reversibility between MV^+ and DQ^+ that the reduction potential of dioxygen in a zeolite supercage lies close to −0.4 V,[10] which corresponds to its reversible potential in aqueous media.[12] Such a value differs significantly from $E° = -1.04$ V in acetonitrile as an aprotic organic solvent. Indeed the unique ease of such a dioxygen reduction in the *solid phase* attests to the remarkable polarization effects available in zeolite matrices.[8] In this regard chemical reactions on zeolite surfaces could be likened to those in an aqueous medium.

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(10) Since the values of $E°$ for PQ^{2+} and DQ^{2+} in the zeolite matrix are unknown, we based our conclusions on $E° = -0.45$ and −0.39 V versus SCE, respectively, in acetonitrile. The corresponding values are −0.43 and −0.38 V in DMF and −0.68 and −0.61 V in water.[11] Compare, also: Gemborys, H. A.; Shaw, B. R. *J. Electroanal. Chem.* **1986**, *208*, 95.

(11) See: Bird, C. L.; Kuhn, A. T. *Chem. Soc. Rev.* **1981**, *10*, 49.

(12) (a) Sawyer, D. T.; Valentine, J. S. *Acc. Chem. Res.* **1981**, *14*, 393. (b) Kasai, P. H.; Bishop, R. J. *Zeolite Chemistry and Catalysis*; Rabo, J. A., Ed.; ACS Monograph 171; American Chemical Society, Washington, DC, 1976, Chapter 6.

Rhodopsin Activation: A Novel View Suggested by in Vivo *Chlamydomonas* Experiments

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The blind mutant strain FN28 of the unicellular alga *Chlamydomonas reinhardtii*, which lacks retinal due to blocking of its carotenoid biosynthesis, is not phototactic; however, phototaxis is restored upon incubation with retinal analogues, the action spectral maxima being dependent on the structure of the analogue.[1] The similarity between *Chlamydomonas* in vivo behavioral maxima and in vitro λ_{max} of bovine rhodopsins reconstituted from corresponding retinals indicates that, as in the case of bovine rhodopsins, the retinal is bound to the opsin through a protonated Schiff base linkage $C{=}N^+H$. It also suggested the two photoreceptor pigments to be similar,[1] a hypothesis supported by genomic studies.[2] Incorporation of over 80 retinal analogues[3] have led to the unexpected finding that *Chlamydomonas phototaxis is restored by retinal analogues where specific double bond isomerizations are blocked and by short acyclic aldehydes including hexenal and hexanal.* Thus activation of the *Chlamydomonas* photoreceptor does not occur via the conventional cis/trans isomerization of the polyene system.

Chlamydomonas phototaxis, which peaks at 503 nm, is well-suited for in vivo assays, because no protein biochemical preparation is required, and it responds only to functional pigments. Photoreceptor activation studies can be carried out with the blind mutant FN68 because of its very low sensitivity for phototaxis.

