Influences on CO and O_2 Binding to Iron(II) Porphyrins

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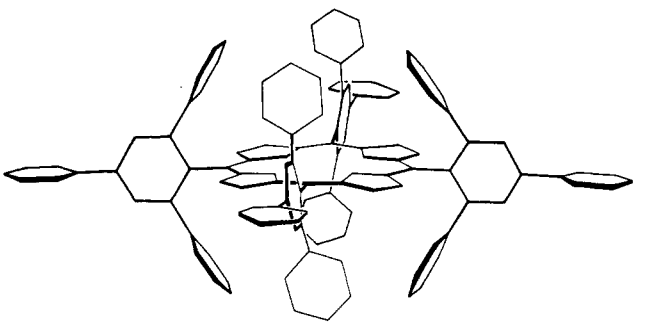
Abstract: Various CO and O_2 affinities have been determined for both an unprotected, "flat" porphyrin ((5,10,15,20-tetraphenylporphyrinato)iron(II)) and a "bis-pocket" porphyrin((5,10,15,20-tetrakis(2',4',6'-triphenylphenyl)porphyrinato)iron(II)) as a function of (1) steric hindrance of a bound imidazole, (2) hydrogen bonding to the bound imidazole, and (3) solvent polarity. Increased steric hindrance beyond a simple 2-methyl substituent has little effect on CO binding. Hydrogen bonding of 1,10-phenanthroline to the bound imidazole has little effect on CO binding, contrary to suggestions made on the basis of imidazolate ligation. Solvent polarity enhances O_2 but diminishes CO affinities, which provides for a new mechanism for CO/O_2 discrimination, an important function in O_2-carrying heme proteins to prevent endogenous CO poisoning.

Our understanding of the mechanisms whereby heme proteins regulate their affinities for small ligands remains incomplete, due to the many factors which contribute to the functioning of the active site.[1] Several model porphyrin complexes are capable of reversible O_2 or CO binding, but quantitative comparisons among these analogues or to the proteins reveal a wide range of observed ligand affinities.[2] Some of the influences that have been suggested to most strongly affect O_2 and CO binding include steric hindrance of axial ligation, local polarity, and hydrogen bonding to the coordinated imidazole, O_2 or CO. The relative importance of these and other contributions remains a matter of some dispute. For example, a controversy has developed over the discrimination of CO and O_2 by heme proteins. We and others have suggested[3] that the heme protein binding site structure disfavors the binding of CO (which is linear and normal to the porphyrin plane in model complexes[4] but tilted or bent in heme proteins[5]) relative to O_2 (which is bent in both cases[6]) as a means of preventing poisoning from endogenously produced CO. Evidence for this proposal comes from the very high CO affinities shown by some iron(II) porphyrin complexes[3c-e] and from the demonstration in other model complexes that steric discrimination of O_2 and CO is possible.[2b,3,8] In contrast, however, kinetic data from very different synthetic analogues have been used to argue against any significant steric contribution to CO and O_2 affinities in heme proteins.[7]

In order to address these apparent discrepancies between different systems, we have measured various CO and O_2 affinities for an unprotected, "flat" porphyrin (FeTPP)[9] and a protected "bis-pocket" porphyrin with completely nonpolar binding sites (FeTTPPP)[9] as a function of (1) steric hindrance in the bound



TTPPP

imidazole, (2) hydrogen bonding to the bound imidazole, and (3) solvation. We have found that increasing steric hindrance beyond a single 2-methyl substituent has little effect on CO binding, that hydrogen bonding of 1,10-phenanthroline to the coordinated imidazole has little effect on CO binding, and that increased local polarity enhances O_2 but diminishes CO affinities. This last observation provides another mechanism for CO/O_2 discrimination and helps explain the differences between various model complexes.

