Internet Electronic Journal of Molecular Design **2004**, *3*, 45–54 ISSN 1538–6414
BioChem Press http://www.biochempress.com

Inter*net* Electronic Journal of Molecular Design

January 2004, Volume 3, Number 1, Pages 45–54

Editor: Ovidiu Ivanciuc

Special issue dedicated to Professor Nenad Trinajstić on the occasion of the 65th birthday
Part 7

Guest Editor: Douglas J. Klein

Quantum Chemical Calculations for Protonated Rhodopsin and Considerations on the Transduction Process in the Retina

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Received: September 22, 2003; Revised: January 15, 2004; Accepted: January 20, 2004; Published: January 31, 2004

Citation of the article:
M. F. S. Pinto and M. Trsic, Quantum Chemical Calculations for Protonated Rhodopsin and Considerations on the Transduction Process in the Retina, *Internet Electron. J. Mol. Des.* **2004**, *3*, 45–54, http://www.biochempress.com.

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Quantum Chemical Calculations for Protonated Rhodopsin and Considerations on the Transduction Process in the Retina[#]

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Received: September 22, 2003; Revised: January 15, 2004; Accepted: January 20, 2004; Published: January 31, 2004

Internet Electron. J. Mol. Des. **2004**, *3* (1), 45–54

Abstract

Our main motivation is to provide some insight in the transduction process in the rod cells of retina upon absorption of light. Models for the prosthetic group of rhodopsin (protonated Schiff base of 11–*cis*–retinal with opsin, named as 11–*cis*–rhodopsin and all–*trans*–rhodopsin in what follows) were studied with various theoretical procedures. The initial geometry of the compounds was first refined with molecular mechanics and further optimized with semi–empirical AM1 and PM3 methods. We also analyzed electrostatic potential charges with *ab initio* methods. The calculations were applied to the ground and the first excited singlet states. For a more comprehensive description of the species under study, accurate configuration interaction calculations for the electronics absorption spectrum of 11–*cis*– and all *trans*–rhodopsin with the aid of the ZINDO/S CI program were performed. The theoretical results were almost identical with the experimental measurements. Finally, we qualitatively comment on the influence of an SH^- group in the absorption spectrum. Our results suggest that the excited state is somewhat less ordered than the ground state, which may have connections with the transduction process.

Keywords. Visual pigments; photoresistor; semiempirical methods; *ab initio* methods.

1 INTRODUCTION

Vertebrates, including humans, have a large number of light sensitive neuron cells in the retina, named rods or cones, depending on the form of their terminal segments [1]. The rods sense low light intensity but cannot discriminate colors, while the cones, less light sensitive, are able to distinguish colors [2]. In these neurons, the electromagnetic energy of light is transformed in electric impulses, which eventually produce the vision phenomenon. The combination of 11–*cis*–retinal, aldehyde derived from vitamin A (retinol), with the protein opsin present in the rods gives origin to the visual pigment rhodopsin.

A photoconducting model for the act of vision had great support for a period of time [3,4].

[#] Dedicated to Professor Nenad Trinajstić on the occasion of the 65th birthday.

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However, if such would be the case, the absorption of a second photon by the same rod would raise the conductivity; the contrary occurs, the absorption of the first photon inactivates the rod until a slow biochemical process of recuperations in dark is finished.

In 1969 Trsic [5] advocated for a photoresistor model for the initiation of the nerve impulse; this proposition was based on semi–empirical quantum chemical methods available at the time (Hückel and ω–technique) and quantum mechanical calculations of transmittance of electrons through the electronic structures of the ground and the excited states of the chromophore group of the rhodopsin molecule. This model was consistent with all the available experimental information, including the fact that a continuous dark flux of Na^+, Ca^{2+} and K^+ ions between the disks containing rhodopsin and the rod membrane was interrupted by the absorption of light [6,7]. Notwithstanding, the naive character of the quantum chemical methods employed at that time hampered the acceptance of that proposition.

