intramolecular rearrangement forms an activated intermediate $H_3Ru_3(CX)(CO)_9$* prior to CO addition. Possibilities for the nature of this intermediate include structures containing (1) one or more terminal hydride ligands, (2) an agostic[4] Ru-H-C bond (Figure 1, structure C), or (3) a fully formed C-H bond and an unsaturated metal site (Figure 1, structure D) which should rapidly react with CO. The third possibility is most likely since (1) and (2) are saturated and unlikely to add CO. Entropies of activation for the limiting rate constants for disappearance of $H_3Ru_3(CX)(CO)_9$ are close to zero [+6 (±2) (Cl), +1.6 (±1.5) (Ph), and −5 (±3) (CO_2Me) eu], consistent with an intramolecular mechanism, and the enthalpies of activation are relatively large [31.4 (±0.9) (Cl), 31.4 (±0.6) (Ph), and 25.1 (±1.0) (CO_2Me) kcal/mol]. The lower value for the activation enthalpy for the CO_2Me derivative may indicate participation of the acyl moiety in the transition state; the rate constant for rearrangement of $H_3Ru_3(CCO_2Me)(CO)_9$ to $H_2Ru_3(CHCO_2Me)(CO)_9$ is the same as that for reductive elimination of methyl acetate in the presence of CO, indicating the same rate-determining step for both reactions.[12]

The deuterium isotope effects, ${}^{H}k_{obsd}/{}^{D}k_{obsd}$, for the reductive elimination from $H_3Ru_3(CX)(CO)_9$ were measured to be 1.00 (±0.05) (3.8 atm, 60 °C) for X = CO_2Me and 0.64 (±0.06) (35 atm, 100 °C) for X = Ph. Isotope effects for reductive eliminations from monometallic complexes fall in the range 1.3-3.3,[1,21] but isotope effects for the formation of hydrocarbons by hydrogenations of carbon or CO on surfaces are frequently less than 1.[13] The inverse isotope effects can be explained by the existence of equilibria involving intermediates in which the force constant of the hydrogen is higher than in the ground state. The calculated equilibrium isotope effects for intermediates having (a) a terminal hydride ligand, (b) a fully formed C-H bond, and (c) an agostic Ru-H-C bond are 0.72, 0.44, and 0.54, respectively.[20] However, normal isotope effects are found for reductive eliminations of molecular hydrogen from clusters having bridging hydrides,[5,17] arguing against (a), and these isotope effects are measured at high CO pressures, where the rate-determining step is formation of $H_3Ru_3(CX)(CO)_9$*, assumed to have a fully formed C-H bond. Then the inverse isotope effect implies the existence of a preequilibrium involving a second intermediate, different than $H_3Ru_3(CX)(CO)_9$* but of the same formulation, most likely having an agostic Ru-H-C bond.[18]

The mechanism shown in Figure 1 fully accounts for the observed rate law and deuterium isotope effects. The structure of intermediate C containing an agostic Ru-H-C bond has precedence in the formation of the isostructural species $HFe_3(HCH)(CO)_9^{1-}$ and $H_3Ru_3(HCEt)(CO)_9^{1+}$.[14,15] Intermediates E and F are based upon the structures of the analogous Os clusters.[16] The slow step in the reductive elimination process is formation of D. The rate law for this mechanism is given by eq 2, of the same form as that found experimentally. At high CO

$$\text{rate} = [k_1k_3k_5P_{CO}/(k_2k_4 + (k_2 + k_3)k_5P_{CO})][H_3Ru_3(CX)(CO)_9] \quad (2)$$

pressures the rate-determining step is formation of the activated intermediate $H_3Ru_3(CX)(CO)_9$* (D) and the observed rate constant becomes k_1k_3/k_2 if $k_2 >> k_3$. Since the isotope effect upon the value of the equilibrium constant k_1/k_2 is <1, the value of ${k_{obsd}}^{H}/{k_{obsd}}^{D}$ will be <1 if the isotope effect upon k_3 is not much > 1.

