a parity rule emerges, in which even n and odd n non-Kekulé acenes are expected to have qualitatively different properties. We have also shown that simple structural arguments can completely rationalize the results obtained from quantitative calculations. We are actively pursuing the experimental characterization of higher non-Kekulé acenes, in order to test the various predictions of the present work.

Acknowledgment. We thank the National Science Foundation for support of this work.

Appendix. Computational Procedures

HMO calculations were performed on an IBM PC/XT or PC/AT with the program HMO acquired from Serena Software, Bloomington, IN. The matrix diagonalization section could not solve structures with a sixfold degeneracy. This should be corrected by increasing the value of the convergence criterion, ρ.

SCF and CI calculations were performed using a PPP π-electron program[20] modified to calculate molecular orbitals for an open-shell configuration.[12] In addition to the standard parameter set,[20] we included a transannular core integral of −0.4 eV for four-membered rings.[21] The parameters are shown in Table V.

SCF calculations were performed for the non-Kekulé acenes up to $n = 6$. The molecular orbitals were optimized for a triplet state for $n = 1$ and 2, a quintet for $n = 3$ and 4, and a septet for $n = 5$ and 6. The calculations were performed using the optimized geometry of $\mathbf{3}_1$ obtained from ab initio calculations including π-space CI[13a] (square four-membered rings; ring CC bonds 1.471 Å; external CC bonds 1.374 Å).

For CI calculations four different types of CI were used. The excitations in each type of CI are (1) excitations within the NBMO's only ("NCI"), (2) excitations within the NBMO's and single excitations to and from the NBMO's ("NSCI"),[12] (3) excitations within the NBMO's and single and double excitations to and from the NBMOs ("NSDCI"), and (4) excitations within the NBMO's and all single excitations ("SCI"). Within each type of CI we also defined different "levels"; for example, a "2-level SCI" would include all single excitations involving the NBMO's and the two next-highest and two next-lowest molecular orbitals. In all the CI calculations, configurations with more than four unpaired electrons were excluded.[22]

Transition frequencies and oscillator strengths were calculated by standard methods for the transitions between the lowest triplet or singlet state and the five next-lowest states of the same multiplicity.

In order to obtain some indication of the reliability of these calculations, we performed calculations on trimethylenemethane (TMM), square cyclobutadiene, and *m*-xylylene. These calculations were done at regular geometries with CC bond lengths of 1.40 Å. The results of these calculations are presented in Table VI, along with values obtained by other means.

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(21) Wirz, J., private communication.

(22) We have also performed several calculations on $\mathbf{3}_1$ and $\mathbf{3}_2$ using all single and double excitations ("SDCI") excluding configurations with more than four unpaired electrons. The trends predicted by such calculations are in complete agreement with the "lower levels" of CI; however, the SDCI calculation may lead to an unbalanced treatment among the states. For $\mathbf{3}_1$, the only configuration with six-unpaired electrons has B_{2u} symmetry and is accessible by double excitation from the ground configuration. Exclusion of this configuration leads to a preferential destabilization of the lower lying $^3B_{2u}$ states relative to all other states. A similar imbalance exists for SDCI calculations on $\mathbf{3}_2$, and for SCI calculations for $\mathbf{3}_3$ and $\mathbf{3}_4$. For this reason, we do not consider the SDCI results to be quantitatively reliable for these structures.

MNDO Barrier Heights for Catalyzed Bicycle-Pedal, Hula-Twist, and Ordinary Cis–Trans Isomerizations of Protonated Retinal Schiff Base

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Abstract: Energy barriers to dark cis–trans isomerization in a protonated retinal Schiff base model in the presence and absence of electrostatic and nucleophilic catalysts have been calculated by the MNDO method. Three general processes—ordinary double bond isomerization, concerted isomerization about two double bonds by bicycle-pedal motion, and one-step double bond and adjacent single bond isomerization by hula-twist motion—are considered. Point negative charges or negatively charged nucleophiles near the protonated nitrogen substantially increase the barrier to cis–trans isomerization over what they would be in the absence of these agents. Negative charge or a nucleophile near C13 lowers the barrier to bicycle-pedal isomerization. Dark isomerization by a hula-twist motion requires greater energy and is not substantially aided by the placement of a negative charge or nucleophile near any of the skeletal atoms in the isomerizing system. The importance of this to the mechanism of dark–light adaption of bacteriorhodopsin is discussed.