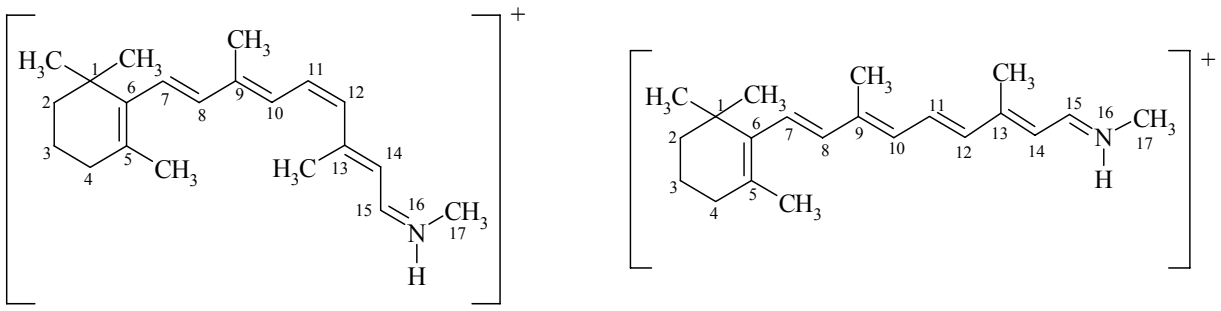
A model for the prosthetic group of rhodopsin (protonated Schiff base of 11–*cis*–retinal with opsin, named as 11–*cis*–rhodopsin in what follows) was studied with various theoretical procedures. The initial experimental geometry of the molecule was first refined with molecular mechanics and further optimized with the semiempirical AM1 and PM3 methods. Charges and bond orders either semiempirical or by *ab initio* methods were also calculated. All the former calculations were also applied to the first excited singlet state and further the ground and the excited states were compared.

The electronic spectra of the isomers 11–*cis* and all–*trans* rhodopsin were also calculated and compared favorably with experimental measurements. Configuration interaction (CI) was employed for this purpose.

2 MATERIALS AND METHODS

The chemical models for the protonated 11–*cis* and all–*trans* chromophores of rhodopsin are shown in Figure 1. Low quality of crystals prevents the obtainment of the X–ray structure of rhodopsin with good resolution. We thus used as initial geometry the structure of 11–*cis*–retinal (6–*s*–*cis*, 12–*s*–*trans)*, assessed by Raman, FTIR and solid–state ^{13}C NMR spectroscopy [8–13] and the all–*trans*–rhodopsin structure. An initial optimization was then performed with molecular mechanics (MM+) as implemented in HyperChem 6.0 [14]. Further, the geometry was refined with AMPAC 6.5 [15], using the AM1 and PM3 methods. Vibrational frequencies were calculated as well in order to check eventual distortions in the geometry. The ZINDO/S CI routine [16–18] was applied for the calculation of the electronic absorption spectra of the systems under study.

The geometry of the excited state of the *cis* isomer was also calculated, both with the ground (frozen) state geometry and with relaxed geometry (optimized for the first excited singlet state). The first excited singlet state was obtained from AMPAC with the option EXCITED.



(*a*) (*b*)

Figure 1. Chemical structures for the protonated 11–*cis* (*a*) and all–*trans* (*b*) rhodopsin chromophores used for the calculations.

The total charge of the system is +1.0. AMPAC 6.5 was also employed to calculate charges, bond orders and energy levels, comparing the ground and the first excited singlet states. Net atomic charges were also calculated with RHF *ab initio* method using STO–3G, 3–21G, 6–31G* and, 6–31G** basis sets, as implemented in the Gaussian 98 program [19].

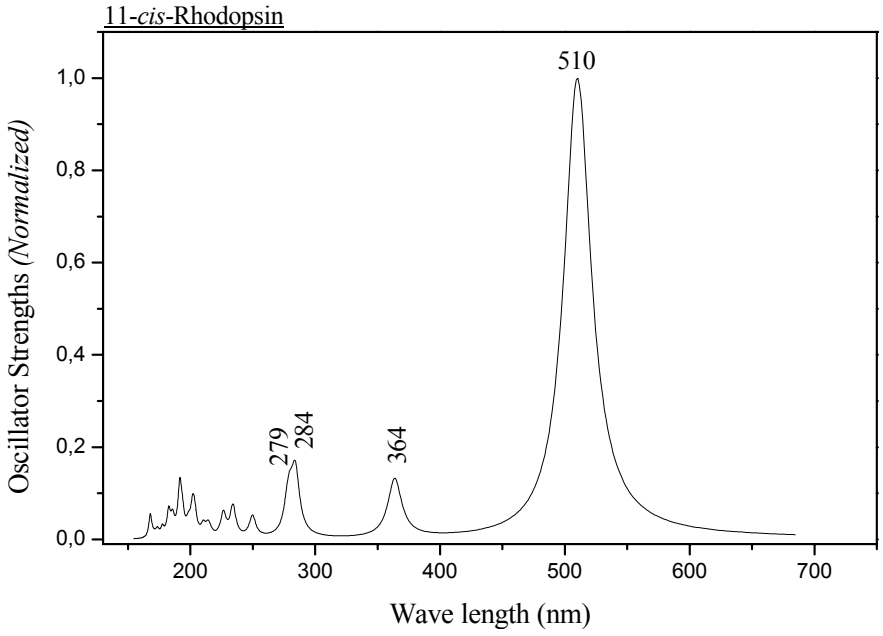


Figure 2. The ZINDO/S CI calculated electronic absorption spectrum of the 11–*cis*–rhodopsin chromophore for the molecular geometry optimization obtained with the PM3 Method. The curves were normalized, giving the value 1.0 to the highest peak.

