Multistep Photoinduced Electron Transfer in a de Novo Helix Bundle: Multimer Self-Assembly of Peptide Chains Including a Chromophore Special Pair[1]

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The importance of protein aggregation in providing environments for charge transport is now well established in natural systems that include the helix bundle that facilitates the light-induced proton pump in bacteriorhodopsin,[2] as well as the membrane protein matrix that houses the reaction center of photosynthetic bacteria.[3] For the latter, the photoactive "special pair" of bacteriochlorophyll molecules, along with primary and secondary electron acceptors, are called upon to self-assemble in a complex array of proteins having significant secondary (e.g., helix) structure in the vicinity of photoreactive partners.[4] A number of recent reports document the exceptional properties of de novo peptides for assembly of non-native groups. These include the porphyrin-laden maquettes,[5] peptides modified with organic moieties or transition-metal complexes capable of photoinduced electron transfer within helical domains,[6,7] and peptide-based assemblies of organic chromophores as dimers reminiscent of the reaction center special pair.[8]

In the present article, we describe a de novo 24-residue peptide having propensity for a high order of self-assembly. Moreover, the synthetic protein is capable of multistep electron-transfer involving an electrostatic docking agent. Other features of the prevailing arrangement include an orientation for peptide chains that allows organization of N-terminal pyrene chromophores as dimers. Photooxidation of the self-assembled peptide bundle using methyl viologen (MV^{2+}) as electron acceptor is followed by charge migration to a remote site on the peptide provided by a tryptophan moiety.

The structure of the target amphipathic peptide, TT1P, is shown as a sequence and helical wheel diagrams in Scheme 1. The design provides for the development of a hydrophobic crevice between helices consisting of leucine and isoleucine residues in a heptad repeat that will favor a head-to-head registration of the α-helices.[9,10] A pyrene chromophore is attached via a short chain at the N-terminus to a leading two-residue span of aspartates (Asp). The latter provide point charge stabilization of helical domains and electrostatic sites for further assembly with external agents. In addition to the essential photochemical properties provided by the N-terminal pendant, the pyrene also plays a structural role in increasing the propensity for peptide aggregation (vide infra).[11] The redox active residue, tryptophan (Trp), is recruited for possible long-range electron transfer with the N-terminus. The plan departs from other designs in that *uncharged* polar residues, serine (Ser) and glutamine (Gln), serve as hydrophilic moieties to be oriented at the periphery of helices. Another peptide, TT1P-W9F, has also been prepared, in which the tryptophan residue is replaced by phenylalanine (Phe), a structurally similar residue that is inert to electron transfer.

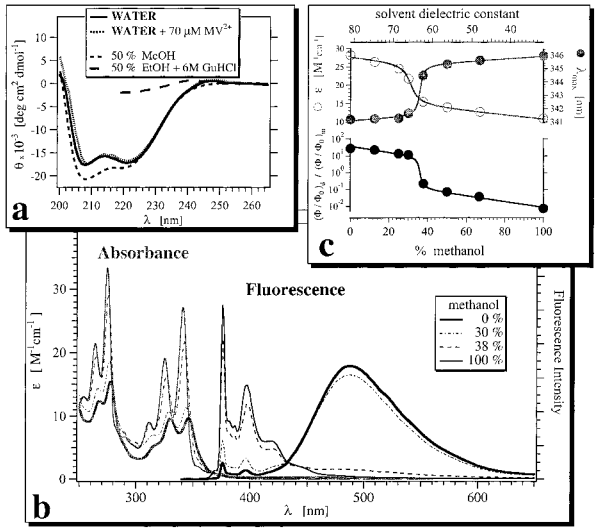
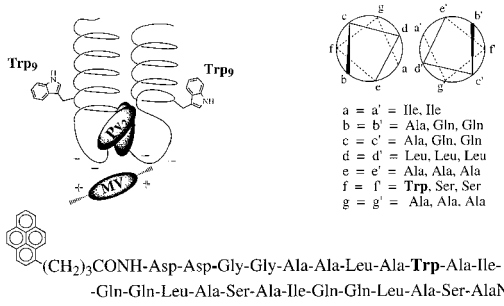


Figure 1. Absorption and emission of 10 μM TT1P including (a) circular dichroism (CD) spectra for various compositions, (b) recorded spectra for various water/methanol mixtures, and (c) titration data for pyrene absorption and monomer/dimer fluorescence ratios (λ_{exc} = 333 nm).