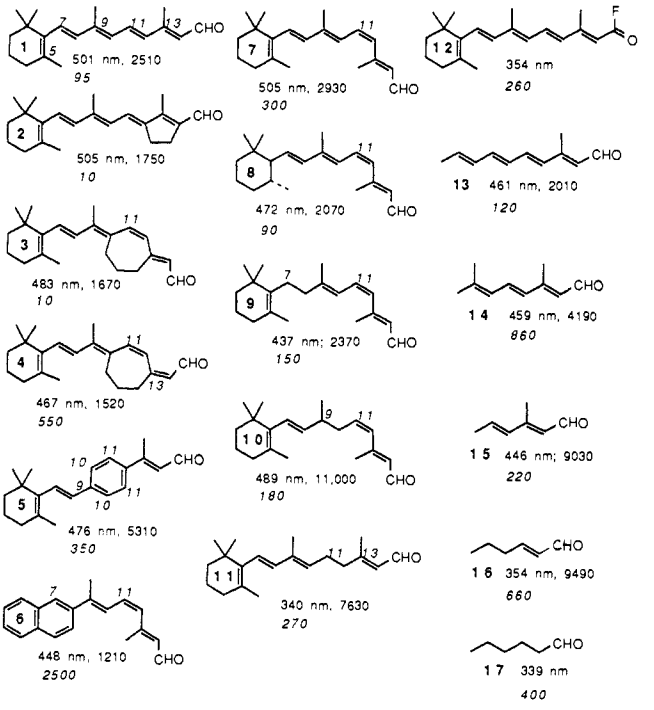

Figure 1. Phototaxis action spectrum data for retinal analogues. Action spectrum maxima in nm, opsin shifts[6] (difference between maxima of phototaxis and protonated Schiff base with *n*-butylamine) in cm^{-1}, and sensitivity in $m^2\ s/10^{18}$ photons (in italics). Compound sources are as follows: **1**, Sigma Chemical Co., St. Louis, MO; **2**, ref 13; **3**, **4**, ref 10b; **5**, ref 14; **6**, synthesized by condensation of *trans*-3-methyl-3β-naphthylpropenal[15] with the anion of trimethylsilylacetone *tert*-butylamine,[16] elongation of C_{18} ketones with diethylcyanomethyl phosphonate, dibal reduction, and HPLC; **7**, ref 17; **8**–**10**, ref 9b; **11**, ref 10c; **12**, ref 5b; **13**, **14**, ref 18 and 19; **15**, reaction of 3-penten-2-one with trimethylsilyl acetaldehyde *tert*-butylimine anion followed by acid hydrolysis;[20] **16**, **17**, Aldrich Chemical Co., Inc.

The threshold phototaxis action spectra in FN68 mutant were measured as described.[1,4] The phototaxis sensitivity (reciprocal of threshold) increases ca. 10 000-fold upon incubation with a retinal analogue, providing high signal/noise ratio. In contrast, enzyme assays used for studies of bovine rhodopsin activation have a dynamic range of ca. 20.[5] Normally, incorporation into *Chlamydomonas* opsin, as measured by restored phototaxis, is as rapid as the incorporation of analogues into bovine opsin in detergent. Representative results of phototaxis maxima, opsin shifts,[6] and sensitivities are listed in Figure 1.[7]

Presumably due to the presence of an isomerase in vivo, incubation of *all-trans*-retinal **1** or 11-*cis*-retinal **7** restores phototaxis around the natural maximum of 503 nm.[1] The natural chromophore has not yet been identified due to the minute amount of extractable chromophore. The mutant was next incubated with retinal analogues in which specific double bond isomerizations were blocked: 13-ene (**2**), 11-ene (**3**, **4**), 9-/11-ene (**5**), and 7-ene (**6**) (Figure 1). All analogues restored activity with reasonable sensitivities indicating that specific double bond isomerization is not required. The only other specific double bond to be considered is the $C{=}N^+H$ linkage. Acid fluoride **12** that forms an amide with the opsin[5b] efficiently restores phototaxis with FN68; thus, $C{=}NH^+$ bond formation and its syn/anti isomerization are not prerequisites for activation in this case. However, the mere presence of a chromophore in the binding site is insufficient for phototaxis recovery; namely, retinonitrile[8a] (CN instead of CHO

(1) Foster, K. W.; Saranak, J.; Patel, J.; Zarilli, G.; Okabe, M.; Kline, T.; Nakanishi, K. *Nature (London)* **1984**, *311*, 756–759.

(2) Martin, R. L.; Wood, D.; Baehr, W.; Applebury, M. L. *Science (Washington, D.C.)* **1986**, *232*, 1266–1269.

(3) (a) Balogh-Nair, V.; Nakanishi, K. *Methods Enzymol.* **1982**, *88*, 496–506. (b) Derguini, F.; Nakanishi, K. *Photobiochem. Photobiophys.* **1986**, *13*, 259–283.

(4) Foster, K.; Saranak, J.; Zarrilli, G. *Proc. Natl. Acad. Sci. U.S.A.*, in press.

