Density Functional Theory Study of the Structure and ^{13}C Chemical Shifts of Retinylidene Iminium Salts

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We present a density functional theory calculation of the structure and ^{13}C chemical shifts of retinylidene molecules which constitute the chromophores of the natural pigments rhodopsin and bacteriorhodopsin. We compare our results with recent X-ray and NMR spectroscopic data on several retinylidene iminium salts characterized by different cation−anion interactions. In agreement with crystallographic data, we find that the amplitude of the bond length alternation between single and double carbon bonds is strongly reduced in the vicinity of the protonated Schiff base nitrogen. The chemical shifts along the carbon chain are in very good agreement with the experimental values for the neutral retinylidene compounds and in fairly good agreement for the charged retinylidene compounds. We find that the ^{13}C chemical shift is mostly affected by the cation−anion distance and to a lesser extent by the presence of hydrogen bonding interaction between the protonated Schiff base of retinal and the counterion. The correlation between ^{13}C chemical shift and atomic charge along the conjugated chain of retinals is found to depend strongly on the specific compound. This result suggests some caution in using atomic charges alone to establish the position of the counterion relative to the chromophore in rhodopsin.

I. Introduction

The chromophores of the natural photoreceptors rhodopsin[1] and bacteriorhodopsin[2] consist of a retinylidene prosthetic group which is linked to the apoprotein opsin via a protonated Schiff base. In both types of photoreceptors, the first photochemical event induced by the absorption of light consists of the isomerization of the retinylidene chromophore.[3] The absorption properties of these proteins are significantly different from those of the free retinals or simple model compounds derived by them, indicating that steric and electrostatic interactions between the positively charged protonated chromophore and protein residues in the binding pocket affect the chromophore structure and tune the electronic properties of the system. The knowledge of the structure is therefore of basic importance for one to understand the properties of these proteins.

Only very recently has a high-resolution crystallographic structure for bacteriorhodopsin (bR) become available,[4−6] whereas it is still missing for rhodopsin. It has been established that in bR there is a key water molecule which forms a hydrogen bond bridge between the nitrogen of the Schiff base and the proton acceptor Asp[8]. The closest oxygen atom of the Asp[85] is located at about 4.2 Å from N.[6]

The available structural information for rhodopsin is indirect: a negative counterion located in the vicinity of the chromophore[7] has been identified as the glutamate protein residue Glu113 by mutagenesis experiments.[8] Resonance Raman spectroscopy and ^{13}C NMR have provided evidence for specific chromophore−counterion interaction in the region C12−C13 of the retinal.[9−12] Recently, solid-state magic angle spinning NMR has been applied to high-precision distance measurements in rhodopsin and in one of its photointermediates,[13,14] providing evidence for distortions of the retinal chromophore induced by the protein. ^{13}C NMR measurements have been used to estimate the counterion position with respect to the retinylidene chromophore of bacteriorhodopsin and rhodopsin.[15−17] These analyses are based on an empirical linear correlation between the ^{13}C NMR chemical shift and the conjugated carbon charge density.[15]

Recently, a series of retinylidene iminium salts were crystallized and their structural and spectroscopic properties were studied by a combination of X-ray, solid-state ^{13}C NMR, and UV spectroscopy.[18,19] The comparative analysis of the ^{13}C chemical shift and of the geometry in these compounds has been used to address the mechanisms by which the electronic properties of the retinal chromophore are modified by its environment in the natural visual pigments.

In this paper we present a density functional theory[20] (DFT) calculation of the structure and ^{13}C NMR chemical shifts[21,22] of several retinylidene compounds. We study the accuracy of DFT in retinal Schiff base systems by comparing the experimental and theoretical ^{13}C NMR chemical shifts for several compounds for which the structure is known. Specifically, we consider two of the crystallized retinylidene iminium salts synthesized in refs 18 and 19, the *N*-*tert*-butyl-retinylidene iminium triflate and the *N*-methyl-*N*-phenyl-retinylidene iminium perchlorate, which, following ref 19, we denote as compounds **3** and **4**, respectively (see Figure 1). Compounds **3** and **4** have a positively charged retinylidene group (with a H^+

