Retinal Chromophores

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Probing and Modeling the Absorption of Retinal Protein Chromophores in Vacuo**

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The wide range of absorption energies observed in visual (425–560 nm) and archaeal (480–590 nm) rhodopsins[1] is one of their most fascinating aspects. This phenomenon has intrigued experimentalists and theoreticians alike even more since it appeared that the retinal protonated Schiff base (RPSB) isomer responsible for such a spectral modulation is the same within each of the two protein classes: archaeal (6s-*trans*,all-*trans*) and visual (6s-*cis*,11-*cis*) rhodopsins.[2,3] Understanding the so called "opsin-shift"[4–6] involves disclosing the delicate role of the environment and would greatly benefit from knowledge of the unperturbed (i.e., gas phase) spectral properties of the isolated chromophore. Rhodopsin's absorption has traditionally been compared with that of the chromophore in methanol solution (λ_{max} = 440 nm), even though this is far from the unperturbed value: a significant spectral variability is recognized in solvents as well ($\Delta\lambda_{max}$ ca. 100 nm) arising from the interactions of the chromophore and its counterion with the solvents.[5] Quantum mechanical (QM) computations could deliver, in principle, such an unperturbed reference value. However, the results for the computed absorptions of isolated RPSBs are spread over a wide range ($\Delta\lambda_{max} > 100$ nm),[7] revealing that the choice of the QM method is still a critical issue.

The determination of an unbiased absorption reference has awaited the capability to produce a stable sample of gas-phase ionic chromophores,[8,9] combined with laser/detecting techniques of suitable accuracy, and the development of theoretical methods that match the resolution of experiments.[10] Herein, we use an improved experimental approach that is able to assess the local structures of the recorded spectra and deliver, for the first time, the intrinsic (i.e., of the unperturbed molecule) high-resolution absorption spectrum of the skewed 6s-*cis* and planar 6s-*trans* RPSB conformations that are implicated in the spectroscopy of visual and archaeal-type rhodopsins, respectively.[2,3,11] Observations are complemented and supported by a new set of advanced MR-MP2 computations that match the experimental accuracy. Besides interpreting experiments, they also define a novel computational benchmark in the field.

Figure 1 displays the RPSBs used in this work. The native chromophore (A; Figure 1) shows an absorption profile that is remarkably broad, ranging from about 450 nm to 650 nm with an essentially flat top extending from 530 nm and 610 nm (Figure 2a). This result is in contrast with earlier reports[9] which show local structures that may be ascribed to changing laser-beam profiles owing to different transverse modes of the optical parametric oscillator (OPO) laser cavity used. To probe the absorption dependence on the β-ionone rotation angle, we measured the absorption of two retinal chromo-

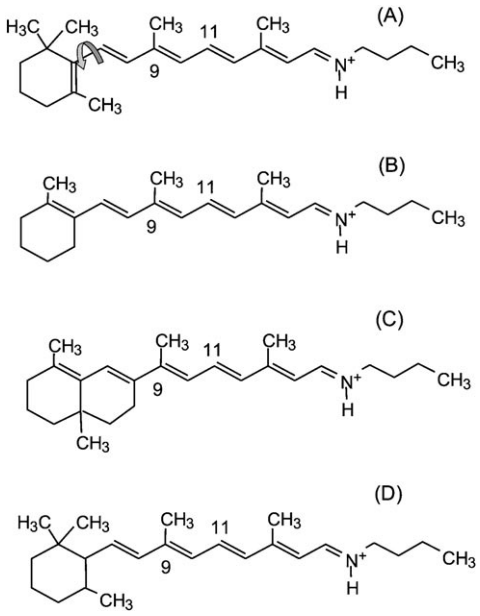

Figure 1. The native system (A) has two almost isoenergetic conformers (indicated by the arrow) with the β-ionone ring in a skewed (i.e., ca. 60° twisted) 6s-*cis* or a planar (i.e., ca. 180°) 6s-*trans* geometry, while both the unlocked (B) and locked (C) models have a stable planar (i.e., ca. 180°) 6s-*trans* structure. D is the C5,C6-dihydro retinal analogue.

