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First computational step towards the understanding of the antioxidant activity of the Phycocyanobilin:Ferredoxin Oxidoreductase in complex with biliverdin IXα

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ABSTRACT

Phycocyanobilin:Ferredoxin Oxidoreductase (PcyA) is a ferredoxin-dependent bilin reductase that converts biliverdin IXα (BV) into 3Z/3E-phycocyanobilin (3Z/3E-PCB) through a four-electron reduction mechanism. Using state-of-the-art QM and QM/QM′ approaches, we found that the propensity of BV to bind PcyA is dominated by electrostatic interactions, especially related to the Arg149 and the Lys221 residues, while H-bonds are formed with His88 and Ser114. Our simulations also reveal that the antioxidant activity is dependent on the intramolecular non-covalent bond interactions. Indeed, we found that the surrounding residues increase the antioxidant character of BV by 2 eV. In addition, the BV antireductant capacity was investigated for the first time demonstrating that it is much more sensitive to the surrounding residues than its antioxidant counterpart.

1. Introduction

Phycocyanobilin:Ferredoxin Oxidoreductase (PcyA) is the most well-known member of the ferredoxin-dependent bilin reductase (FDBR) family. This enzyme plays a pivotal role as light harvesting and photoreceptor pigment in a wide variety of plants. Indeed, it converts biliverdin IXα (BV, see Fig. 1) into a variety of bilins through a very specific reduction mechanism [1]. As shown in Fig. 1, PcyA sequentially reduces the D-pyrrole ring vinyl group and the A-pyrrole ring through a four-electron reduction catalysis of BV to produce 3Z/3E-phycocyanobilin (3Z/3E-PCB) *via* 18^1, 18^2-dihydobiliberdin IXα (18^1,18^2-DHBV), an intermediate two-electron reduced species. 3Z/3E-PCB is a linear tetrapyrrole, member of phytobilins compounds, that are present in the chloroplast of red algae, in colored (brown, red to blueish-green) cryptophytes and in blue/green cyanobacteria.

From both the biological and the chemical points of view, the reduction mechanism of BV into 3Z/3E-PCB is still not perfectly understood, notably because crystallographic data of FDBR have been obtained only once, a decade ago [1]. Let us summarize the current findings. Historically, it was first established that FDBR does not involve organic or metal cofactor(s) that could have played a reductant role [2,3]. In 2004, several bilin radical intermediates were optically detected using absorption and low-temperature EPR spectroscopies, the assignments being performed through ZINDO//AM1 computations of a BV model (vinyl and propionate groups were replaced by methyl groups) [4]. In 2006, Hagiwara and co-workers crystallized the first tertiary structure of FDBR with the PcyA complexing BV (1.54 Å resolution) [1]. The two propionate groups bound to the B- and C-rings were found to be exposed to solution while, in contrast, the D- and A-rings of BV are "buried" inside PcyA (see Fig. 2). Strong hydrophobic interactions taking place through hydrogen-bonds (H-bonds) and salt bridges (SB) stabilize the BV complexation to PcyA. Hagiwara and co-workers suggested a specific role to the PcyA residues presumably involved in the two sequential reduction mechanisms. More precisely, Asp105 was proposed to be the first proton donor as it is (de)protonated in its (minor)major conformation forming several H-bonds (only one) with the D-ring whereas Glu76 would act as the second proton donor residue as it is extremely close to the D-ring vinyl group. It was further hypothesized that the second reduction indirectly involves residues around A-ring (His88, Gln216 and Tyr212) causing a local conformational change

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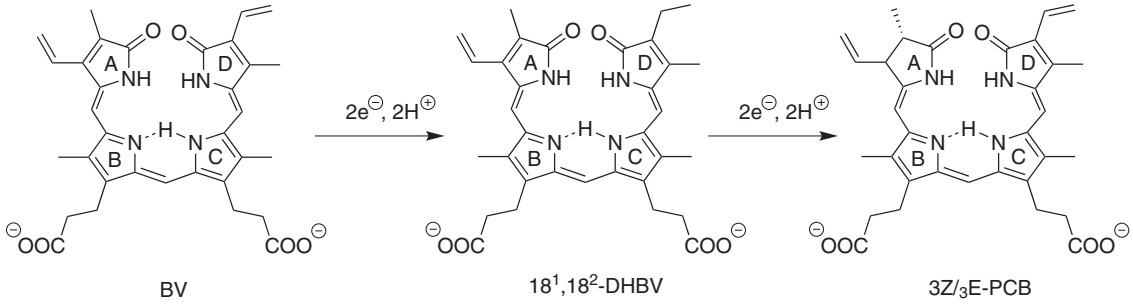

Fig. 1. Representation of BV (or BV+) substrate, as well as 18^1,18^2-DHBV and 3Z/3E-PCB. Radical intermediates are not shown (see Refs. [1,10]).

