Exploring the protonation properties of photosynthetic phycobiliprotein pigments from molecular modeling and spectral line shapes

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Abstract-*In photosynthesis, specialized light harvesting pigment-protein complexes (PPCs) are used to capture incident sunlight and funnel its energy to the reaction center. In Cryptophyte algae these complexes are suspended in the lumen, where the pH ranges between ~5-7, depending on the prolongation of the incident sunlight. However, the pKa of the several kinds of bilin chromophores encountered in these complexes and the effect of its protonation state on the energy transfer process is still unknown. Here, we combine quantum chemical and continuum solvent calculations to estimate the intrinsic aqueous pKas of different bilin pigments. We then use Propka and APBS classical electrostatic calculations to estimate the change in protonation free energies when the bilins are embedded inside five different phycobiliproteins (PE545, PC577, PC612, PC630 and PC645), and critically asses our results by analysis of the changes in the absorption spectral line shapes measured within a pH range from*
4.0 to 9.4. Our results suggest that each individual protein environment strongly impacts the intrinsic pKa of the different chomophores, being the final responsible of their protonation state.

Nature has developed sophisticated and highly efficient molecular architectures to absorb sunlight and convert it into chemical energy, which is finally used by photosynthetic organisms to live and grow. The comprehension of these light-harvesting processes occurring in photosynthetic pigment-protein complexes (PPC) has been an important goal since the first high-resolution structure of the Fenna-Matthews-Olson complex appeared 40 years ago [1]. Cryptomonads are a group of algae which are important primary producers in marine and freshwater environments due to its high quantum yeld. As compared to land plants, the available light that algae can harvest in water environments is significantly reduced. For that reason, cryptomonads' PPC use tunable linear tetrapyrrole chromophores (bilins) covalently bounded to the protein scaffold, which structure and disposition inside the protein have evolved to increase the spatial and spectral cross

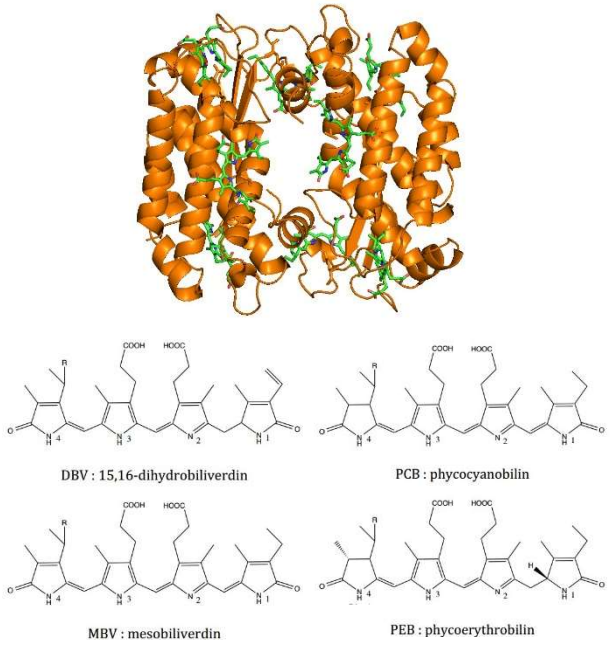

Fig. 1 a) Crystal structure of the pigment-protein complex PC577 and disposition of the containing chromophores (green). b) Structure of the four bilin pigments studied.

affects the local environment of the primary antenna proteins (phycobiliproteins), which are bathed in the lumen. Although many theoretical and experimental studies have been done in order to uncover the basic mechanisms that drive electronic energy transfer in these organisms [3]–[6] and that recently, various PPCs structures have been elucidated (Fig. 1a) [2] (PE545, PC577, PC612, PC630 and PC645), the p*K*a of the chromophores encountered in these complexes and the effect of its protonation state on the energy transfer process is still unknown, and is hard to be determined experimentally because they are covalently bounded to the protein scaffold and they lose their active

cryptomonads these PPCs are suspended in the lumen, inside the intrathylakoid membrane. Under intense illumination, the reaction centers of photosynthetic organisms are capable of redirecting the excess excitation energy by a change in the thylakoid lumen pH, which triggers a biochemical feedback process in which the absorbed energy is dissipated as heat. Unlike in most photosynthetic organisms, in cryptophyte algae, the increased acidification of the thylakoid lumen directly