3 RESULTS AND DISCUSSION

3.1 Electronic Spectra

The ZINDO/S CI routine [16–18] was applied for the calculation of the electronic absorption spectra of the systems under study. The ZINDO/S CI procedure and parameterization is certainly appropriate for the theoretical estimate of the electronic spectrum of these species. The resulting

UV–Visible spectrum the *cis* isomer is shown in the Figure 2 (for PM3 optimized geometry). For the stronger transitions, one finds in the ZINDO/S CI calculation that the HOMO→LUMO determinant has a weight of 83% in the CI expansion. It is also found that the oscillator strength for the first transition is of 1.48. All other transitions have oscillator strength values below 0.21. We can situate the experimental first band, usually called α–band at about 498 nm and the β–band around 340 nm [20–25], as slight variations are found due to different electrostatic or dispersion chromophore–opsin interactions for different species. The agreement is remarkable, specially noting that chromophore–opsin interactions are not accounted for in the above theoretical values. As a matter of fact, the qualitative effect of the opsin specific species in the position of the first band is not difficult to estimate. In a preliminary numerical experiment, we verified that shift in a few nm units can be induced by including a SH^- group in the proximity of the chromophore.

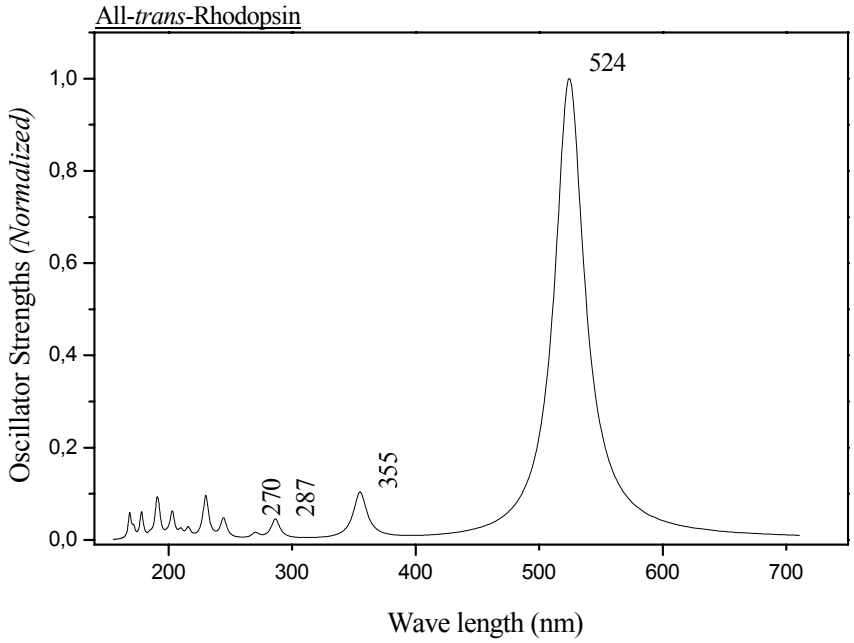


Figure 3. The ZINDO/S CI calculated absorption spectrum of the all–*trans*–rhodopsin chromophore for the molecular geometry optimization obtained with the PM3 Method. The curves were normalized, giving the value 1.0 to the highest peak.

For the *trans* isomer (Figure 3) the first peak is found at 524 nm, thus the shift is bathochromic when going from the *cis* to the *trans* isomer, as expected. This main theoretical absorption band has a weight of 86 % for the HOMO→LUMO determinant and an oscillator strength of 1.81. We remark that the experimental value for this peak is approximately at 543 nm at low temperatures [20–23] and 535 nm at room temperature [24]. Thus, both in the experiment and from theory, the shift is bathochromic when going from the *cis* to the *trans* isomer.

3.2 Comparison between the Ground State and the First Excited Singlet State

Due to the small time elapsed between the absorption of the photon in the retina and the appearance of the signal in the optic nerve, either the excited state itself, or the very first photoproducts after excitation should be responsible for the generation of the electric impulse in the nerve membrane. That is the reason why, if a discussion of the mechanism of the generation of the electric impulse is aimed, the theoretical description of the excited state is of interest. We calculated both the vertical or Frank–Condon (frozen geometry) excited state and the excited state after geometrical relaxation.

