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

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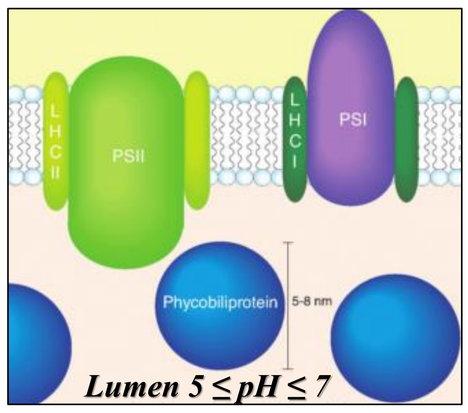
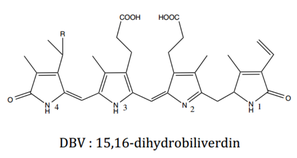
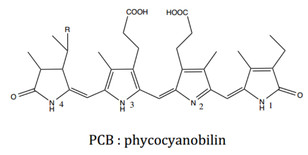
In photosynthesis, specialized light harvesting pigment-protein complexes (PPCs) are used to capture incident sunlight and funnel its energy to the reaction center. The PPCs of cryptophyte algae use tunable linear tetrapyrrole chromophores (bilins) covalently bounded to the protein scaffold, which structure and disposition inside the protein have evolved to increase the spatial and spectral cross section for absorption of incident light.[1] These proteins are suspended in the lumen, where the pH ranges between ~5-7, depending on the prolongation of the incident sunlight. Many theoretical and experimental studies have been done in order to uncover the basic mechanisms that drive electronic energy transfers in these organisms.[2–5] 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: phycocyanobilin (PCB), phycoerythrobilin (PEB), 15,16-dihydrobiliverdin (DBV) and mesobiliverdin (MBV). We then use 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 assess 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. Our study paves the way for accurate structure-based studies on the light harvesting properties of cryptophyte antenna complexes.

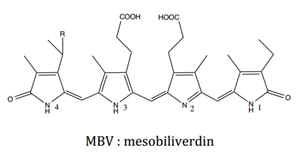
Introduction

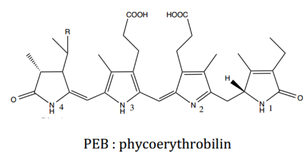
Cryptomonads are a group of algae which are important primary producers in marine and freshwater environments due to its high quantum yeld at very low light conditions. Under intense illumination, the reaction centers of photosynthetic organisms are capable of redirecting the excess excitation energy 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 affects the local environment of the primary antenna proteins (phycobiliproteins), which are bathed in the lumen. Although recently various PPCs structures have been elucidated (PE545, PC577, PC612, PC630 and PC645), the pKa of the containing chromophores is hard to be determined experimentally because they are covalently bounded to the protein scaffold and they lose their active conformation in solution.

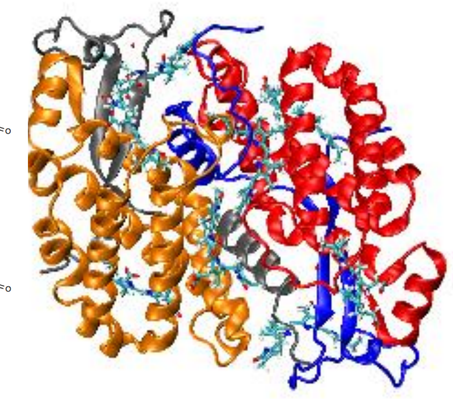












Methodology

We use a thermodynamic cycle to obtain the change in Gibbs free energy of the reaction of deprotonation in solution (ΔG_{aq}), governed by the equilibrium constant of the reaccion (K_a), so the pKa is calculated as follows:

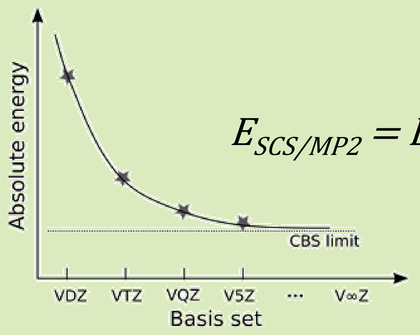
$$HA_{(aq)} \rightarrow A_{(aq)} + H^+_{(aq)} \qquad K_a = \frac{[A_{(aq)}][H^+_{(aq)}]}{[HA_{(aq)}]} \qquad pK_a = -\log K_a$$