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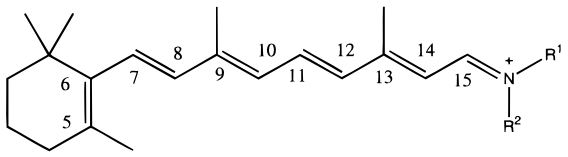
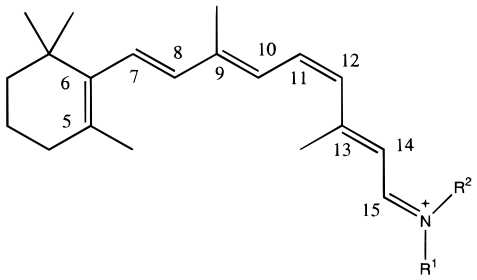


3, R^1=tert-Butyl, R^2=H, X=$CF_3SO_3^-$

4, R^1=Phenyl, R^2=Me, X=ClO_4^-



11-*cis* -RPSB, R^1=CH_2CH_3, R^2=H, X=Cl^-

Figure 1. Schematic structure of the protonated Schiff base of retinal systems studied, showing the conventional numbering system used in this paper.

and a CH_3^+ attached to the N atom, respectively), different counterions, and different hydrogen-bond interactions. We also consider two neutral retinal compounds, the all-trans-retinal,[23] and an 11-cis unprotonated retinylidene Schiff base (11-*cis*-RSB), for which NMR experimental data are available.[24] Furthermore, we compute the ^{13}C NMR chemical shifts for an 11-*cis*-retinylidene protonated Schiff base (11-*cis*-RPSB·Cl) model (see Figure 1) in which a chlorine Cl^- counterion compensates the charge and an ethylimine group is linked to the Schiff base.[25−27] We use the results for this model to study the dependence of the chemical shifts of the conjugated carbon atoms on the cation−anion relative position and on the strength of hydrogen bonding interaction.

In section II, we give some details of the computational approach. Sections III and IV show the results for the structure and for the ^{13}C chemical shifts, respectively. The last section is devoted to conclusions.

II. Computational Methods and Technical Details

We determine the ground state geometry and the NMR chemical shifts describing the electronic structure within DFT.[20] In both types of calculations we use periodic boundary conditions, which correctly account for the interaction between atoms belonging to different periodic cells in crystals. For the crystalline compounds we use the crystallographic lattice vectors as determined experimentally.[18,19] The unit cells of compounds **3** and **4** contain two retinylidene molecules and 142 and 138 atoms, respectively. For the isolated RPSB model we use a large simulation box of 36 × 22 × 22 $(au)^3$ to avoid spurious interactions between periodic replica, as done in previous works.[25,26]

For the geometry optimization we use the generalized gradient approximation (GGA) in the form proposed by Becke[28] and Perdew.[29] Ultra-soft Vanderbilt pseudopotentials[30] are used to describe the interactions of the valence electrons with the inner frozen cores. The Kohn−Sham single particle wave functions are expanded on a plane wave basis set with an energy cutoff of 25 Ry. This energy cutoff provides fully converged results with respect to the basis set for the geometry. We verified that the geometries relaxed with ultrasoft pseudopotentials[30] nearly coincide with those relaxed with norm-conserving pseudopotentials.[31] For example, a comparison between the geometries obtained for compound **3** by using the ultrasoft pseudopotentials[30] with 25 Ry cutoff and the norm-conserving pseudopotentials[31] with 70 Ry cutoff, gives differences in carbon−carbon bond lengths of less than 0.005 Å.

NMR chemical shifts are calculated using the technique described in refs 21 and 22. Such an approach has been successfully applied to periodic systems, such as crystals[22,32] and, with a super-cell technique, to disordered systems such as liquids[33] and amorphous materials.[34] For chemical shift calculations we use norm-conserving pseudopotentials[31] in the Kleinman-Bylander form,[35] and we expand the wave functions on a plane wave basis set with an energy cutoff of 70 Ry. Following the experimental convention, we quote chemical shifts (δ_{TMS}) in parts per million (ppm) relative to the C shift of a liquid sample of tetramethylsilane (TMS) with spherical shape at 300 K. Instead of fixing the absolute shift of liquid TMS by a direct calculation, we take the calculated value for an isolated molecule of benzene to coincide with the experimental (dilute gas) value of 126.9 ppm.[36] To check the importance of the gradient corrections in the description of the retinylidene chemical shifts, we performed a calculation on compound **3**, for the same given geometry, with and without gradient corrections. The effects of gradient corrections on the relative chemical shifts are totally negligible, because the results obtained with the two functionals differ by less than 1 ppm. Thus, in the following, we will only quote chemical shifts computed within the local density approximation (LDA).[37]