Scheme 1



$(CH_2)_3$CONH-Asp-Asp-Gly-Gly-Ala-Ala-Leu-Ala-**Trp**-Ala-Ile-
-Gln-Gln-Leu-Ala-Ser-Ala-Ile-Gln-Gln-Leu-Ala-Ser-AlaNH$_2$

The redox active residue, tryptophan (Trp), is recruited for possible long-range electron transfer with the N-terminus. The plan departs from other designs in that *uncharged* polar residues, serine (Ser) and glutamine (Gln), serve as hydrophilic moieties to be oriented at the periphery of helices. Another peptide, TT1P-W9F, has also been prepared, in which the tryptophan residue is replaced by phenylalanine (Phe), a structurally similar residue that is inert to electron transfer.

Methods for preparation and characterization of the peptides are described in the Supporting Information. Circular dichroism spectra for TT1P in various media are shown in Figure 1a. The results are consistent with the dominance of helix secondary structure for the peptide in water (the results were similar for TT1P-W9F); 6 M guanadinium ion results in denaturation as expected.[12] By fitting the data to published CD spectra,[13] a helical content of 60% was computed for 10 μM TT1P in water.[14,15]

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Absorption and emission spectra for TT1P are also shown in Figure 1b. The salient features include (1) the red shift of the long wavelength absorption from 341 to 346 nm for the pyrene chromophore, (2) a familiar red-shifted emission associated with dimeric or aggregated pyrenes (the "excimer" band centered at 485 nm), and (3) the loss of both absorption and emission features associated with pyrene aggregates on addition of methanol to water solutions (Figure 1c). The fluorescence changes due to pyrene aggregation have been observed[16] under a variety of circumstances, including other reported helix bundle−pyrene conjugate systems[8] and linked-pyrene oligonucleotide probes.[17]

The state of aggregation of TT1P and TT1P-W9F was studied utilizing size-exclusion chromatography, sedimentation analysis, and fluorescence spectroscopy (see Supporting Information). In buffered aqueous media TT1P exists in monomer−dimer−octamer equilibrium with dimerization constant $K_{1,2} = 5.5 \times 10^7$ M^{-1}, and tetramerization constant $K_{2,4} = 1.6 \times 10^{17}$ M^{-3}. Hence, in the micromolar concentration range, where most of the experiments were conducted, the peptides exist predominantly as high-order aggregates (i.e., octamers). Highlighting the role that N-terminal pyrenes play in self-assembly is the finding from sedimentation data that a model 24-mer, TT1B, having an N-terminal butyramide group shows a lower aggregation propensity and a wider distribution of aggregated species.

An indication that photoinduced electron transfer between the pyrene chromophore pair and an external auxiliary acceptor occurs was found in fluorescence quenching experiments using methyl viologen, MV^{2+} (see Supporting Information). The effectiveness of the viologen quencher requires a static quenching mechanism,[18] reflecting the affinity of the viologen to the negative charge patch of the aggregated peptide (i.e., 4 and 16 charges, variously arrayed, on the dimer and the octamer respectively).