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

$\Delta G_{sol}(H^+)$ = -264.0 kcal/mol (1atm) → -265.9 kcal/mol (1M)
$G_{gas}(H^+)$ = -6.28 kcal/mol (1atm)

$$\Delta G_{aq} = -\Delta G_{sol\ AH} + \Delta G_{gas} + \Delta G_{sol\ H+} + \Delta G_{sol\ A-}$$

- **Gas phase Gibbs free energy**: Extrapolation of the MP2 energy to a complete basis set with spin-component scaled MP2 correction of the energy.[6] Final CCSD correction of the energy to recover the correlation between MP2 and CCSD.

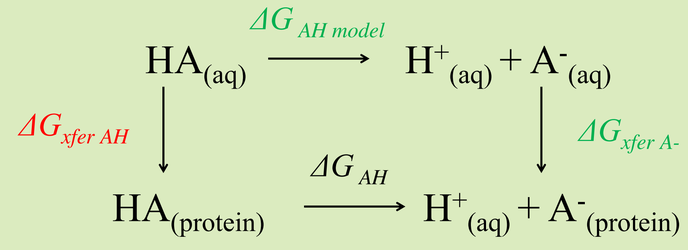


$$E_{CBS} = E_{HF} + E_{Corr} + \Delta CCSD$$

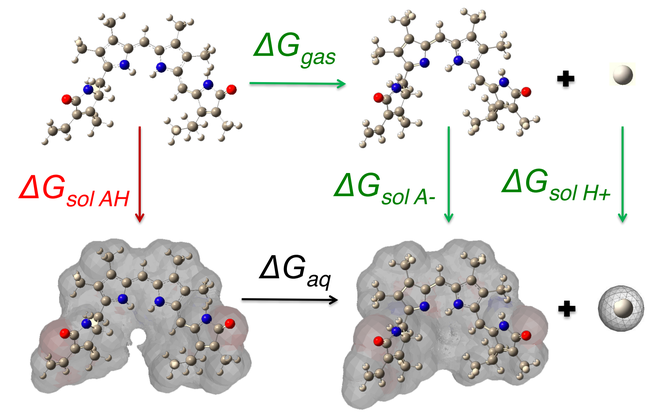
$$E_{SCS/MP2} = E_{HF} + 1/3\,(E_{corr(\alpha\text{-}\alpha)} + E_{corr\,(\beta\text{-}\beta)}) + 6/5\,E_{corr(\alpha\text{-}\beta)}$$

- **Solvation Gibbs free energy**: We use both MST[7] and SMD solvation methods, giving better results the SMD for the neutral species and the MST for the charged ones.

- **pKa values in Proteins:** We use both the Propka server and continuum electrostatics methods (APBS). The latter estimates the transfer free energies from water to the protein local environment using Poisson-Boltzmann energies.



Results



Intrinsic pKas

DBV	dGgas / kcal mol^{-1}	MST-SMD pKa
N4	255.3	17.4
N3	234.3	6.7
N2	233.1	6.3
N1	288.4	29.9

PCB	dGgas / kcalmol-1	MST-SMD pKa
N4	250.2	11.9
N3	238.4	7.1
N2	238.7	7.4
N1	258.1	17.0

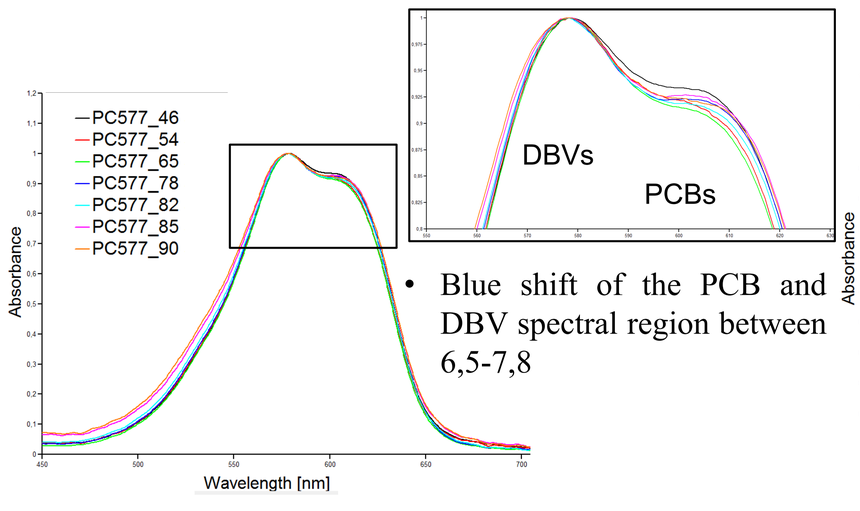
MBV	dGgas / kcalmol-1	pKa
N4	256.6	16.7
N3	236.7	6.6
N2	236.6	6.6
N1	256.2	16.1