Bacteriorhodopsin, the only protein within the purple membrane light-driven proton pump, binds retinal through a protonated Schiff base to its lysine-216.[1] In light-adapted bacteriorhodopsin, bound retinal molecules are in the *all-trans* form, but in the absence of light an isomerization to the 13-*cis* isomer occurs in about half of the molecules. The conversion is really a double isomerization; protonated *all-trans*,15-*anti*-retinal Schiff base isomerizes to the 13-*cis*,15-*syn* isomer.[2] It has been shown that this dark isomerization is a dynamic process with a half-life of about 40 min at ambient temperature.[3]

Another dark but faster cis–trans isomerization takes place in the main body of the bacteriorhodopsin photocycle. Following the first step wherein *all-trans*-retinal is photoisomerized to its 13-*cis* isomer, bound retinal must be rapidly reisomerized to the

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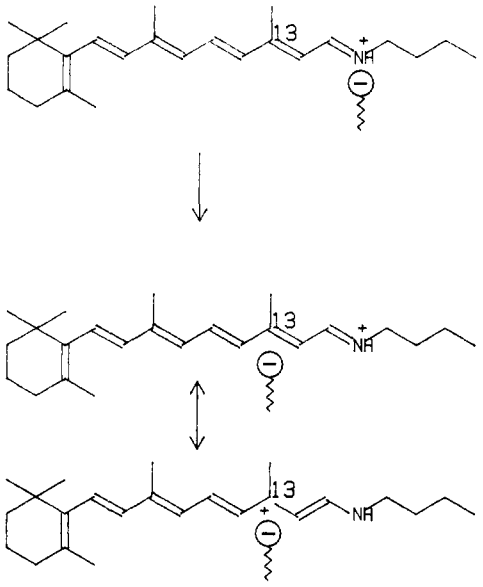
(2) Harbison, G. S.; Smith, S. O.; Pardoen, J. A.; Winkel, C.; Lugtenburg, J.; Herzfeld, J.; Matheis, R.; Griffin, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1706–1709.
(3) Seltzer, S.; Zuckermann, R. *J. Am. Chem. Soc.* **1985**, *107*, 5523–5525.

0002-7863/87/1509-1627$01.50/0

Scheme I



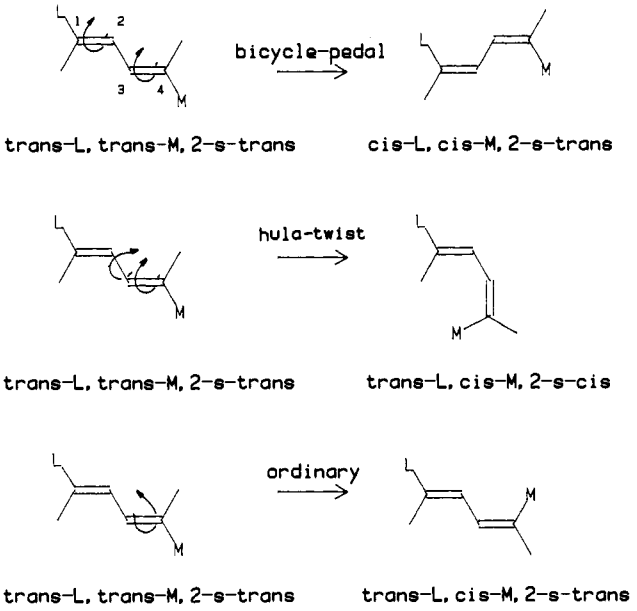
all-trans form to enable repetitive cycling and proton pumping. It does this within 10 ms. While part of the driving force for this specific thermal isomerization may be due to the utilization of energy stored in a strained protein conformation derived from the initial photon absorption and its attendant effects,[4] this cannot be the driving force in the light–dark adaption reaction.