3.2.1 Energy levels

It is interesting to observe the correlation diagram (PM3) for the frontier orbitals for the ground, excited–frozen and excited–relaxed states in Figure 4. For the HOMO–LUMO gap, in the frozen case the two orbitals (now single occupied each) become much closer than in the ground state. After relaxation, the distance between HOMO and LUMO increases but remains smaller than in the ground state. These changes in the HOMO–LUMO gap upon excitation have a first explanation in the fact that self–consistent Hartree–Fock theory for the ground state produces artificial and high–lying virtual levels [see for example ref. 26]. Further, the increase of the gap in the excited state after geometry relaxation implies in a better accommodation of the electronic cloud.

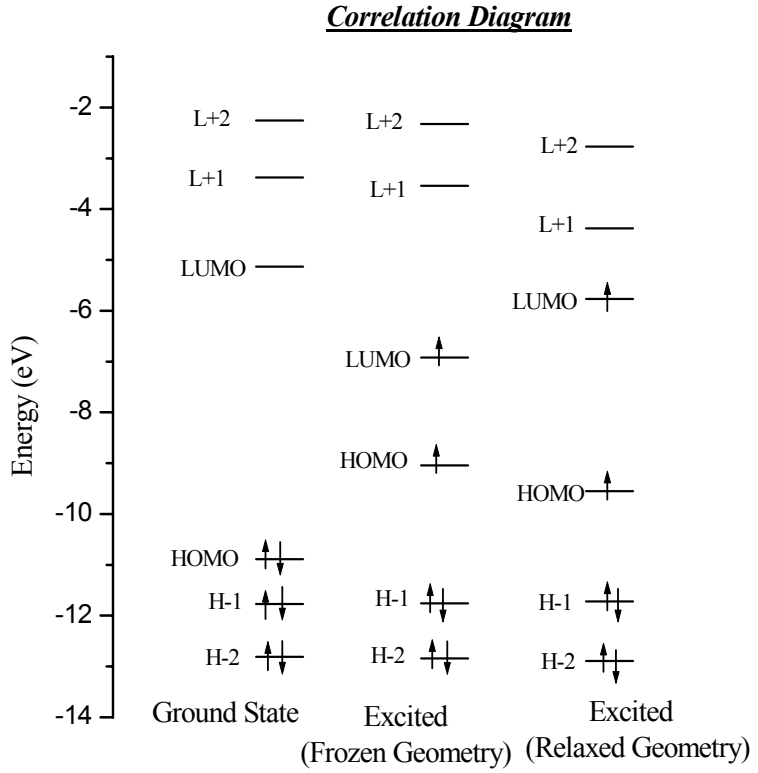


Figure 4. Correlation diagram of the 11–*cis*–rhodopsin chromophore for the ground state, frozen geometry excited state and the excited state after geometrical relaxation, obtained with the PM3 method (AM1 provides the same scheme).

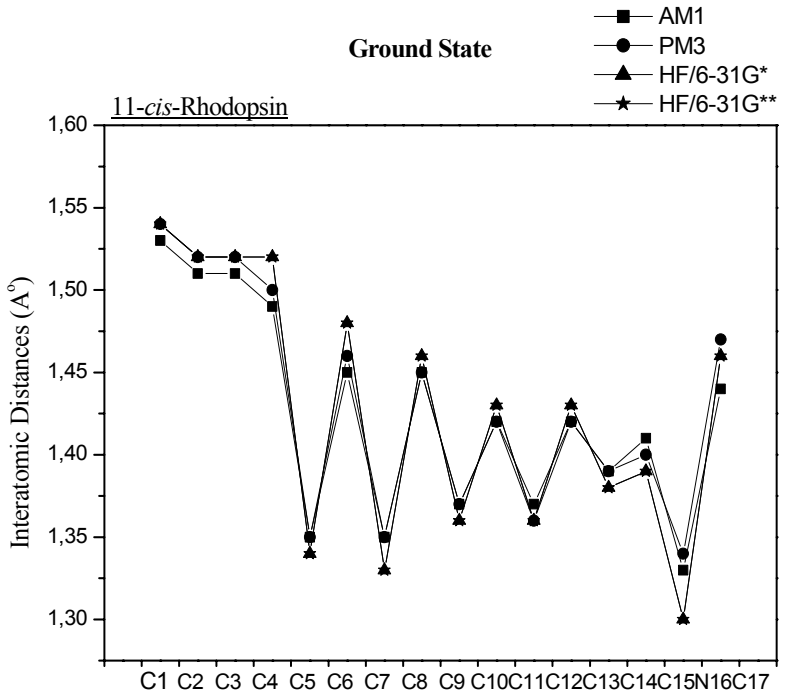


Figure 5. Representation of the 11–*cis*–rhodopsin chromophore interatomic distances calculated for the ground state.