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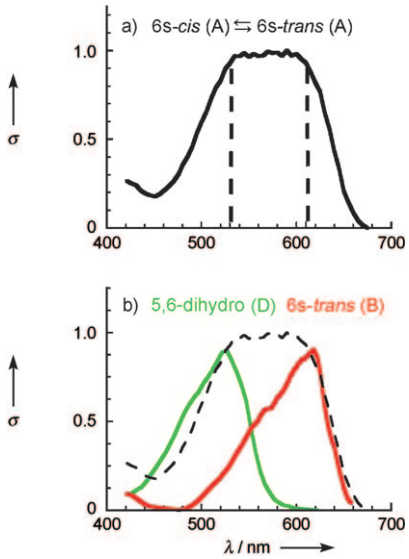


Figure 2. Measured gas-phase absorption cross sections in a) the native protonated Schiff-base chromophore (A) and b) two analogues (B and D: red and green peaks, respectively; the absorption of A is also reported for comparison; dashed curve) measured with identical laser settings (spectra are not normalized, but merely superimposed). The blue (530 nm) and red (610 nm) sides (dashed vertical lines) of the broad absorption maximum of the native compound (A) are taken as the reference gas-phase absorption value for the planar 6s-*trans* and the skewed 6s-*cis* conformers, respectively. Computed vertical $S_0 \rightarrow S_1$ transition energies for the models are found in Table 1.

phore analogues B and C (Figure 1) both of which have a planar 6s-*trans* C5=C6–C7=C8 moiety.[3] The experimental absorption profile of B is shown in Figure 2b. Interestingly, both these compounds display a clear peak (λ_{max} is 618 nm (B) and 630 nm (C)) that falls in the red edge of the native compound spectrum. In contrast, the recorded absorption profile of the C5,C6-dihydro retinal analogue D (Figure 1), also shown in Figure 2b, has a peak at 525 nm which is in the blue side of the plateau. Compound D lacks π-conjugation from the ring end and is thus a model for a retinal chromophore with a fully twisted β-ionone ring (and, in turn, for a skewed highly twisted 6s-*cis* conformer). These results suggest that the β-ionone ring is relatively free to rotate at room temperature so that it explores essentially the whole phase-space and excitation energies span all possible values from fully twisted to fully planar conformations.

To support this scenario, the geometries of the conformational minima in both the native (A) and analogue (B–D) compounds have been optimized at the CASSCF/6-31G* level[12] and their S_0 and S_1 energies computed employing the multireference perturbative CASPT2/ANO-s(C,N[4s3p1d]/H[2s]) approach (Table 1).[10] Interestingly, the S_0 energy difference between the two optimized conformers (the skewed 6s-*cis* with a highly twisted 68° β-ionone ring and the planar 6s-*trans*) is very tiny in A (<1 kcal mol^{-1}), as was previously recognized,[7a] but the spectroscopic implications were not investigated. This finding reveals that both conformations may well be populated under the experimental conditions thus giving the observed broad band: their computed absorptions nicely match the edges of the recorded band shown in Figure 2a. The energy difference, however, is much higher in B (ca. 4 kcal mol^{-1}), in favor of the fully planar 6s-*trans* conformer, which is then the more populated form: this is responsible for the red-shifted and narrower and more-defined absorption peaks observed in B and C. The agreement with the experiments is remarkable for all the studied systems.

