Toward the Synthesis of a Photosynthetic Reaction Center Maquette: A Cofacial Porphyrin Pair Assembled between Two Subunits of a Synthetic Four-Helix Bundle Multiheme Protein

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Our goal is to create simplified synthetic protein-based structures, molecular *maquettes*, of natural redox proteins. Maquettes are intended to better reveal the functional essence of biological structure by avoiding the obscuring complexities that natural proteins inevitably carry for a wide variety of other biological purposes. Feasibility is indicated by the recent syntheses of four-helix bundle proteins that coordinate 1−4 hemes, as designed.[1,2] In one of these studies,[1] two identical homodi-α-helical peptides (α_2) assembled to form $[\alpha_2]_2$ with twofold symmetry and probable all-parallel configuration. This structure provides the foundation for the design and synthesis of maquettes of photosynthetic reaction center proteins of plants and microorganisms. Reaction centers transform solar radiation into electrochemical potential energy, the force that drives the bioenergetics of virtually all life forms on Earth. Several hurdles of design chemistry, proper assembly and functional engineering, must be understood and scaled *en route* to a successful reaction center maquette. This communication focuses on the special pair of chlorophylls of the reaction center that is central to the primary light-activated electron transfer. The special pair was first demonstrated by Norris,[3] much mimicked in chemical structures,[4] and structurally elucidated in the native protein by X-ray crystallography.[5] The reaction center structure reveals the cooperation of two similar twofold symmetric subunits (L and M) in the assembly of the approximately cofacial chlorophyll pair. Here we describe a pair of porphyrins assembled upon association of two α_2 moieties to form $[\alpha_2]_2$.

For this initial study, the free base of 3,8,13,18-tetramethyl-21*H*,23*H*-porphine-2,7,12,17-tetrapropionic (coproporphyrin I; CP) was selected to represent the chlorophyll. All four α-helices of the four-helix bundle had the common sequence (α)-CGGGELWKL**H**EELLKKFEELLKL**H**EERLKKL-$CONH_2$ that was synthesized by solid-phase methodology according to a Fmoc/^{t}Bu strategy. This parent peptide chain was derivatized as CP-α and heterodimerized with another α chain, whose N-terminal amino group was previously capped with an acetyl group, to produce CP-α_2. This molecule, like α_2,[1] self-assembles in aqueous solution to yield the dimeric $[CP\text{-}\alpha_2]_2$.[6,7]

Coproporphyrin I was selected because of its symmetry, its water solubility, and its well-defined but relatively weak tendency to dimerize with approximate cofacial geometry.[8] The dimer to monomer transitions are characterized by a red-shift of the Soret band from 372 to 394 nm, with intensification and an apparent isosbestic point at 378 nm, and parallel general blue-shifts and intensification of the Q_x and Q_y bands in the 500−600 nm region. These changes yielded a CP dimer dissociation constant (K_D) of about 2×10^{-5} M (not shown). In contrast, it was anticipated that the CP dimerization would be greatly promoted in the $[CP\text{-}\alpha_2]_2$ structure, provided that the $[\alpha_2]_2$ is all-parallel, as proposed,[1] and assuming no complications arise from the appendage of CP to α_2. Figure 1A supports these expectations. Only the dimeric spectral signature is evident at 3×10^{-6} M, and examination down to 10^{-8} M (not shown) reveals neither loss of this dimeric spectral signature not appearance of other features. This is consistent with a dimer−monomer K_D value of $<10^{-8}$ M for the CP in the $[CP\text{-}\alpha_2]_2$.

