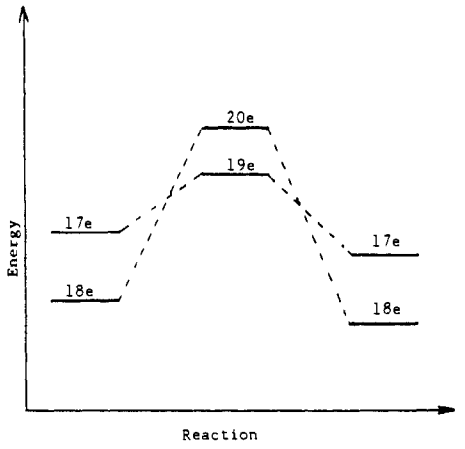

Figure 1. Energy/reaction coordinate diagram for an associative 18e and 17e ligand substitution reaction.

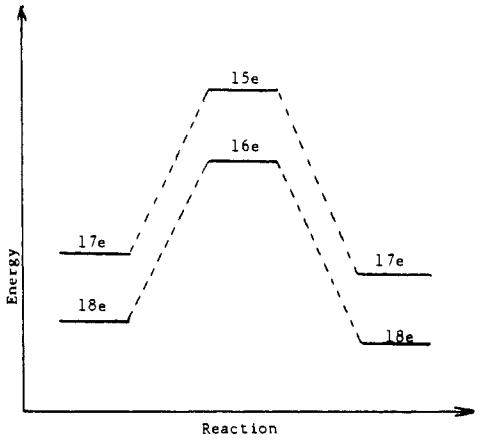

Figure 2. Energy/reaction coordinate diagram for a dissociative 18e and 17e ligand substitution reaction.

Table I. Associative Rates of CO Exchange and EPR Data for $(\eta^5\text{-L})_2$VCO Complexes

complex	k_2,[a] M^{-1} s^{-1}	ν_{CO}, cm^{-1}	A_{iso}, G	ref	steric[b]	electronic[b]
I $(Cp)_2VCO$	ca. 800	1881	28.8	3	+	+
II $(pd)_2VCO$	0.0038[c]	1959	79.1	3	-	-
III $(dmCh)_2VCO$	0.0734	1912	71	9, this work	+	-

[a] At 60 °C. In decalin $[L_2MCO] = 5 \times 10^{-3}$ M for I and II, $[L_2MCO] = 1 \times 10^{-2}$ M for III. [b] Favorable for associative reaction represented +, unfavorable represented -. [c] Reacts mostly by dissociative pathway with $k_1 = 8.1 \times 10^{-6}$ s^{-1}.

associative CO substitution without involving ring slippage. Detailed papers on each of these observations, along with other examples, will be reported later.

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Supplementary Material Available: A plot of the rate of reaction (k_{obsd}) versus concentration of CO which shows the reaction is exclusively associative (1 page). Ordering information is given on any current masthead page.

(12) Miller, G. A.; Therien, M. J.; Trogler, W. C., submitted for publication.

Direct Observation of Superoxide Electron Transfer with Viologens by Immobilization in Zeolite

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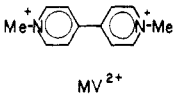
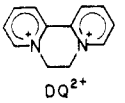
The presence of supercages and channels of various sizes in zeolites has provided the opportunity for shape-selective catalysis.[1] Furthermore such molecular pockets are potentially useful for the immobilization of reactive intermediates that are otherwise subject to rapid diffusive annihilation in solution.[2] It is important however to establish the viability of spectroscopic characterization for such trapped species in a solid matrix. As a test case, we chose the biologically relevant reduction of dioxygen by bipyridinium cation radicals (MV^+ and DQ^+) as produced by photosystem I in

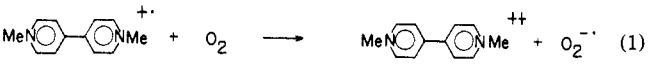


MV^{2+}



DQ^{2+}

chloroplasts during herbicidal action.[3] Although it has been widely accepted that electron transfer to superoxide pertains,[4] i.e.,



(1)

the experimental evidence is indirect,[4,5] owing to the transient character of superoxide in protic media.[6] As a result, little is known about even the reversibility of electron transfer in eq 1.

