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Metal-peptide nanoassemblies

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A new class of metal-peptide nanoassemblies has been prepared by combining the principles of supramolecular coordination chemistry with those of *de novo* protein design.

The preparation of new supramolecular materials having nanometer-scale dimensions has gained in importance due to the potential of such systems to impact many areas of science and technology. This communication describes a new, bioinspired approach for preparing such materials by combining the principles of supramolecular coordination chemistry[1–3] with those of *de novo* protein design.[4–7]

Within the last decade, workers in the field of supramolecular coordination chemistry have exploited the fact that coordination compounds are formed by highly directional metal–ligand bond interactions. It was found that the appropriate use of bridging ligands allows multiple coordination units to be linked together in geometrically precise fashions to produce an interesting family of supramolecular assemblies.[1–3]

In entirely different work, peptide chemists have made significant strides towards the *de novo* design of synthetic proteins having predictable, well-defined structures with nanometer-scale dimensions.[4–7] Among the most well-studied of these are the synthetic, two-stranded α-helical coiled-coils which are modeled after a common protein dimerization motif found in biology.[7] These structures consist of an inter-twining of two α-helices to form a non-covalent left-handed supercoil, and have recently found application in the design of new supramolecular architectures.[8,9] It has been shown that the coiled-coil motif can result from amino acid sequences based on a seven residue heptad repeat, $(abcdefg)_n$, in which hydrophobic amino acids typically occupy positions *a* and *d* of the heptad, hydrophilic residues fill positions *b*, *c*, and *f*, and oppositely-charged residues may occupy positions *e* and *g* to form stabilizing inter-chain salt bridges.[7] Many examples of synthetic coiled-coils have been prepared using this basic design, which can be modified to produce a variety of coiled-coil morphologies, including dimers, trimers, and tetramers, depending on the particular amino acid sequences employed.[10,11]

Our group is developing methods to exploit the directional bonding properties of coordination compounds to orient synthetic coiled-coil dimerization domains in ways that can produce new nanostructured materials. The current approach utilizes disulfide crosslinked coiled-coils as linear bridging ligands joining adjacent *fac*-[$Re(CO)_3$] cores as shown in Fig. 1. The peptide sequence used in this work was based on the IEALEGK heptad repeat, which has been extensively used by our group to prepare a variety of metal-substituted, two-stranded α-helical coiled-coils for electron-transfer studies.[12–14] However, in this work the non-natural amino acid 4-pyridyl alanine (Pal) was placed at position 14 of this sequence, which is the most solvent-exposed *f* position of the second heptad repeat, and a cysteine residue was placed at residue 19 which occupies a *d* position in the hydrophobic core: AcK(IEALEG-K)(IEALEPalK)(IEACEGK)(IEALEGK)GNH_2. These modifications were made in order to incorporate a strong metal binding site into the peptide, and to engineer an inter-chain disulfide bond crosslink to stabilize the coiled-coil structure.

The resulting peptide, hereafter called Pal14C19, was synthesized by solid-phase techniques and purified by reverse-phase HPLC. The oxidized disulfide crosslinked peptide, $Pal14C19_{ox}$, was prepared by treatment with I_2 and also purified by HPLC. Analysis by MALDI-MS confirmed the presence of the oxidized peptide (calculated: 6575.62; observed: 6574.91) in addition to a smaller amount of the reduced peptide monomer (calculated: 3288.81; observed: 3289.50) as being the only two species present in the samples used to prepare the metal-peptide nanoassemblies. The circular dichroism spectrum of $Pal14C19_{ox}$ taken in methanol consists of a positive signal at 194 nm and negative bands at 208 and 222 nm to show that the oxidized peptide dimer is α-helical in nature. These results suggest that the $Pal14C19_{ox}$ peptide may serve as a suitable bridging ligand for preparing supramolecular nanoassemblies.

To create the desired metal-peptide assemblies, samples of $Pal14C19_{ox}$ were reacted in methanol at 37 °C with a limiting amount of $[NEt_4]_2[ReBr_3(CO)_3]$[15] under conditions previously used to form tricarbonyl rhenium(I) complexes of phosphine-derivatized amino acids.[16] $Re(CO)_3$ cores have been extensively used to prepare a variety of molecular square structures having interesting properties.[17] After reacting overnight, a gel-like substance appeared at the bottom of the microfuge tube which could be resolubilized upon shaking. The UV-Vis spectrum of the reaction mixture showed a two-fold increased absorption at 264 nm with a shoulder appearing at 300 nm to confirm the formation of the metal-peptide conjugate as these features correspond to the ligand-centered and metal-to-ligand-charge-transfer bands respectively, of related tricarbonyl rhenium(I) complexes containing pyridyl ligands.[18,19]

