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Porphyrins As Photosensitizers To Enhance Night Vision

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The primary event in mammalian vision is a light-initiated cis-to-trans isomerization of the retinal chromophore bound, via a protonated Schiff base, to a lysine residue in the opsin apoprotein (Figure 1A).[1] This isomerization activates the protein which triggers a series of events resulting in a signal to the brain. This has been a basic tenet of our understanding of vision. Rod−rhodopsin, the opsin−retinal complex responsible for night vision, is a G-protein-coupled receptor, activated by light with an absorbance maximum at 500 nm. As the absorption from rhodopsin is minute above 600 nm, the pigment is not believed to be involved in vision at longer wavelengths.

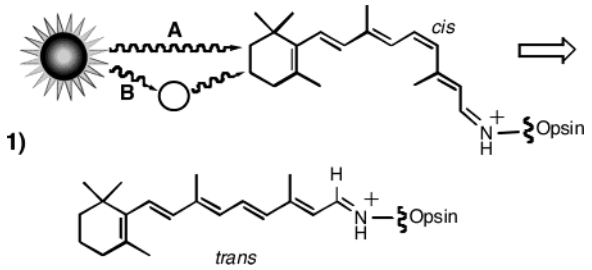
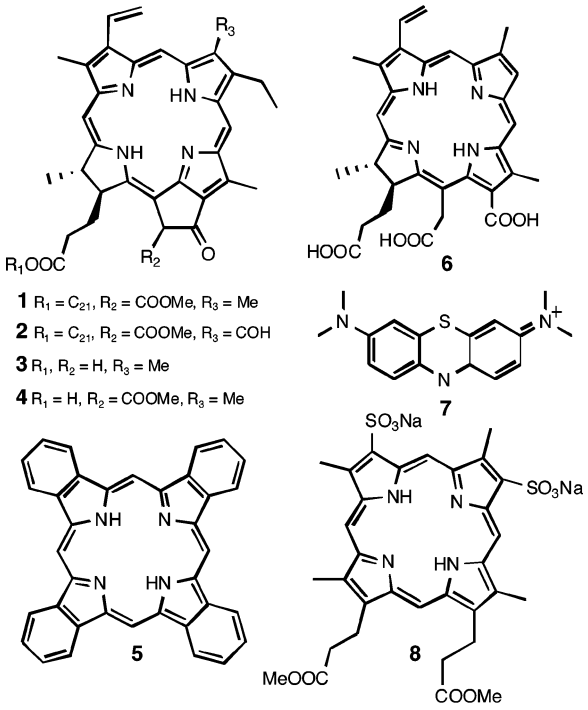


Figure 1. (A) Light of 500 nm excites rhodopsin, initiating isomerization. (B) Longer-wavelength light excites a chlorophyll-derived molecule, shown as a circle, which then transfers its energy to rhodopsin, initiating isomerization.

It has been proposed that the visual signal transduction pathway in a species of deep-sea fish involves the use of photosensitizers.[2] Molecules derived from chlorophyll 650 are thought to absorb longer-wavelength light and transfer the gained energy to shorter-wavelength visual pigments (Figure 1B), thus adding an extra step to their transduction pathway. This is based on the observations that: (i) the fish only possess visual pigments with $\lambda_{max} \leq 545$ nm, (ii) bleaching of its 545 nm pigment with 671 nm light is faster than bleaching with 654 nm light, and (iii) chlorophyll derivatives which have strong absorbances centered at 665 nm have been isolated along with the 545 nm pigment. A triplet−triplet energy-transfer mechanism from the chlorophyll 650 derivatives to the 545 nm pigment has been speculated.[2]

Enhanced visual sensitivity is reported as a common side effect in patients exposed to porphyrins during photodynamic therapy.[3] In related photosynthetic systems found in plants, carotenoids are believed to act as quenchers of chlorophylls and singlet oxygen, in addition to their primary roles as light-harvesting complexes.[4] The quenching involves triplet-state energy transfer from chlorophyll to carotenoid.[4b]

To expand rhodopsin sensitivity into the near-IR we have investigated the bleaching of bovine rhodopsin upon exposure to $\lambda_{max} = 675$ nm light in the presence of various chromophores which are potential photosensitizers with strong absorptions around 665 nm. We report rate enhancements on the order of up to 3 times compared to that for the bleaching of rhodopsin alone with $\lambda_{max} = 675$ nm light.



