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Contribution of bacteriochlorophyll conformation to the distribution of site-energies in the FMO protein

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The structural data for the Fenna–Matthews–Olson (FMO) protein indicate that the bacteriochlorophylls (BChls) display a significant degree of conformational heterogeneity of their peripheral substituents and the protein-induced nonplanar skeletal deformations of the tetrapyrrole macrocycle. As electronic properties of chromophores are altered by such differences, a conformational effect may influence the site-energies of specific pigments and thus play a role in mediating the excitation energy transfer dynamics, but this has not yet been established. The difficulty of assessing this question is shown to be partly the result of the inability of the sequential truncation approach usually employed to account for interactions between the conformations of the macrocycle and its substituents and an alternative approach is suggested. By assigning the BChl atoms to meaningful atom groups and performing all possible permutations of partial optimizations in a full-factorial design, where each group is either frozen in the crystal geometry or optimized *in vacuo*, followed by excited state calculations on each resulting structure (PM6//ZIndo/S), the specific effects of the conformations of each BChl component as well as mutual interactions between the molecular fragments on the site-energy can be delineated. This *factorial relaxation* procedure gives different estimates of the macrocycle conformational perturbation than the approach of sequentially truncating the BChl periphery. The results were evaluated in the context of published site-energies for the FMO pigments from three species to identify how conformational effects contribute to their distribution and instances of cross-species conservation and functional divergence of the BChl nonplanarity conformational contribution are described.

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1. Introduction

Photosynthetic light-harvesting complexes (LHCs) collect energy from sunlight and deliver it to the reaction centers. The pathways of intra- and inter-protein excitation energy transfer (EET) are finely controlled by LHCs to ensure optimum photosynthetic efficiency [1,2]. EET is mediated primarily by the electronic coupling between chromophores as well as the site-energies of the individual pigments (*i.e.*, uncoupled excitation energies). The distance dependence of inter-chromophore electronic coupling is reflected by the overall architecture of LHC proteins [2] through which the chromophore spatial distributions are precisely controlled. In contrast, site-energies are modulated by the local interactions between each pigment and its protein binding-site.

There are many ways in which proteins may influence the absorption characteristics of bound chlorophylls (Chls) and consequently affect EET dynamics. These include the choice of axial ligand, the H-bonding environment, the electrostatic properties of the binding-site [3–6] and for bacteriochlorophylls *a* (BChls a) the degree of coplanarity of the C3-acetyl with the macrocycle plane [7,8]. Additionally, because of the well-known correlation between macrocycle conformation and photophysical properties of porphyrins [9–12], it is possible that protein-induced deformation of bound Chls and BChls also contributes significantly to the site-energy distribution in LHC proteins. The photobiological relevance of this is indicated by Zucchelli *et al.*'s analysis of Chl ring deformation in LHCs [13,14], who presented strong evidence for its role in generating the spectral profile of the main antenna complexes of PSII, and our own work relating it to functional properties of reaction centers [15,16].

The Fenna–Matthews–Olson (FMO) protein is a water-soluble pigment protein complex that mediates EET between the chlorosomes

Abbreviations: AHC, agglomerative hierarchical clustering; BChl, bacteriochlorophyll; Chl, chlorophyll; $E(BChl)_{cryst}$, *in vacuo* SPE of BChl in protein bound conformation; ΔQy[Mac], site-energy perturbation due to macrocycle distortion; d_{ip}, mean absolute deviation of NSD minimum basis model from observed structure for IP normal-modes; d_{oop}, as previous for OOP modes; D_{ip}, NSD total IP distortion; D_{oop}, NSD total OOP distortion; E_{ES}, excited-state energy; EET, excitation energy transfer; EU, experimental unit (*i.e.*, a unit element in a particular factorial experiment); FMO, Fenna–Matthews–Olson protein; HOMO, highest occupied molecular orbital; IP, in-plane; LHC, light-harvesting complex; LUMO, lowest unoccupied molecular orbital; NSD, normal-coordinate structural decomposition; OLS, ordinary least-squares; OOP, out-of-plane; PDB, Protein Data Bank; RC, reaction center; REP, rotational energy profile; RLM, robust linear modelling; SCF, self-consistent field; SPE, single-point energy.

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and reaction centers (RCs) of green bacteria (Fig. 1) [17]. It crystallizes as a C_3-symmetric trimer, thought to resemble the *in vivo* form, where each monomer contains seven BChls a that are packed closely together within the encapsulating protein; notably, there is no apparent symmetry within the monomer [18–22]. These BChls are known to display significant conformational variation with respect to the local geometric parameters of their substituents as well as in the nonplanar conformations of their tetrapyrrole macrocycles. Additionally, an eighth BChl is found in crystal structures from some species, although which species are in possession of BChl 8 remains an open question [23,24]. The dynamics of exciton transfer in the FMO have recently been modelled and suggest alternate pathways wherein BChls 1 or 8 receives excitation energy from the chlorosome baseplate that is then directed through either BChls 2 or 4 and onto BChl 3, through which the FMO excites the RC BChls [24].

