, bathorhodopsin. Early measurements of the isomerization kinetics

REFERENCES AND NOTES


11. Unlike most substances we have studied, the scattering was barely visible on the fluorescent screen and the ambient pressure in the apparatus (about 4 × 10^{-6} Torr) remained unchanged upon opening and closing the oven valve. That diffraction photographs were obtained under such conditions owes to the extremely high scattering power of molecules of COO arising from the high multiplicity of the different distances.


14. The distance parameters are related by $r_i = r_0 - \frac{P_i}{P}$, where $P_i$ is the root-mean-square amplitude of vibration of the atomic pair.


16. This work was supported in part by the National Science Foundation under grant CHE88-10070 to Oregon State University. We are most grateful to G. Chapa-Perez, who made technical contributions to the JMM work, and to R. Boyer, J. Archibald and G. Allison of Oregon State, who built the high-temperature nozzle-oven.

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of the continuum is re-amplified to the microjoule level in a second dye amplifier pumped by the same excimer laser. We use a standard pump-probe technique in which a femtosecond pump pulse excites the rhodopsin sample, and the resulting changes in absorption are measured with a probe pulse, which is delayed in time with respect to the pump. The 35-fs pump pulse at 500 nm comes directly from the amplifier. The 10-fs probe pulse is created by splitting off part of the pump and focusing it in an optical fiber to generate a spectrally broadened and chirped pulse. This pulse is then compressed by means of a sequence of gratings and prisms for phase compensation (16). The 450- to 580-nm bandwidth of the probe pulses allows us to resolve the spectral dynamics of the rhodopsin molecule following excitation by the narrow-band (<15 nm) pump pulses.

The rhodopsin sample is prepared by isolating rod outer segments from bovine retinas (10). The isolated outer segments are purified by ultracentrifugation in a continuous sucrose gradient, lysed with cold water, and pelleted by additional centrifugation. Rhodopsin from 400 retinas is solubilized (5% Ammonyx-LO, 20 mM MOPS, pH 7.4) and then concentrated to an optical density of 15 OD/cm (at 500 nm) using Amicon CF25 centriflo membrane cones. A 3-ml sample was flowed through a 300-μm wire-guided jet at a sufficient velocity to ensure complete replacement of the sample between each pair of pump-probe pulses. The 35-fs pump pulses are focused on the rhodopsin jet to an energy density of ~400 μJ/cm² (~70-μm spot size). At these pump fluences, only ~10% of the exposed sample is bleached by the pump. The weaker probe pulses are similarly focused on the sample (~30 μJ/cm²). Transient changes in absorption (ΔT/T) are measured with two different techniques. Time-resolved measurements at specific wavelengths are obtained by spectrally filtering the probe pulse (after passing through the sample) and combining differential detection with lock-in amplification. Differential spectral measurements over the entire bandwidth of the 10-fs probe pulse are made with a spectrometer and a dual diode array detector. In all measurements, the maximum signal (ΔT/T) is a few percent, and the linearity is verified in order to avoid saturation effects.

The transient change in absorption is measured at 500, 535, 550, and 570 nm, following excitation of rhodopsin by a 35-fs pulse at 500 nm (Fig. 2). Measurements at 500 nm probe the initial bleach and partial recovery of the ground state absorption of 11-cis rhodopsin. At early times, we observe a transient excited-state absorption as evidenced by the negative differential signal (ΔT/T<0) near zero delay. The arrival of the pump pulse induces an absorption at ~500 nm, assigned to the S₁→S₃ transition (Fig. 1), which interferes with the ground-state bleach signal. As the wavepacket created in the first excited state moves away from the Franck-Condon region, the excited-state absorption disappears and the full bleach of the rhodopsin absorption is revealed by 125 fs. A rapid partial recovery of the initial bleach at 500 nm is observed, which has a time constant of ~250 fs. The subsequent long-time recovery of the bleach occurs with a time constant of ~8 ps.

Fig. 1. Schematic ground-state and excited-state potential energy surfaces for the 11-cis → 11-trans isomerization in rhodopsin, adapted from (14). The reaction path of the photosomerization is indicated by the nonadiabatic potential surfaces (broken lines).

Fig. 2. Transient absorption measurements of 11-cis rhodopsin at various wavelengths following a 35-fs pump pulse at 500 nm (~10-fs probe).
Transient absorption changes measured at 550 nm and 570 nm reveal the kinetics of photoprotein formation. At 570 nm, near the peak absorption of the photoprotein, we observe a rapidly developing absorption ($\Delta T/T < 0$) which reaches a maximum by 200 fs. Beyond 200 fs there is very little change in the absorption, indicating that the photoprotein is formed on this time scale. This conclusion is supported by the rapid appearance of absorption at 550 nm, on the blue side of the photoprotein absorption band. At this wavelength however, there is a 100 fs delay before the absorption develops. Between 200 fs and 1 ps, the absorption at 550 nm gradually increases, and then remains unchanged out to 6 ps delay.