We find that MV^+ and DQ^+ can be efficiently incorporated into zeolite-Y. Thus when the solid was slurried with 10^{-5} M $MV^+PF_6^-$ under an inert argon atmosphere, the blue solution was bleached, and the color was transferred to the colorless zeolite-Y. Small amounts of $MV^+PF_6^-$ were successively added until the acetonitrile solvent remained slightly blue, even after prolonged stirring.[7] The total amount of $MV^+PF_6^-$ incorporated in the brilliant blue zeolite corresponded to roughly 20% occupancy of the supercages.[8] A bright green zeolite was obtained by similarly

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(6) (a) Farrington, J. A.; Ebert, M.; Land, E. J.; Fletcher, K. *Biochim. Biophys. Acta* **1973**, *314*, 372. (b) Moreover the ESR spectrum of O_2^- is not observable in solution owing to its degenerate ground state. See: Kasai, P. H.; Bishop, R. J., Chapter 6 in ref 8b.

(7) To ensure completely anhydrous conditions, sodium zeolite-Y was initially dehydrated fully at 200 °C for 5 h at 10^{-5} Torr. After $PO^{\bullet+}$ and $DQ^{\bullet+}$ were introduced via rigorously purified and anhydrous acetonitrile, the blue and green zeolites were further activated at 150 °C for 2 h at 10^{-5} Torr.

(8) See, however: (a) Rabo, J. A.; Kasai, P. H. *Prog. Solid State Chem.* **1975**, *9*, 1. (b) Rabo, J. A. *Zeolite Chemistry and Catalysis*; Rabo, J. A., Ed.; ACS Monograph 171; American Chemical Society, Washington, DC, 1976; Chapter 5.

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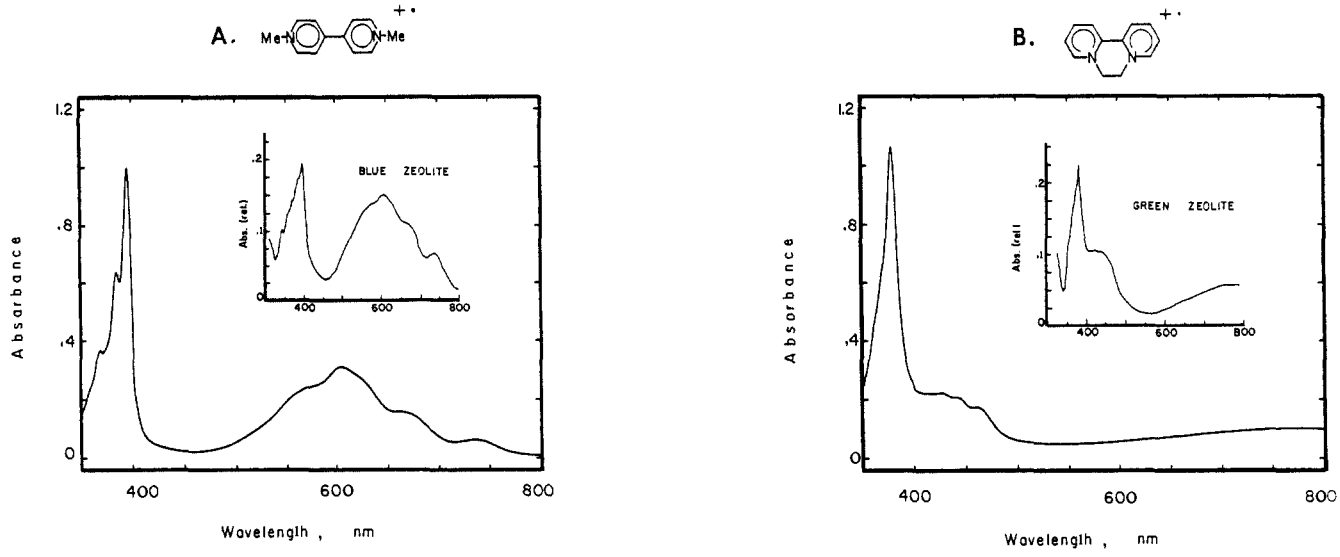


Figure 1. Comparison of the absorption spectra of (left) 2.5 × 10^{-5} M $MV^+PF_6^-$ and (right) 3.8 × 10^{-5} M $DQ^+PF_6^-$ in acetonitrile solution with the diffuse reflectance spectra of blue and green zeolites shown in the insets.

