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The Role of the β-Ionone Ring in the Photochemical Reaction of Rhodopsin

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Time-dependent density functional theory (TDDFT) calculations on the photoabsorption process of the 11-cis retinal protonated Schiff base (PSB) chromophore show that the Franck−Condon relaxation of the first excited state of the chromophore involves a torsional twist motion of the β-ionone ring relative to the conjugated retinyl chain. For the ground state, the β-ionone ring and the retinyl chain of the free retinal PSB chromophore form a $-40°$ dihedral angle as compared to $-94°$ for the first excited state. The double bonds of the retinal are shorter for the fully optimized structure of the excited state than for the ground state suggesting a higher cis−trans isomerization barrier for the excited state than for the ground state. According to the present TDDFT calculations, the excitation of the retinal PSB chromophore does not primarily lead to a reaction along the cis−trans torsional coordinate at the $C_{11}-C_{12}$ bond. The activation of the isomerization center seems to occur at a later stage of the photo reaction. The results obtained at the TDDFT level are supported by second-order Møller−Plesset (MP2) and approximate singles and doubles-coupled cluster (CC2) calculations on retinal chromophore models; the MP2 and CC2 calculations yield for them qualitatively the same ground state and excited-state structures as obtained in the density functional theory and TDDFT calculations.

I. Introduction

Rhodopsin is the photoreceptor protein responsible for vision and 11-cis retinal is the photoabsorbing chromophore embedded in rhodopsin. All carbon−carbon bonds in the conjugated retinyl chain of the chromophore are in the trans position except between C_{11} and C_{12}. The photoabsorption reaction involving the 11-cis retinal chromophore has been extensively studied.[1−4] In the sixties, it was proposed that the primary absorption of the photon leads to an isomerization at the cis position yielding an all-trans retinyl chain.[5,6] The isomerization model was later questioned when it became apparent that the first intermediates in the absorption process were formed within 6 ps after excitation. Such a short time was considered to be too fast for a cis−trans isomerization process.[7] However, later spectroscopic studies supported the isomerization model,[8] which has become the generally accepted model, after it was shown that the fixation of the double bond between C_{11} and C_{12} in the cis conformation leads to a passivation of the photo receptor.[8−12] The strongest indication for the cis−trans isomerization step was obtained by blocking the isomerization process by connecting C_{10} and C_{13} with a CH_2 group thus forming a cyclopentene ring. The observed slow rate of the internal conversion in the photo reaction of the 11-cis-blocked five-membered retinal was taken as a strong indication of the cis−trans isomerization as the primary reaction step.[13]

The photochemistry of bacteriorhodopsin involves the isomerization of all-trans retinal to 13-cis retinal. The reaction has many similarities to that of rhodopsin but takes place on the picosecond time scale, making it much easier to experimentally observe early photo products.[14,15] Fourier transformed optical absorption spectra of deuterated 11-cis retinal chromophores showed that the $C_{11}-C_{12}$ bond is not involved in the initial step of the excitation-state dynamics,[4] and that the excited-state dynamics of 13-trans-locked bacteriorhodopsin appears to be similar to that of the native bacteriorhodopsin up to 100 fs.[16] Recent femtosecond spectroscopic studies of the primary events in retinal-modified bacteriorhodopsin analogs indicate that the isomerization of the retinyl chain is preceded by preparatory reaction steps.[17−20] In the femtosecond spectroscopic studies of native and blocked retinal chromophores, it was found that the restriction of the retinal motion at the isomerization center does not change the initial photocycle mechanism.

The difference in the electron density between rhodopsin and bathorhodopsin, which is the first intermediate of the photo reaction that can be trapped, has very recently been deduced from X-ray measurements.[21] The density difference was found to show strong positive and negative areas at the β-ionone ring and at the $C_{11}-C_{12}$ bond suggesting that the photoisomerization process is not just a simple one-bond flip.[21] Spectroscopic studies of rhodopsins also support this notion.[22−24] Nakamichi and Okada found that the dihedral angle around the $C_{11}-C_{12}$ bond changes by 90° from about $-40°$ in rhodopsin to $-155°$ for bathorhodopsin.[21] The excitation-state dynamics measurements by Akiyama et al.[16] suggest two other reaction steps before the incomplete isomerization forming bathorhodopsin occurs. The almost temperature-independent initial reaction step occurring within 20 fs can most likely be assigned to Franck−Condon relaxation.[4,16,25] Mathies et al.[24] found that the absorption of a photon takes the chromophore into the Franck−Condon region, and after that the wave packet evolves

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along Franck−Condon coupled modes such as C−C stretches and methyl rock. The hydrogen-out-of-plane and carbon backbone torsions are not excited by the initial absorption; instead these modes are activated 200−300 fs after the photon absorption by the intramolecular vibrational energy redistribution mechanism.[24]