Experimental Section

All solvents used were purchased as reagent grade or better. Each was purified[10] in the following manner, subsequently freeze–thaw–degassed, and stored under N_2 in a Vacuum-Atmospheres inert atmosphere box

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(9) (a) Abbreviations used in this paper include: 2-EtIm, 2-ethylimidazole; Hb, hemoglobin; Im, imidazole; 2-*i*-PrIm, 2-isopropylimidazole; Im-chelated-protoheme, protoheme-*N*-[3-(imidazolyl)propyl]amide; $M = P_{1/2}(O_2)/P_{1/2}(CO)$; 2-MeIm, 2-methylimidazole; 1,2-Me_2Im, 1,2-dimethylimidazole; Me_4Im, 1,2,4,5-tetramethylimidazole; $P_{1/2}$, partial pressure of gas at half saturation; phen, 1,10-phenanthroline; TpivPP, 5,10,15,20-tetrakis[*o*-(pivalamido)phenyl]porphyrinate(2-), the "picket-fence" porphyrin; TPP, 5,10,15,20-tetraphenylporphyrinate(2-); TTPPP, 5,10,15,20-tetrakis(2,4,6-triphenyl)porphyrinate(2-). (b) The synthesis and characterization of the "bis-pocket" porphyrin (H_2TTPPP) and its complexes is reported elsewhere.[9c] (c) Suslick, K. S.; Fox, M. M. *J. Am. Chem. Soc.* **1983**, *105*, 3507.

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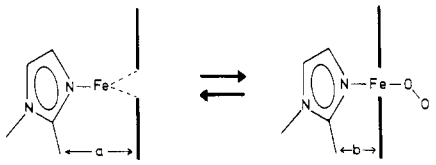

Figure 1. Out-of-plane displacements of 5- and 6-coordinate iron(II) porphyrins.

(<1 ppm of O_2 or H_2O). Mesitylene was treated with silica gel, dried initially with $CaCl_2$, and distilled from sodium. Toluene and benzene were distilled from sodium benzophenone ketyl. Chlorobenzene was washed with concentrated H_2SO_4, aqueous Na_2CO_3, and water, dried with $CaCl_2$, and finally distilled over P_2O_5. *o*-Dichlorobenzene was washed with concentrated H_2SO_4, dried with $CaCl_2$, and distilled from CaH_2. Purity of the solvents was crucial to avoid trace concentrations of ligands which would perturb the four-coordinate Fe^{II}TTPPP optical spectrum. Imidazoles were obtained from Aldrich, Fluka (Me_4Im), or BASF-Germany (2-*i*-PrIm), distilled or sublimed, and recrystallized at least three times under inert atmosphere (<1 ppm of O_2). The removal of trace impurities (e.g., unhindered imidazoles) is critical; recrystallization was continued until constancy of K_B and $P_{1/2}$ was achieved. 1,10-Phenanthroline was multiply recrystallized from benzene under inert atmosphere (<1 ppm of O_2). Prepurified O_2, CO, and compressed air were purcheased from NCG Industrial Gases, and the 5.25% O_2 in N_2 mixture was purchased from Union Carbide.

Samples for spectrophotometric titrations were made under N_2 in the inert atmosphere box.[9c] Spectra were recorded on a Hitachi 100-80A spectrophotometer equipped with thermostated cell holder and bath <±0.2 °C). Data analysis required only one limiting spectrum and is described elsewhere.[2a,11] For base binding studies, varying amounts of the imidazole of interest were added to a stock toluene solution of the four-coordinate iron(II) porphyrin. K_B values were determined at seven wavelengths and varied by less than 10% within any single titration and from run to run. For O_2 or CO binding studies, concentrations of the porphyrins were $\approx 4.0 \times 10^{-6}$ M, and imidazoles were chosen to ensure >99% five-coordinate complex formation; titrations were made by direct injection of known partial pressures of CO or O_2 into a 50-mL tonometer. $P_{1/2}$ values were determined at six wavelengths and varied by <±5% within a single run and by <±10% from run to run. Good isosbestic points were observed, and in all cases >90% reversibility was demonstrated by vacuum degassing.

Results and Discussion

Proximal Steric Effects. The influence of proximal steric hindrance (i.e., restraint of imidazole coordination) has been of long standing interest both in heme proteins and in synthetic analogues.[1] This concept lies at the core of the Perutz mechanism of Hb cooperativity. The use of 2-methyl-substituted imidazoles to hinder imidazole coordination and greatly diminish trans ligation of O_2 or CO has been well-established for synthetic analogues,[2] as shown schematically in Figure 1. Structural studies of five- and six-coordinate iron porphyrin complexes of both heme proteins and of synthetic analogues show[6] that the out of porphyrin plane displacement shown by five-coordinate, deoxy complexes is typically 0.5 Å, while upon O_2 or CO binding, this diminishes to <0.1 Å. Thus the steric contact between the 2-methyl substituent and the porphyrin is substantially increased upon oxygenation.[6b] No systematic studies have been reported, however, on the effects of varying amounts of steric hindrance in such systems.