Further examination of the CP dimer−monomer equilibrium when associated with $[CP\text{-}\alpha_2]_2$ made use of the solvent trifluoroethanol (TFE). TFE is considered to disrupt quaternary and tertiary protein structure stabilized by hydrophobic interactions without decreasing the helicity of the single-stranded α-helices.[9,10] Figure 1B shows that the spectral transitions displayed by $[CP\text{-}\alpha_2]_2$ on addition of TFE are consistent with a dimer to monomer transition. Independent measurements done on free CP in solution show that, in the range studied, TFE itself has minimal effects on the spectral properties of the dimer or monomer forms. In addition, circular dichroism (data not shown) of $[CP\text{-}\alpha_2]_2$ in fully aqueous medium showed a ratio $\theta_{222}/\theta_{208} = 1.00$, whereas in aqueous TFE (4.6 M, 32 %), it was 0.85, consistent with the dissociation of the α-helical coiled-coil of $[CP\text{-}\alpha_2]_2$ into monomeric α-helices of CP-α_2.[10,11] Overall, these results show that the parallel dimeric structure of $[\alpha_2]_2$ stabilizes the dimerization of CP by ≥1000-fold (≥4 kcal/mol) in $[CP\text{-}\alpha_2]_2$. We have examined $[CP\text{-}\alpha_2]_2$ for its ability to coordinate iron protoporphyrin IX as reported for $[\alpha_2]_2$.[1] Up to four hemes are incorporated without causing a change in the CP dimer spectrum (not shown). This adds weight to the view that the CP pair in $[CP\text{-}\alpha_2]_2$ is an appendage positioned in the aqueous phase *outside* the all-parallel four-helix structure that may be represented as shown in Figure 2.

Compared with the reaction center protein from *Rhodopseudomonas viridis*,[5] $[CP\text{-}\alpha_2]_2$ remains much simpler and offers a robust frame on which to build a reaction center maquette. Based on our current understanding of the sharp requirements for the engineering of the high-yield light-driven

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(6) Before α was cleaved, the peptide−resin was split into two for different derivatization of the N-terminal cysteine (C). In one batch, the cysteine was acetylated with acetic anhydride, and in the other it was acylated with an approximate 5-fold excess of the performed 1-hydroxybenzotriazole active ester of CP. In the latter case, the active propionate ester was prepared by reacting CP·2HCl with equimolecular amounts of 1-hydroxybenzotriazole and *N*,*N*′-diisopropylcarbodiimide in the presence of 2-fold excess of *N*,*N*′-diisopropylethylamine. Both peptides were cleaved with trifluoroacetic acid/ethanedithiol/water (92:6:2) and purified to homogeneity by preparative reversed phase HPLC. Heterodimerization was performed reacting the 5-nitro-2-pyridylsulfenyl (pNpys) derivative[7] of the thiol group of acetyl-α with CP-α to produce the 62-residue CP-α_2 peptide. The intermediate peptides and the final product were characterized by analytical HPLC, UV−vis (indole and/or CP absorption), and laser-desorption mass spectrometry. In addition, size exclusion chromatography (Superose 12, 1 cm × 30 cm, elution buffer 50 mM Tris at pH 8.5 with 100 mM NaCl, 1 mL/min) revealed that CP-α_2 assembles into the dimeric four-helix bundle $[CP\text{-}\alpha_2]_2$ protein.

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(11) CD was carried out in 50 mM phosphate buffer (pH = 8), 100 mM NaCl at a concentration of 2.3−3.4 μM in a 2 mm quartz cuvette.