In this work, the Franck−Condon step of the photoabsorption reaction is studied by performing density functional theory (DFT) and time-dependent density functional theory (TDDFT) calculations on the ground state and the first excited state of the 11-cis retinal protonated Schiff base (PSB) chromophore. The retinyl chain is terminated by a protonated Schiff base yielding a net charge of +1 for the chromophore. Additional insights are obtained from analogous studies of the retinal PSB chromophore blocked in the cis form at C_{11} by a five-membered ring. Similar results also were obtained in DFT and TDDFT studies of the 11-cis retinal PSB blocked by a seven-membered ring, the all-trans retinal PSB, and the 13-cis retinal PSB.[26] Similarities in the structure relaxation for the Franck−Condon region are expected from the low isomerization selectivity of the free chromophore in solution.[15] Therefore, the present results are compared not only with experimental values for rhodopsin, but also with experimental data for bacteriorhodopsin. The reliability of the employed DFT calculations was assessed by performing comparative studies on retinal model compounds at the second-order Møller−Plesset (MP2) and approximate singles and doubles coupled-cluster (CC2) levels.

II. Computational Methods

The molecular structures of the ground and first excited states of the 11-cis retinal PSB and the 11-cis-blocked five-membered ring retinal PSB were optimized at the DFT level using Becke's three-parameter hybrid functional[27] with the Lee−Yang−Parr correlation functional[28] (B3LYP). For the excited state, the molecular structures were optimized at the TDDFT[29,30] level using the B3LYP functional. The molecular structures of the ground state of the native retinal molecule and of the retinal model compounds were optimized at the second-order MP2 level employing the resolution of the identity (RI) approach.[31] For the first excited state of the retinal model compounds, the molecular structures were optimized at the approximate singles and doubles (CC2) level employing the RI method.[32,33] The structures of the retinal chromophores obtained at the DFT and TDDFT levels were confirmed to be energy minima by calculating the harmonic vibrational frequencies using numerical differences. The Karlsruhe triple ζ basis sets augmented with one set of polarization functions (TZVP) were used.[34,35] For the retinal model compounds, we employed the newer Karlsruhe triple ζ basis sets, denoted def2-TZVP.[36] All calculations have been done with TURBOMOLE.

III. Results

A. Native and 11-cis-Blocked Retinal Chromophores. The molecular structures of the ground and the first excited states of the native 11-cis retinal PSB chromophore and of the 11-cis-blocked five-membered ring retinal PSB chromophore optimized at the B3LYP level are shown in Figure 1 and 2. The changes in the molecular structures of the native and 11-cis-blocked retinals upon excitation are very similar. The double bonds in the retinyl chain are slightly shorter in the excited-state structures than in the ground state. The single bonds of the retinyl chain are longer than in the ground state. The methyl group at C_5 and one methyl group at C_1 participate in the Franck−Condon relaxation process whereas the second methyl group at C_1 seems to be inactive. The distance from C_5 to the methyl group changes from 150.6 to 148.1 pm in the native retinal and from 150.9 to 148.2 pm in the cis-blocked one. In the native retinal, the bond distances to the methyl groups at C_1 change from 154.5 and 154.4 pm in the ground state to 157.0 and 154.5 pm for the optimized structure of the first excited state. This could explain why the minimum requirement for a β-ionone ring analogue that can activate rhodopsin is a structure that includes a methyl group at C_1 or C_5.[38,39]

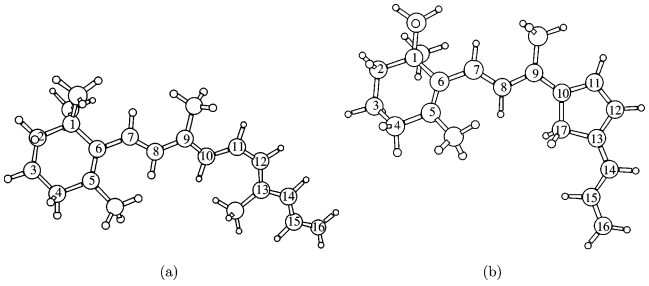

Figure 1. The molecular structure of the ground state of (a) the native 11-cis retinal PSB and (b) the 11-cis-blocked retinal PSB chromophores optimized at the B3LYP level.

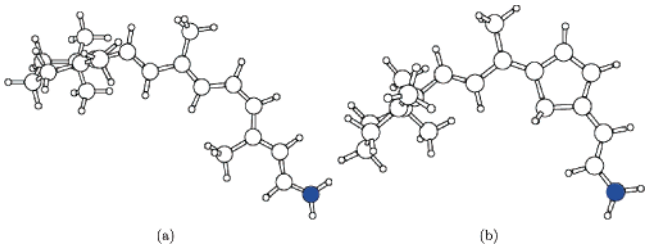

Figure 2. The molecular structure of the first excited state of (a) the 11-cis retinal PSB and (b) the 11-cis-blocked retinal PSB chromophores optimized at the B3LYP level. The nitrogen atom is marked with dark blue.