In all experiments the bleaching rates of bovine rhodopsin were measured using UV−vis spectroscopy by monitoring the absorbance at 500 nm corresponding to that for the Schiff base. A 0.009 mmol solution of bovine rhodopsin (90% in ROS suspension) solublized in 5% dodecyl-β-D-maltoside[5] in phosphate-buffered saline was used for all bleaching experiments. The curve marked rhodopsin in Figure 2 depicts a bleaching rate set to 1.0, for the initial 30 min of bleaching of bovine rhodopsin, with $\lambda_{max} = 675$ nm light at 25 °C.

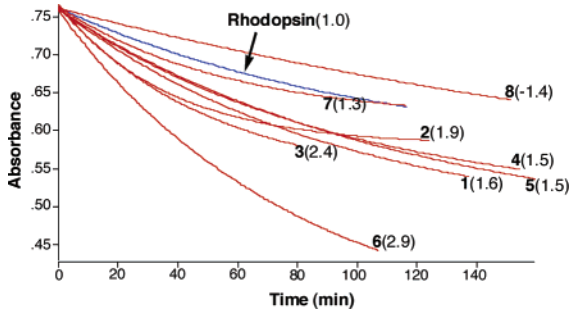


Figure 2. Bleaching rates for a 0.009 mmol solution of bovine rhodopsin in 5% dodecyl-β-D-maltoside phosphate-buffered saline, in the presence of compounds **1**−**8** and for rhodopsin alone, upon exposure to $\lambda_{max} = 675$ nm light. Rates were measured by UV−vis spectroscopy by measuring absorbance at 500 nm over time. Numbers in brackets are normalized rates for the first 30 min.

Figure 3 shows the UV−vis spectra, collected over 80 min, for the above solution of rhodopsin in the presence of porphyrin **3**.

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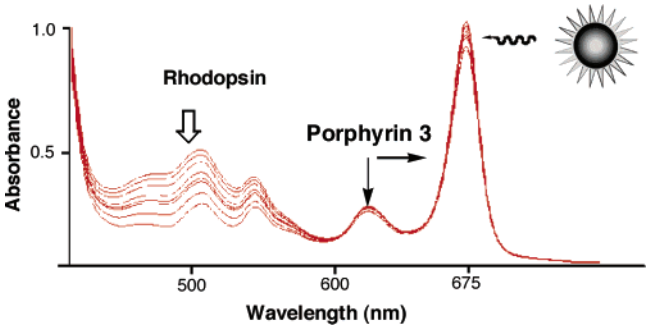

Figure 3. UV−vis spectrum, taken over 80 min, monitoring the decrease in absorbance at 500 nm (rhodopsin), for irradiation of a 0.009 mmol solution of bovine rhodopsin, in the presence of compound **3**, with λ_{max} = 675 nm light.

Absorbances at 625 and 675 nm are from **3**, while that at 500 nm is mainly from rhodopsin and a small absorbance due to **3**. Upon exposure to λ_{max} = 675 nm light, the absorbance at 500 nm decreased 2.4 times faster compared to that for bleaching rhodopsin alone, as shown by curve **3** in Figure 2.[6] A negligible decrease at 500 nm was observed when a solution of porphyrin **3** was irradiated without rhodopsin or with the apoprotein opsin.