Here, we combine quantum chemical and continuum solvent electrostatic calculations to build a thermodynamic cycle and obtain the change in the Gibbs free energy of the reactions of deprotonation in solution (ΔG_{aq}), governed by the equilibrium constant of the reaction (K_a), so the p*K*a is calculated using (1).

$$pK_a = \frac{\Delta G_{aq}}{RT\ln(10)} \quad (1)$$

We extrapolate the MP2 energy to a complete basis set and we also applied the spin-comonent scaled MP2 correction of the energy proposed by Grimm [7]. We used both MST [8] and SMD solvation methods, giving better results the SMD for the neutral species and the MST for the charged ones, so we performed an average of both sovation free energies to finally obtain the intrinsic p*K*a of each protonable site of each bilin chromophore (PCB, PEB, DBV and MBV) (Fig. 1b, Table I).

Table I. Gibbs free energies in the gas phase and intrinsic p*K*as for each kind of bilin chromophore.

DBV	ΔG_{gas} (kcal mol^{-1})	pKa	PCB	ΔG_{gas} (kcal mol^{-1})	pKa
N4	255.3	17.4	N4	250.2	11.9
N3	234.3	6.7	N3	238.4	7.1
N2	233.1	6.3	N2	238.7	7.4
N1	288.4	29.9	N1	258.1	17.0
MBV	ΔG_{gas} (kcal mol^{-1})	p*K*a	PEB	ΔG_{gas} (kcal mol^{-1})	p*K*a
N4	256.6	16.7	N4	249.4	13.3
N3	236.7	6.6	N3	235.4	6.8
N2	236.6	6.6	N2	234.1	6.5
N1	256.2	16.1	N1	290.4	30.0

Then, we used both Propka server and continuum electrostatic methods (APBS) to estimate the change in the Gibbs free energy of the reaction of deprotonation of the two central pyrrole rings, which are the only ones susceptible to undergo deprotonation, in each particular environment inside each protein (PC577, PC612, PC630, PC645 and PE545). If we observe the crystal structure, we can see that all chromophores instead of MBVs, are coordinated under the two central pyrrole rings by an aspartic or glutamic acid, presumably stabilizing the protonated form of the chromophore with p*K*as ranging between 6 to 7, while the MBVs present p*K*as ranging between 4-5. As we can assume an error of ± 1 or 2 p*K*a units, we finally asses our results by analysis of the changes in the experimental absorption spectral line shapes measured within a pH range from 4.0 to 9.4 (See Fig. 2).

Our results suggest that each individual protein environment strongly impacts the intrinsic p*K*a of the different chomophores, being the final responsible of their protonation state. So, if we observe the experimental spectra, between 5.4 and 8.2 (Fig. 2) there are no apparent changes in the DBVs and PCBs spectral region, while from 6.5 to 7.8 there is a shift of the absorption lineshape within the MBVs region. So, we can assume that we are underestimating the results 1.5 – 2 p*K*a units, and that all chromophores are protonated at a working pH of 7.5, being the MBVs the first ones to deprotonate due to the lack of an stabilizing group coordinating the two central pyrrole rings.

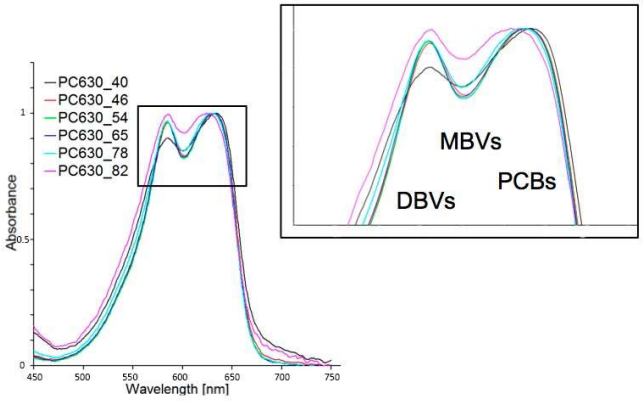

Fig. 2 Absorption spectrum of the antenna protein PC630 at different pHs between 4.0 and 8.2.

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