The most apparent difference between the molecular structures of the ground state and the first excited state is the dihedral angle between the retinyl chain and the β-ionone ring. For the ground-state structure, the ring and the chain form a dihedral angle of −39°, whereas in the first excited-state the ring and the chain are perpendicular against each other with a torsion angle of −94°. This applies to both the native and the 11-cis-blocked chromophores. For the free chromophore, the retinyl chain is practically planar in the ground state as well as in the first excited state. The TDDFT calculations do not provide any indications of a cis−trans isomerization of the C_{11}−C_{12} bond in the Franck−Condon region. The activation of the isomerization center seems to occur at a later stage of the photo reaction as also recently observed in femtosecond spectroscopy measurements.[24] The more pronounced bond length alternation of the retinyl chain obtained in the calculations suggests a higher cis−trans isomerization barrier in the excited-state than for the ground-state structure. The calculated C−C distances are given in Table 1 and 2.

The retinyl chain of the 11-cis-blocked chromophore is strongly bent as compared to the native retinal chromophore. For the cis-blocked chromophore, the C_6, C_{11}, and N_{16} atoms form an angle of 95°, whereas in the native chromophore the angle is 139°. Such a strong distortion of the retinyl chain can affect the functional ability of the protein.

The present calculations indicate that the photo reaction begins with a Franck−Condon relaxation involving the β-ionone ring and that the isomerization center is activated at a later stage. However, steric effects might prevent the twisting of the

TABLE 1: The C−C Distances (in pm) for the Ground State (GS) and First Excited State (1ES) of the 11-cis Retinal PSB Chromophore Calculated at the B3LYP Level[a]

bond	GS(B3LYP)	1ES(B3LYP)	GS(MP2)
C_1-C_6	155.1	150.1	152.8
C_1-C_2	154.3	154.5	153.0
C_2-C_3	152.1	152.3	151.7
C_3-C_4	152.5	152.5	152.0
C_4-C_5	150.9	148.3	150.6
C_5-C_6	136.5	142.5	136.3
C_6-C_7	144.8	149.2	145.0
C_7-C_8	136.6	133.3	136.2
C_8-C_9	142.7	147.1	143.0
C_9-C_{10}	139.5	135.9	138.4
$C_{10}-C_{11}$	140.0	143.6	140.7
$C_{11}-C_{12}$	139.4	137.8	138.4
$C_{12}-C_{13}$	140.6	141.8	141.0
$C_{13}-C_{14}$	141.0	140.9	139.6
$C_{14}-C_{15}$	137.7	137.5	138.4
$C_{15}-N_{16}$	133.5	135.3	132.6
$C_5-C_6-C_7-C_8$	−38.9	−94.3	−45.1

[a] The MP2 ground state structure also is reported. The $C_5-C_6-C_7-C_8$ torsion angles (in degrees) of the β-ionone ring also are given. The numbering of the atoms is shown in Figure 1.

TABLE 2: The C−C Distances (in pm) for the Ground State (GS) and the First Excited State (1ES) of the 11-cis-Blocked Five-Membered Ring Retinal PSB Chromophore Calculated at the B3LYP Level[a]

bond	GS	1ES
C_1-C_6	154.8	150.1
C_1-C_2	154.8	154.5
C_2-C_3	152.0	152.4
C_3-C_4	152.1	152.5
C_4-C_5	150.4	148.2
C_5-C_6	137.5	142.4
C_6-C_7	144.2	148.9
C_7-C_8	137.0	133.6
C_8-C_9	142.7	146.7
C_9-C_{10}	139.1	136.1
$C_{10}-C_{11}$	140.7	144.1
$C_{11}-C_{12}$	139.2	137.9
$C_{12}-C_{13}$	139.9	140.5
$C_{13}-C_{14}$	140.2	140.1
$C_{14}-C_{15}$	137.4	137.3
$C_{15}-N_{16}$	133.6	135.5
$C_{10}-C_{17}$	152.2	153.4
$C_{13}-C_{17}$	151.3	151.7
$C_5-C_6-C_7-C_8$	−23.6	−109.8

[a] The $C_5-C_6-C_7-C_8$ torsion angles (in degrees) of the β-ionone ring also are given. The numbering of the atoms is shown in Figure 2.

β-ionone ring relative to the retinyl chain, in case the chromophore is embedded in the protein. The effect of the protein has not been considered in this study because we believe that it is of ultimate importance to have a proper description of the free chromophore and to understand its processes before environmental effects are taken into account.

The vertical excitation energy for the native retinal chromophore calculated at the B3LYP level is 2.34 eV (529 nm). For the 11-cis-blocked retinal chromophore, the corresponding excitation energy is 2.29 eV. These energies can be compared to the recently measured gas-phase value of 2.03 eV for 11-cis-retinal PSB.[40] The optimization of the first excited-state involving a large change in the torsion angle at the β-ionone ring also results in a large Franck−Condon shift of 1.44 eV. The vertical deexcitation energy from the first excited state to the ground state of the native retinal chromophore is 0.90 eV. For the native chromophore, the zero-point vibrational corrections for the ground and excited states are 12.43 and 12.34 eV, respectively. The corresponding values for the cis-blocked one are 11.86 and 11.72 eV. Thus, the zero-point vibrational energy corrections correspond to a redshift of the calculated absorption maximum at 530 nm by 20−30 nm.