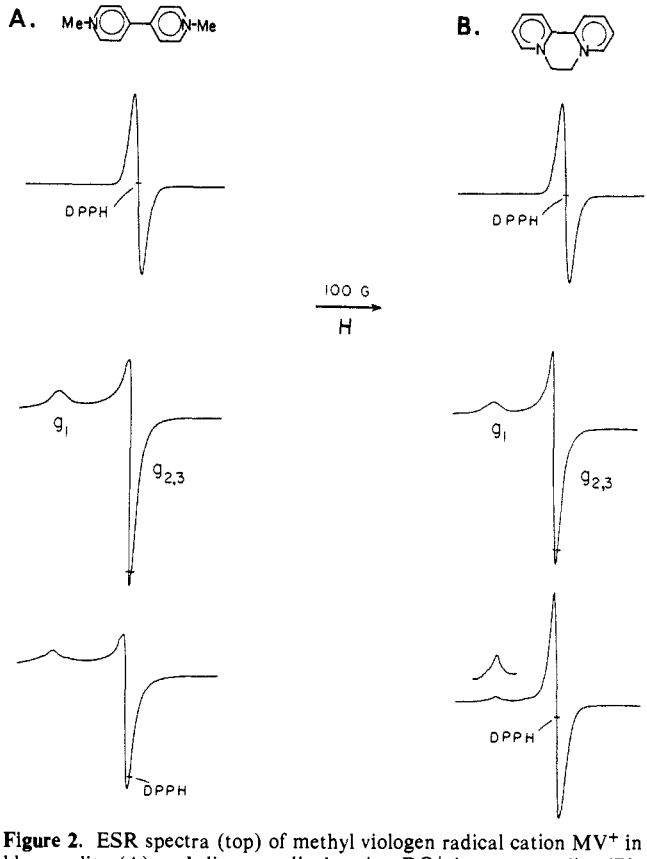
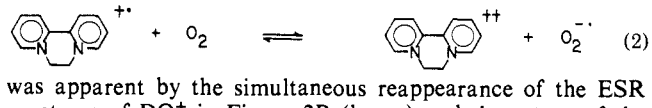


Figure 2. ESR spectra (top) of methyl viologen radical cation MV^+ in blue zeolite (A) and diquat radical cation DQ^+ in green zeolite (B), calibrated with DPPH $\langle g \rangle$ = 2.0037. Superoxide (middle spectra) produced from the introduction of dioxygen. After evacuation at <1 Torr (lower spectra).

treating NaY with $DQ^+PF_6^-$. The diffuse reflectance spectra in Figure 1 of the blue and green zeolites were the same as the absorption spectra in solutions of MV^+ and DQ^+, respectively. Both sets differed from the diffuse reflectance spectra of crystalline purple-black $MV^+PF_6^-$ and green-black $DQ^+PF_6^-$, which only showed featureless absorptions over the entire spectral range owing to their dimeric nature in the solid.[3a] As such, the blue and green zeolites can be regarded as "solid solutions" of MV^+ and DQ^+ that exist as discrete monomeric cation radicals—presumably due to the limited space within the supercage. This conclusion is supported by the isotropic ESR spectra of MV^+ ($\langle g \rangle$ = 2.0030) and DQ^+ ($\langle g \rangle$ = 2.0029), respectively, in Figure 2A,B (top). The ESR spectra with ΔH_{pp} = 17 G were unchanged at 77 K, and the isotropic g values were the same as those of MV^+ and DQ^+ in acetonitrile.