The chromophore remains bound to the protein and shielded from solvent throughout these processes and, consequently, the protein is most likely the catalyst in these isomerization processes. Moreover, cis–trans isomerization of the retinal moiety, catalyzed by the protein, is regiospecific. Only the *all-trans* and 13-*cis* isomers of retinal are observed in native bacteriorhodopsin. Catalyzed isomerizations of free retinal or retinal Schiff bases in solution lead to several isomers. How the protein accomplishes these dark isomerizations is important to the understanding of the purple membrane cycle and may be pertinent to the specific dark regeneration of 11-*cis*-retinal and rhodopsin in the visual cycle.

We have previously suggested that cis–trans isomerization of the protonated retinal Schiff base in the absence of light may be brought about by its interaction with a negatively charged amino acid residue which would provide nucleophilic or electrostatic catalysis.[3] Properly placed electrostatic or nucleophilic catalysts would operate to reduce double bond orders and increase adjacent single bond orders thereby to facilitate rotation about reactant double bonds.[5] One of the two negative point charges near retinal is believed to be adjacent to the positively charged Schiff base nitrogen. The suggestion has been made that this negative charge could be provided by the carboxylate group of aspartate-212. Aspartate-212 and lysine-216, the important lysine binding retinal through a protonated Schiff base linkage, are expected to be almost one above the other in an α-helical structure. *The movement of the carboxylate group (driven by dynamic microconformational changes in the protein) from the protonated nitrogen to the vicinity of C13 of the retinal would lead to stabilization of an intermediate which could readily undergo facile internal rotation (see Scheme I).*[3] Cis–trans isomerization about the C13–C14 reactant double bond could be catalyzed by such a process. Within this scheme the carboxylate group is suggested to be found, most of the time, near the protonated nitrogen but moves to the C13 position for a finite time and then returns to its position near nitrogen. Consequently, at any one time, only a small fraction of the bacteriorhodopsin molecules have their aspartate-212 carboxylate anions near the C13 position of the bound retinal.

(4) Warshel, A.; Barboy, N. *J. Am. Chem. Soc.* **1982**, *104*, 1469–1476.
(5) Feliu, A. L.; Smith, K. J.; Seltzer, S. *J. Am. Chem. Soc.* **1984**, *106*, 3046–3047.

Scheme II



At present there are three models to rationalize the dynamics and stereochemistry of cis–trans isomerization (see Scheme II). The first involves concerted isomerization about two double bonds bonded to the same single bond, by a **bicycle-pedal motion**.[6] Second is the recently introduced **hula-twist** motion involving simultaneous twisting about a double bond and a directly adjacent single bond.[7] Lastly is the **ordinary** isomerization which involves rotation about one specific double bond converting it from cis to trans and vice versa. In view of the tight protein environment surrounding the bound retinal, isomerization by the former two models appears more feasible than the last because isomerization by the hula-twist and bicycle-pedal motions requires less protein reorganization during the process than isomerization by rotation about just one bond.

It is of interest to determine how realistic any of these models may be in rationalizing dark isomerization of the chromophore catalyzed by an aspartate residue. An approach to this question, which is described in this paper, uses the MNDO[8] method to calculate energy barriers for a 0° to 180° rotation (cis ⇌ trans) by each of the model processes under different conditions. Previous reports of MNDO calculations have demonstrated success in the ability of this method to calculate barriers to internal rotation in various systems.[9] Energy barriers to internal rotation about the C–C double bonds of ethylene (62.5 kcal/mol)[9a] and propylene

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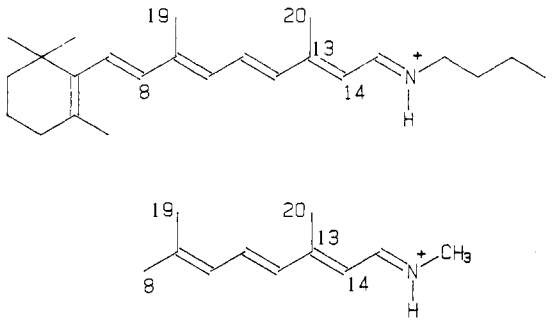

Figure 1. Above, $C_{24}H_{37}N^+$: protonated *all-trans*-retinal-lys-216 Schiff base (only tetramethylene fragment of lys-216 shown). Below, $C_{11}H_{18}N^+$: model for protonated *all-trans*-retinal-lys-216 Schiff base.