III. Results: Ground-State Structure

The ground-state structures obtained within our DFT approach for the all-trans-retinal, the unprotonated 11-*cis*-RSB, and the 11-*cis*-RPSB·Cl have been already presented and discussed in previous works.[26,38] Here we recall that the protonation has a strong effect on the carbon−carbon bond length alternation (BLA) in the conjugated chain of retinal: Although the neutral compounds present clear bond alternation along the entire carbon chain, the 11-*cis*-RPSB·Cl model for the chromophore of rhodopsin shows a strong reduction of BLA in the terminal region close to the Schiff base nitrogen.[26]

The crystallographic structures of the retinylidene compounds **3** and **4** described in refs 18 and 19 give us the opportunity to check the accuracy of the DFT in describing the effect of protonation on the BLA in the conjugated carbon chain. This is the first time a direct comparison with an accurate crystallographic structure for a protonated Schiff base of retinal is performed.

Starting from the crystallographic structures,[18,19] we have relaxed the atomic coordinates. Actually, because the X-ray analysis cannot determine the position of the hydrogen atoms, we have first optimized the position of the H atoms keeping all the other atoms fixed and then we let everything relax. The configuration obtained after the H relaxation is used as the experimental structure to compute the ^{13}C chemical shifts. The configuration obtained after the full relaxation is then used as the theoretical structure to compute the ^{13}C chemical shifts.

Overall, our fully relaxed structures agree well with the X-ray data, with a standard deviation of 0.03 Å. The largest displacements are observed for the oxygen atoms of the perchlorate

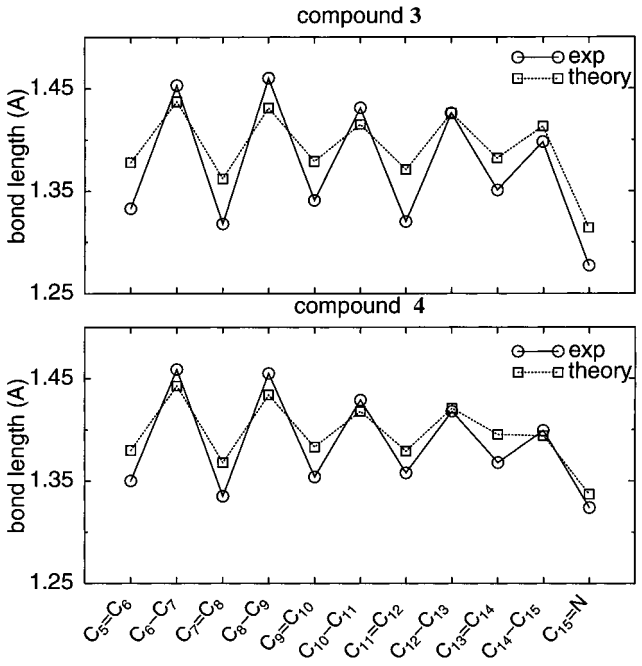


Figure 2. C−C bond lengths along the conjugated chain for the charged compounds **3** and **4**. The theoretical predictions (squares) are compared with the experimental data (circles).

counterion in compound **4**. This is not surprising because experimentally the perchlorate anion was found to be rotationally disordered,[18] therefore, we just observe a reorientation of the anion with respect to the retinal.

In Figure 2, we show the carbon−carbon and carbon−nitrogen bond length along the conjugated chain for compounds **3** and **4** and compare our results with the experimental diffraction data.[19] It is clearly seen that the GGA functional (as well as the LDA functional) underestimates the BLA. This well-known error is more pronounced in infinite conjugate chains[39] than in short conjugated chains, such as retinals,[38,40] where the bond alternation is stabilized by the chain terminations. Incidentally, it was recently shown that the polarizability and nonlinear optical properties of conjugated chains are poorly described by the currently available exchange-correlation potentials.[41] Despite the underestimation of the BLA, the effect of the protonation and of the methylation is reproduced by the theory: the BLA is in fact strongly reduced in the vicinity of the Schiff base as an effect of the presence of a positive charge.

