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Rational design of a zinc phthalocyanine binding protein

Andrew C. Mutter [a], Jessica A. Norman [a], Michael T. Tiedemann [c], Sunaina Singh [a], Sha Sha [a], Sara Morsi [a], Ismail Ahmed [b], Martin J. Stillman [a], Ronald L. Koder [a,*]

[a] Department of Physics, The City College of New York, New York, NY 10031, United States
[b] Department of Biochemistry, The City College of New York, New York, NY 10031, United States
[c] Department of Chemistry, The University of Western Ontario, Ontario, Canada

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ABSTRACT

Phthalocyanines have long been used as primary donor molecules in synthetic light-powered devices due to their superior properties when compared to natural light activated molecules such as chlorophylls. Their use in biological contexts, however, has been severely restricted due to their high degree of self-association, and its attendant photoquenching, in aqueous environments. To this end we report the rational redesign of a de novo four helix bundle di-heme binding protein into a heme and Zinc(II) phthalocyanine (ZnPc) dyad in which the ZnPc is electronically and photonically isolated. The redesign required transformation of the homodimeric protein into a single chain four helix bundle and the addition of a negatively charge sulfonate ion to the ZnPc macrocycle. To explore the role of topology on ZnPc binding two constructs were made and the resulting differences in affinity can be explained by steric interference of the newly added connecting loop. Singular binding of ZnPc was verified by absorption, fluorescence, and magnetic circular dichroism spectroscopy. The engineering guidelines determined here, which enable the simple insertion of a monomeric ZnPc binding site into an artificial helical bundle, are a robust starting point for the creation of functional photoactive nanodevices.

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1. Introduction

Phthalocyanines offer a highly absorbent chromophore with excellent chemical properties, making them ideal molecules to act as primary donors in artificial light-powered devices (Torre et al., 2007; Rawling and McDonagh, 2007). They have been used extensively in synthetic charge separation constructs due to their chemical robustness relative to chlorophyll and porphyrin derivatives, in particular their resistance to photodecomposition. Furthermore, their ease of synthesis and their long wavelength, high molar absorptivity action spectrum, with *Q*-band maxima at near-infrared wavelengths of 650 nm and higher, are ideal for solar energy conversion.

Phthalocyanines have seen little translation into practical application for a simple reason: even in relatively hydrophobic solvents such as chloroform, they are extremely prone to self-association, forming stacked, columnar aggregates with poor photophysical properties (Schutte et al., 1993). To take full advantage of their superior spectrochemical properties phthalocyanines must be maintained in 'splendid isolation', avoiding their assembly into cofacial aggregates (Brewis et al., 2000). Simple modifications such as macrocycle sulfonation, while making monomeric phthalocyanines more soluble in hydrophilic solvents, do little to inhibit the progressive accumulation of stacked phthalocyanines as the cofacial stacking interaction is highly favorable energetically. Designed proteins, which offer a unique matrix which can specifically bind phthalocyanines in isolation, promise to provide a solution to this problem. Furthermore, as the distances between donor and acceptor sites in light activated electron transfer process are critical factors in both the lifetime and yield of charge separation in proteins (Punnoose et al., 2012), it is important that we develop methods to tightly bind a ZnPc cofactor at a specified site in an artificial protein.

We have chosen to start with the homodimeric four α-helix bundle protein HP7, which binds two heme cofactors at two bis-histidine heme ligation sites with one histidine ligand originating from each helix (See Fig. 1) (Koder et al., 2009; Zhang et al., 2013, 2011a). This protein is derived from a series of progenitor proteins which started with the prototype maquette protein H10H24 (Robertson et al., 1994), a tetrameric four α-helix bundle protein and progressed through the design intermediates BB (Gibney et al., 1997) and HP-1 (Huang et al., 2004). As H10H24 has been shown to bind a wide array of porphyrins, including heme A (Gibney et al., 2000), iron mesoporphyrin IX, and iron 1-methyl-2-oxo-mesoheme XIII (Shifman et al., 2000), it seemed likely that HP7 would be able to incorporate a cofactor with a significantly different macrocyclic structure such as ZnPc (See

* Corresponding author.
E-mail address: koder@sci.ccny.cuny.edu (R.L. Koder).

