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A BLUE SHIFT OF PROTONATED RETINAL SCHIFF BASE. A MODEL STUDY FOR BACTERIORHODOPSIN

Mordechai Sheves*[†] and Timor Baasov
Department of Organic Chemistry, The Weizmann Institute of Science
Rehovot, Israel

ABSTRACT: A blue shift was observed in the absorption maxima of protonated retinal Schiff base. The shift is due to electrostatic interaction of a non-conjugated positive charge, located in the vicinity of the ionone moiety, with the polyene chromophore.

Visual pigments consist of a chromophore covalently bound to an apoprotein, opsin. Biochemical extraction studies have shown that in all pigments the chromophore is 11-cis retinal,[1,2] the parent aldehyde (retinal) of vit A, which is bound to the ε-amino terminal of a lysine residue of the apoprotein opsin via a protonated Schiff base (SBH^+) linkage.[3,4] The 11-cis retinal $\underline{SBH^+}$ formed from n-butylamine has its absorption maximum at 440 nm in methanol, whereas the maxima of visual pigments from various sources have maxima as far to the red as 580 nm. These red shifts (in cm^{-1}) from 440 nm, which are due to the effects of the protein environment were called "opsin shift".[5]

The purple membrane is a novel light energy transducing membrane which constitutes part of the plasma of *Halobacterium halobium*. The color of the purple membrane is due to a pigment which has its absorption maxima at 560 nm, and has all-trans retinal as its chromophoric group linked to a lysine[6] through a protonated Schiff base.

Recently, Nakanishi[5,7] proposed an external point charge model, which places, in addition to a counter-anion near the Schiff base iminium nitrogen, a second negative charge. In bovine rhodopsin, where the opsin shift is 2730 cm^{-1}, the second negative charge is located in the vicinity of carbons 12-14 of the chromophore. In bacteriorhodopsin (opsin-shift-4870 cm^{-1}), the second negative charge is located in the vicinity of the ionone moiety.

In order to find out if a non-conjugated charge in the vicinity of the ionone ring can affect the absorption maxima of retinyl-iminium polyene, we synthesized compounds $\underline{1}$, $\underline{2}$ and $\underline{3}$.

β-Ionone was brominated with NBS in refluxing CCl_4 for 1 hour and then was reacted at room temperature in THF with dimethylamine for 12 hours, to give amino-keto $\underline{4}$.[8] Condensation with sodium salt of triethyl phosphonoacetate at 25°C gave amino-ester $\underline{5}$. Reduction with diisobutylaluminium hydride followed by oxidation with MnO_2 afforded amino-aldehyde $\underline{6}$.[9] A second condensation of $\underline{6}$ with sodium salt of diethyl 4-phosphono-3-methyl-crotononitrile gave nitrile

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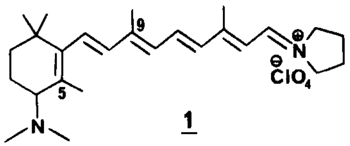
[†] Bat Sheva Fellow 1981-1983



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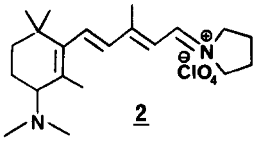

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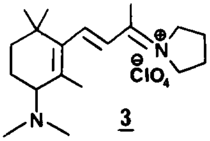

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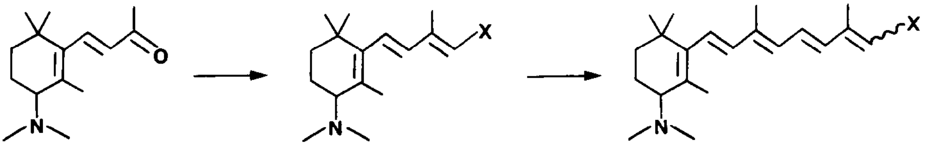
7, which after reduction with diisobutylaluminium hydride gave amino-aldehyde 8[10] (as a mixture of two isomers). Compounds 4, 6 and 8 were condensed with pyrrolidine perchlorate to give 3, 2 and 1, respectively.



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5 X=CO_2Et

6 X=CHO

7 X=CN

8 X=CHO

Compound 1 absorbs at 443 nm in EtOH. Protonation of the amino group at C_4 caused a blue shift to 430 nm. This blue shift (683 cm^{-1}) increased to 1109 cm^{-1}, when the absorption maximum was taken in $CHCl_3$.

The blue shifts observed by protonation of the amino group at C-4 increased as the length of the polyene decreased. Compound 2 exhibited a blue shift of 3218 cm^{-1} in EtOH and 3540 cm^{-1} in $CHCl_3$, while 3 gave a blue shift of 3740 cm^{-1} in $CHCl_3$ (Table).

These results show that a non-conjugated charge in the vicinity of the ionone ring can influence the absorption maxima of retinyl iminium polyene by electrostatic interaction through space.[11] A positive charge located ca. 3Å from C-5 caused a blue shift of 1109 cm^{-1} (in $CHCl_3$). This shift is smaller than the one observed in a compound having a positive charge located ca. 3Å from carbon 9[12] (ca. 2150 cm^{-1}).