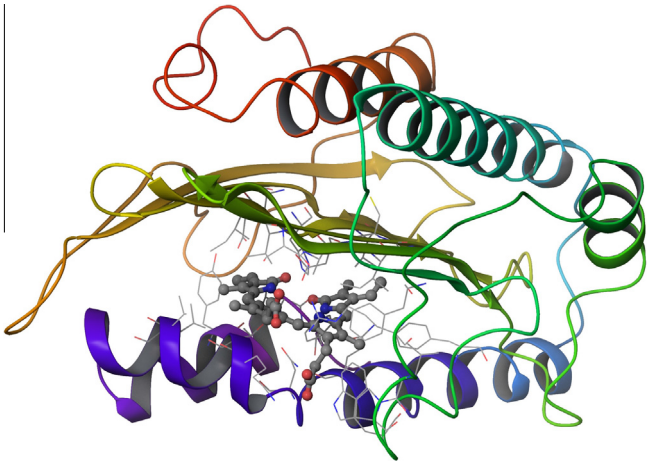

Fig. 2. Representation of the BV embedded into the PcyA protein: the protein is displayed in cartoons, the main residues within 3 Å are shown as wireframes while the BV ligand is shown in balls and sticks. For the sake for clarity, all hydrogen atoms have been omitted.

allowing to capture the second proton from the re-protonated Asp105 (proton transfer from His88 or from the amide group of B- or C-ring to the deprotonated Asp105). In the same time, Tu et al. identified that the Asp-His pair might also tune the second reduction process [5]. The same group of authors revisited the mechanism in 2007 and suggested the presence of new intermediates characterized by the protonation of the carbonyl oxygen atoms in the A- and D-pyrrole rings [6]. In 2009, Stoll and workers used electron paramagnetic resonance and *g* tensor Density Functional Theory (DFT) calculations to characterize protonated species of the BV radical. These computations were performed on the BV, Glu76, His88 and Asp105 system (structure extracted from the D105N diffraction data) in gas phase. In that model system, all heteroatoms were frozen and the propionate groups were replaced by methyl groups [7]. The new intermediate proposed in Ref. [7] presents the C–OH of the A-ring oriented towards His88 while the C–OH of the D-ring is directed to the oxygen atom of the A-ring. In 2010, the group of Higawara suggested that Glu76 is playing a role in the proton donation process while His88 would be important for the second reduction step, in agreement with the previous works [8]. Interestingly, NMR analysis allowed assessing the role of the protonation state of histidine residues as well as the dynamics of an "axial" water molecule on the first reduction step [9]. Very recently, an insights into the proton transfer mechanism of BV reduction were obtained by Unno et al. thanks to the first determination of the actual position of hydrogen atoms through neutron diffraction [10]. These authors: (i) found that BV exists in both neutral (three N pyrrole rings are protonated) and protonated (four N pyrrole rings are protonated) forms; (ii) determined that the protonation state of Asp105 is related to nature of the BV species; (iii) assessed the protonation state of the key histidine residues; (iv) found that the "axial" water molecule is only present when BV is neutral; and (v) detected the presence of a hydronium ion in the vicinity of BV.

The available computational information is more scarce. Indeed, only a few works were performed to explore the BV:PcyA complex, and most of them were focused on spectroscopic properties (absorption spectra and *g*-tensor) only [4,7]. These studies were typically performed on quite small BV model systems with propionate groups replaced by methyl groups, without accounting for vicinal water molecules nor considering the BV relaxation in the PcyA surrounding. Therefore, the present contribution is the first study devoted to the BV:PcyA complex. More precisely, accurate quantum mechanical (QM) and QM:QM′ calculations have been performed to understand the role of the surrounding residues on both the stabilization BV and its oxidation and reduction processes. The antioxidant [antireductant] capacity was evaluated *via* the electro-donating power (ω^-) [electro-donating power (ω^+)] that more accurately describes these phenomena than the usual ionization potential (IP) [electronic affinity (EA)] [11]. Indeed, the ω parameters account for the propensity of a molecule to donate or to accept a charge within a complex environment. We underline that the antioxidant strength cannot be directly obtained from experimental data due to the large impact of the surroundings. Consequently, theoretical calculations emerge as a necessary tool in the present framework.

This article is organized as follows: first we explore the full relaxation of BV into PcyA with QM:QM′, secondly a pairwise analysis is performed to unravel the key residues stabilizing the ligand, and thirdly both the antioxidant and antireductant abilities of BV are investigated.

2. Computational methods

The starting coordinate set of the Phycocyanobilin:Ferredoxin Oxidoreductase used in our molecular dynamic simulations was obtained from protein structural models derived from X-ray measurement [1], available in the Protein Data Bank (PDB code: 2D1E, 1.51 Å resolution). The structure was solvated in a cubic box of 9 Å padding and a total ion concentration 0.15 mol/L (chloride and sodium ions mixture). The protonation states were set up using PROPKA3.0 [12,13] on the PDB2PQR online server [14] and following Ref. [10]. In our computations we took into account the major conformation, that is, we considered a neutral BV (the $-2|e|$ charge is solely due to propionate groups), protonated Glu76, Asp105 and Arg149, and a singly protonated His88 on its N_δ, all other residues were straightforwardly assigned. In order to relax the system, a minimization was carried out using the all-atom CHARMM27 forcefield [15] combined with the set of parameters developed by Kaminski et al. for two similar