(60.3 kcal/mol),[9b] calculated by the semiempirical MNDO method, agree closely with experimental values of 65 and 61.3 kcal/mol, respectively. Calculated and observed barriers to internal rotation about the central C–C single bond of perfluorobutadiene agree well too.[9c] Very good agreement is also found between observed and calculated heats of cis–trans isomerization of 2-butene and 1,3-pentadiene.[9a] While present calculated and experimental energies can be expected to differ by a few kilocalories, it is likely that the relative energies obtained for sets of related systems will be more reliable.

The protonated *all-trans*-retinal Schiff base structure ($C_{24}H_{37}N^+$) was truncated to give the model ($C_{11}H_{18}N^+$) used in the present calculations (Figure 1) in order to reduce the time of computation. Without sacrificing any important nitrogen interactions, an *N*-methyl group was chosen to simulate the tetramethylene group of lysine. Since isomerizations about the C13–C14 and C15–N double bonds are primarily addressed in this study, it was deemed sufficient to include in the model only the hydrogens and heavy atoms between C8 and the *N*-methyl group; groups more distant than these would have little to no effect if they were also included. Included also are the methyl groups at C13 and C9 designated as C20 and C19, respectively. Barriers to rotation about the C13–C14 double bond alone, concerted double isomerization about the C13–C14 and C15–N bonds by a bicycle-pedal motion, and concerted rotation about the C13–C14 double bond and C14–C15 single bond (hula-twist) were calculated in the *absence* of a negative charge or nucleophile and in their *presence* at different positions in the model. As a starting point, standard bond lengths and bond angles were chosen. Bond lengths[10] and bond angles[10] in and around the isomerizing bond(s), however, were optimized in each calculation to achieve minimum energy for each increment of bond rotation. Except for the hydrogens of the methyl groups, the remaining atoms of the model occupy a single plane in the initial reactant. In most of the calculations the point negative charge (electrostatic catalysis) or a negatively charged nucleophile (nucleophilic/electrostatic catalysis) was placed 2.5 Å above or below the specific atoms on the side of the plane opposite to the path that nearest neighbor atoms make during the bond rotation process. In some calculations acetate ion was used to simulate the presence of the carboxylate ion of aspartate. Since little difference was found between the effect of acetate and chloride, chloride ion was used in subsequent calculations because of the shorter computational time required.

Bicycle-Pedal Double Cis–Trans Isomerization. In Table I are shown the calculated energies required to reach the top of the barrier under different conditions in concerted double cis–trans isomerization by bicycle-pedal motion about the C13–C14 and C15–N bonds. Also shown are the calculated energy differences between the *all-trans*-,15-*anti* and 13-*cis*,15-*syn* isomers in the presence and absence of negative charge or ion located at different positions in the model. In the bicycle-pedal process the integrity of the plane, incorporating atoms C14, C15, and the hydrogens directly bonded to them, H14 and H15, is maintained. This plane is rotated simultaneously about the C13–C14 and C15–N bonds so that the C15–C14–C13–C12 dihedral angle is always equal to the C14–C15–*N*-methyl-C dihedral angle. Heats of formation were calculated in 30° increments of dihedral angle between 0° and 180°, representing the initial *all-trans*,15-*anti* system isomerizing to the 13-*cis*,15-*syn* isomer.[10] Over this range, heats of formation increased monotonically until an angle of 90° was reached and then decreased to a new minimum at 180°.

Table I. Calculated Energy Barriers for Concerted Cis–Trans Isomerization about C13–C14 and C15–N by Bicycle Pedal Motion[a]

catalyst	2.5 Å from	$\Delta H^{\ddagger}_{(0\rightarrow 90°)}$, kcal	$\Delta H_{(0\rightarrow 180°)}$, kcal
none		24.6	4.4
negative charge	C13	20.8	4.6
negative charge	N	62.2	5.0
AcO^-	C13	16.0	4.2
Cl^-	C13	15.2	4.5
Cl^-	N	50.4	5.0

[a] Protonated *all-trans*,15-*anti*,14-*s*-*trans*-retinal Schiff base model is converted to the 13-*cis*,15-*syn*,14-*s*-*trans* isomer.