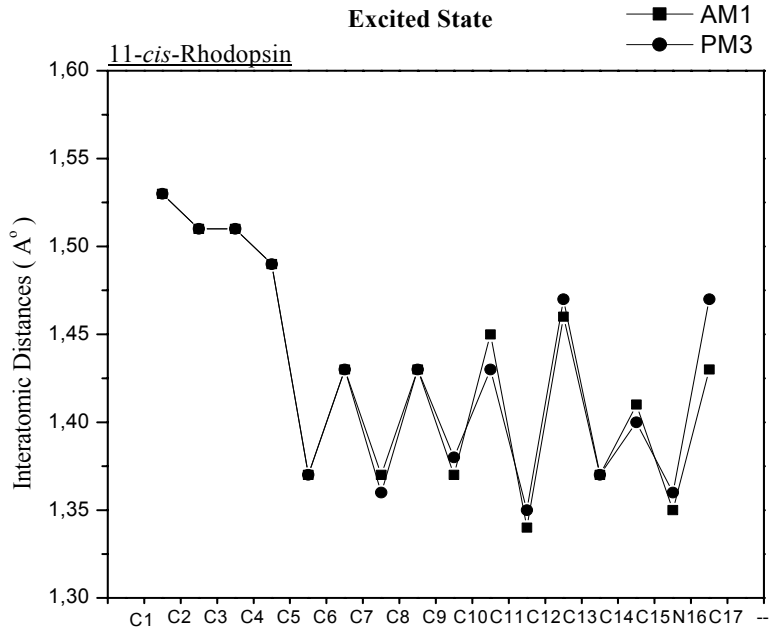


Figure 6. Representation of the 11–*cis*–rhodopsin chromophore interatomic distances calculated for the excited state (relaxed geometry).

3.2.2 Interatomic distances, bond orders and charges

The geometries of the ground and the excited (relaxed) states are shown in Figures 5 and 6, respectively. Although there are no drastic changes, two modifications may be observed: (*a*) the very neat C–C bond length alternation in the ground state is slightly reduced (*i.e.* double bond lengths are slightly decreased while single bond lengths increase) after absorption of the photon; (*b*) on the other hand, the C_{15}–N_{16} distance increases from *ca*. 1.33 Å to about 1.35 Å (AM1) and from *ca*. 1.34 Å to about 1.36 Å (PM3). We calculated the charge density distribution for the ground and the first excited singlet states for the 11–*cis*–rhodopsin chromophore with AM1 and PM3 methods and also, for comparison propose, with the *ab initio* RHF method using STO–3G, 3–21G, 6–31G* and 6–31G** basis sets, as implemented in the Gaussian 98 program [19]. On the other hand, charges were calculated with both the Mulliken analysis and as derived from electrostatic potential calculation (ESP) [27,28]. No doubt, some numerical variances are encountered, but similar trends can be observed in all cases. We choose to illustrate the charge distribution with ESP as calculated with AM1. Figures 7 and 8 compare the ESP ground and excited state (relaxed geometry) charges for 11–*cis*–rhodopsin chromophore, respectively. One can observe a crucial difference between the two states: for the excited state there is a clear disruption (from carbon atom C_9 to atom C_{15}) in the neat alternation of charges in the carbon chain found in the ground state. Similar qualitative trends are found for other calculated charges. This decrease in alternation upon excitation would not be a result of the very minor geometry change (we are discussing stages well before the *cis–trans* isomerization) but of electron density rearrangement, since the former trends are present for the frozen geometry excited state as well.