In conclusion, reductive elimination of CH_3X from $H_3Ru_3(CX)(CO)_9$ proceeds by sequential formation of the three C-H bonds, at least two of which are formed by intramolecular processes. An inverse isotope effect for reductive elimination suggests a preequilibrium between species containing Ru-H-Ru and Ru-H-C bonds.

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Supplementary Material Available: Table of kinetic data for reductive elimination of CH_3X from $H_3Ru_3(CX)(CO)_9$, X = Ph, Cl, and CO_2Me, table of analysis of mass spectral data from the crossover experiment, and calculations of equilibrium isotope effects (4 pages). Ordering information is given on any current masthead page.

5-(Trifluoromethyl)bacteriorhodopsin Does Not Translocate Protons

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We wish to report that a proper fit of the retinal chromophore in the bacteriorhodopsin (bR) binding site is necessary for proton pumping. Bacteriorhodopsin[1], the pigment contained in the purple membrane of *Halobacterium halobium*, functions as a light-driven proton pump and converts solar energy into a proton gradient that is coupled to ATP synthesis.[2] The retinal chromophore is covalently linked to Lys-216[3] of the apoprotein via a protonated Schiff base (SBH^+) linkage.[4,5] There are two forms of bR,[2,6] the light-adapted bR^{LA} absorbing at 570 nm and the dark-adapted bR^{DA} absorbing at 560 nm, the chromophores of which are respectively *all-trans*-retinal and a 1:1 mixture of *trans*- and 13-*cis*-retinal. Both forms undergo a photocycle but only bR^{LA} translocates protons. The red shift to 570 nm in bR from 440 nm (in MeOH) in retinal/butylamine-SBH^+ has been attributed to electrostatic interactions between the protonated chromophore and the protein within the binding site (external point charge model, Figure 1).[7,8]

The present studies with 5-(trifluormethyl)(5-TFM)-5-norretinal[9] were undertaken in order to investigate the effect of electronic perturbation on the point charges near the β-ionone ring. Incubation of retinal oxime free apo-membrane[10] with *trans*-5-TFM-retinal (HEPES buffer, pH 7.0) in the dark for 60 min resulted in smooth formation of pigment (5-TFM-bR), the "dark-regenerated" pigment, λ_{max} 465 nm (Figure 2a). Irradiation

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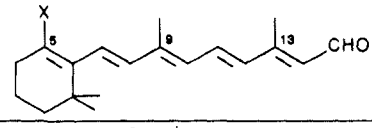
Table I. Absorption Maxima of Chromophores, Pigments, and Derivatives[a]



X	-CHO (MeOH)	SB (MeOH)	SBH^+ (MeOH)	pigment (HEPES)	opsin shift,[b] cm^{-1}	H^+ pumping
CH_3	380	365	440	568	5100	yes
CF_3	360	350	418	465	2400	no

[a] -CHO, aldehyde; SB, *n*-butylamine Schiff base; SBH^+, *n*-butylamine protonated Schiff base. [b] The opsin shifts denote the difference in cm^{-1} between the *n*-butylamine SBH^+ (in MeOH) and the pigment absorptions.[7]

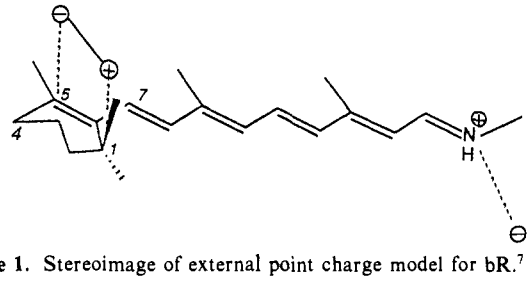


Figure 1. Stereoimage of external point charge model for bR.[7]

of the 5-TFM-bR for 20 min with a 1000-W tungsten lamp equipped with heat filters and a OG430 Schott cutoff filter gave the LA form which absorbs ca. 15% weaker but at the same wavelength (Figure 2b); leaving the light-adapted pigment in the dark for 24 h reverses the change to give the DA form with intensified absorption at 465 nm. Methylene chloride denaturation/extraction[11] followed by HPLC revealed the chromophores to be as follows: dark regenerated, all-*trans*; LA, 70% 13-*cis* and 30% all-*trans*; DA, 30% 13-*cis* and 70% all-trans. Note the difference in composition of the LA and DA species from those of natural bR.