PEB	dGgas / kcalmol-1	pKa
N4	249.4	13.3
N3	235.4	6.8
N2	234.1	6.5
N1	290.4	30.0

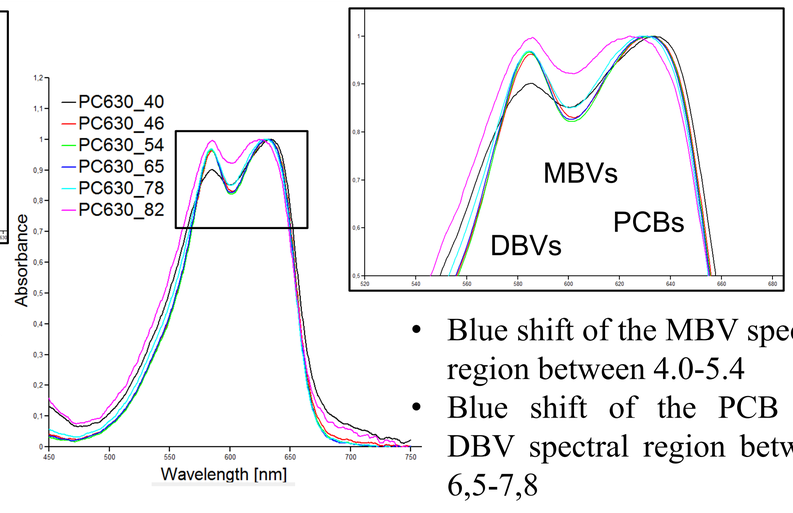
Protein pKas

PC630	$\alpha\alpha'\beta_2$			pKa	
		propKa	APBS	propKa	APBS
Chain	Residue	N2		N3	
A	MBV_19	4.93	3.63	5.69	3.87
B	DBV_50-61	7.24	5.53	7.32	5.98
B	PCB_158	7.91	6.59	7.79*4.68	6.53
B	PCB_82	7.28	5.66	7.12	5.55
C	MBV_19	4.72	4.60	5.11*4.05	4.60
D	DBV_50-61	6.85	6.31	7.06	6.53
D	PCB_158	8.07*4.68	6.40	7.74*4.51	6.36
D	PCB_82	7.26	5.86	6.97	5.69

PC577	$\alpha_2\beta_2$			pKa	
		propKa	APBS	propKa	APBS
Chain	Residue	N2		N3	
A	PCB_20	7.82	7.30	7.89	7.28
B	DBV_50-61	7.22	8.02	7.64	8.11
B	PCB_158	8.02	7.26	7.87	7.20
B	PCB_82	7.39	6.29	7.12	6.16
C	PCB_20	7.78	7.51	7.94	7.23
D	DBV_50-61	7.22	7.73	7.66	8.40
D	PCB_158	8.10	7.39	7.84	7.35
D	PCB_82	7.31	6.23	7.11	6.03

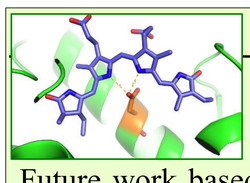


- Blue shift of the PCB and DBV spectral region between 6,5-7,8



- Blue shift of the MBV spectral region between 4.0-5.4
- Blue shift of the PCB and DBV spectral region between 6,5-7,8

Conclusions & Future work



Our results indicate that MBVs are unprotonated, whereas DBVs and PCBs are protonated at the range of pHs relevant for photosynthesis.

Future work based on polarizable QM/MM calculations are being performed to investigate if the observed spectral shifts are indeed due to a change in the bilin protonation state, or related to changes in the protonation pattern of the Asp residue coordinating most bilins.

MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

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