Measurements at 535 nm, on the red edge of the 11-cis rhodopsin absorption band and on the blue edge of the photoprotein absorption, are more complicated. The excited-state ($S_1 \rightarrow S_0$) absorption induced by the pump ($\Delta T/T < 0$) is apparent at short times between 0 and 100 fs. This induced absorption does not appear instantaneous as is the case at 500 nm, but is delayed by 50 fs. The delay is attributed to the dynamics of the excited-state absorption resulting from spreading or motion (or both) of the wavepacket excited on the $S_1$ surface as it leaves the Franck-Condon region. By 100 fs we observe the appearance of the ground-state bleach, and by 200 fs the photoprotein absorption begins to dominate and the signal changes sign. The behavior at longer times (1 ps) at 535 nm results from a slow recovery of the ground-state absorption (as observed at 500 nm). In addition, we observe oscillatory behavior at all probe wavelengths, though this is particularly evident at 500 nm and at 570 nm (between 0 and 200 fs).

Complementary information about the isomerization kinetics is provided by the differential spectral measurements (Fig. 3). At 535 fs delay, the increase in absorption between 490 and 540 nm results from the excited-state ($S_1 \rightarrow S_0$) transition which dominates the bleach signal at short times. The initial appearance of photoprotein is indicated by the differential absorption observed between 540 and 580 nm. Between 33 fs and 200 fs, the photoprotein absorption increases, and the initial rhodopsin bleach between 470 and 540 nm becomes evident. The filling in of the bleach signal is observed at longer delays, consistent with the time-resolved measurements (Fig. 2). The photoprotein absorption at 570 nm remains unchanged after 200 fs, indicating that the isomerization is complete on this time scale.

The blue shift of the isosbestic point (from 540 to 515 nm) with increasing delay is most likely due to vibrational cooling of both the rhodopsin and photoprotein ground states, as well as conformational relaxation. Although we cannot exclude the possibility that some residual excited-state population contributes to the recovery of the bleach at long times, our interpretation is supported by the fact that vibrational cooling and conformational relaxation are known to occur in the related pigment, bacteriorhodopsin, on a similar time scale, ~3 ps (17). Differential absorption spectra at 6 ps delay show the residual bleach between 470 and 515 nm and the photoproduct absorption between 515 and 580 nm.

These femtosecond measurements have temporally resolved the isomerization of the 11-cis retinal prosthetic group in rhodopsin. The rapid increase in absorption between 540 and 580 nm shows that the all-trans photoprotein is formed in only 200 fs. This confirms earlier suggestions that the photo-sensitivity and high quantum yield of rhodopsin require a rapid non-radiative isomerization (12, 13). Furthermore, the non-exponential kinetics observed at 550 nm and 570 nm (Fig. 2) indicate that the isomerization cannot be described by a first-order process. The appearance of the excited-state absorption at ~500 nm at short times is consistent with theoretical models of the electronic structure of the 11-cis chromophore in rhodopsin (14, 18). The rapid disappearance of this absorption is most likely due to the speed of the torsional motion. The lack of any obvious evidence for stimulated emission from the excited state also indicates that the torsional wavepacket on the excited-state surface rapidly moves out of the Franck-Condon region. In addition, the 100- to 200-fs oscillatory behavior in the time-resolved measurements at 500 nm, as well as at 535, 550, and 570 nm indicates that non-stationary vibrational states are excited by the short pulses (19). The vibrational frequency of these oscillations (~135 cm$^{-1}$) is consistent with known low-frequency torsional modes of rhodopsin (12), suggesting that excited-state torsional oscillations (14, 15) may modulate the appearance of photoprotein. However, impulsive Raman excitation of the rhodopsin ground state can also contribute to coherent vibrational oscillations in these measurements (20). Detailed analysis of these oscillations may provide a basis for accurate modeling of the photochemical potential surfaces (21).

The difference spectra at long delays are in reasonable agreement with previous measurements made with substantially longer pulses (7, 22, 23). Difference spectra at 6 ps delay (Fig. 3) indicate an isosbestic (~515 nm) which is within the range of values previously reported. Although our measurements of the differential absorption maxima (~500 nm and ~550 nm) are slightly shifted from what others have observed, this may be due to measurement uncertainty or the shorter wavelength (500 nm) of the excitation pulses used here, or both. In addition, the signal ratio $\Delta A_{\text{short}}/\Delta A_{\text{rhodopsin}} = 3$ measured at 40-ps delay (not shown), is consistent with single-photon excitation (23). Because we use pulses that are shorter than the ground-state recovery and photoprotein-formation times, with fluxes (~400 mJ/cm² or ~0.1 photons absorbed per molecule) that are much lower than previous studies, the possibility of multiphoton photolysis is minimized. Finally, because the time scale of our measurements is faster than the photo-→bathorhodopsin transition, we assume that the initial photoprotein is the intermediate, bathorhodopsin, identified by Shichida et al. (7).