The blue shift observed is probably caused by destabilization of the excited state relative to the ground state. Theoretical calculations[13,14] on protonated retinal Schiff base predicted that the charge on the nitrogen is positive and that it is smaller in the excited state than in the ground state. This phenomena is due to positive charge migration toward the ring upon excitation of protonated retinal Schiff base. Thus, an external positive charge in the vicinity of the ring will destabilize the excited state relative to the ground state. The bigger blue shift observed in a compound carrying a positive charge in the vicinity of carbon 9 than the one

Table: λ_{max}/nm of Protonated Schiff Bases

Chromophore	pH	λ_{max}	Solvent	$\Delta\nu/cm^{-1}$ [a]
1	8	443	EtOH	
1	2[b]	430	EtOH	683
1	8	467	$CHCl_3$	
1	2[b]	444	$CHCl_3$	1109
2	8	378	EtOH	
2	2[b]	337	EtOH	3218
2	8	393	$CHCl_3$	
2	2[b]	345	$CHCl_3$	3540
3	8	343	$CHCl_3$	
3	2[b]	304	$CHCl_3$	3740

[a] $\Delta\nu$ between pH 8 and pH 2 in the same solvent.

[b] Acidified with HCl.

observed for compound 1 suggests that in the excited state the density of positive charge at carbons 8-10 is larger than at carbon 5. The increasing blue shifts observed in compounds 1, 2 and 3 (by protonation of the amino group), suggest that the density of positive charge at carbon 5 in the excited state increases as the polyene becomes shorter. In a shorter chromophore, the charge is distributed between a smaller number of carbons, resulting in larger density of positive charge at carbon 5.

The spectroscopic information obtained encourages the synthesis of a compound carrying a negative charge at carbon 5, which will allow the evaluation of the external point charge model for bacteriorhodopsin.

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References and Notes

1. a) G. Wald, Nature (London), 1968, 219, 800.
   b) R. Hubbard and A. Kropf, Proc. Natl. Acad. Sci. U.S.A., 1958, 44, 130.
2. V. Balogh-Nair and K. Nakanishi, in "Stereochemistry". Tamm, Ch., Ed., Elsevier Biomedical Press: Amsterdam, 1982, Chapter 7, 283-334.

3. R. Callender and B. Honig, An. Rev. Biophys. Bioorg., 1977, 6, 33.
4. K.J. Rothschild and H. Marera, Proc. Natl. Acad. Sci., U.S.A., 1982, 79, 4045.
5. K. Nakanishi, V. Balogh-Nair, M. Arnaboldi, K. Tsujimoto and B. Honig, J. Am. Chem. Soc., 1980, 102, 7945.
6. D. Oesterhelt and W. Stoeckenius, Nature (London), New Biol., 1971, 233, 149.
7. B. Honig, U. Dinur, K. Nakanishi, V. Balogh-Nair, M.A. Gawinowicz, M. Arnaboldi and M.G. Motto, J. Am. Chem. Soc., 1979, 101.
8. ^{1}H n.m.r. ($CDCl_3$) δ 1.05 (6H, s), 1.79 (3H, s), 2.22 (6H, s, $(CH_3)_2N$), 2.32 (3H, s), 3.08 (1H, t, J 8 Hz, 4-H), 6.11 (1H, d, J 16.5 Hz, 8-H), 7.25 (1H, d, J 16.5 Hz, 7-H).
9. ^{1}H n.m.r. ($CDCl_3$) δ 1.03 (6H, s), 1.75 (3H, s), 2.23 (6H, s, $(CH_3)_2N$), 2.32 (3H, s), 3.09 (1H, t, J 8 Hz, 4-H), 5.95 (1H, d, J 8 Hz, 10-H), 6.17 (1H, d, J 16.2 Hz, 8-H), 6.75 (1H, d, J 16.2 Hz, 7-H), 10.14 (1H, d, J 8 Hz, 11-H).
10. ^{1}H n.m.r. ($CDCl_3$) δ 1.04 (6H, s), 1.76 (3H, s), 2.02 (3H, s), 2.33 (3H, s), 3.09 (1H, t, J 8Hz, 4-H), 5.96 (1H, d, J 8 Hz, 14-H), 6.2-6.9 (4H, m, 7-H, 8-H, 10-H, 12-H), 10.11 (1H, d, J 8 Hz, 15-H).
11. a) M. Sheves, K. Nakanishi and B. Honig, J. Am. Chem. Soc., 1979, 101, 7086.
    b) I. Tabushi, Y. Kuroda and K. Shimokawa, J. Am. Chem. Soc., 1979, 101, 4759.
12. M. Sheves, T. Baasov and N. Friedman, J. Chem. Soc. Chem. Comm., in press.
13. a) H. Suzuki, T. Komatsu and T. Kato, J. Phys. Soc. Jap., 1973, 34, 156.
    b) H. Suzuki, T. Komatsu and H. Kitajima, J. Phys. Soc. Jap., 1974, 37, 177.
14. B. Honig, A.D. Greenberg, U. Dinur and T.G. Ebrey, Biochemistry, 1976, 15, 4593.

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