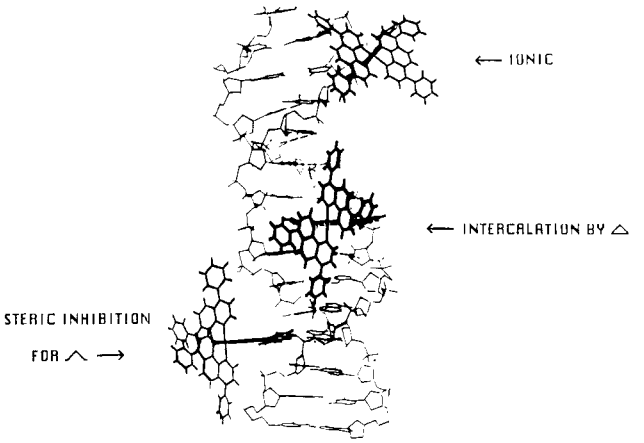

Figure 9. Enantiomeric selectivity for $Ru(DIP)_3^{2+}$ in binding to B-DNA. Top: Ionic binding of Λ isomer to the sugar–phosphate backbone. Middle: Intercalation of Δ isomer into the double helix. Bottom: Steric inhibition of intercalation of Λ isomer into the helix.

$(DIP)_3^{2+}$. This conclusion is further supported by polarization measurements in which higher values for polarization were observed for the Δ isomer than for the Λ isomer upon binding to DNA (Table III). It is clear from these experiments then that significant enantiomeric discrimination of Δ relative to Λ occurs in binding to B-DNA.

The enantiomeric selectivity governing binding of $Ru(DIP)_3^{2+}$ enantiomers to B-DNA is best understood in terms of the intercalative model depicted in Figure 9. When intercalated into the right-handed helix, the disposition of non-intercalated ligands of the Δ isomers matches the helical asymmetry of the right-handed DNA groove. For the Λ isomer the orientation of the ligand opposes the helical groove, and steric constraints completely preclude binding of Λ-$Ru(DIP)_3^{2+}$, with the large bulk of its non-intercalated ligands, within the helix. The Λ isomer may, however, bind electrostatically to the DNA helix, and in Figure 9, for the Λ isomer, such an electrostatic association, or ion condensation, is depicted. This binding mode, altering the surrounding water structure of the metal, is also likely to alter its emission properties. The increase in excited-state lifetimes might be expected to be smaller than that for intercalative binding, however. Quenching of condensed ruthenium cations by ferrocyanide would also likely be greater than that for intercalatively bound cations. The loosely held, ionically bound species, which may even be exchanging rapidly with free ruthenium, are significantly more accessible to the anionic quencher than the intercalatively bound species. Emission polarization results are consistent with this model as well, since rigidly bound intercalation of the Δ isomer would give rise to a polarization higher than that for the electrostatically bound Λ isomer.

Table IV. Binding Characteristics of Ruthenium Complexes with DNA

	methods of analysis			mode of binding	
probe	emission enhancement	$[Fe(CN)_6]^{4-}$ quenching	steady-state polarization	inter-calation	ionic binding
I	none	linear	none	–	– (?)
II	yes	biphasic	yes	+	+
III Δ	yes	biphasic	yes	+	+
III Λ	yes	linear	(no)	–	+

Conclusion

Modes of binding each of these metal complexes to the DNA helix may be distinguished readily by taking advantage of the different photophysical properties of ruthenium(II). A summary of these modes and the methods of analysis used for these determinations are given in Table IV. It is our conclusion that binding of $Ru(bpy)_3^{2+}$ to DNA is negligible, either by intercalation or by electrostatic interactions. In contrast both $Ru(phen)_3^{2+}$ and $Ru(DIP)_3^{2+}$ show a strong affinity for DNA. Here two modes of binding are evident and are assigned to intercalation and electrostatic association. Significant enantiomeric selectivity in intercalation is found for $Ru(DIP)_3^{2+}$, and it is the Δ isomer that binds preferentially by intercalation. For these complexes, the emission lifetimes are increased significantly upon binding to DNA, just as are the emission intensities. With use of ferrocyanide anion, the quenching of emission from these probes in the presence of DNA can clearly distinguish between bound and free forms and indeed between the rigid intercalatively bound form and the loose electrostatic component. Emission polarization provides additional evidence in support of these different binding modes.