The protonated Schiff base, in the absence of a negative point charge or ion, exhibits a barrier to double cis–trans isomerization by a bicycle-pedal motion of about 25 kcal (Table I). The barrier is increased substantially if a point negative charge or a negative ion is placed 2.5 Å above the nitrogen. A negative charge close to the nitrogen clearly acts to stabilize the chromophore against thermal isomerization. If the negative charge or ion moves, as a result of dynamic microconformational changes in the protein, to the vicinity of C13 (see Scheme I), the barrier to isomerization about the two double bonds, by bicycle-pedal motion, is drastically lowered by 35–42 kcal to values which are reasonable for facile thermal isomerization at ambient temperature. In the planar reactant, a point negative charge, 2.5 Å above N, when moved to within 2.5 Å of C13, polarizes the local π-system so as to induce greater positive charge at C13 and, at the same time, reduces the C13–C14 and C15–N bond orders by about 23% and increases the C14–C15 bond order by 33% (Table II). If instead of a bare negative charge chloride ion is moved from N to C13 the effect is essentially the same but more pronounced. The C13–C14 and C15–N bond orders are decreased 27–31% and the C14–C15 bond order increased 46%. Bicycle-pedal motion is strongly facilitated by this excursion of negative charge or nucleophile.

It is important to note that by bringing a negative charge or negative ion to the vicinity of C13 of the protonated retinal Schiff base model, only the skeletal double and single bond orders between C13 and the protonated nitrogen are altered in a way to facilitate concerted isomerization about the C13–C14 and C15–N bonds. Double and single bond orders on the other side of C13, e.g., the C11–C12 double and C12–C13 single bonds, are hardly altered (see columns 3 and 4, Table II) and therefore the barrier to rotation about the C11–C12 double bond would remain high in spite of the presence of a negative charge or negative ion at neighboring C13.

Is the movement of the nucleophile from the vicinity of the protonated Schiff base nitrogen to C13, however, energetically feasible? Heats of formation were calculated for the planar model with chloride ion 2.5 Å directly above each of the four skeletal atoms important in the bicycle-pedal process. The system with chloride ion moving from N to C15, to C14, and then to C13 exhibits an alternation but a net decrease in energy. The heats of formation for chloride above N, C15, C14, and C13 are 89.3, 71.8, 88.3, and 80.9 kcal, respectively. Neglecting other factors at this time, it would appear that the movement of chloride ion from N to C13 can be accomplished with little cost in energy.

The dependence of barrier height on distance of the negative charge or ion from C13 is shown in Table III. It is interesting that the barrier to bicycle-pedal motion increases only very slowly with distance of the nucleophile or negative charge from C13 so that even at 4.5 Å the barrier is still 5–7 kcal below what it is in the absence of a nucleophile and low enough to allow double

(10) The following bond lengths—C13–C14, C14–C15, C15–N, and H_N–N— and angles—C20–C13–C12, C14–C13–C12, H14–C14–C13, C15–C14–C13, H15–C15–C14, and N–C15–C14—were optimized in the calculations.

isomerization to occur readily at ambient temperature.[11]

Isomerizations about One Double Bond. Calculated barriers to isomerization about one double bond in the absence and in the presence of chloride ion 2.5 Å above N or 2.5 Å below C13 are shown in Tables IV and V. In one set of calculations the barrier to isomerization about the C13–C14 bond was calculated while allowing the C20–C13–C14–H14 dihedral angle to vary in increments of 30°.[10] Similar calculations were carried out for isomerization about the C15–N bond where the H15–C15–N–H_N dihedral angle was changed by similar increments.[10] In both cases barriers increased monotonically to a maximum when the dihedral angle equalled to 90° and then decreased to a new minimum at 180°. Here again stabilization against isomerization about either the Schiff base linkage or about the C13–C14 double bond is provided by the presence of a negative charge or negative ion near N. Birge and Hubbard,[12] using an INDO–CISD MO method, previously noted a similar increase in barrier height to internal rotation about the C11=C12 bond upon placing a counterion near the C15=N group. Chloride, 2.5 Å below C13, reduces the individual barriers to rotation about the C13–C14 and C15–N double bonds to values which would make it likely, in the absence of other structural restrictions, for them to isomerize easily.

