Dielectric Medium Effects on Absorption Maxima of Protonated Retinylidene Schiff Bases as Models of Rhodopsin

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Dielectric medium effects on absorption maxima of protonated retinal Schiff bases (PSB) are studied using a reaction field theory incorporated into the INDO/S method. The calculation shows that a counterion, such as carboxylate, has no explicit role in regulating the absorption maximum of PSB even in a low dielectric medium.

Visual pigment, rhodopsin (Rh), possesses a chromophore retinal bound to apoprotein opsin via protonated Schiff base linkage.[1] The absorption maximum of the chromophore exhibits a large bathochromic shift compared with those of protonated retinylidene Schiff bases (PSB) in solution. It has been believed that such a spectral shift are caused chiefly by electrostatic effects of counterion (i. e., external charge) placed along the conjugated chain.[2] A variety of theoretical studies have been addressed to building of a detailed molecular model.[3] Recently, for example, Smith et. al.[4] determined the spatial arrangement of the counterion relative to the PSB moiety in Rh, a model which could consistently explain the experimental data for the absorption maximum and ^{13}C NMR chemical shifts of Rh. However, most of those studies have given no apparent attention to effects of the surrounding protein matrix as a dielectric medium. The importance of dielectric response in proteins has been well substantiated since the pioneering work of Warshel et. al.[5] Here, we report that dielectric medium effects cause a significant amount of blue-shift to the absorption maximum of PSB with and without a counterion, and hence the absorption spectrum of Rh may not be explained only by the conventional picture such as the external point charge model.

Several types of reaction field theory have been proposed.[6] Among them, the matrix inversion boundary element method, developed by our group[7] and Tomasi's group,[8] may be one of the most reliable and advanced methods. This method has successfully applied to elucidating ground state properties of molecules in condensed phase. In particular, recent studies have demonstrated that dielectric effects of protein matrices provide a significant contribution to enzymic reactions of serine protease and carbonic anhydrase.[9,10] In this study, our reaction field theory is applied to properties involving excited states. The theory was incorporated into the INDO/S molecular orbital program,[11] which can handle single-excitation configuration interaction (SCI) . We took into account configurations whose 0th order transition energy is up to 10 eV. This threshold was chosen so that the calculation for the lowest π-π^* transition of some retinal derivatives reproduces the corresponding experimental values. The geometries of the molecules studied (Figure 1) were optimized with the PM3 method[12] which is incorporated in the program MOPAC 6.01.[13] Then, the dihedral angles for C5-C6-C7-C8 and C11-C12-C13-C14 of 11-*cis*-retinal moiety were fixed at 45° and -140°, respectively.[4] Compound **1** was used as a model of 11-*cis*- PSB. **1** has the same chain length as retinal, but no ionone ring. The dihedral angles for the C5-C6-C7-C8 and C11-C12-C13-C14 linkages were taken to be identical with those for 11-*cis*-retinal, respectively.

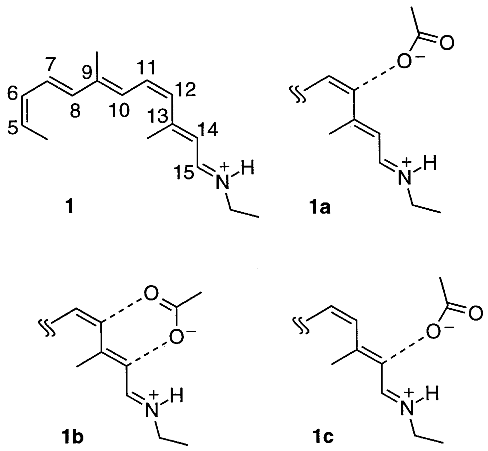

Figure 1. Molecular structure of the compound studied as a model of 11-*cis*-retinylidene PSB. **1a-c** are variations in the relative position of counterion.

Table 1. Calculated absorption maxima for 11-*cis*-Retinal PSB and its model compounds (in nm)

	$\varepsilon=1$	$\varepsilon=4$
11-*cis*-retinal PSB	537.5	427.8
with carboxylate	501.3	429.1
Model Compound **1**	516.8	418.3
with carboxylate	494.6	419.5

First, according to Smith et. al.,[4] carboxylate was placed as a counterion so that one oxygen atom was distant from C12 by 3.0 Å, and the angle of O-C12-H12 was 60°. Table 1 lists the wavelengths of the lowest π-π^* transition of 11-*cis*- PSB with such a counterion, together with those for 11-*cis*- PSB without counterion. When dielectric constant ε equals one (in vacuo state), the wavelength of the PSB-HCOO- system is blue-shifted by ~40 nm than that of the PSB cation, and is in good agreement with that of Rh (498 nm) and also with Smith's calculated results (503 nm). With an increase in ε, the absorption maxima of both PSB with and without counterion are blue-shifted. When ε is four, their wavelengths become nearly equal to each other. The absorption maxima of **1** with and without carboxylate are also shown in Table 1. Although lack of the ionone ring slightly affects the value of the absorption maxima, such a structural truncation cause no serious influence for the tendency of e-dependence of the absorption maxima.