Acknowledgment. The authors thank the National Science Foundation, the Army Office of Research, and the National Institutes of Health for the generous support of this research. We thank Dr. Charles Doubleday for providing the software employed in our single photon counting analyses, Dr. I. R. Gould for initial help in the experiments, and A. T. Danishefsky for the preparation of compounds.

Communications to the Editor

Dynamic Cis–Trans Isomerization of Retinal in Dark-Adapted Bacteriorhodopsin

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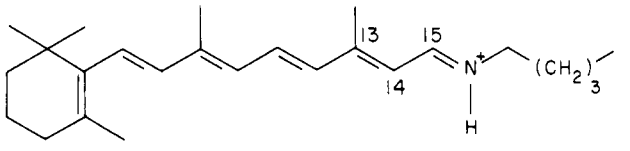
The purple membrane, the light-driven proton pump of *Halobacterium halobium*, contains a proteinic pigment, bacteriorhodopsin (bR), present in trimeric form, wherein the retinal is bound to lysine 216 of bR via a protonated Schiff base I.[1] Retinal,



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which in light-adapted bR (bR^{LA}) is ~100% all-trans, isomerizes in the dark to a resting, dark-adapted form, bR^{DA}, containing a ~1:1 bound mixture of *all-trans*- and 13-*cis*-retinal (eq 1). It

$$bR^{LA}\ (\sim 100\%\ \text{all-trans}) \underset{h\nu}{\overset{\text{dark}}{\rightleftharpoons}} (\sim 1{:}1\ \text{13-cis/all-trans})\ bR^{DA} \quad (1)$$

has been shown recently that two adjacent double bonds cis–trans isomerize in reaction 1. The chromophore of bR^{LA} is protonated 13-*trans*,15-*anti*-retinylidene Schiff base (I); in bR^{DA} half the

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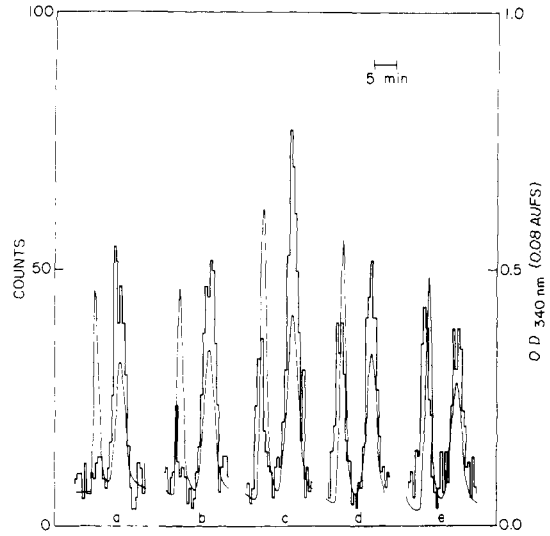

Figure 1. HPLC radioactivity traces of CH_2Cl_2 extracts of bR^{DA} reconstituted at 25 °C with a 1:1 mixture of [15-^{3}H]-*all-trans*- and unlabeled 13-*cis*-retinal taken at (a) 0, (b) 20, (c) 45, (d) 100, and (e) 240 min after combination.

retinal is the protonated 13-cis,15-syn isomer.[2] Our recent discovery of an enzyme-catalyzed one-step double-cis–trans isomerization of 6-keto-2,4-heptadienoates,[3] proceeding by a bicycle-pedal motion,[4] prompted us to investigate this apparent double isomerization because (1) while equilibrated mixtures of retinals in polar and nonpolar solvents contain 20–25% of 13-cis,[5] 13-*cis*-retinal, puzzlingly, reaches ~50% in bR^{DA} and (2) the kinetics of dark adaptation has been suggested to deviate from first order;[6] the question arises as to whether a 1:1 mixture of isomers is a stabilizing factor for the protein in its dark-adapted form. Are there sites in bR^{DA} that prefer to bind 13-cis and others that prefer to bind all-trans and once these sites are satisfied the membrane is in its most stable form and isomerization stops? The following experiments, however, indicate that double-cis–trans isomerization in equilibrated bR^{DA} continues as a first-order process.