In order to probe the steric rigidity of heme–imidazole interactions, we have examined a series of substituted imidazoles with increasing steric bulk, as given in Table I. If we extend Tolman's steric model for phosphorus ligands,[15] we may define minimum and maximum cone angles for least and most crowded conformations of the substituted imidazoles; these were determined from CPK models with a $M-N_{Im}$ distance of 2.1 Å. The increasing steric hindrance must lie between these estimates and, as one would expect, K_B (the formation constant for the 5-coordinate complex) decreases with increasing steric bulk. A previous study[13e] of *relative* binding of 1-*n*-butylimidazole vs. 1,2-Me_2Im to Fe^{II}heme(CO) is consistent with our observations, as is the binding of 2-alkylimidazoles to iron(III) and manganese(III) porphyrins.[13f,g] Interestingly, the binding of a sixth ligand, as reflected by $P_{1/2}$(CO), to the 5-coordinate complexes does not show as well-defined behavior. As noted before,[2] 2-methyl-substituted imidazoles dramatically reduce sixth site ligand affinities compared to unhindered imidazole (by 10^3). The further progression up through Me_4Im, or even no fifth nitrogenous base,[13d] results in very modest diminution to CO affinity, in spite of the change in spin states[16] ($S = 1$ for FeTPP vs. $S = 2$ for FeTPP(2-MeIm)). *Clearly, the initial effect of steric bulk is very high, but further increases do not influence trans ligation significantly.*

Table I. Steric Influences on CO Binding

system[a]	θ_{max},[b] deg	θ_{min},[b] deg	K_B, M^{-1}	$P_{1/2}$(CO), torr
FeTPP(Im)	100	100	*c*	0.002[d]
FeTPP(2-MeIm)	125	115	25 000[e]	0.14
FeTPP(1,2-Me_2Im)	125	115	27 000[f]	0.27
FeTPP(2-EtIm)	135	115	18 000	0.33
FeTPP(2-*i*-PrIm)	135	120	8 900	0.20
FeTPP(Me_4Im)	150	130	4 800	0.54
FeTPP				1.6[g]

[a] In toluene, 300 K unless otherwise noted. Imidazole concentrations for $P_{1/2}$(CO) determinations were selected to give initially >99% of the five-coordinate complex (i.e., $>100/K_B$). [b] Cone angles as described in text. [c] A value of ~ 4500 M^{-1} has been determined by deconvolution of consecutive equilibria (ref 13b). The dramatic change in pK_a of Im compared to any 2-alkyl-substituted imidazole precludes a meaningful comparison of K_B (ref 13c). [d] Benzene, 298 K, ref 13a. [e] This work; similar value reported at 298 K, ref 13b. [f] 298 K, ref 2e. [g] 293 K, ref 13d.

Table II. Influence of Imidazole Hydrogen Bonding

system	solvent	$P_{1/2}$(CO), torr (300 K)
FeTPP(2-MeIm)	toluene	0.14
FeTPP(2-MeIm)	toluene + 0.18 M phen	0.20
FeTPP(1,2-Me_2Im)	toluene	0.27
FeTPP(1,2-Me_2Im)	toluene + 0.18 M phen	0.34

Imidazole Hydrogen Bonding. Since many heme proteins have an iron-coordinated histidine, the N_δ–H of which can be involved in hydrogen bonding to other residues, it has been suggested that changes in such hydrogen bonding could in part account for changes in O_2 and CO affinities, such as Hb cooperativity.[17] Indeed, many physical properties of synthetic analogues change upon deprotonation of or hydrogen bonding to coordinated imidazoles,[18] including a decrease in the reduction potential,[18a,f] a