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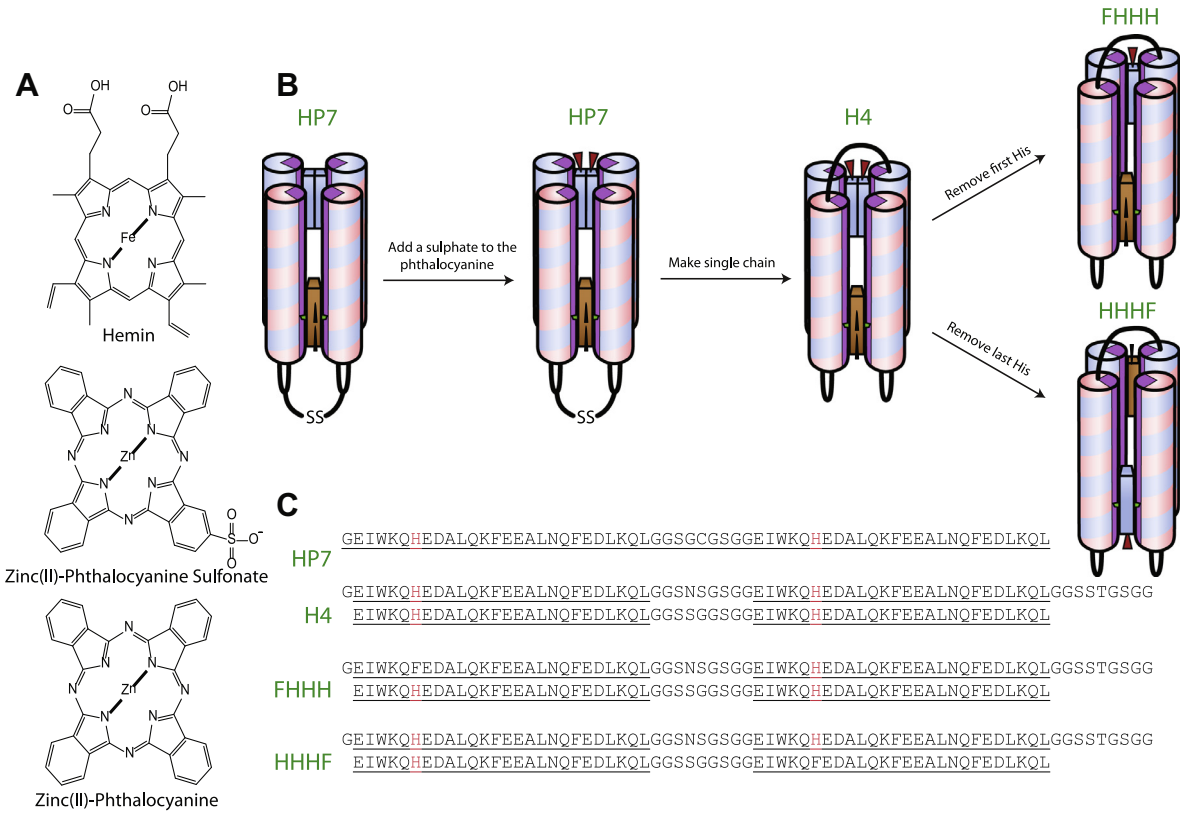

Fig.1. Schematic overview of the design pathway used to make a heme phthalocyanine dyad protein, (A) Structure of the cofactors used. (B) The design pathway leading to FHHH and HHHF proteins. (C) Primary structure of each protein used in this work.

Fig. 1A). Furthermore, Noy and coworkers have recently reported that HP7 binds zinc bacteriochlorophyllide as mixed monomers and dimers at each binding site (Cohen-Ofri et al., 2011). Here we report the changes in HP7 necessary to enable the binding of an isolated ZnPc cofactor and the creation of a ZnPc-heme heterocomplex. To our knowledge, this is the first report of the creation of an artificial phthalocyanine binding protein.

2. Materials and methods

2.1. Materials

Hemin (Fluka), Zinc Phthalocyanine (ZnPc) (Aldrich) and Pyocyanine (Cayman Chemical) were purchased from the indicated companies and used without further purification. All other chemicals were from VWR, Inc. The pET32a vector containing the gene for HP7 (Koder et al., 2006) was the kind gift of P.L. Dutton. Construction of the HP7-H7F mutant was reported earlier (Zhang et al., 2011b). Molecular biology reagents were from New England Biolabs.

2.2. Synthesis of Monosulphonated Zinc Phthalocyanine (ZnPcS)

ZnPcS was synthesized using a the method of d'Alessandro et al. (2005) replacing the Ru(II)Cl_2 with Zn(II)Cl_2: Briefly, a 1:1 M mixture of 4-sulfophthalic acid and zinc chloride with 0.1 equivalents ammonium molybdate were dissolved in water. To this mixture 3.5 mol excess of phthalic anhydride and forty parts urea were added and heated until a homogeneous solution was achieved. 1.5 mL aliquots of the mixture were transferred to 20 mL ampoules which were flame sealed under vacuum. The ampoules were then heated in a sand bath behind a blast shield to 200 °C for 3 h then 260 °C for 3.5 h.

Solid crude product was removed from the ampoules and ground to a powder, washed with brine and then extracted with ethanol. This results in a mixture of ZnPc sulfonates. Purified ZnPcS was obtained after flash chromatography using an ethyl acetate:methanol gradient from 10:0 to 9:1. ZnPcS was further purified by crystallizing from dimethylformamide/methylene chloride. Purity was confirmed by HPLC-MS using a Vydak Inc. analytical C18 column running a 5% phosphoric acid pH 5.0/methanol gradient. Yield = 2%. λ_{abs}max ($CDCl_3$) = 672 nm. ESIMS: *m/z* 737.2 ($M^+ + 1$).

2.3. Cloning, protein expression and purification

Genes encoding HHHH (Fig. 1E) were synthesized (Biomatik Corp., Cambridge, ON) with an N-terminal TEV cut site and inserted between the BamHI and XhoI restriction sites of pET32a (+) (Novagen) as previously described Koder et al. (2006). HHHF and FHHH expression vectors were created by site directed mutagenesis using Stratagene quick change kit. All proteins, expressed from the plasmids described above as fusions with thioredoxin, were grown and purified as published previously for HP7 (Koder et al., 2006). Briefly, 2L cultures of BL21(DE3) *Escherichia coli* containing the appropriate plasmid were induced at an OD_{600} of 1.0 with 0.5 mM isopropyl-β-thiogalactopyranoside and shaken for 5 h at room temperature. Cells were collected by centrifugation, lysed using a French press, and proteins purified using a Ni-nitrilotriacetic acid column (Qiagen, Inc.) according to manufacturer's instructions. The fusion protein was dialyzed into 50 mM Tris–HCl, 1 mM DTT, pH 8.0 and then cleaved overnight with His6-tagged TEV protease (Invitrogen, Inc.). The reaction mixture was filtered through Ni-nitrilotriacetic acid resin, and the purified proteins concentrated by centrifugation. All stages of purification were monitored by SDS–PAGE. Pure proteins were dialyzed into either 250 mM

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