bR was photobleached with hydroxylamine;[7] all subsequent operations were carried out in dim red light. The membrane was washed exhaustively with 2% bovine serum albumin (BSA) in 0.1 M HEPES buffer,[8] at which time the apomembrane showed no perceptible retinal oxime absorption. BSA washings were followed by washes with buffer. The titer of the membrane for *all-trans*-retinal was determined spectrophotometrically and then 0.9 equiv of a 1:1 mixture of isotopically labeled *all-trans*-[9] and unlabeled 13-*cis*-retinal was combined with apomembrane (pH 7.25, 0.1 M HEPES) in the dark to form bR^{DA}. Periodic determinations of isomer and label distributions were by HPLC on dried CH_2Cl_2 extracts of aliquots (~10 nmol) of the reconstituted bR^{DA}.[11] In early experiments deuterium was used as the label; in later, more extensive experiments tritium was used. Sample HPLC traces and radioactivity assays, for the experiment starting with [15-^{3}H]-*all-trans*-retinal, are shown in Figure 1. It is readily seen that bR, reconstituted with a 1:1 mixture of 13-*cis*- and *all-trans*-retinal, to initially provide bR^{DA}, continues to isomerize its retinal in the dark. The mole fraction of 13-*cis*-retinal, however, is observed to remain relatively constant at 0.48 ± 0.03, throughout.

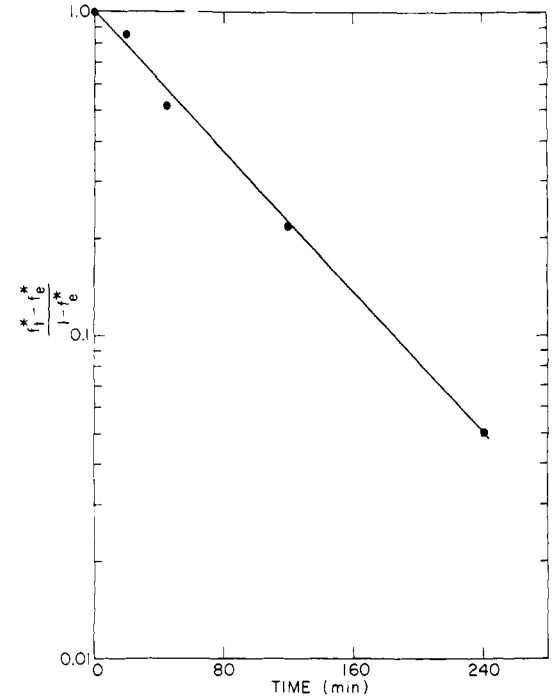

Figure 2. Plot of the fraction of radioactivity remaining in the all-trans isomer vs. time (r = 0.99).

The kinetic expression for a unimolecular reversible reaction,

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$$

is given by $\ln [(A_o - A_e)/(A_t - A_e)] = (k_1 + k_{-1})t$, where A_o, A_e, and A_t are the concentrations of A at $t = 0$, ∞, and t, respectively. A similar expression holds for isotopic exchange

$$\ln [(A_o^* - A_e^*)/(A_t^* - A_e^*)] = \ln [(1 - f_e^*)/(f_t^* - f_e^*)] = (k_1 + k_{-1})t$$

where the asterisks are for isotopically labeled species and the f's refer to the fraction of original (total) activity in isomer A. The results, plotted in Figure 2, indicate that although the retinal isomers are at chemical equilibrium cis–trans isomerization continues. The observed rate constant ($=k_1 + k_{-1}$), 1.27×10^{-2} min^{-1}, compares favorably with that measured (1.07×10^{-2} min^{-1}) spectrophotometrically at 592 nm for the kinetics of dark adaptation (eq 1) of a reconstituted aliquot of the same apomembrane fraction. Good first-order kinetics are followed.

Since retinal is surrounded by protein and lipid it would appear that catalyzed cis–trans isomerization is provided by the protein.