TABLE 3: The Excitation Energies (in eV) of the Native 11-cis Retinal PSB, 13-cis Retinal PSB, All-trans Retinal PSB, and the 11-cis-blocked Five-Membered Ring Retinal PSB Chromophores Calculated at the B3LYP Level

molecule	state	structure	energy	osc str
11-cis retinal PSB	1ES	GS	2.34	1.24
11-cis retinal PSB	2ES	GS	3.10	0.45
11-cis retinal PSB	1ES	1ES	0.90	0.00
11-cis retinal PSB	2ES	1ES	2.72	1.29
13-cis retinal PSB	1ES	GS	2.29	1.13
13-cis retinal PSB	2ES	GS	3.11	0.56
13-cis retinal PSB	1ES	1ES	0.88	0.00
13-cis retinal PSB	2ES	1ES	2.66	1.18
all-trans retinal PSB	1ES	GS	2.34	1.40
all-trans retinal PSB	2ES	GS	3.13	0.59
all-trans retinal PSB	1ES	1ES	0.87	0.00
all-trans retinal PSB	2ES	1ES	2.73	1.51
11-cis-blocked retinal PSB	1ES	GS	2.36	0.92
11-cis-blocked retinal PSB	2ES	GS	3.29	0.23
11-cis-blocked retinal PSB	1ES	1ES	1.06	0.00
11-cis-blocked retinal PSB	2ES	1ES	2.69	0.71

The obtained excitation and fluorescence energies including the oscillator strengths are summarized in Table 3. The oscillator strength for the transition between the first excited state and the ground state in the perpendicular conformation, i.e., for the excited-state structure, is only 10^{-4}; the Franck−Condon relaxation of the molecular structure of the first excited state efficiently prevents undesired luminescence processes.

The characteristic features in the Franck−Condon relaxation of 11-cis retinal PSB are reproduced in the excited-state optimizations of the bacteriorhodopsin chromophore and its photo product, i.e., for all-trans retinal PSB and 13-cis retinal PSB, respectively.[26]

B. Retinal Model Compounds. The accuracy of the DFT and TDDFT calculations was checked by comparing the optimized ground-state and excited-state structures of the all-trans polyene (C_8H_{10}) with the molecular structures obtained in MP2 and CC2 structure optimizations. For the ground state, the C−C distances obtained at the B3LYP and MP2 level agree very well. The bond distances differ by less than 0.5 pm. The bond lengths calculated at the complete active space self-consistent field (CASSCF) level are also in close agreement with the present results.[41] The bond length alternation is somewhat larger at the CASSCF level. The B3LYP, CC2, and CASSCF calculations yield for the first excited state largely equal C−C distances with a maximum deviation of 0.9 pm between the B3LYP and CC2 bond lengths; the largest difference between the B3LYP and CASSCF bond distances is 0.8 pm. The calculated C−C distances for C_8H_{10} are compared in Table 4. The optimization of the ^1B_u state of all-*trans*-1,3-butadiene at the B3LYP TDDFT level leads to a structure of C_s symmetry as also obtained in correlated ab initio calculations.[42,43] For all-*trans*-1,3,5-hexatriene, all-trans-1,3,5-octatetraene, and all-trans-1,3,5,7-decapentadiene, the B3LYP TDDFT optimization of the ^1B_u states does not break the C_{2h} symmetry, which is in agreement with previous calculations at ab initio correlation levels.[41,44] The C−C bond distances and excitation energies of the polyenes are compared to literature values in Table 4. Judging by these calculations, it can be concluded that reliable molecular structures are obtained for the ground and first excited state of the conjugated hydrocarbon chain at the B3LYP DFT and B3LYP TDDFT level, respectively.

TABLE 4: Comparison of the C−C Distances (in pm) Obtained at Different Computational Levels for the Ground (^1A_g) and First Excited State (^1B_u) of all-*trans*-1,3,5-Hexatriene, all-*trans*-1,3,5,7-Octatetraene, and all-*trans*-1,3,5,7,9-Decapentaene[a]