IV. Results: ^{13}C Chemical Shifts

In this section we will show the results for the ^{13}C chemical shifts in several retinal compounds. We will first check the accuracy obtainable within DFT for compounds whose structure is known, and then we study the effect induced by displacing the counterion position and by the presence and the strength of a hydrogen bond.

Neutral Retinal Compounds. Before analyzing the spectroscopic properties of different retinylidene iminium salts, we checked the accuracy of our approach in the simpler case of neutral retinals where the problem of positive charge delocalization is absent.

We show in Figure 3 the computed ^{13}C chemical shifts of the all-trans-retinal. We considered the skewed 6-s-cis conformation—which is the most common crystalline form—in the optimized geometry within our theoretical approach.[38] The agreement with the experimental values[24] is very good, with the exception of the ^{13}C-5 chemical shift. It is known that the

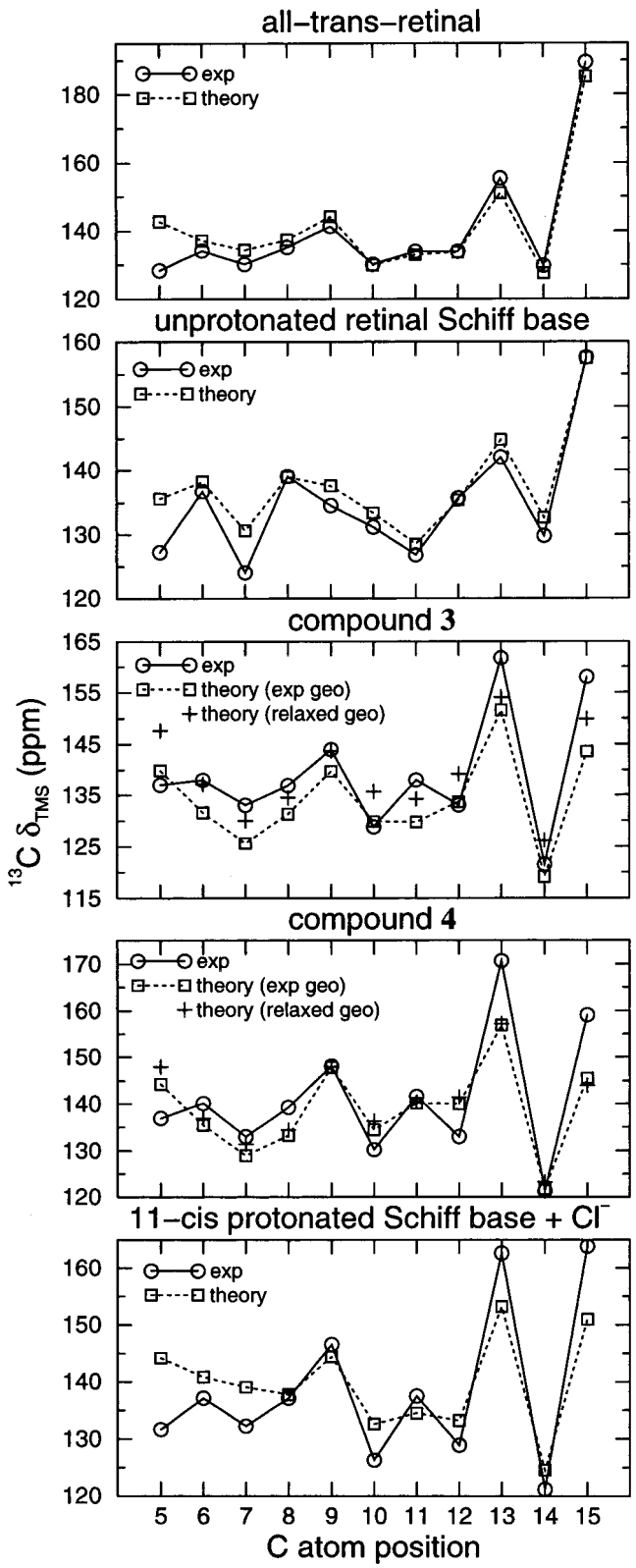


Figure 3. Computed isotropic ^{13}C chemical shifts for neutral and charged retinal compounds vs experimental data. Notice that different *y*-axis scales have been used in different panels in order to optimize the resolution.