SDS-PAGE experiments were performed to help characterize the ability of the metal-peptides to form supramolecular nanostructures. As shown in Fig. 2, the metal-peptide reaction mixture contained several discrete higher molecular-weight species. Comparison with molecular weight standards including both the $Pal14C19_{ox}$ disulfide crosslinked dimer and the related Pal-14 apopeptide, which has no cysteine residue and travels as a peptide monomer under the denaturing PAGE conditions, shows the existence of metal-peptide assemblies having approximate molecular weights of *ca.* 6, 14, 21, and possibly 27 kDa, in addition to a small amount of monomeric peptide. These results are confirmed by the MALDI-MS results presented in Fig. 3 which show a progression of higher molecular weight metal-peptide assemblies containing from 1 to at least 4 coiled-coil subunits. The additional peaks seen in the spectrum can be attributed to the formation of

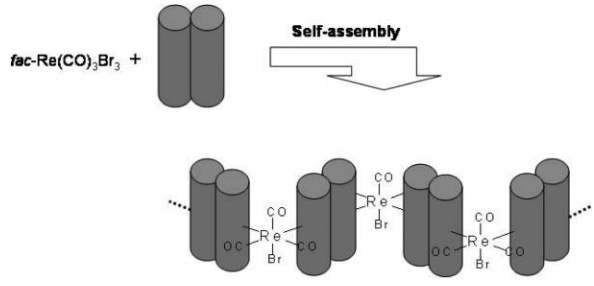

Fig. 1 Design of metal-peptide nanoassemblies in which the directional bonding properties of an octahedral rhenium complex are used to orient the self-assembly of peptide coiled-coils in a predetermined fashion.

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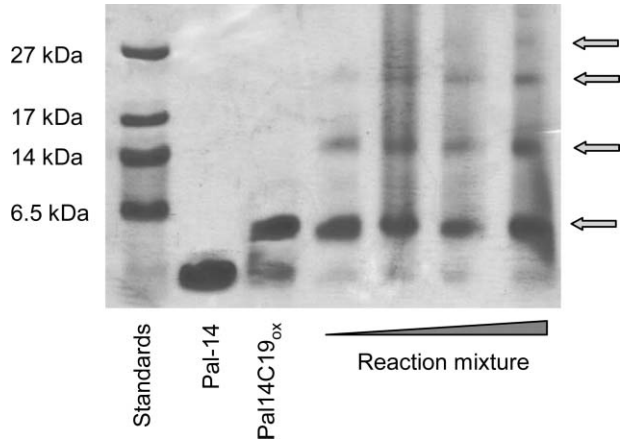

Fig. 2 SDS-PAGE results showing the presence of discrete higher molecular weight metal-peptide assemblies upon reacting $Pal14C19_{ox}$ and $[NEt_4]_2[ReBr_3(CO)_3]$ in methanol.

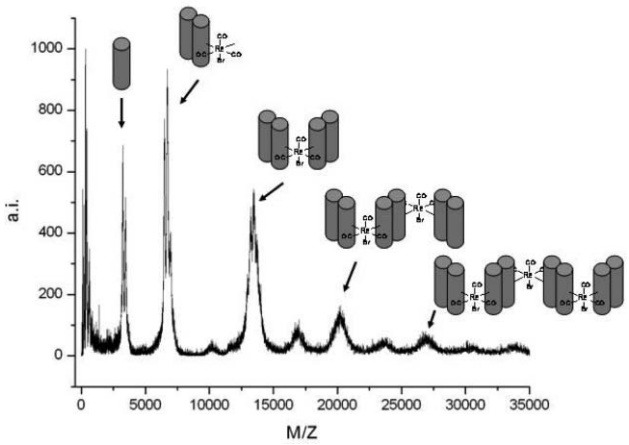

Fig. 3 MALDI-MS of the metal-peptide reaction mixture showing a progression of higher molecular weight species.

metal-peptide conjugates containing reduced Pal14C19 monomers as chain termination sites.

The results presented here demonstrate how metal-binding coiled-coil peptides can be used to direct the assembly of discrete, higher-order metal-peptide conjugates according to the principles of supramolecular coordination chemistry. Ongoing work is directed towards further characterizing the structures of these assemblies and exploring the use of different metal complexes along with other types of *de novo* designed peptide structures to prepare interesting metal-peptide nanoassemblies.

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