level	state	C1−C2	C2−C3	C3−C4	C4−C5	C5−C6	EE[b]
hexatriene							
B3LYP/ def2-TZVP	^1A_g	133.7	144.6	134.6			4.72[c]
MP2/ def2-TZVP	^1A_g	134.3	144.6	135.0			5.31[d]
CASSCF/ ANO-DZP[e]	^1A_g	134.6	145.6	135.6			
octatetraene							
B3LYP/ def2-TZVP	^1A_g	133.8	144.3	135.0	143.7		4.04[c]
MP2/ def2-TZVP	^1A_g	134.3	144.4	135.5	143.8		4.61[d]
B3LYP/ 6-31G**[f]	^1A_g	134.4	144.8	135.5	144.1		
CASSCF/ ANO-DZP[e]	^1A_g	134.7	145.9	135.9	146.4		
decapentaene							
B3LYP def2-TZVP	^1A_g	133.9	144.2	135.1	143.4	135.4	3.56
MP2/ def2-TZVP	^1A_g	134.4	144.3	135.5	143.5	135.7	4.12[d]
CASSCF/ ANO-DZP[e]	^1A_g	134.7	146.3	136.0	145.6	136.3	
hexatriene							
B3LYP/ def2-TZVP	^1B_u	138.4	140.4	142.4			4.20
CASSCF/ ANO-DZP[e]	^1B_u	138.5	140.0	140.6			
octatetraene							
B3LYP/ def2-TZVP	^1B_u	136.9	141.1	140.5	139.7		3.61
CC2/ def2-TZVP	^1B_u	137.8	140.5	141.4	139.0		4.07
CASSCF/ ANO-DZP[e]	^1B_u	137.2	141.0	139.7	139.3		
decapentaene							
B3LYP/ def2-TZVP	^1B_u	136.1	141.7	139.9	139.9	140.3	3.19
CASSCF/ ANO-DZP[e]	^1B_u	136.5	141.9	139.0	139.7	139.5	

[a] The excitation energies to the $1B_u$ state (EE in eV) are also reported. [b] The experimental 0−0 excitation energies of hexatriene and octatetraene are 4.93 eV[73] and 4.41 eV[74]. [c] Excitation energies also are reported in ref 75. [d] The excitation energy is calculated at the CC2 level. [e] Ref 41. [f] Ref 76.

A more realistic model system in this context can be obtained by replacing one of the $-CH_2$ units of the polyene with an isoelectronic protonated Schiff base $-NH_2^+$. In addition we chose, as in the retinyl chain, the third C−C bond from the Schiff base to be in the cis position yielding 2-*cis* $C_7H_8NH_2^+$. The molecular structures of the ground and the first excited state of 2-*cis* $C_7H_8NH_2^+$ are shown in Figure 3. For this retinyl chain model, the B3LYP and MP2 structures for the ground state also agree well. For the excited state, the B3LYP and CC2 bond distances differ by at most 3 pm. Qualitatively, the same structures are obtained at the B3LYP and MP2/CC2 levels. For the ground state, the bond length alternation for 2-*cis* $C_7H_8NH_2^+$ is smaller than for the all-trans polyene. The obtained C−C and C−N distances for 2-*cis* $C_7H_8NH_2^+$ are given in Table 5. A comparison of the obtained bond lengths shows that the largest structural differences between the ground and the first excited state is the long C_4-C_5 bond of 148.2 pm calculated at the B3LYP level and the −95° twist of the torsional angle around it. At the CC2 level, the corresponding bond length is 145.6 pm with a −80° twist around the same formal single C−C bond. This result indicates that Havinga's nonequilibrating excited rotamers (NEER) principle[45−47] is not valid for the studied Schiff base species. The NEER principle states that the barrier for rotations around single bonds is enhanced, and the barrier for rotation around double bonds is lowered when the excited state can be described as a single excitation from the highest occupied molecular orbital to the lowest unoccupied one. The ground-state energy of the twisted structure calculated at the CC2 level is only 0.88 eV larger than for the planar one. The loss in the self-consistent field (SCF) energy due to destruction of the conjugation of the C−C bonds is only 0.98 eV, and the correlation energy of the twisted structure is somewhat larger than for the planar one. However, at the CC2 level, the difference in the Franck−Condon relaxation is 1.07 eV in favor of the twisted conformation making it 0.12 eV below the planar structure. The CC2 excitation energy for the twisted structure is 2.38 eV as compared to 3.62 eV for the planar one. The excitation energy for the twisted structure is thus significantly smaller than obtained for the untwisted one even though one from a simple particle in the box argument would believe the opposite.[48] At the CCS level, the difference in the Franck−Condon shifts is only 0.61 eV. Thus, the CCS calculations suggest that the structure of the first excited state is largely planar, showing the importance of an accurate treatment of the electron correlation.

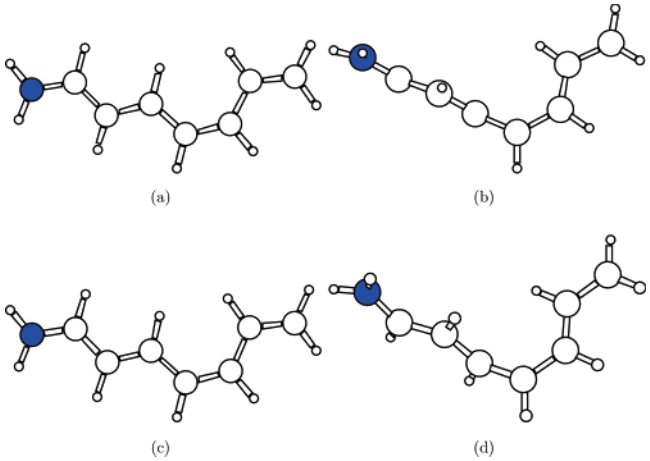

Figure 3. The molecular structures of (a) the ground state of 2-*cis* $C_7H_8NH_2^+$ optimized at the B3LYP level, (b) the first excited state of 2-*cis* $C_7H_8NH_2^+$ optimized at the B3LYP level, (c) the ground state of 2-*cis* $C_7H_8NH_2^+$ optimized at the MP2 level, and (d) the first excited state of 2-*cis* $C_7H_8NH_2^+$ optimized at the CC2 level. The nitrogen atom is marked with dark blue).