The ca. 20-nm hypsochromic shift of 5-TFM-retinal and derivatives as compared to the parent *trans*-retinal and derivatives (Table I) can be accounted for by the electronegative character of the TFM group which would destabilize the excited state. However, a dramatic difference of 100 nm is seen in the TFM and natural pigments, both of which contain similar chromophores with six double bonds. The resonance Raman spectrum of 5-TFM-bR (DA) shows a band at 1650 cm^{-1}, which upon deuteration shifts to 1621 cm^{-1}; the chromophore is thus attached to the protein by an SBH^+ linkage.[12] The position of the Schiff base peak, as well as the deuteration shift, is quite different from the shift of 1642 to 1625 cm^{-1} in the native pigment.[12] Since these two parameters have been shown to be independent indices of the Schiff base environment,[12,13] the following differences between 5-TFM-bR and native bR suggest the Schiff base environments to be quite different; namely, in the TFM analogue the wavelength is higher in the protonated and lower in the deuterated species as compared to natural bR. The 100-nm blue shift in the λ_{max} of the analogue pigment can be rationalized by the model shown in Figure 1. Namely, the electronegativity and/or the bulk of the 5-TFM group hinders the approach of the β-ionone ring to the vicinity of the negative charge. Thus, although the Schiff base is protonated, the small opsin shift of 2400 cm^{-1} (Table I) suggests that the chromophore is not properly locked in the natural binding site; the geometry of the 6-s bond is also unknown for this pigment.[7,8]

To test the proton pumping ability, soybean phospholipid vesicles containing 5-TFM-bR (prepared by sonication method[14]) were

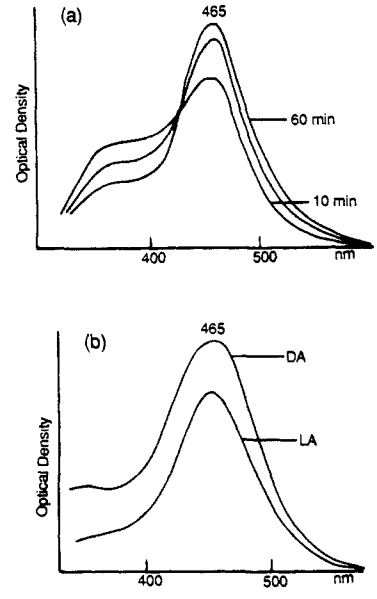


Figure 2. (a) Reconstitution of 5-TFM-bR. (b) Light and dark adaptation of 5-TFM-bR.

irradiated (conditions same as for light adaptation) and the pH change was monitored by a glass electrode. The proton pumping of this analogue was negligible compared to natural bR measured under similar conditions. Furthermore, absorption measurements carried out on hydrated films at 70 K showed formation of a red-shifted species, λ_{max} 495 nm (yield ~3%), which was stable up to 200 K; however, there was no indication for formation of an "M" type intermediate. These properties of the 5-TFM analogue are in contrast with those of the 13-TFM analogue[15] which absorbs at the long wavelength of 624 nm and retains proton pumping and normal photocycling ability; presumably the chromophore in this analogue is located in a binding site close to that of the natural (due to lack of electrostatic or steric hindrance). We previously showed that blocking of the *trans*/13-*cis* isomerization by a $-CH_2-CH_2$ bridge across C-11 and C-14 blocks proton pumping,[16] while preliminary studies with dihydro-bR analogues showed that the full unsaturated polyene system is also necessary for efficient pumping.[17] Current studies indicate that a proper fit of the chromophore in the binding site is required for proton translocation as well. A study of the photocycle process is being pursued with this analogue to clarify the proton-pumping phenomenon.

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