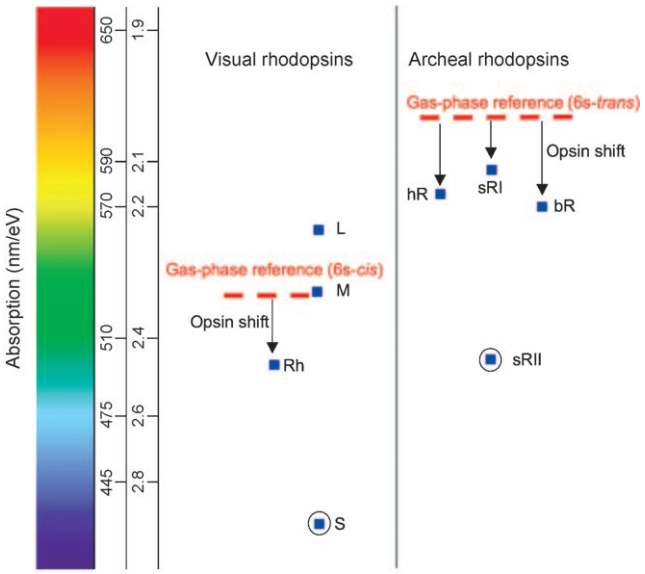
New reference wavelengths for the intrinsic absorption of the two RPSB conformers found in visual and archaeal rhodopsins are thus derived, with maximums at approximately 530 (6s-*cis*) and 610 nm (6s-*trans*), respectively (Figure 2a). It is apparent that only a small blue-shift is observed on going from the gas-phase cationic chromophore to bR (hR, sRI) or Rh (≤ 30 nm), it is almost negligible for the M-cone pigment, and is even reversed for L (see Scheme 1). Notably, this result reveals that opsin masks the counterion and eliminates its electrostatic interaction with the cationic chromophore, thus smoothing most of the counterions's blue-shifting activity. In addition, other protein dipoles that might blue shift the absorption are not operative. However, there are exceptions: sRII and the S-cone visual pigment absorb at significantly blue-shifted values (Scheme 1). This finding suggests that the counterion and protein dipoles are not masked in these two cases, or alternatively, that a further deconjugation in the chromophore chain occurs in the protein pocket.

It is worth noting that to date no retinal protein has been revealed that absorbs red shifted to the gas-phase 6s-*trans* reference value of 610 nm. The "blue membrane" form of bR absorbs at 605 nm while the most red-shifted intermediate detected in bR photocycle (denoted as O) absorbs at approximately 610 nm. It is apparent that in these species the chromophore behaves as in the gas phase. In other words, the electrostatic blue-shifting effect of the counterion[13] and

Table 1: Computed ground state and vertical $S_0 \rightarrow S_1$ transition energies.[a]

Compound	6s-*trans*		6s-*cis*		Abs_{exp}
	E	Abs_{calcd}	*E*	Abs_{calcd}	
A	0.6	620, 46.2, 2.00	0.0	547, 52.3, 2.27	530–610, 46.9–54.0, 2.03–2.34
B	0.0	612, 46.7, 2.03	3.86	566, 50.5, 2.19	618, 46.3, 2.01
C[b]		642, 44.5, 1.93			630, 45.4, 1.97
D[c]	Abs_{calcd}: 514, 55.7, 2.41				525, 54.5, 2.36

[a] CASPT2/ANO-s ground-state relative energies (*E*, in [kcal mol^{-1}]) and vertical $S_0 \rightarrow S_1$ absorptions (Abs_{calcd} in [nm], [kcal mol^{-1}], [eV]) are computed at the CASSCF/6-31G* level for optimized geometries of the chromophores (A–D): a *N*-methyl terminal is used. Experimental absorption maxima (Abs_{exp} in [nm], [kcal mol^{-1}], [eV]) are also reported. Underlined entries highlight the conformer(s) contributing to the recorded values. [b] This molecule was synthesized as a 6s-*trans* form. [c] 6s-*trans* and 6s-*cis* conformers cannot be assigned for D which exists as a single form, absorbing at the reported value ([nm], [kcal mol^{-1}], [eV]).