The potential curve for the torsion around the single bond in 2-*cis* $C_7H_8NH_2^+$ is shown in Figure 4. The potential curve was calculated at the CC2 level by fixing the torsional degree of freedom and optimizing the rest of the internal coordinates. The

TABLE 5: The C−C and C−N Distances (in pm) Obtained at Different Computational Levels for the Ground (GS) and First Excited State (1ES) of 2-*cis* $C_7H_8NH_2^+$

level	state	N1−C2	C2−C3	C3−C4	C4−C5	C5−C6	C6−C7	C7−C8
MP2/def2-TZVP	GS	130.8	139.7	137.1	141.1	136.7	143.3	134.5
B3LYP/def2-TZVP	GS	131.6	139.3	137.8	140.9	137.1	143.1	134.4
CC2/def2-TZVP	1ES	135.9	138.9	139.5	145.6	140.6	140.0	138.2
B3LYP/def2-TZVP	1ES	135.2	140.6	136.1	148.2	138.1	140.9	137.9

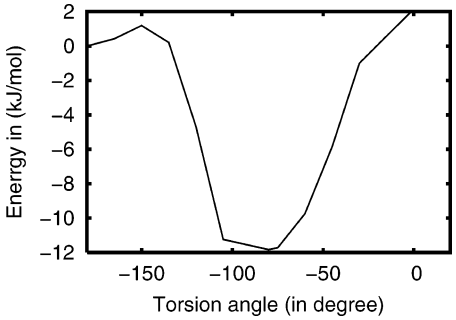

Figure 4. The potential energy curve (in kJ/mol) as a function of the torsion around the single bond of 2-*cis* $C_7H_8NH_2^+$ where the twist occurs.

perpendicular structure is seen to be 12 kJ/mol below the planar one and the two structures are separated by a tiny barrier of about 1 kJ/mol, whereas the torsion motion is barrierless at the B3LYP TDDFT level. Most likely, the tiny barrier disappears at ab initio levels when considering higher-order correlation effects. A small potential barrier of 1 kJ/mol has no chemical consequences, whereas the optimization algorithms might have difficulties to find the minimum. We can conclude here that the results obtained at the B3LYP TDDFT level are thus supported by the calculations at the CC2 level.

Page and Olivucci[49] studied the molecular structures of the ground state of linear Schiff base polyenes at the CASSCF, CASPT2, MP2, and B3LYP levels. Their calculations showed that the CASSCF calculations on $C_{10}H_{12}NH_2^+$ yield a significantly larger bond length alternation than that obtained at the other computational levels, whereas very similar molecular structures were obtained at the CASPT2, MP2, and B3LYP levels. At the CASSCF and CASPT2 levels, they obtained a smaller bond length alternation for the ground state than for the first excited state thus supporting the results of the present study. In the structure optimizations, they constrained the Schiff base polyenes to be planar by assuming C_s symmetry. This could be the reason why they did not obtain any twisted conformation in the CASPT2 study.

The Franck−Condon relaxation mechanism also was studied for a retinal model compound consisting of the β-ionone ring with the retinyl chain replaced by a $-CH{=}NH_2^+$ unit. The molecular structures optimized at the B3LYP and CC2 levels are shown in Figure 5. The B3LYP and MP2 optimizations of the ground-state structure of the model retinal chromophore yielded a planar orientation of the $-CH{=}NH_2^+$ unit relative to the β-ionone ring; at the B3LYP level, the $C_5-C_6-C_7-N_8$ dihedral angle is 0° and the MP2 value is 17°. The TDDFT B3LYP optimization of the first excited state leads to a rotation of the $-CH{=}NH_2^+$ unit (or of the β-ionone ring) yielding a dihedral angle of −79°. At the CC2 level, the dihedral angle is 64°. A perpendicular orientation of the Schiff base is obtained at both the CC2 and the B3LYP TDDFT levels. For the retinal model compound, the Franck−Condon relaxation mechanism is analogous to the one obtained for the chromophore with a full retinyl chain. The change in the molecular structure due to Franck−Condon relaxation is similar to the twisted intramolecular charge transfer (TICT) mechanism that has been found to be responsible for the double fluorescence of 4-(dimethyl)-aminobenzonitrile.[50,51]

IV. Discussion

The excitation of the retinal chromophore to the first excited state is known to involve a significant change in the dipole