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In our previous report[3] an earlier proposed enzyme mechanism of nucleophilic catalysis[13] was shown to lead to an intermediate capable of bicycle-pedal motion and consequently to a one-step double-cis–trans isomerization. Similarly, attack of a nucleophile at C13 of I,[14] could, by bicycle-pedal-motion, reversibly yield the 13-cis,15-syn isomer after loss of the nucleophile. An alternate mechanism involving the movement of a counteranion from the region of the protonated imine to the vicinity of C13 of I also seems reasonable. This could induce transitory localization of the positive charge of I at C13 thereby reducing the C13–C14 and C15–C16 bond orders and allow concerted rotation about these bonds.[15] Aspartate 212 might fulfill either catalytic role, i.e., as a nucleophile or to provide electrostatic stabilization[16] during charge localization at C13. Apparent support for this idea comes from pH–rate data of dark isomerization.[17] Within the pH range 6.5–10.0 there is a (broad, shallow) bell-shape curve which has been verified in our laboratory and which could be accommodated by the interaction of a carboxylate ion with a protonated Schiff base. Further studies are required to establish the mechanism.

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Insertion of Elemental Sulfur into Tungsten–Carbon Bonds[1]

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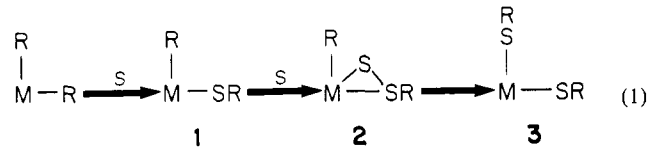
Insertion reactions of metal–carbon σ bonds are an integral and important part of transition-metal organometallic chemistry.[2] However, few such insertions involving elemental sulfur as the intervening species have been described.[3] We now wish to report the sequential transformations shown in eq 1



(1)

(where M = $(\eta^5\text{-}C_5H_5)W(NO)$ and R = CH_2SiMe_3) which constitute the first documented examples of this type of reactivity.

The new complexes **1–3** may be synthesized in high yields (i.e. >80% isolated) by employing the experimental conditions summarized in Scheme I. Hence, treatment of $(\eta^5\text{-}C_5H_5)W(NO)(CH_2SiMe_3)_2$[4] in toluene with a stoichiometric amount of elemental sulfur results in the formation of the thiolato complex **1**, which is ultimately obtained as a red-violet solid by crystallization from hexanes. Further treatment of **1** with an equimolar amount of sulfur in THF affords the alkylperthio complex **2**, which is isolable as an orange-red, crystalline solid from hexanes. When **2** is maintained in toluene at 35 °C for 12 h, it converts to the red dithiolate complex **3**, which may also be purified by crystallization from hexanes. Each of the complexes **1–3** may also be synthesized directly from $(\eta^5\text{-}C_5H_5)W(NO)(CH_2SiMe_3)_2$ as shown in Scheme I.

The complexes **1–3** are diamagnetic solids which are freely soluble in common organic solvents to give moderately air-sensitive solutions. Their spectroscopic properties[5] are consistent with their possessing monomeric "piano-stool" molecular structures, a fact that has been confirmed by single-crystal X-ray crystallographic analyses of **1** and **2**.[6] In particular, their solid-state molecular geometries indicate that the organosulfur ligands are attached to the tungsten centers by essentially single W–S bonds (~2.4 Å).[6] Hence, **1** is best viewed as being a 16-electron complex, whereas **2** can be formulated as an 18-electron species in which the $\eta^2\text{-}S_2R$ ligand functions as a formal 3-electron donor.

Given these facts, the sequential conversions summarized in eq 1 may then be viewed as occurring in the manner depicted in Scheme II. The original $(\eta^5\text{-}C_5H_5)W(NO)R_2$ reactant, a demonstrated 16-electron Lewis acid,[4] first coordinates S_8. This coordination renders the coordinated S atom prone to intramolecular nucleophilic attack by one of the alkyl groups,[7] a process that is accompanied by the concomitant expulsion of the residual sulfur fragment which equilibrates with the S_8 reagent in solution.[8] The resulting 16-electron complex **1** may coordinate a further molecule of S_8. Once bound, the coordinated S atom undergoes intramolecular attack by the SR ligand (a stronger nucleophile than the R group) to afford **2**. Upon warming, **2** converts to **3** (possibly also a 16-electron complex) by transfer of a sulfur atom, but on the basis of the present evidence no inferences concerning the mechanism of this step may be drawn. Nevertheless, since each of the transformations presented in eq 1 can be effected independently, it is clear that the activation barrier increases for each sequential step.

Mechanisms similar to that presented in Scheme II may well be operative for the analogous insertion reactions involving elemental oxygen[9] and selenium[10] for which intermediate species have neither been isolated nor detected spectroscopically. Consistent

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