Isomerization by the Hula-Twist Motion. Finally calculations of the barriers for the hula-twist motion should be mentioned. This motion of simultaneous rotation about the C13–C14 double bond and the C14–C15 single bond was proposed to rationalize the ability of the protonated retinal Schiff base to undergo cis–trans isomerization about one double bond in the confined environment by the protein. By such a motion protonated *all-trans*,15-*anti*,14-*s-trans*-retinal Schiff base would be converted to its 13-*cis*,15-*anti*,14-*s-cis* isomer and vice versa. It should be recognized that although both the one-bond isomerization motion and the hula-twist motion, when applied to the *all-trans*,15-*anti* reactant model, form 13-*cis*,15-*anti* isomers, they are different. One-bond isomerization forms the 14-*s-trans* while the hula-twist isomerization leads to the less stable 14-*s-cis* isomer. In the model reactant structure (Figure 1) the H14–C14–C13–C12 and H15–C15–C14–H14 dihedral angles are 0° and 180°, respectively. An isomerization of the *all-trans*,15-*anti* isomer changes these dihedral angles to 180° and 0°, respectively. Simultaneously, the H14–C14–C13–C12 dihedral angle was reduced and the H15–C15–C14–H14 dihedral angle was increased in steps of 30° from their values in the reactant toward their values in the product. Heats of formation for each of these structures in the absence and presence of chloride were calculated.[10] As shown in Table VI, barriers to thermal isomerization via the hula-twist motion are very high and relatively independent of whether chloride is near C13, C14, C15, N, or even completely absent.

Concluding Remarks. The present calculations suggest that the movement of a negatively charged nucleophile, e.g., the carboxylate anion of a nearby aspartate residue, from the vicinity of the nitrogen to, for example, C13, is associated with a catalytic effect in cis–trans isomerization in the retinal protonated Schiff base of bacteriorhodopsin. Part of the catalytic effect is due to polarization and hence changes in bond orders of the π-system by a nearby negative charge, but an additional enhancement of this effect is also brought by partial electron donation to the π-system by the nucleophile. Thus a nucleophile near C13 catalyzes double isomerization about the C13–C14 and C15–N bonds by bicycle-pedal motion, and ordinary isomerization about the C13–C14 or C15–N bonds. Neglecting other protein interactions, the results of MNDO calculations suggest that only about 5 kcal/mol more is required to effect isomerization by bicycle-pedal motion than is required for ordinary cis–trans isomerization.

As for single isomerization by the hula-twist motion, the energy required appears exceedingly high for a ground-state process in the absence or even in the presence of negative charge or nucleophile in the vicinity of the isomerizing bond. It is probably only energetically possible for excited-state processes.

The role of negative ions in lowering double bond rotational barriers in retinal Schiff bases has been studied previously by two other groups.[13,14] In one model[13] an anion near the protonated Schiff base nitrogen and an acid group near retinal's cyclohexenyl ring were suggested to be provided by the protein. As one coordinate in the calculation of the energy barrier surface for rotation about any one particular double bond by ordinary isomerization, the lengths of all double bonds were allowed monotonically to approach those of single bonds while the lengths of interconnecting single bonds were allowed to approach those of double bonds. A complete alteration of original double and single bond lengths results in transfer of positive charge from nitrogen to the six-membered ring of retinal which induces deprotonation of the acid group near the six-membered ring and protonation of the anionic group near the nitrogen. In effect, negative charge placed at the six-membered ring stabilizes, to some degree, the conversion of all double bonds to single bonds and single bonds to double bonds thereby reducing the barrier to rotation about the original double bonds. Since all double bonds were increased in length it would appear that all barriers to rotation about original double bonds would be lowered. That, however, would lead to a loss of regiospecificity in the isomerization process. In the present study movement of the negative charge or negative ion from the region of the protonated nitrogen to the vicinity of C13 reduces only the bond orders of the C13–C14 and C15–N double bonds. It has little effect on the C11–C12 double bond order (Table II) and can account for the regiospecificity in the isomerization process.