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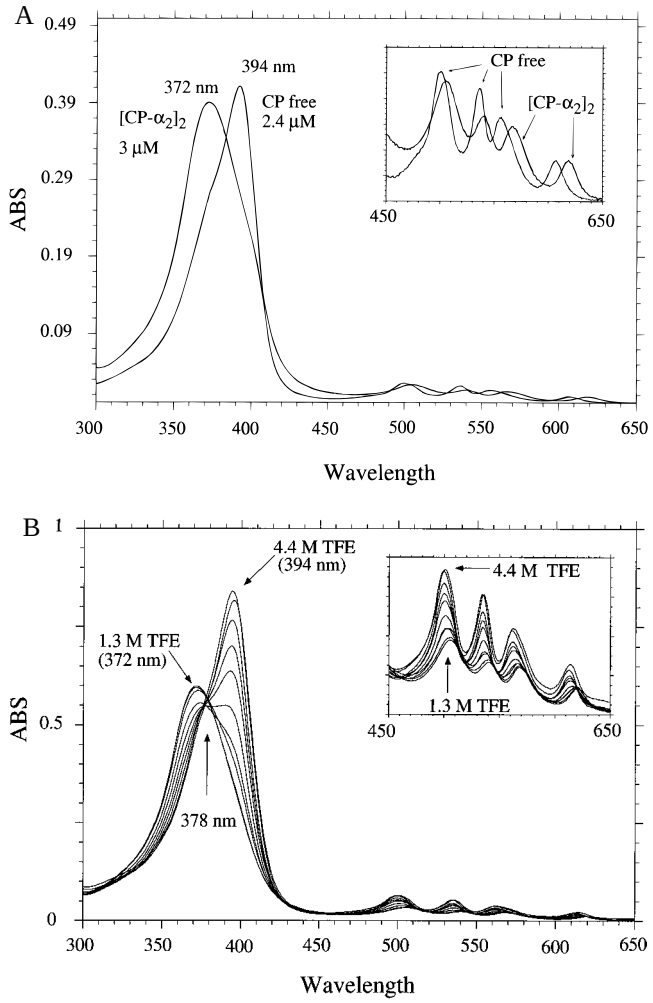

Figure 1. (A) UV−vis spectra in aqueous buffer (50 mM Tris, 100 mM NaCl, pH 8.5) of free CP and $[CP\text{-}\alpha_2]_2$ at the concentrations indicated. The inset shows the Q_x-Q_y region. (B) UV−vis monitoring onf the dissociation of $[CP\text{-}\alpha_2]_2$ into CP-α_2 by addition of trifluoroethanol (TFE) to a 4.5 μM buffered (50 mM Tris), 100 mM NaCl, pH 8.5) solution of $[CP\text{-}\alpha_2]_2$. Traces recorded are (in M) 1.3, 1.8, 2.3, 2.5, 2.7, 3.0, 3.2, 3.6, 4.0, and 4.4, corresponding to 10−32% TFE. The inset shows the Q_x-Q_y region. The concentrations carry an uncertainty (up to 15% overestimate) resulting from the overlapping spectra of the porphyrin and the indole.

electron transfer found in the native reaction center[12] and the determined radiative decay of the excited state CP in the $[CP\text{-}\alpha_2]_2$ ($\sim 10^{-8}$ s, unpublished results with L. Jahn and R. M. Hochstrasser), the structure shown in Figure 2 is expected to have a quantum yield of <0.01. Future work on modified $[CP\text{-}\alpha_2]_2$ structures will test the effects of moving the hemes and the light-excitable CP into closer proximity, as in the reaction center.

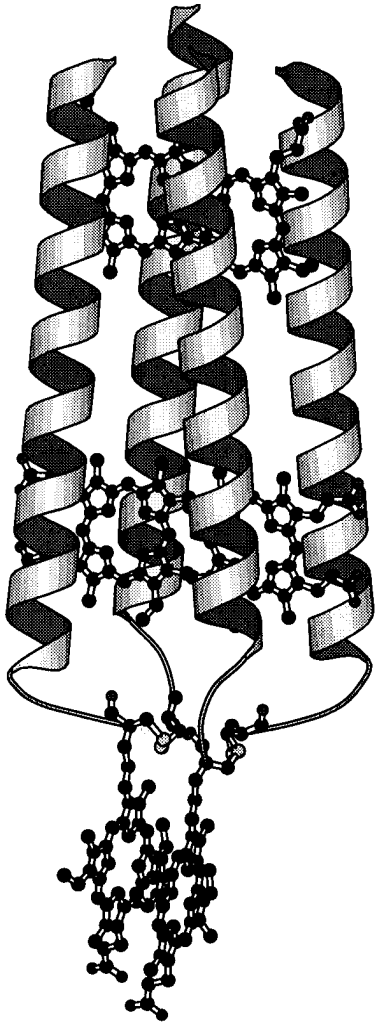

Figure 2. Three-dimensional ribbon representation of the photosynthetic reaction center maquette $[CP\text{-}\alpha_2]_2$. The cofactors are displayed as stick-and-balls models.

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