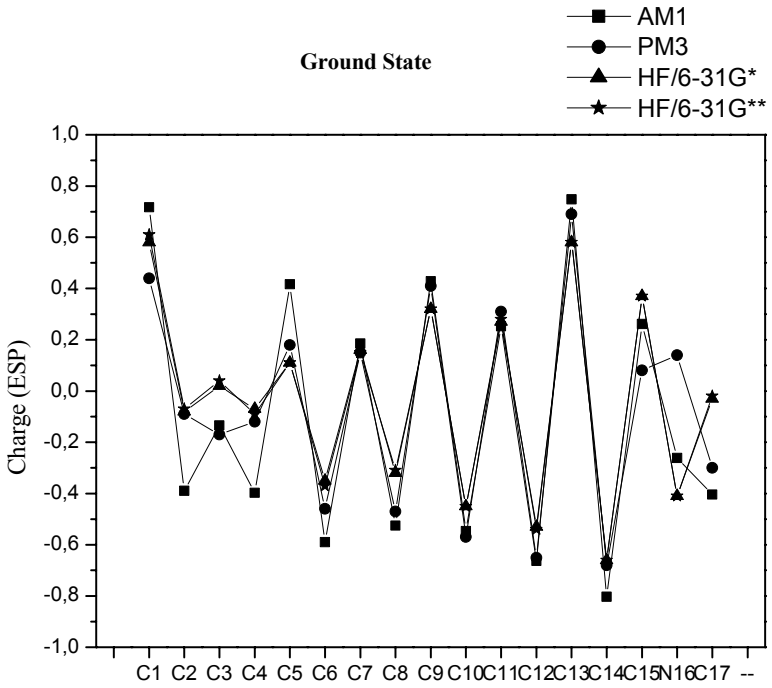


Figure 7. Representation of the 11–*cis*–rhodopsin chromophore charges calculated for the ground state.

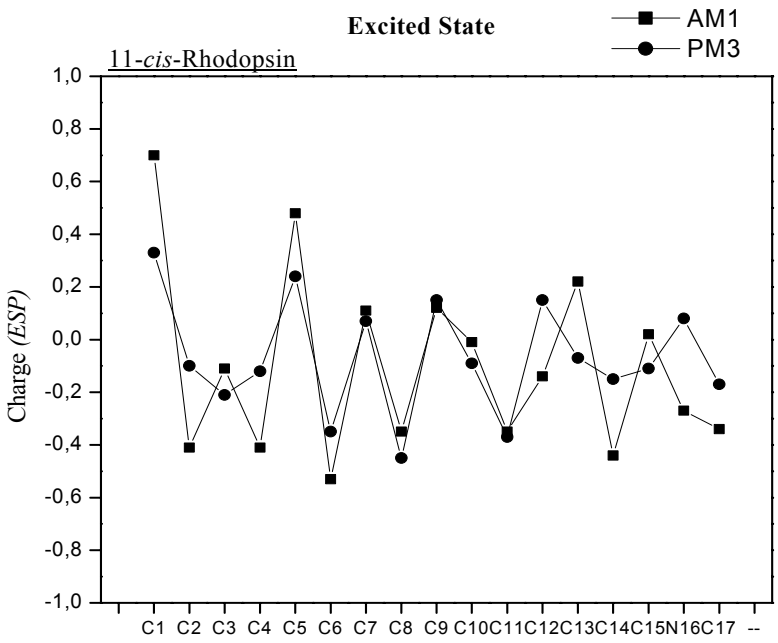


Figure 8. Representation of the 11–*cis*–rhodopsin chromophore charges (ESP) calculated, for the excited state and relaxed geometry.

We found a decrease in single–double bond alternation, *i.e*., conjugation in the skeleton of the 11–*cis* isomer upon electronic excitation. This trend is detected for atom–atom distances, bond orders and, in a more enhanced manner, for the charge distribution. This effect is caused rather by electron density rearrangement after the absorption of light than due to change in geometry. These results would suggest why the *cis* → *trans* isomerization is facilitated in the excited state. But our finding is also suggestive of a decrease in charge density alternation order in the excited state, which could thus be a poorer electric conductor than the ground state. The experiment by Mergulhão *et al*. [29] shows exactly the same behavior for a protonated polymer. Moreover, the accepted model for the visual information generation, as described by Rieke *et al*. [7], has the very same photoresistance mechanism.

4 CONCLUSIONS

Two tasks were undertaken in this study: (*a*) To provide an accurate description of the electronic absorption spectra of 11–*cis*– and all–*trans* rhodopsin chromophores; (*b*) To compare the ground and first excited singlet states of the protonated chromophore of 11–*cis*–rhodopsin in the hope to bring some insight for the process of the generation of the visual signal after absorption of light by the rods in the retina. Theoretical electronic absorption spectra for the 11–*cis* species and the all–*trans* isomer were calculated with the ZINDO/S CI method. The resulting simulated spectra agree remarkably with experimental measurements.