(5) (a) Fukada, Y.; Shichida, Y.; Yoshizawa, T.; Ito, M.; Kodama, A.; Tsukida, K. *Biochemistry* **1984**, *23*, 5826–5832. (b) Calhoon, R. D.; Rando, R. R. *Biochemistry* **1985**, *24*, 3029–3034.

(6) Nakanishi, K.; Balogh-Nair, V.; Arnaboldi, M.; Tsujimoto, K.; Honig, B. *J. Am. Chem. Soc.* **1980**, *102*, 7945–7947.

(7) Details to be published elsewhere.

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in **1**), which cannot bind to the protein, although competitively reduced the rate of retinal incorporation, was found to be inactive.[8]

The cells were also incubated with 11-*cis*-dihydroretinals **8–11** and acyclic series **13–16** containing the same number of double bonds. The action spectral maxima of restored phototaxis are dependent on the conjugation length in both series. The action spectra peaks resulting from incorporation of tetraenals **8/13** were at 472/461 nm, trienals **9/14** at 437/459 nm, dienals **10/15** at 489/446 nm, and monoenals **11/16** at 340/354 nm. These aldehydes are linked to the photoreceptor via a C=N$^+$H bond as in other retinal proteins, since the maxima undergo progressive blue shifts with shorter conjugation, and the trend of phototaxis maxima restored by the acyclic series is similar to the absorption maxima of 11-*cis*-dihydroretinals in bovine rhodopsin.[9] The sharp change in opsin shifts[6] that one observes between the trienals and dienals, e.g., 2370 cm^{-1} in **9** versus 11 000 cm^{-1} in **10**, suggests that a negative charge may be near the terminus of the conjugated system -$C_{(11)}$=$C_{(12)}$-$C_{(13)}$=$C_{(14)}$-$C_{(15)}$=NH$^+$-, near C-10 or C-11.[7] Hexenal **16** and even hexanal **17** (!) incorporated readily and, surprisingly, were as sensitive as other retinals, including 11-*cis*-retinal **7**. The maximum for hexanal **17** (339 nm) is at a shorter wavelength than that of hexenal **16** (354 nm); this together with its red shift compared to hexanal in solution shows hexanal is also bound through C=N$^+$H.

The activity of hexanal corroborates other results which suggest that a route other than cis–trans isomerization is possible for activation of *Chlamydomonas* rhodopsin. Upon photoexcitation of rhodopsins, electron density is redistributed toward the imine (C=N) end of retinal causing a variety of events including cis–trans isomerization. Although it is established that cis–trans isomerization occurs with visual pigments,[10] it is not known whether this is the event responsible for visual transduction. Most experiments have measured the changes that retinal undergoes in the regulatory (binding) site and not the activation of the peripheral enzymatic site responsible for initiating the visual cascade.

Two mechanisms for the triggering of *Chlamydomonas* rhodopsin are conceivable. *Charge redistribution in the photoexcited state, where the electron density moves toward N*,[11] acts (i) directly to activate rhodopsin or (ii) indirectly, via the possible subsequent step of syn/anti isomerization of the C=N$^+$H bond, to trigger phototaxis. In view of the positive phototaxis response of analogue **12**, direct activation is more likely. Because of the possible homology among eukaryotic rhodopsins,[2,12] a mechanism similar to that occurring in *Chlamydomonas* may be involved in triggering the activity of other rhodopsins. These aspects are currently under investigation.

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(8) (a) Liu, R. S. H.; Asato, A. E. *Tetrahedron* **1984**, *40*, 1931–1969. (b) Chromophores that neither restored phototaxis nor affected incorporation of *trans*-retinal include methyl ketones, e.g., β-ionone and the C_{18} ketone (13-one in **1**), and aldehydes that carry a ring 5-6 carbons removed from C=N$^+$H, e.g., β-ionylideneacetaldehyde.