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Table III. Solvent Dependence of CO and O_2 Affinities

system[a]	solvent	π^{*b}	$P_{1/2}(CO)$, torr	$P_{1/2}(O_2)$, torr	M^c
FeTPP(1,2-Me_2Im)	mesitylene	0.41	0.23		
	toluene	0.54	0.27[d]		
	chloro-benzene	0.71	0.38		
	o-dichloro-benzene	0.80	0.68		
FeTTPPP(1,2-Me_2Im)	mesitylene	0.41	0.0080	640[e]	80 000
	toluene	0.54	0.0091	508[e]	55 800
	benzene	0.59	0.0092	473[e]	51 400
	chloro-benzene	0.71	0.012	299[e]	24 900
	o-dichloro-benzene	0.80	0.016	227[e]	14 200
FeTpivPP(1,2-Me_2Im)[f]	toluene	0.54	0.0089	38	4 280
Hb (human, T-state)[g]	H_2O, pH 7	1.09	~0.3	~40	~150

[a] 300 K unless otherwise noted. [b] Solvent polarity scale, ref 12. [c] $M \equiv P_{1/2}(O_2)/P_{1/2}(CO)$. [d] Other determinations of this $P_{1/2}(CO)$ are, at 296 K, 0.14 ± 0.02 torr, which is within experimental error of this value upon extrapolation to 300 K; ref 2c–e. [e] Reference 9c. [f] 298 K, ref 3d,e. [g] 298 K, ref 14.

diminution of the stability constants of ferric porphyrin imidazole and imidazolate complexes,[18b,c] and most importantly a drop in CO affinities of ferrous porphyrin *imidazolate* complexes.[18d,e] These studies suggest, but have not unambiguously demonstrated, the influence of proximal imidazole hydrogen bonding on O_2 or CO affinities of iron(II) porphyrins.

We can provide *direct* evidence on this question by using 1,10-phenanthroline (phen) to hydrogen bond to the coordinated 2-MeIm. It has been previously established that phen forms strong hydrogen bonds to coordinated imidazoles,[18a,19] and yet does not displace the imidazole ligand (we have confirmed this: K_B for FeTPP + phen is $<5\ M^{-1}$). Thus, the effect of the addition of phen to FeTPP(2-MeIm) on its CO affinity is a good measure of the influence of proximal hydrogen bonding. As shown in Table II, only a slight decrease in CO affinity occurs, which cannot, however, be due to hydrogen bonding, since FeTPP(1,2-Me_2Im) (which cannot form a hydrogen bond) shows the same decrease. This slight decrease in CO affinity is most likely due to the increase in solvent polarity which 0.18 M phen causes, rather than an interaction with the bound imidazole (see below).

Thus, we have found *no evidence for the significant influence of proximal hydrogen bonding on the functional property of CO binding* to a 5-coordinate iron(II) porphyrin complex. Such an effect may be expected to be greater on O_2 binding; further work is underway.

Solvation. In comparisons between heme proteins and synthetic analogues and among synthetic analogues, the influence of solvation on O_2 and CO binding presents a serious potential problem to interpretation of such data.[1d,f] Two aspects of solvation need to be considered: (1) the relative solvation energies of the five- and six-coordinate complexes and (2) the change in these relative solvation energies as the solvent is changed.[20] It is generally accepted, for example, that increased solvent polarity increases O_2 affinities due to the stabilization of the expected charge separation in such complexes.[1] The situation for CO binding, however, is confused. No thorough studies have been made, but in anecdotal reports, as solvent polarity increases, CO affinities sometimes decrease,[7c,21] and sometimes increase.[2e,g] The solvent systems in these reports vary widely, are often ill-defined solvent mixtures, and differ dramatically in their ability to coordinate, to hydrogen bond, and to act as Lewis acids.

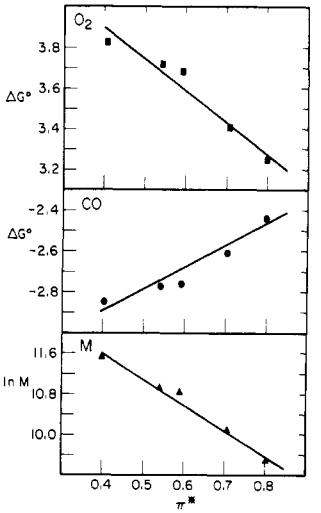

Figure 2. Solvent polarity effects on O_2 and CO binding. O_2: $\Delta G°$ (kcal/mol) for FeTTPPP(1,2-Me_2Im) + O_2 at 300 K vs. π^*. CO: $\Delta G°$ (kcal/mol) for FeTTPPP(1,2-Me_2Im) + CO at 300 K vs. π^*. M: ln [M] for FeTTPPP(1,2-Me_2Im) at 300 K vs. π^*. Solvents, in order of increasing π^* values, are meistylene, toluene, benzene, chlorobenzene, and *o*-dichlorobenzene; standard state is 1 torr.