^{13}C-5 chemical shift depends strongly on the torsional angle between the cyclohexene ring and the conjugated chain.[24] Therefore, the error on the C-5 site may be explained by the underestimation of this torsional angle in the geometry used. If we exclude the C-5, the root mean square (rms) error in the chemical shifts along the conjugated chain is less than 3 ppm.

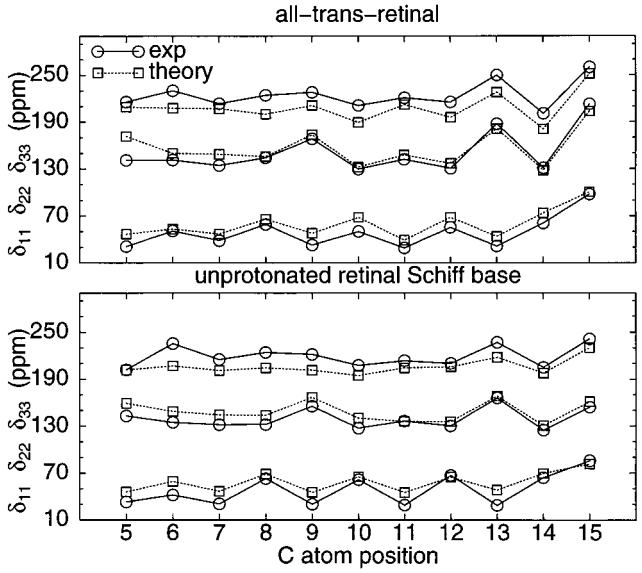


Figure 4. Computed principal values of ^{13}C chemical shift tensor for neutral retinal compounds vs experimental data.

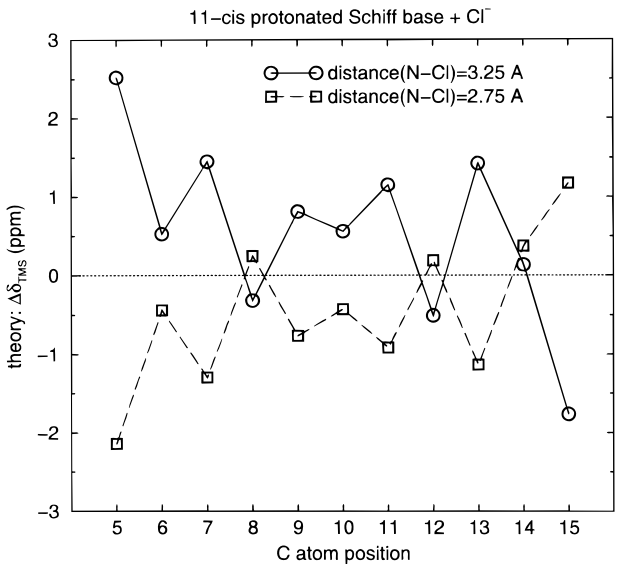


Figure 5. Variation of the computed chemical shifts for the 11-cis protonated Schiff base as a function of the distance between the Cl^- counterion and the N atom. We show the chemical shift differences for the N−Cl^- distances of 3.25 and 2.75 Å, with respect to a reference case with a N−Cl^- distance of 3.0 Å.

In Figure 3 we show the ^{13}C chemical shifts for an unprotonated 11-*cis*-RSB compound. The geometry used for the calculation was obtained in a previous work,[26] whereas the experimental chemical shifts are taken from ref 24, Table 5, with ethylimine chain termination, which is the same termination used in our model. Once more, in this neutral system the agreement with the experiment is good, with a rms error of 3.7 ppm. If we do not include in the average the result for the C-5 site, the rms error is 2.8 ppm. We notice that the largest errors are found for the carbon sites close to the cyclohexene ring, whereas the agreement improves significantly for the carbon atoms close to the Schiff base.