Calculations of the lowering of the C13–C14 rotational barrier caused by placing a negative ion at different positions along the polyene chain of a model for a protonated retinal Schiff base have also been reported.[14] Although the C13–C14 barrier is lowest when an anion is near C9 rather than C11 or C13, it would appear that an anion at C9 would have the disadvantage of also lowering the C11–C12 and the C9–C10 barriers thereby reducing the regiospecificity of the isomerization reaction. In the area where the present and previous studies overlap, the conclusions are the same.

The protein affects the isomerization process in ways that are not taken into account in these calculations. MNDO calculations are carried out as if the reactants were in a vacuum ($\epsilon = 1.0$) when they are really in a pocket of the protein. It is believed that the environment surrounding the protonated Schiff base linkage is, except for the aspartate group, predominantly composed of the side chains of neutral amino acid residues. Differing estimates of the dielectric constant in the microenvironment of an ion pair within a protein, such as the aspartate-212-protonated Schiff base nitrogen couple, have been made recently. Warshel et al.[16] argue that a protein region around an ion pair is substantially polar. Honig and Hubbell,[17] however, find that such ion pairs can be stable in proteins in regions with dielectric constants of only 2–4. In any case, the electrostatic catalytic effects calculated here for a region of $\epsilon = 1$ will have to be reduced in regions of higher effective dielectric constant.

The protein environment is unaccounted for in another way and that is the steric restrictions on retinal imposed by the protein. This is most serious in the barriers calculated for ordinary double bond isomerizations shown in Tables IV and V since these motions

(11) A note of caution should be introduced here. Self Consistent Field was achieved readily by the standard MNDO method for all cases presented in Table III except for the 90°-twisted structure with chloride ion at 7 Å from C13. For this case it was necessary to resort to the SHIFT method of the MOPAC program whereby the unoccupied MOs are raised by some set value during the calculation and then this increment is removed after SCF is achieved.

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Table II. Effect of Position of Negative Charge or Negative Ion on C13–C14, C14–C15, and C15–N Bond Orders in the Reactant

catalyst	2.5 Å from	bond orders				
		C11–C12	C12–C13	C13–C14	C14–C15	C15–N
none		1.667	1.150	1.457	1.282	1.396
negative charge	N	1.803	1.052	1.712	1.08	1.610
negative charge	C13	1.703	1.12	1.34	1.44	1.24
chloride	N	1.807	1.049	1.722	1.077	1.598
chloride	C13	1.774	1.081	1.194	1.572	1.159

Table III. Effect of Varying the Distance between the Catalyst and C13 on the Calculated Barriers to Bicycle-Pedal Motion about the C13–C14 and C15–N Bonds[a]

catalyst	distance from C13, Å	$\Delta H^{\ddagger}_{(0\rightarrow 90°)}$, kcal
negative charge	2.5	20.8
negative charge	3.5	20.0
negative charge	4.5	19.8
negative charge	6.0	21.5
negative charge	7.0	22.4[b]
none		24.6
Cl^-	2.5	15.2
Cl^-	3.5	16.6
Cl^-	4.5	18.1
Cl^-	6.0	19.9
Cl^-	7.0	20.2
AcO^-	2.5	16.0
AcO^-	4.5	17.0

[a] Protonated *all-trans*,15-*anti*,14-*s-trans*-retinal Schiff base model is converted to the 13-*cis*,15-*syn*,14-*s-trans* isomer. [b] See ref 11.

Table IV. Calculated Barriers for One-Bond Rotation about C13–C14[a]

catalyst	2.5 Å	$\Delta H^{\ddagger}_{(0\rightarrow 90°)}$, kcal	$\Delta H_{(0\rightarrow 180°)}$, kcal
none		15.8	−0.4
Cl^-	N	38.0	0.2
Cl^-	C13	11.6	−0.1

[a] Protonated *all-trans*,15-*anti*,14-*s-trans*-retinal Schiff base model is converted to the 13-*cis*,15-*anti*,14-*s-trans* isomer.

