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Self-Assembly of Peptide Porphyrin Complexes: Toward the Development of Smart Biomaterials

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Peptides and proteins have been studied as potential biomaterials because of the strict amino acid sequence control afforded by synthetic chemical methods and their ability to fold and self-assemble into well-defined three-dimensional structures, ultimately leading to nanoscale assemblies with tailor-made structures and properties.[1] For example, peptides have been engineered to self-assemble into nanofilaments with structures that respond to solution conditions.[2] Chemical components capable of inducing folding by tightly binding to peptides can impart novel environmental responsiveness and functionalities not typically seen in purely peptidic materials, expanding the utility of engineered peptide assemblies. For example, adding porphyrin, or chemically similar moieties, to a peptide assembly imparts the ability to catalyze and photosensitize chemical reactions, store oxygen, transport electrical charge, or transfer molecular excitation energy.[3−6]

In this study, we demonstrate that an anionic porphyrin, *meso*-tetrakis(4-sulfonatophenyl)porphine ($TPPS_4$), induces a coiled-coil structure in a designed peptide, Cp3K-N, resulting in a tightly bound porphyrin−peptide pair. The amino acid sequence for Cp3K-N (Figure 1) is derived from a longer peptide sequence, Cp3K, designed to self-assemble into one-dimensional coiled-coil nanofilaments.[2] Cp3K-N contains three lysines spaced three residues apart from one another. Isoleucines and leucines spaced three and four residues apart, respectively, act as determinants for dimeric coiled-coil motifs.[7] Solutions containing Cp3K-N and $TPPS_4$ in 10 mM Tris-HCl at pH 7.6 were studied with UV−vis spectroscopy (UV−vis), circular dichroism spectroscopy (CD), and analytical ultracentrifugation (AU).[8]

Evidence for binding of the porphyrin to the peptide, resulting in induced α-helical content in the peptide, is shown by UV−vis (Figure 1a) and CD (Figure 1b) measurements, respectively. As seen in previous studies of porphyrin binding to peptides and nucleotides, the absorbance of the porphyrin Soret band at 413 nm decreases with increasing peptide concentration.[9−11] Unlike many of these studies, however, a blue-shifted peak appears at 403 nm upon porphyrin−peptide binding rather than a red-shifted peak, and the porphyrin Q_x bands are red-shifted upon binding (Figure 1a, inset).[9−11] These indications of a strong Cp3K-N/$TPPS_4$ interaction are accompanied by significant changes in the Cp3K-N CD spectra (Figure 1b) which indicate that the largely unfolded structure of Cp3K-N in the absence of $TPPS_4$ is converted into an α-helical structure with increasing stoichiometric ratio of $TPPS_4$ to Cp3K-N. At a 1:1 stoichiometry, the peptide is largely in a helical conformation (∼82%) as judged by the ellipticity of the band at

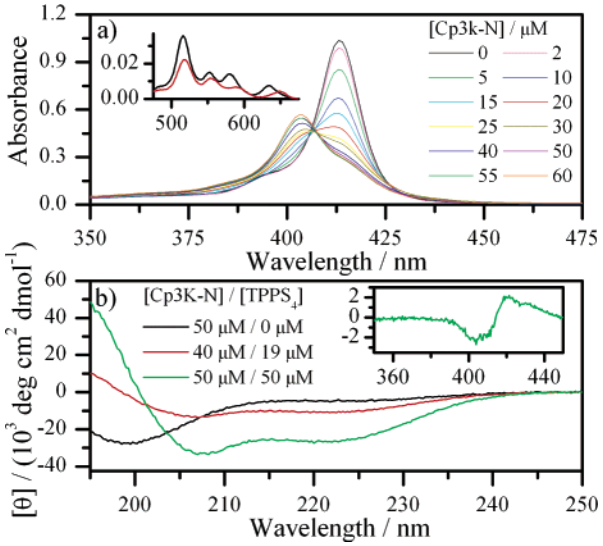

Figure 1. (a) Series of absorbance spectra taken from solutions containing various Cp3K-N concentrations and [$TPPS_4$] = 20 μM (inset: porphyrin Q-bands). (b) CD spectra from various Cp3K-N/$TPPS_4$ solutions (inset: porphyrin Soret band). The amino acid sequence of Cp3K-N is Ac−IQQLKNQIKQLLKQ−$CONH_2$.

222 nm and the spectral shift of the higher-energy band to 208 nm.[12] The porphyrin Soret band (Figure 1b, inset) also shows CD, indicating the existence of a specific porphyrin binding site on the helical peptide involving perhaps close porphyrin−porphyrin interaction.