Our comparison of the ground and the excited state indicates some decrease in conjugation in the last case. These results give support to the early findings of Trsic [5], although, curiously enough,

this trend seemed more enhanced in the old calculations. The present theoretical results allow the consideration that the ordered conjugated ground system is a better electrical conductor than the excited defect species. This is certainly consistent with the fact that absorption of light stops the dark current and the Ca^{2+}, K^+ and Na^+ ion flow [6,7]. A very enlightening analogy is provided by protonated polyanilines, in which a light impulse produces a decrease in current, with the possible creation of excited defect species [29]. The former conclusions are consistent with some long time well established notions by Professor Wald. Rhodopsin in the rods is virtually in the solid state [30] and there are millions of rhodopsin molecules in each rod. If there is an ionic dark current between the rods and the cell membrane, the rods ought to conduce electrical charges. The time elapsed between the absorption of light and the detection of an electric impulse in the optical nerve is shorter than the initiation of isomerization [31]. Thus, optical information is due to a very early phenomenon. A rod has many compartments, and the absorption of a photon by one of them stops the dark current and initiates the bleaching process [32]. Further happens a slow biochemical sequence of events, which eventually recovers that particular rod. (We all have the experience that after a very intense light exposure, many rods simultaneously excited, we remain blind for several seconds). It is licit to presume that the compartment is a section of the rod membrane containing one or several rhodopsin molecules. This work puts forward some plausible arguments for a possible mechanism for the interruption of the dark current after light absorption in the rods of the retina.

Acknowledgment

The authors acknowledge financial support from the Brazilian Agencies CAPES, CNPq and FAPESP.

5 REFERENCES

[1] H. Lodish, A. Berk, S. L. Zipursky, P. Matsudaira, D. Baltimore and J. E. Darnell, *Molecular Cell Biology*, Freeman, New York, 1999.
[2] D. L. Nelson and M. M. Cox, *Lehninger Principles of Biochemistry*, Worth, New York, 2000.
[3] B. Rosenberg, Photoconduction in Hindered Cis–Isomer of β–Carotene and Its Relation to Theory of the Visual Receptor Process. *J. Opt. Soc. Am.* **1961**, *51*, 238–240.
[4] B. Rosenberg, R. A. Orlando and J. M. Orlando, Photoconduction and Semiconduction in Dried Receptors of Sheep Eyes. *Arch. Biochem. Biophys.* **1961**, *93*, 395–398.
[5] M. Trsic, May Rhodopsin be a Photoresistor? *An. Real Soc. Esp. Fís. Quím.* **1969**, *65*, 833–836.
[6] F. Rieke and D. A. Baylor, Molecular Origin of Continuous Dark Noise in Rod Photoreceptors. *Biophys. J.* **1996**, *71*, 2553–2572.
[7] F. Rieke and D. A. Baylor Single–Photon Detection by Rod Cells of the Retina. *Rev. Modern Phys.* **1998**, *70*, 1027–1036.
[8] K. A. Bagley, V. Balogh–Nair, A. A. Croteau, G. Dollinger, T. G. Ebrey, M. K. Hong, K. Nakaganishi and J. Vittitow, Fourier Transform Infrared Difference Spectroscopy of Rhodopsin and its Photoproducts at low Temperature, *Biochemistry*, **1985**, *24*, 6055–6071.
[9] L. C. P. J. Mollevanger, A. P. M. Kentgens, J. A. Pardoen, J. M. L. Courtin, W. S. Veeman, J. Lugtenburg and W. J. De Grip, High–Resolution Solid–state ^{13}C NMR Study of Carbon C–5 and C–12 of the Chromophore of bovine Rhodopsin. Evidence for a 6–*s*–*cis* Conformation With Negative–Charge Perturbation Near C–12, *Eur. J. Biochem.* **1987**, *163*, 9–14.
[10] A. R. Oseroff and R. H. Callender, Resonance Raman Spectroscopy of the Retinylidene Schiff's Base Counterion in Bovine Rhodopsin, *Biochemistry*, **1974**, *13*, 4243–4248.
[11] S. O. Smith, I. Palings, V. Copié, D. P. Raleigh, J. Courtin, J. A. Pardoen, J. Lugtenburg, R. A. Mathies and R. G. Griffin, Low–Temperature Solid–state ^{13}C NMR Studies of the Retinal Chromophore in Rhodopsin, *Biochemistry*