(9) (a) Honig, B.; Dinur, U.; Nakanishi, K.; Balogh-Nair, V.; Gawinowicz, M. A.; Arnaboldi, M.; Motto, M. G. *J. Am. Chem. Soc.* **1979**, *101*, 7084–7086. These earlier studies used 9-cis- rather than 11-*cis*-dihydroretinals due to synthetic ease. (b) For 11-cis series, see: Koutalos, Y.; Ebrey, T. G.; Tsuda, M.; Park, M.-H.; Lien, T.; Odashima, K.; Shimizu, N.; Derguini, F.; Nakanishi, K.; Honig, B., submitted to *Biophys. J.*

(10) (a) Hubbard, R.; Kropf, A. *Proc. Natl. Sci. U.S.A.* **1958**, *44*, 130–139. (b) Akita, H.; Tanis, S. P.; Adams, M.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1980**, *102*, 6370–6372. (c) Gawinowincz, M. A.; Balogh-Nair, V.; Sabol, J. S.; Nakanishi, K. *J. Am. Chem. Soc.* **1977**, *99*, 7720–7721. (d) Shichi, H. *Biochemistry of Vision*; Academic Press: New York, 1983.

(11) Salem, L.; Bruckmann, P. *Nature (London)* **1975**, *258*, 526–528.

(12) (a) Findlay, J. B. C.; Pappin, D. J. C. *Biochem. J.* **1986**, *238*, 625–642. (b) Findlay, J. B. C. *Photobiochem. Photobiophys.* **1986**, *13*, 213–228.

(13) Fang, J.-M.; Carriker, J. D.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 5162–5164.

(14) Kolling, E.; Gartner, D.; Oesterhelt, D.; Ernst, L. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 81–82.

(15) Akhtar, M.; Jallo, L.; Johnson, A. H. *Chem. Commun.* **1982**, 44–46.

(16) Croteau, A.; Termini, J. *Tetrahedron Lett.* **1983**, *24*, 2481–2484.

(17) Denny, M.; Chun, M.; Liu, R. S. H. *Photochem. Photobiol.* **1981**, *33*, 267–269.

(18) M.-Szeweykowska, M.; Pardoen, J. A.; Dobbelstein, D.; Van Amsterdam, L. J. P.; Lugtenburg, J. *Eur. J. Biochem.* **1984**, *140*, 173–176.

(19) Jayathirtha Rao, V.; Zingoni, J. P.; Crouch, R.; Denny, M.; Liu, R. S. H. *Photochem. Photobiol.* **1985**, *41*, 171–174.

(20) Corey, E. J.; Enders, D.; Bock, M. G. *Tetrahedron Lett.* **1976**, 7–10.

$[Na_2Fe_{18}S_{30}]^{8-}$: A High-Nuclearity Cyclic Cluster Generated Solely by Iron–Sulfur Bridge Bonding

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Since its introduction,[1] spontaneous self-assembly of iron–sulfur clusters from an iron salt, a sulfide source, and thiolate, halide, or areneoxide as a terminal ligand has proven to be a highly productive synthetic method. Clusters containing the cores $[Fe_2S_2]^{2+}$,[2-4] linear $[Fe_3S_4]^{1+}$,[4] cubic $[Fe_4S_4]^{2+,1+}$,[1,2,5] prismatic $[Fe_6S_6]^{3+,2+}$,[6] and $[Fe_6S_9]^{2-}$[4,7] have been obtained in good yield. Use of tertiary phosphine as a terminal ligand, usually in the presence of thiolate or halide, redirects the reaction pathways to new clusters containing basket $[Fe_6S_6]^{2+,1+}$,[8] stellated octahedral $[Fe_6S_8]^{2+,1+}$,[9] and monocapped prismatic $[Fe_7S_6]^{3+}$[10] cores. Further examination of the effects of different potential terminal ligands in assembly systems has afforded a remarkable cluster of unprecedented structure.