In conclusion, we have time-resolved the spectral dynamics of the primary step in vision. Our results indicate that the cis→trans isomerization of rhodopsin is essentially complete in only 200 fs. This observation has important implications for the photochemistry of vision. First, 200 fs is faster than typical vibrational dephasing and relaxation times (19), suggesting that the photochemistry occurs from a vibrationally coherent system. Indeed, we see indications of
coherent vibrational oscillations which are rapidly damped (at 570 nm) as the photosensitive isomerization process. The significance of such vibrational coherence in the photochemistry of vision has been discussed in several theoretical studies (14, 15). Second, the speed of the isomerization process is consistent with the traditional picture of photochemistry which assumes vibrational relaxation in the excited state followed by partitioning to photoproduc
t and to reactant (24). Our results indicate a highly barrierless transition in the formation of photoproduc, suggesting that the isomerization follows a nonadiabatic potential surface (broken lines in Fig. 1) which reflects the strong coupling between the rhodopsin excited-state and the ground-state of the photoproduc. This presents experimental evidence for a new paradigm for visual photochemistry that may be relevant for a variety of photochemical and photobiological processes.

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Targets for Dioxin: Genes for Plasminogen Activator Inhibitor–2 and Interleukin-1β

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Dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD), a widespread environmental contaminant, may elicit its effects by altering gene expression in susceptible cells. Five TCDD-responsive complementary DNA clones were isolated from a human keratino
cyte cell line. One of these clones encodes plasminogen activator inhibitor–2, a factor that influences growth and differentiation by regulating proteolysis of the extracellular matrix. Another encodes the cytokine interleukin-1β. Thus, TCDD alters the expression of growth regulatory genes and has effects similar to those of other tumor-promoting agents that affect both inflammation and differentiation.

TCDD IS AMONG THE MOST TOXIC pollutants known, and it is a prototype for a large class of halogenated aromatic hydrocarbons (1). It is carcinogenic (2) and teratogenic (3) in rodents, but effects of exposure to TCDD in humans are less well understood (4). In humans the most common adverse response to TCDD is chloracne (5). The pathogenesis of this disease is disorder is characterized by altered patterns of proliferation and differentiation in the epidermis and cutaneous appendages, resulting in interfollicular hyperkeratosis and acantho
sis and squamous metaplasia of the epidermal lining of the sebaceous glands, with subsequent hair follicle atrophy (6). These effects and others associated with exposure to TCDD in both humans and in other animals are believed to occur through the high-affinity binding of TCDD to the aryl hydrocarbon receptor (AhR) and subsequent changes of gene expression in responsi
ive cells (1, 7, 8). The first TCDD-responsive gene to be isolated and the most extensively studied one is cytochrome P450 (CYP1A1). Increased transcription of CYP1A1 (P450A1A) requires accumulation of the TCDD-AhR complex in the nucleus and interaction of the complex with specific DNA response elements (DRE) (7).

To elucidate the events occurring in hu
man cells in response to treatment with TCDD, we used differential hybridization to isolate five different TCDD-responsive cDNA clones. Two of these clones, 18 and 142, represent full-length cDNAs (Table 1). One clone was identified as CYP1A1 by cross-hybridization to a CYP1A1 probe (9). The kinetics of induction of mRNA for each clone was determined by Northern (RNA)

**Table 1. Isolation of TCDD-responsive clones.** A cDNA library was prepared from RNA isolated from a subclone (c122) of the human keratinocyte cell line SCC-12F (18) treated for 6 hours with TCDD (10 μM) and CHX (10 μg/ 

<table>
<thead>
<tr>
<th>Clone</th>
<th>Isolates*</th>
<th>cDNA size (bp)</th>
<th>mRNA size (kb)</th>
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<tr>
<td>CYP1A1</td>
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<td>2050</td>
<td>3.0</td>
</tr>
<tr>
<td>18</td>
<td>5 (4)</td>
<td>2100</td>
<td>1.9</td>
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<td>141</td>
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<tr>
<td>142</td>
<td>1 (0)</td>
<td>1400</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* Indicates the number of times a related cDNA clone was independently isolated. The value in parentheses indicates the number isolated in Screen I only. † After we performed gel electrophoresis, sizes were determined by comparison with DNA or RNA standards (Gibco-BRL), as appropriate.