Factors Affecting the Rate of Thermal Isomerization of 13-*cis*-Bacteriorhodopsin to All-Trans

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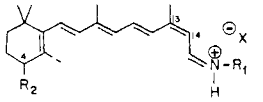
Bacteriorhodopsin (bR), the pigment of *Halobacterium halobium* contains in its dark-adapted form 13-*cis*- and *all-trans*-retinal iminium salt chromophores in thermal equilibrium. The light-adapted form, which contains only the *trans*-retinal isomer, undergoes, under the influence of light, a photocycle involving a photochemical isomerization to the 13-cis isomer, followed by several events which include a rapid thermal isomerization of 13-cis to all trans.[1] Photochemical and thermal isomerization occur also in visual pigments of vertebrates. Thus, the mechanism by which protonated retinal Schiff bases (PRSB) undergo thermal isomerization is of significant importance.[2,3] In this study, we suggest that the activation energy for thermal isomerization of 13-cis PRSB to all-trans PRSB and isomerization of the C═N⁺ bond is reduced (relative to retinal or its Schiff base) by charge delocalization. The protein can control the barrier for isomerization by interaction through space of the retinal skeleton with nonconjugated external charges and by separation of the positively charged nitrogen from its counteranion. These effects play a role in reducing the barrier for thermal isomerization of 13-*cis*-bR to all trans which allows a fast isomerization to complete the photocycle.



1 R_1 = n-Bu; R_2 = H

2 R_1 = t-Bu; R_2 = H

3 R_1 = t-Bu; R_2 = $-\overset{\oplus}{N}H(CH_3)_2\overset{\ominus}{X}$

4 R_1 = $(CH_2)_2\overset{\oplus}{N}H(CH_3)_2$; R_2 = H

X

a) CF_3COO
b) CCl_3COO
c) $CHCl_2COO$
d) CH_2BrCOO
e) Cl
f) Br
g) ClO_4

It was shown that excess of TFA (trifluoroacetic acid) red shifted the absorption maximum of PRSB in nonprotic solvents[4-6] due to the homoconjugation effect which weakens the interaction of the counteranion with the positively charged nitrogen, enhancing charge delocalization. This effect should increase bond alternation and may cause fast isomerization of 13-cis to trans PRSB. Thus, the *n*-butylamine Schiff base of 13-*cis*-retinal (**1a**) was protonated with 1 equiv of TFA in $CDCl_3$ and the isomerization was followed by ^1H NMR. Only C═N⁺ isomerization from the anti to the syn isomer was observed.[7,8] However, by increasing TFA concentration to 0.35 M, an instant C═N⁺ isomerization occurred followed by a relatively fast isomerization ($t_{1/2}$ = 12 min) of 13-cis to all trans until thermal equilibrium of ca. 2:8 was reached (Figure 1). Fast isomerization to all-trans was also observed using the *tert*-butylamine 13-*cis*-PRSB **2**, however, no C═N⁺ isomerization was detected using a 0.35 M concentration of TFA (Figure 2).

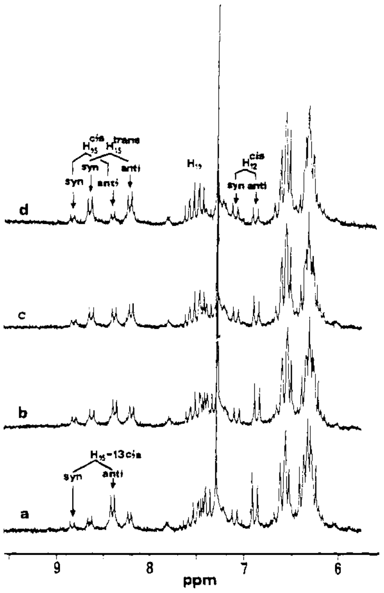


Figure 1. Isomerization of **1a** at 25 °C using excess TFA (0.35 M) to its all-trans isomer in $CDCl_3$, followed by vinyl proton NMR spectra: (a) 3 min, (b) 8 min, (c) 13 min, (d) 28 min.