1987, *26*, 1606–1611.
[12] I. Palings, E. Pardoen, J. Winkel and C. Van Der Berg, Assignment of Fingerprint of Vibrations in the Resonance Raman Spectra of Rhodopsin, Isorhodopsin and Bathorhodopsin: Implication for Chromophore Structure and Environment, *Biochemistry* **1987**, *26*, 2544–2656.
[13] I. Pepe, Rhodopsin and Phototransduction, *J. Photochem. Photobiol. B: Biol*. **1999**, *48*, 1–10.
[14] HYPERCHEM For Windows, version 6.0, Ontario, Hypercube, Inc. 2000.
[15] AMPAC, version 6.5, Semichem, Shawnee, Inc. 2000.
[16] W. P. Anderson, T. R. Cundari and M. C. Zerner, An Intermediate Neglect of Differential–Overlap Model for 2nd Row Transition–Metal Species, *Int. J.Quantum Chem*. **1991**, *39*, 31–45.
[17] W. P. Anderson, W. D. Edwards and M. C. Zerner, Calculated Spectra of Hydrated Ions of the 1st Transition–Metal Series, *Inorg. Chem*. **1986**, *25*, 2728–2732.
[18] M. C. Zerner, G. H. Loew, R. F. Kirchner and U. T. Mueller–Westerhoff, Intermediate Neglect of Differential–Overlap Technique for Spectroscopy of Transition Metal–Complexes – Ferrocene, *J. Am. Chem. Soc*. **1980**, *102*, 589–599.
[19] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. LIU, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. AL–Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head–Gordon, E. S. Replogle and J. A. Pople, Gaussian, Inc., Pittsburgh PA, **1998**.
[20] T. Yoshizawa and O. Kuwata, Vision: Photochemistry; in: *Organic Photochemistry and Photobiology*, CRC Press, New York, 1995, *197*, pp. 1493–1499.
[21] J. Nathans, Rhodopsin: Structure, Function and Genetics, *Biochemistry* **1992**, *31*, 4923–4931.
[22] T. Yoshizawa and G. Wald, Prelumirhodopsin and the Bleaching of Visual Pigments, *Nature* **1963**, *197*, 1279–1286.
[23] D. O. Cowan and R. L. Drisko, *Elements of Organic Photochemistry*, Plenum Press, New York, 1976.
[24] A. S. Waggoner and L. Stryer, Induced Optical Activity of the Metarhodopsins, *Biochemistry* **1971**, *10*, 3250–3254.
[25] M. J. Burke, D. C. Pratt, T. R. Faulkner and A. Moscowitz, An Analysis of the Absorption and Circular Dichroism of Some Visual Pigments, *Exp. Eye Res*. **1973**, *17*, 557–572.
[26] A. Szabo and N. S. Ostlund, *Modern Quantum Chemistry*, McGraw–Hill, New York, 1989.
[27] R. E. Bruns, P. H. Guadagnini and A. A. D. Souza, Cargas Atômicas em Moléculas, *Química Nova* **1996**, *19*, 148–155.
[28] K. B. Wiberg and P. R. Rablen, Comparison of Atomic Charges Derived via Different Procedures, *J. Comput. Chem*. **1993**, *14*, 1504–1518.
[29] S. Mergulhão, R. M. Faria, G. F. L. Ferreira and J. Sworakowski, Transport of Holes in Uniformly and Non–uniformily Protonated Poly (*o*–methoxyaniline), *Chem. Phys. Lett*. **1997**, *269*, 489–493.
[30] G. Wald and P. K. Brown, Human Rhodopsin, *Science* **1958**, *127*, 222–226.
[31] G. Wald, J. Durell and R. C. C. S. George, The Light Reaction in the Bleaching of Rhodopsin, *Science* **1950**, *111*, 179–181.
[32] G. Wald, On the Mechanism of the Visual Threshold and Visual Adaptation, *Science* **1954**, *119*, 887–892.

Biographies

Melissa Fabíola Siqueira Pinto was born in Manaus, Brazil in 1978. In 1996, she initiated her undergraduate studies at the Federal University of Pará in Belém and concluded in 2001, at the Federal University of São Carlos, São Paulo. In 2003, she concluded the Master degree at the Instituto de Química de São Carlos and is now initiating her PhD work with Professor Milan Trsic. She published one paper in Journal of Molecular Structure: Theochem.

Milan Trsic was born in Belgrade, Serbia in 1937. In 1948 he arrived in Chile with his parents where he obtained a professional degree in Chemistry and Pharmacy in 1961. In 1966 he concluded a PhD degree with Professor Raymond Daudel in Paris. During 1972–73 he was a PD fellow with Professor Per–Olov Löwdin in Uppsala. From 1974 to 1978 he was a Research Associate in Calgary, Alberta, Canada. He holds a position at the University of São Paulo, Brazil at the São Carlos Campus since 1978, where he became a Full Professor in 1991. He has published more than 80 papers, two book chapters and edited two books. His main interest have been perturbation theory, excited states, sulfur nitrides and, presently, various chemical species with technological interest. He is one of the authors at the origin of what is known as the Generator Coordinate Hartree–Fock Method.