Treatment of a solution of 9.0 mmol of anhydrous $FeCl_3$ in 70 mL of methanol at 5 °C with a solution of 27 mmol of Na[PhNC(O)Me] in 60 mL of methanol (generated in situ from equimolar acetanilide and sodium metal) afforded a yellow mixture, which was stirred for 30 min at 5 °C. A slurry of 12 mmol of Li_2S in 70 mL of methanol was slowly added, the reaction mixture was filtered after 1 h, and 9.0 mmol of (*n*-Pr_4N)Br in 15 mL of methanol was added to the dark green filtrate. Over a 5-day period a dark crystalline solid separated, which was collected, washed (ethanol), and dried in vacuo to afford 1.59 g

(1) (a) Herskovitz, T.; Averill, B. A.; Holm, R. H.; Ibers, J. A.; Phillips, W. D.; Weiher, J. F. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 2437. (b) Averill, B. A.; Herskovitz, T.; Holm, R. H.; Ibers, J. A. *J. Am. Chem. Soc.* **1973**, *95*, 3523.

(2) Berg, J. M.; Holm, R. H. In *Iron–Sulfur Proteins*; Spiro, T. G., Ed.; Interscience: New York, 1982; Chapter 1.

(3) (a) Reynolds, J. G.; Holm, R. H. *Inorg. Chem.* **1980**, *19*, 3257. (b) Cleland, W. R., Jr.; Averill, B. A. *Inorg. Chem.* **1984**, *23*, 4192. (c) Han, S.; Czernuszewicz, R. S.; Spiro, T. G. *Inorg. Chem.* **1986**, *25*, 2276.

(4) Hagen, K. S.; Watson, A. D.; Holm, R. H. *J. Am. Chem. Soc.* **1983**, *105*, 3905.

(5) (a) Christou, G.; Garner, C. D. *J. Chem. Soc., Dalton Trans.* **1979**, 1093. (b) Hagen, K. S.; Watson, A. D.; Holm, R. H. *Inorg. Chem.* **1984**, *23*, 2984. (c) Saak, W.; Pohl, S. *Z. Naturforsch.* **1985**, *40B*, 1105. (d) Müller, A.; Schladerbeck, N.; Bögge, H. *Chimia* **1985**, *39*, 24. (e) Rutchik, S.; Kim, S.; Walters, M. A. *Inorg. Chem.* **1988**, *27*, 1515.

(6) (a) Coucouvanis, D.; Kanatzidis, M. G.; Dunham, W. R.; Hagen, W. R. *J. Am. Chem. Soc.* **1984**, *106*, 7998. (b) Kanatzidis, M. G.; Hagen, W. R.; Dunham, W. R.; Lester, R. K.; Coucouvanis, D. *J. Am. Chem. Soc.* **1985**, *107*, 953. (c) Kanatzidis, M. G.; Salifoglou, A.; Coucouvanis, D. *Inorg. Chem.* **1986**, *25*, 2460.

(7) (a) Christou, G.; Sabat, M.; Ibers, J. A.; Holm, R. H. *Inorg. Chem.* **1982**, *21*, 3518. (b) Strasdeit, H.; Krebs, H.; Henkel, G. *Inorg. Chem.* **1984**, *23*, 1816.

(8) (a) Snyder, B. S.; Reynolds, M. S.; Noda, I.; Holm, R. H. *Inorg. Chem.* **1988**, *27*, 595. (b) Snyder, B. S.; Holm, R. H. *Inorg. Chem.* **1988**, *27*, 2339. (c) Reynolds, M. S.; Holm, R. H. *Inorg. Chem.*, submitted for publication. (d) Snyder, B. S.; Holm, R. H., unpublished results.

(9) (a) Agresti, A.; Bacci, M.; Cecconi, F.; Ghilardi, C. A. *Inorg. Chem.* **1985**, *24*, 689. (b) Cecconi, F.; Ghilardi, C. A.; Midollini, S.; Orlandini, A.; Zanello, P. *J. Chem. Soc., Dalton Trans.* **1987**, 831.

(10) Noda, I.; Snyder, B. S.; Holm, R. H. *Inorg. Chem.* **1986**, *25*, 3851.

0002-7863/88/1510-6589$01.50/0