In Table III, we present a systematic survey of the influence of solvation and solvent polarity on O_2 and CO binding. We utilized a closely analogous solvent series to minimize the complications of hydrogen bonding, solvent coordination, or variation of types of solvent–solute interaction.[12]

As we have noted before,[9c] due to the nonpolar nature of the binding site of our bis-pocket porphyrin, O_2 affinities are low (i.e., large $P_{1/2}$) compared to synthetic analogues with a more polar environment, in keeping with the poorer stabilization of the charge separation associated with Fe–O_2 bonding. In sharp contrast, however, the CO affinities of our bis-pocket porphyrin iron(II) complex, Fe(II)TTPPP(1,2-Me_2Im), are *not* diminished relative to other 1,2-Me_2Im complexes, thus demonstrating little charge separation for Fe–CO bonding. As seen in Figure 2, a strong linear correlation exists between the $\Delta G°$ of ligand binding for both O_2 and CO and the solvent polarity, as represented by the empirical solvation scale π^*, which measures the relative ability of the solvent to stabilize charge separation.[12] As solvent polarity increases, O_2 affinities increase, *but* CO affinities *decrease*. Exactly the same trend is observed for CO affinities of FeTPP(1,2-Me_2Im), demonstrating that the protecting pockets are not involved in this effect.

It should be noted that while the solvent polarity trends are present in both the flat and the bis-pocket porphyrin, the absolute $P_{1/2}(CO)$ values are quite different. This too is probably a solvation effect and has been discussed in detail elsewhere.[2g,11a] Briefly, it appears that there is a preferential solvation of the 5-coordinate, flat porphyrin complex relative to its CO adduct; such preferential solvation is minimized by the protecting pockets, thus enhancing observed CO affinities. Alternatively, a "stiffness" factor has been suggested[22] to originate from a putative steric congestion of the protected pocket porphyrins in their five-coordinate, domed state. A definitive choice between these is not yet possible.

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(20) As has been noted often in detail before,[1f,2a,3d,3e] using $P_{1/2}(CO)$ eliminates from consideration any influence of solvation on the free dissolved CO. Since the activity of the dissolved CO in different solvents is constant at constant ambient CO partial pressure, solvent dependences of solvation of the dissolved CO, or of its apparent concentration or of its Henry's law constants, are irrelevant.

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The comparison between polarity influences on O_2 and CO binding is quite intriguing. In the bis-pocket porphyrin, where both $P_{1/2}(O_2)$ and $P_{1/2}(CO)$ can be determined in a range of solvents, ln M (which is proportional to the differences in the free energies of the O_2 and CO complexes) correlates well with solvent polarity, as shown in Figure 2. Therefore, *this iron complex discriminates against CO binding as binding site polarity increases*. If it is accepted that O_2 affinities increase with polarity due to stabilization of charge separation, then it is reasonable to suggest that the dipole moment of the 6-coordinate CO complex must be smaller than the combined moments of free CO and the 5-coordinate complex. This suggests another mechanism by which heme proteins may differentiate between O_2 and CO and indicates the importance of the distal binding sites polarity to the ligand affinities of heme proteins.

These observations may also be used to explain in large measure the discrepancies between previous synthetic analogues: those with only moderately polar pockets in nonpolar solvents[3] (e.g., "picket-fence" porphyrins) will have much larger M values than those with open binding sites in polar media[7] (e.g., "chelated protoheme" in aqueous micelles). In addition, our observations are consistent with the change in M reported for the open-faced chelated protoheme in micellar water compared to benzene.[7c,21]

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Registry No. FeTPP(Im), 67077-69-4; FeTPP(2-MeIm), 48243-44-3; FeTPP(1,2-Me_2Im), 72186-60-8; FeTPP(2-EtIm), 90555-51-4; FeTPP(2-*i*-PrIm), 90583-04-3; FeTPP(Me_4Im), 90583-05-4; FeTPP, 16591-56-3; FeTTPPP(1,2-Me_2Im), 90583-06-5; CO, 630-08-0; O_2, 7782-44-7.