Laser flash photolysis experiments were carried out using a Q-switched Nd:YAG laser and methods that have been previously described.[19] On photolysis of the model compound, pyrenebutanoic acid in Ar-purged water in the presence of MV^{2+}, phototransients assigned to the viologen radical ion ($MV^{+\cdot}$, λ_{max} = 395 nm[20]) and the pyrene radical cation (λ_{max} = 460 nm[21]) were observed as shown in Figure 2. Similar irradiation of TT1P and 0.5 mM MV^{2+} provided the 395 nm viologen transient, but failed to show the pyrene derived intermediate (460 nm) in the time domain of 100 ns to 100 μs. Importantly however, a new transient was observed that absorbed in the 530−600 nm region and overlapped a viologen band centered at 605 nm.[20] This species, assigned to the tryptophan derived radical cation,[22] peaks at 550 nm in the μs time domain and decays, along with the viologen transient, at very long times (>1 ms). Transients in the 400−650 nm spectral region are not observed for the mutant peptide, TT1P-W9F, absent the redox-active Trp moiety, in the time domain of 1 μs and above, a result consistent with the effective competition of back-electron transfer between pyrene$^{+\bullet}$ and viologen$^{+\bullet}$ moieties that are complexed within the peptide bundle domain and have no avenue for charge entrainment.

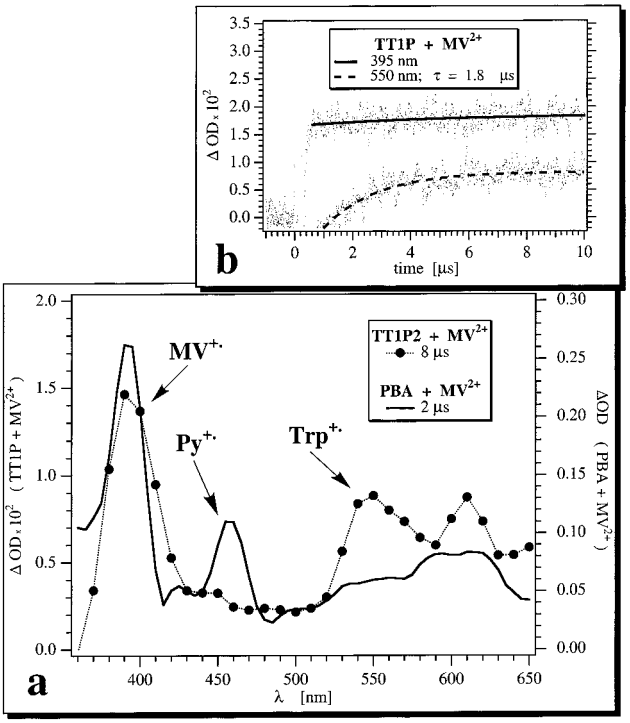

Figure 2. Transient spectra (a) and growth/decay curves (b) observed on laser flash photolysis of 50 μM TT1P in the presence of 500 μM MV^{2+} and 150 μM pyrenebutyric acid (PBA) in the presence of 2.5 mM MV^{2+} (λ_{ex} = 355 nm, 10 ns pulse).

The results are consistent with a mechanism of photoreduction of viologen that is docked to the peptide and the transfer of cationic charge to a remote Trp residue. A rate constant for the transport of charge to the helix bundle domain has been measured ($k = 5.5 \times 10^5$ s^{-1}) by monitoring the onset of transient absorption at 550 nm (Figure 2). Although other models will have to be examined in order to carry out a "pathway" analysis[23] of this result, it is possible that the magnitude for this rate constant requires a mechanism of electronic coupling that extends beyond through-σ-bond coupling and potentially involves the hydrogen bond network of the helix bundles (see Supporting Information).

The prevailing structural and photochemical model that results from this study (Scheme 1) is highlighted by the steps of self-assembly of peptide strands and subunits, complexation with an auxiliary electron-transfer agent, quenching via electron transfer of a primary chromophore pair, and charge entrainment within a synthetic protein domain. We believe this to be the first report of a de novo peptide system that is capable of high-order aggregation, preserved secondary structure, and photoinduced charge separation and transport within the helix bundle.

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Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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