The FMO protein is considered an excellent model system for the study of photosynthetic EET in general and its optical spectra have been the subject of substantial theoretical analysis [7,8,25–28]. Due to the availability of high-resolution structural data [18–24,29–32] a number of these studies have focused on predicting the spectral characteristics and EET dynamics directly from the atomic structure. Initially, Fajer and coworkers in 1990 [26] employed the semi-empirical ZIndo/S method. The structural models derived from the experimental coordinates consisted of the BChls *in vacuo* subject to various levels of truncation, with and without axial ligand and, where relevant, including nearby charged- and aromatic residues. However, whilst this established an idea of the overall effect of the presence of a particular perturbation (*e.g.*, removal of the acetyl introduces an almost systematic blue-shift) they did not obtain a simple relationship between individual conformational parameters and the calculated site-energy and instead implied the presence of, "*a nonadditive relationship between the influences of framework distortion and acetyl orientations*" [26]. Modern work has culminated in approaches with greater computational sophistication that provide more or less *ab initio* site-energies that yield simulated spectra in good agreement with experiment, yet the individual site-energies and the dominant modes of influence by the protein are still contentious [7,8,25,27].

Thus, there is still debate regarding the BChl site-energies in the FMO with respect to their values as well as to the details of the factors that affect their heterogeneity suggesting that there is still cause to suspect a functional role for the simultaneously observed variation in macrocycle conformations that have been reported since some of the earliest crystal structures [10,26]. While it would be interesting to uncover exactly how the conformational properties of BChls contribute to its properties it is no trivial matter to separate individual contributions such as local functional group conformation from the effect of the overall macrocycle conformation, suggesting that the standard approach of sequential truncation is not necessarily adequate. Furthermore, sequential truncation is inherently flawed, as porphyrin macrocycle distortion and substituents influence each other [33]. Here we attempted to address this issue by employing a more sophisticated experimental design to model precisely how the individual BChl site-energies are influenced by protein-induced conformational variation.

In addition, previously we emphasized the necessity of critically assessing all relevant structural data when one intends to investigate the effects of conformational differences between protein-bound tetrapyrrole cofactors since these features are often close to the positional error limits of protein crystallography [15,16]. Thus, we first consider the extent of BChl macrocycle conformational variation observed in these data *via* a comparative analysis of the normal-coordinate structural decompositions (NSD) [34] of the pigment conformations afforded by each structure. Although this aspect of the study remains inconclusive owing to the high-degree of inter-structure conformational variation, its potential to bias our study is considered and mitigated by applying the approach to multiple crystal structures.

In summary, we report the development and application of a computational approach to delineate the effects of the conformations of the individual molecular components and substituents of BChl pigments upon their unperturbed excitation energies to assess the role of conformational control in modulating this EET parameter in the FMO protein. The paper is organized as follows: following the experimental details (§2) we begin by describing macrocycle conformational variation amongst the FMO BChls via normal-coordinate structural decomposition (NSD) of the BChls from available crystal structures of the complex (§3.1). Next, we present empirical evidence that demonstrates the presence of a regulatory effect of the BChl substituents upon the influence of macrocycle distortion (§3.2.1) and similarly an effect of macrocyclic nonplanarity upon the influence of the C3-acetyl dihedral angle (§3.2.2). We then discuss the theoretical justification of our computational approach (§3.3.1) and describe the results in terms of: the conformational factors most influencing the site-energies calculated for all pigments over all crystal structures (§3.3.2); general trends in the relative importance of the conformational influence of the different components of BChl (§3.3.3); how inter-pigment variation of the most important conformational factors identified may contribute to the distribution of published site-energies for the complexes from three species (§3.3.4), with detailed consideration of a particularly biologically relevant site-energy difference provided in SI §3.3.4 A; and lastly, how the results of the truncation procedure differ from those obtained using our method by a direct comparison of the two approaches (§3.3.5). Finally, we discuss the performance and reliability of our computational design (§4.1) as well as the results obtained from each analysis stage as a whole (§4.2) and conclude by highlighting our most general results (§5).

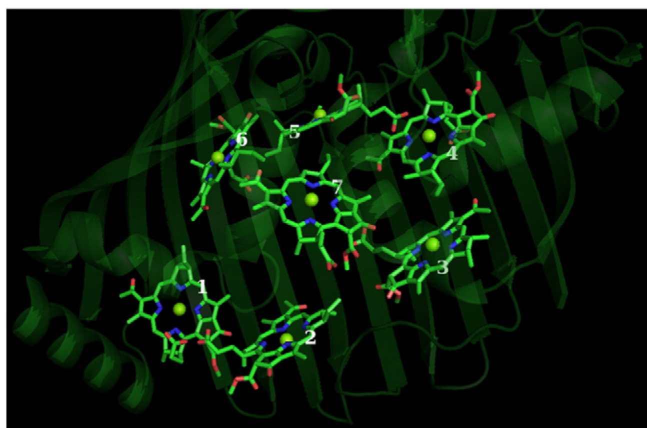

Fig. 1. The BChl arrangement in the Fenna–Matthews–Olson protein (phytyl chains have been truncated for clarity). Drawn using PyMol and the coordinates from PDB ID: 3BCL [21]. BChl 8 it not present in this structure although when present is situated outside of the encapsulating β-sheets, close to BChl 1.

2. Methods

2.1. Factorial relaxation (fragment-based partial optimizations)

It is useful to overview our experiment in juxtaposition to Fajer and coworkers' [26] wherein the protein-induced structural perturbations of the FMO BChls on their site-energies were modelled by progressively truncating the BChl structures. We argue that there are two caveats inherent to this approach: 1) Each truncation produces a new chemical entity that will behave differently under conformational perturbation (*e.g.*, the rotational energy profile of the C3-acetyl would be qualitatively different upon truncation of the C2-Me group); and 2) The sequence of truncation influences the outcome of the experiment (*e.g.*, if we sequentially truncate the C7-methyl and C8-ethyl then ΔE of the *first* removal would include the additional strain energy of their interaction

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