In Figure 4 we compare the principal values of the chemical shielding tensor for the all-trans-retinal and for the unprotonated 11-*cis*-RSB compound with the available experimental data.[24] Again, the agreement is quite good for all the components: note in particular how the odd−even oscillation observed in δ_{11} from C-5 to C-14 is well reproduced.

Retinylidene Iminium Salts: Compounds 3 and 4. In the charged compounds the location of the counterion has a strong influence on the ^{13}C chemical shifts. Indeed, the experimental NMR spectra obtained in solution or for crystalline samples are clearly distinct.[19] Furthermore, the spectra on crystalline samples depend on counterion type.[19] To compare theory with experiment it is therefore essential to consider a case in which the geometry, i.e., the counterion location, is known precisely.

We compute the chemical shifts in the retinylidene iminium salts **4** and **3** fully taking into account the periodic crystalline lattice,[21,22] and compare our results with the solid-state NMR spectra[19] in Figure 3. We show the ^{13}C chemical shifts both for the experimental geometry (square) and for the fully relaxed structure (plus). The agreement with the experiment for these compounds is not as good as for the neutral systems of the previous section. In both compounds the rms error is around 7 ppm. We note also that the error is more evenly distributed over all the sites of the conjugated chain, in contrast to the neutral systems of the previous section where the largest errors were mainly localized close to the ring.

The chemical shifts computed with the optimized geometry are almost rigidly shifted upward. This effect is quite small for compound **4**, being in average of 1.4 ppm, and larger for compound **3**, with an average shift of about 5.0 ppm. However, the overall agreement with the experimental data does not improve upon geometry optimization. Even though the structural relaxation has a relatively large effect on the BLA along the conjugated chain in compound **4** (see Figure 2), this is not reflected in a significant change in the ^{13}C chemical shifts. The effect of the relaxation is more important in the compound **3** where the counterion is closer to the retinylidene group; specifically, the N−O distance goes from 2.85 Å in **3** to 4.5 Å in **4**.

11-cis Protonated Schiff Base of Retinal. Here we describe the results obtained for the 11-cis protonated Schiff base of retinal with a Cl^- counterion.[12,26] In these calculations we modify the position of the counterion with respect to the retinal to analyze separately the effect on the ^{13}C chemical shift of hydrogen bonding interaction and of cation−anion distance.

By optimizing the position of the Cl^- in the vicinity of the Schiff base, we obtain a minimum energy configuration for a N−Cl distance of about 3.0 Å with the Cl^- aligned along the N−H direction, thus forming a hydrogen bond. In Figure 3 we show the ^{13}C chemical shifts along the carbon chain obtained for this configuration, and we shall use this result as a reference for different N−Cl distances. The experimental values[42] have been obtained for a 11-*cis*-RPSB chloride salt in $CDCl_3$ solution, which has the same counterion as used in our model compound. In this case we do not know the exact experimental position of the counterion and the theoretical optimized geometry in the vacuum at 0 K should be taken only as a reasonable guess for the experimental structure. Thus, the comparison between theoretical and experimental NMR spectra is less stringent in the present case than for the well-characterized crystallized compounds **3** and **4**. The rms deviation with respect to the experimental values of the chemical shift is 7 ppm, thus the same error which is observed for the salts **3** and **4** discussed above. This is quite satisfactory because here we are mostly interested in trends as a function of the position of the counterion.

We then displaced the position of the Cl^- ion of ±0.25 Å from the nitrogen to simulate the effect of a stronger (weaker) hydrogen bond. In Figure 5 we show the chemical shift differences with respect to the case at a distance of 3.0 Å. The