Scheme 1. Absorptions of archaeal rhodopsins (proton/chloride ion pumps bacteriorhodopsin (bR, $\lambda_{max}=570$ nm)/halorhodopsin (hR, $\lambda_{max}=576$ nm), and the two phototaxis proteins, sensory rhodopsin I (sRI, $\lambda_{max}=587$ nm) and rhodopsin II (sRII, λ_{max} ca. 500 nm: ppR-(NpsRII) is 500 nm, sRII is 480-500 nm)), and visual rhodopsins (the twilight vision rhodopsin pigment (Rh, $\lambda_{max}=498$ nm) and the three color vision cone pigments—blue (S, $\lambda_{max}=425$ nm), green (M, $\lambda_{max}=533$ nm), and red (L, $\lambda_{max}=560$ nm). These values are compared to that of the corresponding retinal conformer (6s-*cis* and 6s-*trans* for archaeal and visual rhodopsins, respectively) in vacuo (dashed red lines). A residual (blue-shift) counterion effect (black arrows) can generally be addressed to the opsin-shift in Rh and bR (hR, sRI). Anomalous blue-shifted pigments (S and sRII) are marked with a circle. Note that the reference gas-phase absorption value for the planar 6s-*trans* conformer is chosen as the red edge of the broad native (A) absorption band maximum reported in Figure 2a and the skewed 6s-*cis* conformer as the blue edge.

protein dipoles are completely eliminated through, for example, an effective hydrogen-bonding network to the counterion.

In conclusion, instead of the previously employed absorption in methanol, we suggest that the unperturbed gas-phase RPSB absorptions reported in this work should be used as new starting points for the "opsin effect" (and, more generally, RPSB spectral properties in different environments). We have shown that in some retinal proteins, including the archetypal visual and archaeal systems, twilight vision rhodopsin pigment (Rh) and bacteriorhodopsin (bR), the absorption is close to that recorded in the gas phase. The precise gas-phase spectroscopy of RPSB may indeed be essential for understanding the exceptional (that is, most reactive, efficient, and fast)[14] photoisomerizing operation of retinal pigments.

Experimental Section

Methods

Spectroscopy: The ions were first trapped in a cylindrical ion trap at room temperature. After 100 ms accumulation time the ions were extracted, accelerated to 22 keV, mass and charge-analyzed by a magnet, and directed into the ELISA storage ring.[8] After about 40 ms of storage the chromophore ions were exposed to a 3 ns laser pulse of tunable wavelength. The many milliseconds of storage in vacuum prior to photoexcitation ensure that the experiment begins with a molecule occupying the electronic ground state S_0. Each ion bunch was only exposed to one laser pulse. Photoabsorption brings the molecule into the first excited state of the same spin multiplicity S_1. Internal conversion (IC) transfers excess electronic energy into vibrational energy. The energy increase per absorbed photon is 1.9–2.5 eV for an absorption band at 500–650 nm. This produces a hot ground-state chromophore ion that dissociates and hence yields a neutral and a positively charged fragment. The absorption cross section σ was obtained as the time-integrated counts of neutral photo fragments normalized to the number of photons in the laser pulse and to the number of ions in the ion bunch. With respect to the previously applied equipment,[9] the experimental technique has been improved herein in several ways by using 1) a new laser with better beam quality, 2) an active control of the laser-pulse energy to avoid saturation, and 3) an additional detector to include the possible prompt action of the photo excitation.

Synthesis: Chromophores B–D were synthesized using previously described methods.[3,4,15]

Computations: CASSCF/6–31G* geometry optimizations[12] and multireference perturbative CASPT2/ANO-s(C,N[4s3p1d]/H[2s]) computations[10] are both performed using a full active space of π electrons and orbitals and employing, respectively, a single-state (S_0) wavefunction and a three-roots (equally weighting S_0, S_1, and S_2) state average wavefunction with a 0.2 imaginary level shift.

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