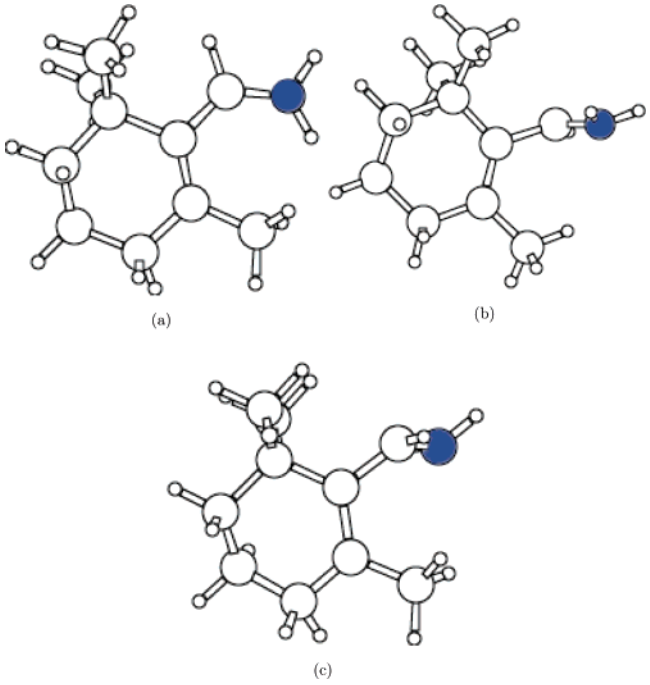

Figure 5. (a) The molecular structure of the ground state of the β-ionone ring with the retinyl chain replaced by a $-CH{=}NH_2^+$ unit optimized at the B3LYP DFT level. The molecular structure of the first excited state optimized at the (b) B3LYP TDDFT and (c) CC2 levels, respectively. The nitrogen atom is marked with dark blue.

moment corresponding to an electron transfer from the β-ionone ring toward the Schiff base.[52] At the B3LYP level, the charge transfer changes the dipole moment from 13.9 D for the ground state to 5.3 D for the optimized first excited state. The change in the dipole moment of 8.6 D can be compared to the experimental value of 12.7 ± 1.4 D.[52] The sensitivity of the charge transfer due to external perturbations was assessed by studying the excitation process with the chromophore stabilized by two methanol molecules and also by stabilizing the protonated Schiff base with a point charge of charge −1. The corresponding dipole moments obtained for the retinal chromophore with one methanol molecule hydrogen bonded to the Schiff base and one to the β -ionone ring are 14.4 and 6.3 D. The change in the dipole moment is only 0.5 D smaller in the presence of the two methanol molecules. Thus, to simulate the large blue shift of 150 nm observed for the retinal chromophore in methanol[40] requires more than two solvent molecules. Stabilization of the Schiff base by a negative point charge does not significantly affect the excitation process either. In that case, the dipole moment for the ground state is 13.5 D as compared to 5.4 D for the fully optimized structure of the first excited state.

The first excited-state seems to be well described at the B3LYP level even though it is known that TDDFT might have difficulties to accurately treat states involving long-range charge transfer.[53,54] TDDFT calculations have a tendency to give too low energies for charge transfer states.[51] The B3LYP and CC2 calculations on the retinal model compound yield qualitatively the same Franck−Condon mechanisms even though the excited-state structures slightly differ. At both levels, the substituent with the protonated Schiff base twists from a planar conformation to perpendicular position. This is exactly the same mechanism as obtained for the retinal chromophore at the B3LYP level. For the model compound, the distance between the Schiff base and the β-ionone ring is significantly shorter than in the retinal chromophore. Therefore, in this case charge transfer

problems eventually should play a less significant role than for the molecule with the full-conjugated retinyl chain. Perhaps, a more definitive answer would be given by the corresponding CC2 calculation. However, the CC2 optimization of the molecular structure of the first excited state of the full retinal chromophore consisting of 51 atoms in C_1 symmetry is a formidable task but feasible if enough computational resources are available. A single-point CC2 calculation using the molecular structure of the first excited state optimized at the B3LYP TDDFT level yields an energy that is only 0.36 eV larger than that obtained for the ground-state structure optimized at the MP2 level. Such a small energy difference indicates that a fully optimized twisted conformation can indeed lie below the untwisted one also at ab initio levels.

The full retinal chromophore embedded in the protein also has been studied at the CASSCF level.[55] The CASSCF calculations yield a torsion angle of −54° between the β-ionone ring and the retinyl chain, which can be compared to the Hartree−Fock (HF SCF) value of −58°. In the most recent study by Cembran et al., an even larger dihedral angle of −68° was obtained.[56] The experimental values for the β-twist angle in the protein are −30.3°, −31.9°, [57] and −44° [58] suggesting that the torsion angles obtained at the HF SCF and CASSCF levels are too large. The validity of comparisons to the calculated values in the gas phase is of course limited. However, the β-ionone angle of −45° for the free retinal PSB chromophore obtained at the MP2 level is closer the experimental value than the CASSCF one. The MP2 structure should be accurate, because according to the D1 diagnostics[59] 11-cis retinal PSB having a D1 value of 0.05 is dominated by one configuration. The active space used in the CASSCF calculations might be too small for a correct description of the correlation effects at the β-ionone ring. It is important to remember that CASSCF calculations on retinals are extensive and that they are not routinely employed on molecules of this size. They can thus suffer from unexpected computational difficulties and do not necessarily provide accurate reference data for retinal chromophores.