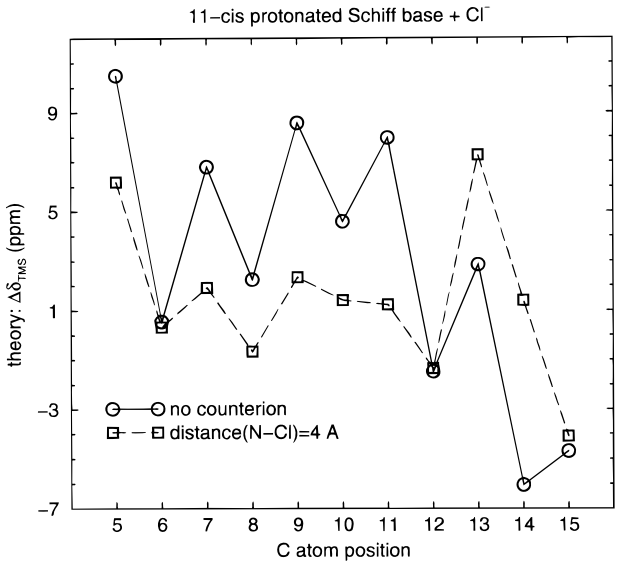


Figure 6. Variation of the computed chemical shifts for the 11-cis protonated Schiff base as a function of the distance between the Cl^- counterion and the N atom. We show the chemical shift differences for the N−Cl^- distance of 4.0 Å and for a case with no counterion, with respect to a reference case with a N−Cl^- distance of 3.0 Å.

first comment is that the effect is quite small and is significant only for the odd-numbered carbon sites along the chain. It is noticeable that the largest shift is observed for the C-5.

In ref 19, a chemical shift difference as large as ≈6 ppm is observed at the C-13 site between the retinylidene iminium salts **2** and **3**. This difference is attributed to a different hydrogen bonding interaction in the two compounds, the N−H···O distance being 0.09 Å longer in compound **2**. Our results indicate that a weaker hydrogen bond actually induces downfield chemical shifts of odd-numbered carbons of the chain, but the effect is much smaller than that observed by Elia et al.[19] even though we considered a larger displacement of 0.25 Å. We conclude that a stronger hydrogen bond alone cannot explain the observed chemical shift difference.

In Figure 6 we show the effect of further increasing the anion−cation distance. When the counterion is at a distance of 4.0 Å (squares) we observe a considerable chemical shift difference, particularly at the C-13 and C-5 sites. The effect at this distance is mostly electrostatic because the hydrogen bond interaction should be negligible. When we move the counterion at "infinite" distance (circles), the chemical shift difference is noticeable also at the odd-numbered carbon site far from the nitrogen. This result is consistent with a positive charge more delocalized over the entire conjugated chain. Indeed, it was previously found that the main effect of the presence of the counterion is to localize the positive charge close to the Schiff base and to increase the BLA along the retinal chain.[26]

Correlation between Atomic Charge and Chemical Shift. The existence of a regularity in the chemical shift change ($\Delta\delta$) of the carbon in RPSB as a function of the counterion distance, and the correlation of $\Delta\delta$ with changes in atomic charge densities ($\Delta\rho$),[15] have been used in building models for the interaction between the chromophore of rhodopsin and/or bacteriorhodopsin and its protein environment.[15,17,24] Specifically, this correlation was used to determine the optimal counterion distance which would reproduce the chemical shift data, with atomic charges usually computed by semiempirical quantum mechanical methods.[15−17] However the charge−chemical shift correlations have been directly verified in ref 15 only for the two neutral compounds also considered in this work.

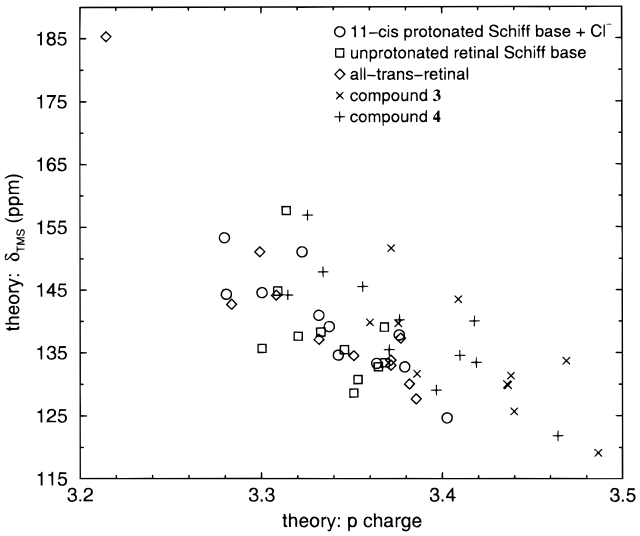


Figure 7. Computed ^{13}C chemical shifts as a function of the *p* component of atomic charges for different compounds.