The blue zeolite was bleached instantly at −78 °C upon the introduction of dioxygen. This dramatic color change was accompanied by the disappearance of the ESR spectrum of MV^+. The concomitant appearance of the anisotropic ESR spectrum of superoxide with g_1 = 2.084, and $g_{2,3}$ = 2.005 is shown in Figure 2A (middle). The value of g_1 for O_2^- was consistent with it being paired with a monovalent cationic center,[9] either in MV^{2+} or in a nearby Na^+ center of the supercage. This ambiguity was more clearly resolved with g_1 = 2.065 for the superoxide generated in the green zeolite ($g_{2,3}$ = 2.003). The magnitude of this g shift is clearly consistent with an organic cation pair. Moreover the value of g_1 = 2.065 is intermediate between $g_1 \sim 2.08$ for univalent cations such as Na^+ and $g_1 \sim 2.05$ for divalent cations such as Ca^{2+} and Ba^{2+} in zeolites.[9] Such a g value accords with the ion-pair interaction of superoxide with both proximate cationic centers in DQ^{2+}, unlike the situation with MV^{2+} (vide supra) in which the cationic centers are far apart.

Bleaching of blue and green zeolites by dioxygen occurred at significantly different rates. Whereas the blue zeolite lost all of its color instantly at −78 °C, the loss of the gree color required more than 10 min under the same conditions. Such a variation in rate accords with the different oxidation potentials of MV^+ and DQ^+ with E° = −0.45 and −0.39 V versus SCE, respectively. Indeed the difference of ΔE° = 0.06 V was sufficient to render the electron transfer to dioxygen *irreversible* with MV^+ and *reversible* with DQ^+. Thus the ESR spectrum of superoxide from MV^+ was unchanged when the tube was evacuated to <1 Torr, as shown by the comparison of Figure 2A (middle) and (lower). By way of contrast, the ESR spectrum of superoxide from DQ^+ essentially disappeared when the tube was evacuated, and reversible electron transfer, i.e.,



(2)

was apparent by the simultaneous reappearance of the ESR spectrum of DQ^+ in Figure 2B (lower) and the return of the colorless solid to the original green color. Repetition of this cycle occurred more than a dozen times without apparent diminution of the ESR signals or degradation of the colors. Such a reversible electron transfer from superoxide ion pairs is unprecedented. Furthermore, we infer from the observed difference in superoxide

(9) Lunsford (Lunsford, J. H. *Catal. Rev.* **1973**, *8*, 135) describes the variations in the superoxide g tensors with cationic charge on the counterion.

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.

Acknowledgment. We thank T. M. Bockman for the bipyridinium salts and helpful suggestions and the National Science Foundation and the Robert A. Welch Foundation for financial assistance.

(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.

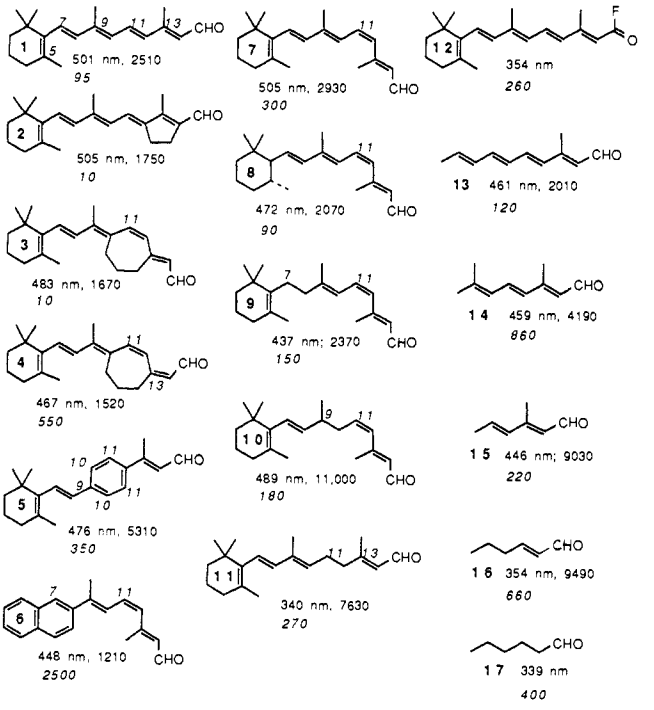

Figure 1. Phototaxis action spectrum data for retinal analogues. Action spectrum maxima in nm, opsin shifts[6] (difference between maxima of phototaxis and protonated Schiff base with *n*-butylamine) in cm^{-1}, and sensitivity in $m^2\ s/10^{18}$ photons (in italics). Compound sources are as follows: **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

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(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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