Catalysis of Vitamin A Aldehyde Isomerization by Primary and Secondary Amines

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Abstract: Model studies were conducted to quantitatively assess the role of Schiff base formation in catalyzing thermal isomerizations of vitamin A aldehydes (retinaldehyde), processes that are known to be critical for vertebrate vision and proton pumping in certain halophilic bacteria. Schiff base formation by itself, between vitamin A aldehydes and either saturated or aromatic amines, does not strongly enhance the measured thermal rates of isomerization. However, protonation of the Schiff bases strongly enhances their rates of isomerization; at 65 °C the first-order rates of thermal isomerization of 11-*cis*-retinal, the *n*-butylamine Schiff base, and the aniline Schiff base are 2.4×10^{-6}, 8.0×10^{-6}, and 2.8×10^{-6} s^{-1}, respectively. At 25 °C the HCl-catalyzed rates of isomerization of the *n*-butylamine Schiff bases of 11-*cis*-, 13-*cis*-, and 9-*cis*-retinal are 2×10^{-2}, 3×10^{-2}, and 9.4×10^{-4} s^{-1}, respectively. However, the rates of these isomerization reactions appear to be dependent on the strength of the conjugate base because base catalysis is probably required. Trifluoracetic acid proved to be a much weaker catalyst than HCl. Under conditions of approximately equal protonation, the first-order rates of isomerization of the *n*-butylamine and aniline Schiff bases of 11-*cis*-retinal are 2.6×10^{-6} and 7.9×10^{-4} s^{-1} at 25 °C. This result is most easily understood in terms of the greater nucleophilicity of chloride vs. trifluoroacetate. Adding nucleophilic bases to the protonated primary amine Schiff bases to enhance the rate of isomerization is not possible because the deprotonation of the Schiff base renders base catalysis ineffective. However, Schiff bases formed with secondary amines, such as piperidine, can obviate this problem because their positive charge cannot be neutralized by proton transfer. It is shown here that piperidine also catalyzes the isomerization of vitamin A aldehydes with a pseudo-first-order rate constant of $k = 4.1 \times 10^{-5}$ s^{-1} at 37 °C, but here the rate-limiting step is Schiff base formation itself, rather than the isomerization reactions. The model studies reported here suggest that the physiological mechanism of vitamin A aldehyde isomerization will involve positively charged Schiff base formation followed by nucleophilic attack on the relevant carbon–carbon double bond. The fact that biological molecules, such as reduced flavins and phosphatidylethanolamine (PE), catalyze the isomerizations of the vitamin A aldehydes is in accord with this view.

The mechanisms by which retinoid isomers can be catalytically interconverted are of considerably interest biologically. The initial event in vertebrate visual transduction involves the capture of a photon by rhodopsin, resulting in the isomerization of 11-*cis*-retinal, bound to opsin via a protonated Schiff base, to its all-*trans* congener.[1,2]. Subsequent hydrolysis of the *all-trans*-retinal–opsin Schiff base leads to the formation of *all-trans*-retinal and opsin. This overall process is called bleaching and is the only step in vision where light is directly involved. In order for rhodopsin regeneration to occur, *all-trans*-retinal, or a derivative thereof, must be thermally isomerized to its 11-*cis* congener. This bleaching/regeneration cycle is central to vision and dark adaptation and is an important component in the mechanisms which allow for maintainance of vision over a range of up to perhaps 10 log units in background light intensities.[3] Furthermore, in certain halophilic bacteria, a photochemical transformation of the *all-trans*- to the 13-*cis*-retinylidine Schiff base, probably in the protonated form, coupled with a thermal back reaction, is central to proton pumping and energy production in these organisms.[4] Finally, 13-*cis*-retionic acid is an important drug in the treatment of acne and other skin diseases and appears also to undergo thermal isomerization in vivo to its all-*trans* congener.[5] Of interest here are model systems through which the retinals can be thermally isomerized at ambient temperature which can serve as reference points for the in vivo isomerization mechanisms, whether the latter by enzymatic in nature or not.

To begin with, it is useful to consider the energetics of the isomerization reactions of 11-*cis*-retinal. It is noteworthy that 11-*cis*-retinaldehyde is thermally isomerized with an activation energy of approximately 25 kcal/mol.[6] A lowering of this ac-

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