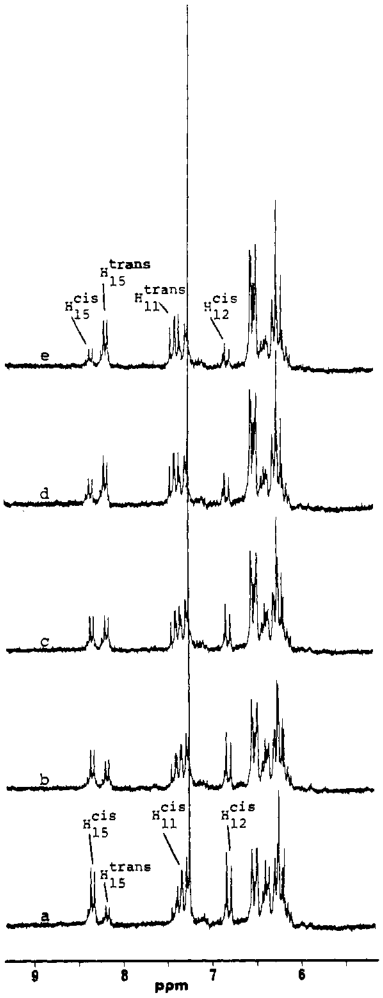


Figure 2. Isomerization of **2a** at 25 °C using 0.35 M TFA to its all-trans isomer in $CDCl_3$, followed by vinyl proton NMR spectra: (a) 3 min, (b) 8 min, (c) 12 min, (d) 17 min, (e) 30 min.

[†] Incumbent of the Morris and Ida Wolf Career Development Chair, in perpetuity established in memory of their parents by Mr. and Mrs. Edwin L. Fishman, Mr. and Mrs. Howard M. Wolf, and Mr. and Mrs. Marvin Mills.

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For further investigation of the effect of carboxylate anions on the isomerization rate, we protonated **2** with trichloroacetic acid (TCA), dichloroacetic acid, and bromoacetic acid (all up to 0.35 M solution of the corresponding acid). We clearly observed a decrease in the isomerization rate of the $C_{13}=C_{14}$ double bond ($t_{1/2}$ = 1 and 13 h for **2b** and **2c**) and no isomerization in the case of bromoacetic acid.

The interaction between the positively charged nitrogen and its counteranion can also be controlled by introducing counteranions of different sizes. Thus, protonation of RSB with $HClO_4$ in $CDCl_3$ caused a red shift relative to HCl.[9] Similarly, *n*-butylamine 13-cis PRSB salt of perchlorate underwent isomerization to all-trans (in $CDCl_3$) with $t_{1/2}$ = 50 min, while with corresponding chloride and bromide salts $t_{1/2}$ is 8 h. Charge delocalization can also be influenced by changing the substituent on the nitrogen. Substitution of the *n*-butyl group by *tert*-butyl slowed down the isomerization. *tert*-Butyl perchlorate salt of 13-cis showed only 20% isomerization after 10 h at 25 °C, while HCl salt **2e** exhibited a negligible amount of isomerization. All 13-cis PRSB salts described above exhibited no isomerization of C=C bond (after 6 h at 25 °C) but did show that of $C=N^+$ using methanol as a solvent.

Interaction through space with external charges may influence charge delocalization controlling the rate of thermal isomerization[10,11] of $C_{13}=C_{14}$ cis to trans. To investigate this possibility, we followed the thermal isomerization (in $CDCl_3$) of **3a** salt[12] carrying a positive charge in the neighborhood of the β-ionone ring. We could not observe either C=C or $C=N^+$ isomerization even after 5 h at 25 °C, using a 0.6 M concentration of TFA. On the contrary, a positive charge in the vicinity of the positively charged nitrogen **4a**[13] enhanced the thermal isomerization of 13-cis PRSB to all trans. This isomerization was observed even using 0.35 M solution of bromoacetic acid in $CDCl_3$ or TFA in CD_3OD ($t_{1/2}$ = 66 min), conditions that failed to isomerize 13-cis PRBS **1**.