Table V. Calculated Barriers for One-Bond Isomerization about C15–N[a]

catalyst	2.5 Å	$\Delta H^{\ddagger}_{(0\rightarrow 90°)}$, kcal	$\Delta H_{(0\rightarrow 180°)}$, kcal
none		21.2	
Cl^-	N	48.6	
Cl^-	C13	9.4	4.4

[a] Protonated *all-trans*,15-*anti*,14-*s-trans*-retinal Schiff base model is converted to the *all-trans*,15-*syn*,14-*s-trans* isomer.

Table VI. Calculated Barriers to Isomerization via the Hula-Twist Motion about C13–C14 and C14–C15 Bonds[a]

catalyst	2.5 Å	$\Delta H^{\ddagger}_{(0\rightarrow 90°)}$, kcal	$\Delta H_{(0\rightarrow 180°)}$, kcal
none		50.2	5.2
Cl^-	N	50.2	4.9
Cl^-	C13	39.9	
Cl^-	C14	49.3	
Cl^-	C14[b]	54.1	
Cl^-	C15	65.7	

[a] Protonated *all-trans*,15-*anti*,14-*s-trans*-retinal Schiff base is converted to the 13-*cis*,15-*anti*,14-*s-cis* isomer. [b] Cl^-–C14 distance optimized at the top of the barrier and that distance (2.14 Å) used throughout.

would require substantial reorganization of the protein pocket. It is believed that the energies for these processes are substantially underestimated by the present calculations. Neglect of the protein environment is less important in bicycle-pedal and hula-twist motions for cis–trans isomerization. In the absence of consideration of the protein environment, MNDO calculations suggest that bicycle-pedal motion, catalyzed by the presence of the carboxylate anion of an aspartate side chain at C13, would require an additional 5 kcal or less to accomplish its process than that necessary to carry out rotation about only one double bond. In view of the restrictive environment the lowest energy path for double isomerization would appear to be bicycle-pedal motion rather than two one-step isomerizations.

The conclusions drawn here are also applicable, with minor modification, to the rhodopsin system. In both rhodopsin and bacteriorhodopsin negative charges, furnished by amino acid side chains, are believed to be near the nitrogens of the respective protonated retinal Schiff bases.[18] The present calculations show that a point negative charge or nucleophile near the protonated nitrogen substantially increases the individual barriers to dark isomerization about either the C15–N or the C13–C14 bond. Similarly, the barrier to concerted isomerization about the C15–N and C13–C14 bonds via a bicycle-pedal motion is increased, relative to what it would be if the negative charge or ion were absent.

Of the five most stable cis–trans isomers of retinal, 11-*cis* is the least stable and highly reactive toward dark isomerization.[19] In the absence of light, isomeric integrity of 11-*cis*-retinal is enhanced by combination with opsin. The shielding of bound retinal from external agents and the van der Waals attractions between it and the amino acid side chains of the "protein pocket" are commonly believed to be responsible for the enhanced stability of the chromophore. This is probably only part of the stabilization. Maintaining a negative charge or nucleophile near the nitrogen would appear to stabilize all bound isomers of protonated retinal Schiff bases thereby preventing dark thermal isomerization about double bonds.

The present calculations demonstrate that catalysis of cis–trans isomerization about the C13–C14 bond of protonated retinal Schiff base could be achieved by bringing a negative charge or ion near C13 and by removing the negative charge from the vicinity of N. Similar catalysis for isomerizing about the C11–C12 bond is anticipated if the negative charge or ion were brought instead to the vicinity of C11. Thus rhodopsin could be catalytically regenerated by the interaction of a negatively charged group of the protein with C11 of the bound *all-trans*-retinal. It would appear, however, that such a process would be too slow to account for the rapid regeneration in higher forms of life. Nevertheless, it is interesting that in addition to the negative charge at N a second negative charge is predicted to be near C12–C14 in the bovine rhodopsin structure.[18a]

Acknowledgment. This research was carried out under contract DE-ACO2-76CH00016 with the U.S. Department of Energy and supported by its Division of Chemical Sciencies, Office of Basic Energy Sciences. The author would like to thank Dr. Marshall Newton for his encouragement in carrying out this study and Dr. Richard Stanton for his help in setting up the MOPAC program. The author also thanks one of the referees for pointing out ref 13, 14 and 16.

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