Similar rate measurements were performed for compounds **1**−**8**, the results of which are shown in Figure 2. Curves 1−8 in Figure 2 show rhodopsin bleaching rates in the presence of compounds **1**−**8**, respectively. Chlorin e_6, **6**, showed the greatest rate enhancement, 2.9 times faster compared to that for rhodopsin. Chlorophyll derivatives **1**−**4** and the tetrabenzoporphine, **5**, all gave rate enhancements greater than 1.5. Methylene blue, **7**, which does not possess the porphyrin architecture, gave the smallest rate enhancement. The sodium sulfate porphyrin, **8**, gave a decreased rate of bleaching (−1.6); this is in accordance with the observation that sodium dodecyl sulfate denatures the protein.[5]

Energy transfer from compounds **1**−**8** to rhodopsin may occur through the singlet (A) or triplet (B) excited states of **1**−**8** as shown in Figure 4. Singlet energy transfer would be unfavorable since there is minimal overlap between the emission spectrum of compounds **1**−**8** around 675 nm and the absorption spectrum of rhodopsin centered at 500 nm. This was further confirmed by fluorescence measurements. The fluorescence spectra measured at 25 or −196 °C, of chlorin e_6 (**6**), the fastest accelerator, showed no change in the presence of as much as 2 equiv of rhodopsin, indicating little communication between the pigment and the singlet excited state of chlorin e_6.

The rate of energy transfer from the triplet states of compounds **1**−**8** (B, Figure 4) depends on their triplet-state energies, lifetime and quantum yield, and orbital overlap (direct or indirect) between **1**−**8** and the Schiff base. Compounds **1**−**8** all have similar triplet-state energies which are between 31 and 33 kcal/mol.[7] The triplet-state energy of rhodopsin is 40 kcal/mol,[8] making triplet energy sensitization slightly uphill. However, porphyrins **1**−**6** exhibit long-lived triplet states ranging from 0.8 to 1.0 ms and quantum yields above 95%,[7] making triplet−triplet sensitization possible. Methylene blue, **7**, has a significantly shorter-lived triplet state (0.5 ms) and a lower quantum yield (52%).[7a] The smaller increase in bleaching rate for **7**, however, may also be a result of a lower binding constant to rhodopsin compared to those for compounds **1**−**6** and thus a smaller orbital overlap.

Oxygen quenches the chlorin e_6 triplet at a rate of 1.9×10^9 M^{-1} s^{-1}. [9] However, no change in bleaching rates was observed when a 1:1 mixture of chlorin e_6 or pheophytin *b*, **2**, to rhodopsin was irradiated with 675 nm light in oxygen-poor or -saturated (1.45 mmol O_2) solutions. This observation suggests electron-transfer quenching of the excited states of compounds **1**−**8** by rhodopsin.[4c]

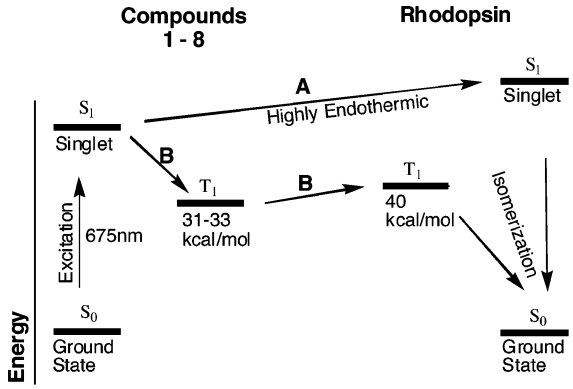

Figure 4. Energy-level diagram depicting two energy-transfer pathways from compounds **1**−**8** to rhodopsin: singlet−singlet (A) and triplet−triplet (B).

We have shown that the use of photosensitizers by nature to enhance rod outer-segment sensitivity to long wavelength light may be a general mechanism. Evidence suggests that the porphyrins act as photosensitizers and excite the visual pigment via electron or triplet-state energy transfer. These mechanisms suggest that rhodopsin possesses a pocket, proximal to the Schiff base, so that porphyrins act as photosensitizers. We are currently investigating this photosensitizer mechanism to enhance rod sensitivity in vitro.

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