Strong interaction between the counteranion and the positively charged nitrogen will stabilize the positive charge on the nitrogen and will decrease charge delocalization. This effect will slow down the isomerization rate of 13-cis to trans. Excess TFA will weaken the interaction between the carboxylate anion and the positive charge due to the homoconjugation effect, causing enhancement of charge delocalization. Similarly, this effect can be achieved by introducing a large counteranion like perchlorate or periodate. Carboxylate anions exhibiting a weak homoconjugation effect will cause slow isomerization. Indeed, we observed an isomerization rate in the order TFA, TCA, dichloroacetic acid, and bromoacetic acid. In all these cases we observed $C=N^+$ isomerization, indicating sufficient charge distribution along this bond to allow relatively fast thermal isomerization. Methanol, a leveling solvent, will cancel the effect of the counteranion on the positively charged nitrogen[9] as well as the homoconjugation effect. However, it will stabilize the positive charge on the nitrogen by strong solvolysis and thus will reduce charge delocalization toward the $C_{13}-C_{14}$ double bond, resulting in a slow isomerization. The positive charge on the nitrogen can also be stabilized by a *tert*-butyl group attached to the nitrogen.[14] Thus, no isomerization was observed in **2e** and **2g**.

External positive charge in the neighborhood of the β-ionone ring reduces charge delocalization[12,15] and bond alternation. Therefore, no thermal isomerization was observed in **3a**. A positive charge close to the nitrogen will cause the opposite effect. Thus, isomerization in **4** was observed, even in methanol, which prevents isomerization in the case of **1** and **2**.

In this study, we have shown that thermal isomerization of 13-cis to all-trans PRSB is controlled by charge delocalization, which is influenced by interaction through space with external charges and by the nature of the counteranion. This isomerization is accompanied by a fast $C=N^+$ isomerization of anti to syn.[16] Thus, our results strongly support the suggestion that 13-cis bR can isomerize smoothly to all trans due to interaction with a negative charge in the vicinity of the ring which will increase charge delocalization.[10,11,17] The isomerization can also be enhanced by separation of the positively charged nitrogen from its counteranion.[18-20] It should be noted that steric hindrance imposed by the protein might also influence the barrier for thermal isomerization. Our results point strongly to a protonation of the M_{412} intermediate of bR prior to thermal isomerization from 13-cis to all trans.[21]

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Registry No. *anti*-**1**, 68737-92-8; *syn*-**1a**, 92216-31-4; *syn-trans*-**1a**, 92098-19-6; *anti*-**1e**, 68737-89-3; *syn-trans*-**1e**, 92098-24-3; *anti*-**1f**, 92098-25-4; *syn-trans*-**1f**, 92098-26-5; *anti*-**1g**, 92098-22-1; *syn-trans*-**1g**, 92098-23-2; *anti*-**2**, 92098-20-9; *anti-trans*-**2a**, 92216-33-6; *syn-trans*-**2b**, 92098-21-0; *syn-trans*-**2c**, 92125-63-8; *anti*-**2d**, 92098-34-5; *anti*-**2g**, 92098-27-6; *syn-trans*-**2g**, 92125-64-9; *anti*-**3a**, 92098-29-8; *anti*-**4a**, 92098-31-2; *syn-trans*-**4a**, 92098-33-4.

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Synthesis and Characterization of a Homoleptic Actinide Alkyl. The Heptamethylthorate(IV) Ion: A Complex with Seven Metal-Carbon σ Bonds

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Although interest in preparing homoleptic actinide peralkyls dates back to the 1940s,[1] all synthetic attempts (principally involving uranium) have resulted in thermally unstable and largely uncharacterized/uncharacterizable products.[2-4] We reasoned

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