CASSCF calculations in combination with second-order perturbation theory (CASPT2) is a popular means to study molecular excitation processes.[60] For small organic molecules, the approach undoubtly has been very successful.[61,62] However, for more extended molecules it has indeed some serious limitations.[63] Because of the rapidly increasing size of the configuration interaction space with the number of active electrons and the number of the active orbitals, their numbers must be severely restricted at the cost of the reliability of the calculation; the selection of the active space might introduce a biased description of the system. Until recently, one has studied model retinal compounds and focused mainly on the isomerization center to circumvent the problems with the exponential growth of the size of the CASSCF calculations.[53,64−68] Based on their CASSCF calculations, Olivucci et al.[64,66,67,69,70] have suggested a two-state, two-mode model, according to which the relaxation from the Franck−Condon region takes place via a shallow energy plateau with CC-stretching motions toward a planar saddle point. Of course, such studies can provide no information about movements of the β-ionone ring.

In the most recent CASSCF calculations on the full retinal chromophore, the active space has been chosen to consist of 12 electrons in 12 orbitals.[55,56] This is the smallest acceptable active space correlating only the π electrons of the conjugated double bonds. Yet, the experimentally obtained change in the electron density upon excitation indicates significant changes of the electronic structure in the vicinity of the β-ionone ring,[21] suggesting that more electrons correlating the β-ionone ring need to be included in the active space. In the CASSCF optimization of the molecular structure, the $\sigma-\pi$ interactions are considered at an uncorrelated level and these effects are taken into account later by employing single-point CASPT2 calculations. Obviously, such a CASSCF/CASPT2 study on the full retinal chromophore might also yield inaccurate molecular structures and properties. Program implementations of CASPT2 gradients are rare,[71] whereas in the CASPT2 studies on retinal chromophores, the molecular structures are often optimized at the CASSCF level and dynamical electron correlation effects are accounted for by using single-point CASPT2 calculations. Sekharan al.[72] pointed out that geometry optimizations at the CASPT2 level are still computationally too expensive for retinals.

A recent CASPT2 study on the retinal chromophore employing an atomic natural orbital (ANO) basis set and a MP2/6-31G** optimized structure yielded excitation energies of 2.05 and 2.84 eV for the two lowest excited states.[72] In a previous CASPT2 study employing a 6-31G* basis set and a CASSCF/6-31G* optimized structure, the corresponding excitation energies were 2.28 and 3.49 eV, respectively.[68] The use of a slightly larger basis set red-shifted the two first transitions by 0.23 and 0.65 eV, respectively. The first excitation energy obtained in the CASPT2/ANO calculation is in excellent agreement with the experimental value obtained in a recent molecular beam measurement,[40] whereas the energy of the second excited state is 0.34 eV smaller than the experimental result.

V. Conclusions

The present DFT and TDDFT calculations show that the Franck−Condon step of the photochemical event of the retinal PSB chromophore involves a 60° change in the dihedral angle between the β-ionone ring and the retinyl chain. The Franck−Condon relaxation increases the bond length alternation making the conjugated double bonds in the retinyl chain somewhat shorter for the excited state than for the ground state. The increased bond length alternation indicates that the energy barrier for the cis−trans isomerization at C_{11} might be even higher in the excited state than in the ground state suggesting that the dynamics of the isomerization reaction is more involved than a one-bond flip.

The calculations do not provide any information about the photoreaction mechanism after the Franck−Condon step. One can speculate that the wave packet moves along the retinyl chain to the isomerization center as also suggested by Mathies et al.[24] Steric effects might hinder the torsional motion of the β-ionone ring in the protein, but the angular momentum of the ring twist can be transferred along the retinyl chain to the $C_{11}-C_{12}$ double bond assisting the isomerization step.

The reliability of the DFT calculations was assessed by calculations on retinal model compounds. MP2 and CC2 calculations yielded for them qualitatively the same ground state and excited-state structures as obtained at the DFT and TDDFT levels, respectively. The changes in the molecular structure upon excitation are found to be very similar for the native and 11-cis-blocked chromophores as well as for the other retinal isomers. The photoreaction seems to begin with a Franck−Condon relaxation involving a twist of the β-ionone ring relative to the retinyl chain. Thus, the present computational study indicates that the excitation of the retinal chromophore does not primarily lead to a reaction along the cis−trans torsional coordinate. The activation of the isomerization center seems to occur at a later stage of the photo reaction. These findings also

are supported by recent femtosecond spectroscopic and excited-state dynamics studies on rhodopsins.[4,16−20,22,23,25]

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Supporting Information Available: Cartesian coordinates for the molecules are available free of charge via the Internet at http://pubs.acs.org.

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