Titrimetric experiments (Figure 2) illustrate the strength and stoichiometry of the Cp3K-N/$TPPS_4$ interaction. Excesses of Cp3K-N or $TPPS_4$ beyond the stoichiometry of 1:1 produce no further decrease in Soret band absorbance or increase in peptide α-helical content. Given the apparent two-state behavior observed in the UV−vis experiment (as judged by the isosbestic point), we treat the data using a simple binding model to obtain a dissociation constant of 2.09 ± 0.46 μM.[8] This K_d is comparable to those measured for peptides specifically engineered to bind porphyrins, though those peptides lack the readily identifiable structural transition that accompanies peptide binding seen here.[10]

Adding 0.15 M NaCl significantly reduces the α-helix content observed in porphyrin−peptide mixtures, suggesting that binding has a significant electrostatic component involving interactions between the porphyrin sulfonates and the peptide lysines (Figure 3). No binding is seen when a cationic porphyrin, meso-tetra(*N*-methyl-4-pyridyl)porphine, is used.[8] The reversible electrostatic porphyrin attachment seen here, an important paradigm for self-assembling systems, offers distinct advantages over irreversible porphyrin−peptide incorporation strategies wherein peptide chains

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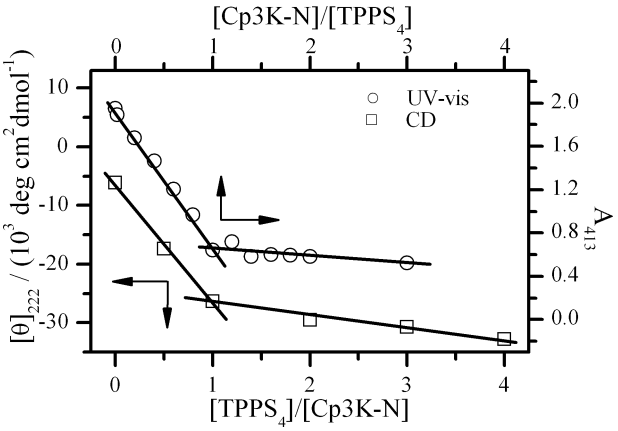

Figure 2. Dependence of absorbance (at 413 nm) and ellipticity (at 222 nm) on stoichiometric ratio from two titration experiments. In the absorbance measurements, [$TPPS_4$] was fixed at 50 μM and [Cp3K-N] was varied, whereas, in the CD measurements, [Cp3K-N] was fixed at 100 μM and [$TPPS_4$] was varied. Lines have been added as a guide to the eye.

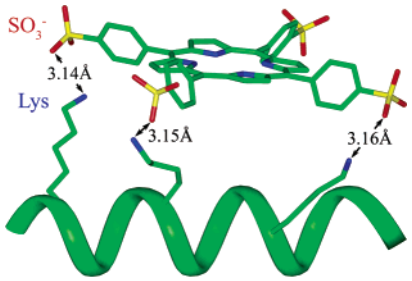

Figure 3. Model of a 1:1 complex of $TPPS_4$/Cp3K-N. The closest distances between the anionic sulfonate groups and the cationic lysines are labeled.[7]

have been covalently attached to porphyrins or ligated to the metal centers of metalloporphyrins;[4a,13] such work has been reviewed extensively.[14] For example, the electrostatic mode of binding allows incorporation of various metals into the porphyrin centers making the porphyrin−peptide complexes useful as catalysts, photosensitizers, or metal scavenging materials.[3]

Longer peptide sequences (i.e. Cp3K) are known to dimerize, forming a coiled-coil.[2,7] The ability of shorter sequences derived from Cp3K (i.e., Cp3K-N), induced to form an α-helix by $TPPS_4$, to form higher order assemblies was determined using AU sedimentation equilibrium measurements. When prepared individually, both Cp3K-N and $TPPS_4$ sedimented at their covalent molecular weights. Global analysis of data sets collected at three speeds for a porphyrin−peptide mixture revealed an apparent molecular weight of 6710 ± 330 Da. This suggests higher-order oligomerization, assuming a 1:1 complex stoichiometry with a theoretical molecular weight of 2696 Da. The square roots of variance obtained by numerically fitting AU data using several theoretical models are shown in Table 1.[8]

Consistent with the speed dependence of the apparent molecular weight, an indication of polydispersity, two-species models fit the data better than any single-species model.[8] The data are consistent with dimeric coiled coils decorated with two porphyrins that weakly associate most likely through porphyrin−porphyrin interactions. The

Table 1. Square Roots of Variances (SQOV) Calculated by Fitting AU Sedimentation Equilibrium Data to Various Solution Models ($TPPS_4$/Cp3K-N)

one-state models		equilibrium models	
ratio	SQOV (×10^{-3})	ratio	SQOV (×10^{-3})
1:1	15.0	1:1−3:3	2.86
2:2	5.40	1:1−4:4	3.37
3:3	5.35	2:2−3:3	2.79
4:4	12.9	2:2−4:4	2.83[a]

[a] Value does not significantly change if a 1:1−2:2−4:4 model is used.

ability of $TPPS_4$ to specifically bind and induce the assembly of a coiled coil offers the promise of creating responsive materials that are electronically and photonically active. Our previous work on longer peptides that form one-dimensional micron-sized polymers suggests that, with the incorporation of porphyrin derivatives, we can form long porphyrinic-peptide arrays capable of electron or excitation energy transfer.[2,5,6]

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Supporting Information Available: Experimental information available as pdf file. This material is available free of charge via the Internet at http://pubs.acs.org.

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