Using compound **1**, we examined the following four models

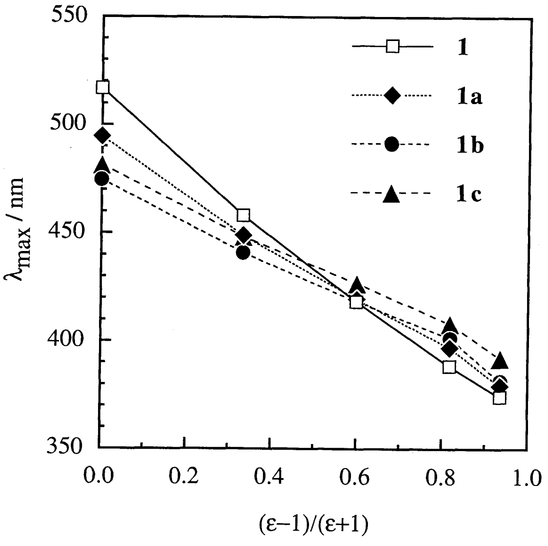

Figure 2. Calculated absorption maxima as a function of a factor (ε-1)/(ε+1).

different in the relative position of the carboxylate to the conjugated chain: (1) one carbonyl oxygen is located near C12 and the other is in the direction opposite to the polyene chain (as similar to Smith's model, **1a** in Figure 1), (2) one carbonyl oxygen is located near C12 and the other near C14 (**1b**), (3) one carbonyl oxygen is located near C14 and the other is in the direction opposite to the polyene chain (**1c**), (4) PSB cation. In Figure 2, the absorption maxima are plotted as a function of a factor $f(\varepsilon)=(\varepsilon-1)/(\varepsilon+1)$. In all the cases, the absorption maxima shifts to blue in nearly-linear fashion as $f(\varepsilon)$ increases, although the magnitudes of the slopes are different. The absorption maxima of all the species studied converges around 420 nm when the dielectric constant of medium is around four (or $f(\varepsilon)=0.6$). This indicates that the electrostatic effect of the counterion almost dissipates even in a low dielectric medium, corresponding to diethylether.

It remains unclear what value of dielectric constant should be given to the protein part of Rh. In general, it is likely to be reasonable that only the contribution of atomic polarizability to the dielectric constant of protein is 2.5-3.0.[14] In recent study using the Poisson-Boltzmann electrostatics, the dielectric constant of bacteriorhodopsin, an analogue of Rh, was assumed to be four.[15] Then, the pKa's of side chains of amino acids were successfully reproduced. According to those studies, it seems to be reasonable that the dielectric constant of Rh is about four.

Consequently, it may be said that the counterion has no explicit role in regulating the absorption maximum of the chromophore in Rh. This finding may be forced to demand serious modification to the famous external charge model of Rh.

References and Notes

1 For example, review: R.R.Birge, *Annu. Rev. Phys. Chem.*, **41**, 683 (1990).
2 B.Honig, U.Dinur, K.Nakanishi, V.Balogh-Nair, M.A. Gawinowicks, M.Arnaboldi, and M.G.Motto, *J. Am. Chem. Soc.*, **101**, 7084 (1979).
3 For example: a)K.Nakanishi, V.Balogh-Nair, M.Arnaboldi, K.Tsujimoto, and B.Honig, *J. Am. Chem. Soc.*, **102**, 7945 (1980); b)J.R.Tallent, E.W.Hyde, L.A.Findsen, G.C.Fox, and R.R.Birge, *J. Am. Chem. Soc.*, **114**, 1581 (1992); c)Y.Beppu and T.Kakitani, *Photochem. Photobiol.*, **59**, 660 (1994).
4 M.Han and S.O.Smith, *Biochemistry*, **34**, 1425 (1995).
5 A.Warshel and M.Levitt, *J. Mol. Biol.*, **103**, 227 (1976).
6 See for review: J.Tomasi and M.Persico, *Chem. Rev.*, **94**, 2027 (1994).
7 H.Hoshi, M.Sakurai, Y.Inoue, and R.Chujo, *J. Chem. Phys.*, **87**, 1107 (1987).
8 R.Cammi and J.Tomasi, *J. Comput. Chem.*, **16**, 1449 (1995).
9 T.Furuki, M.Sakurai, and Y.Inoue, *J. Phys. Chem.*, **99**, 12047 (1995).
10 M.Sakurai,T.Furuki, and Y.Inoue, *J. Phys. Chem.*, **99**, 17789 (1995).
11 M.Rajzmann and P.François, QCPE **11**, 382 (1979).
12 J.J.P.Stewart, *J. Comput. Chem.*, **10**, 209 (1989).
13 J.J.P.Stewart and J.Frank, MOPAC Ver 6.01, Seilar Reserch Laboratory, U.S.Air Force Academy, Colorado Springs, CO 80840-6528, 1989.
14 H.Nakamura, T.Sakamoto, and A.Wada, *Protein. Eng.*, **2**, 177 (1996).
15 D.Bashold and K.Gerwert, *J. Mol. Biol.*, **224**, 473 (1992).