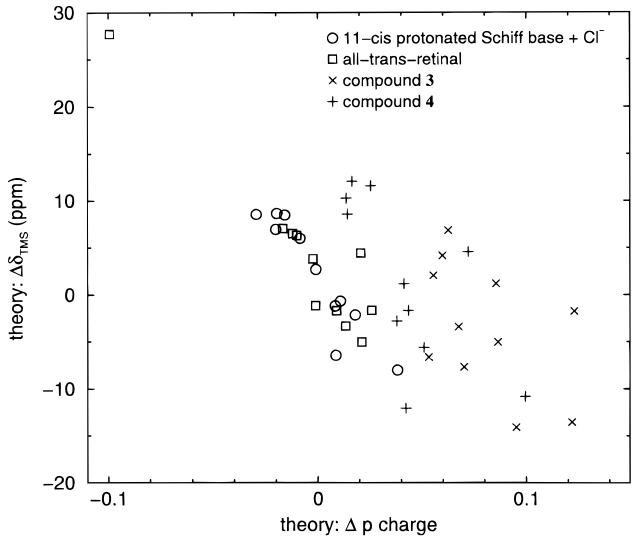


Figure 8. Variation of the computed ^{13}C chemical shifts as a function of the variation of the *p* component of atomic charges for different compounds, using as reference the values obtained for the unprotonated retinal Schiff base.

The validity of the same correlation in a charged situation is thus only speculative.

Here we explore the existence of this correlation for the retinylidene compounds considered in this study. For each atom we have defined *s* and *p* atomic charges. These are defined as the integrals, in a sphere of radius 1.0 bohr centered at the atomic positions, of the square of *s* and *p* components of occupied wave functions. The resulting values have been normalized by the integral of the square of 2*s* and 2*p* wave function, respectively, of the isolated C pseudoatom, in a sphere of the same radius.

In Figure 7 we show the computed chemical shifts for the carbon atoms from C-5 to C-15 as a function of the corresponding *p* atomic charge. A linear fit of these data gives a large standard deviation of 7.14 ppm, with a slope of −147.6 ppm/electron. The inclusion of the *s* charge does not improve the correlation.

It has been noted that the correlation is higher in a differences plot compared to absolute values.[15] In Figure 8 we use the neutral unprotonated 11-*cis*-RSB compound as a reference and we plot the variation of the chemical shift as a function of the

variation of the *p* charge. In agreement with the conclusion of ref 15, we find a high linear correlation between the variation of the chemical shift and the variation of the charge for the other neutral compound, the all-trans-retinal. The same linear correlation well describes the data for the 11-cis protonated Shiff base. However, in the charged compounds **3** and **4** the correlation is much weaker. In this case, a linear fit considering all the compounds still has a large standard deviation of 5.8 ppm, a slope of −126.5 ppm/electron, and a y_{int} of 4.6 ppm. The large deviation observed in the charged compounds suggests that the charge correlation cannot be safely used to determine the counterion location in the rhodopsin and/or bacteriorhodopsin.

Conclusion

We have used DFT to compute the equilibrium structure and the ^{13}C NMR chemical shifts for several retinylidene compounds. The main results of this work can be summarized as follows: (i) In agreement with crystallographic data, we find that the amplitude of the bond length alternation between single and double carbon bonds is strongly reduced in the vicinity of the protonated Schiff base nitrogen. (ii) The chemical shifts along the carbon chain are in very good agreement with the experimental values for the neutral all-trans-retinal and unprotonated retinal Schiff base and in fairly good agreement for protonated retinylidene compounds. We speculate that the larger deviations observed for the charged systems are due to some deficiency of the currently available density functionals in describing the polarizability of conjugated molecular chains.[41] (iii) The ^{13}C chemical shifts are much more sensitive to long-range electrostatic cation−anion interactions than to the strength of hydrogen bond interactions. (iv) In contrast to what was assumed in previous calculations, in some of the charged compounds we find a poor correlation between atomic charges and chemical shift differences. Thus, one should use some caution in using this correlation to extrapolate geometry constraints as the distance of the counterion in rhodopsin.

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