Coordination Crosslinking of Helical Substituted Oligoamide Nanorods with Cu(II)

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Substituted oligoamides are short sequences of unnatural amino acids. Oligoamides made entirely of β^3 amino acids yield helical monomers that, if N-acylated, assemble into nanorod structures *via* a supramolecular assembly motif. Crosslinking these structures using a secondary binding motif would deliver a unique hierarchical nanostructured material. In this work, this possibility is explored by attempting coordination crosslinking of oligoamides WKLWEL (KE) and WELWEL (EE) (where the letters denote the analogous α-amino acids) with Cu(II). Atomic force microscopy revealed specific morphologic changes in KE but not in EE. Small angle neutron scattering confirmed a specific change in the typical size of the KE assemblies in solution upon Cu(II) addition. Vibration spectroscopy measurements revealed that Cu(II) can coordinate to the amine moieties of the side chains, without direct effect on the backbone amides. Fast drying of the sample lead to oligoamide templated crystallization of $CuCl_2$. It was revealed that metal coordination takes place in dilute solutions, whereas the high dielectric constant of the concentrated salt solution upon drying leads to an ionic interaction between deprotonated sidechain amine moieties, yielding a composite structure defined by charge interactions. The former is the first realization of a metallosupramolecular framework structure.

Keywords: spontaneous assembly; metal coordination; metallosupramolecular framework; substituted oligoamides; 14-helix

Introduction

Substituted oligoamides based on β^3 amino acids exhibit a range of unique helical secondary structures.[1-5] β^3 amino acids are synthetic analogues of natural α amino acids, differing in an extra methylene group in the carbon backbone, therefore the same nomenclature and coding may be used to describe the side chains.[6-8] The properties of such oligoamides show marked differences to natural peptides: the presence of the extra methylene group gives greater flexibility to the molecule, and as such, substituted oligoamides are known to form multiple helices named after the number of atoms per helical turn.[7-14] The 14-helix is the

most common structure, where the hydrogen of the backbone amide of amino acid i binds to the carbonyl oxygen of amino acid $i+3$.[3, 4, 9, 11, 15, 16] This fold exhibits much greater structural stability than the α-helix of proteinogenic amino acids due to the higher conformational flexibility of the backbone and the resulting reduced steric strain in the helical loop.[1, 3, 6, 7, 10, 11] The 14-helix structure is unique among substituted oligoamides (including natural peptides) due to its pitch of ≈3 residues, which aligns every 4th residue such that it creates three 'faces' of the helix.[3-5, 17-19] This symmetry yields an ideal building block for the design of complex nanostructures.[20-22]

One dimensional assembly of the 14-helices can be achieved by the acylation of the N-terminus of the oligoamides, yielding a head-to-tail 3-point hydrogen-bonding motif that copies the intramolecular 14-helix hydrogen bonding pattern to the termini.[1, 17-19, 23-25] The resulting nanorods are stable in a range of solvents and may also assemble into a wide variety of superstructures, such as fibrils, rope-like bundles, dendritic, and mesh-like structures.[17-19, 23-25] These superstructures form through a variety of interactions including hydrophobic attractions, van der Waals interactions, and supramolecular H-bonding interactions, the strength and propensity of which are strongly dependent on the oligoamide primary structure (i.e. side chains and cross section geometry), as well as the chemical and physical properties of the solvents used.[17-19, 23-25] The diversity of superstructures available from oligoamides coupled with the high level of control achievable via molecule design and assembly conditions makes substituted oligoamides remarkably robust candidates for the design of spontaneously assembling nano-materials.[1, 9, 17-19, 23-25] In particular, the stability of the 14-helix raises the possibility of using metal coordination to crosslink the supramolecular nanorod assemblies without interfering with the primary supramolecular assembly motif; yielding a hitherto unprecedented metallosupramolecular framework (MSF) structure.

The dimensions of these materials pose a challenge to structural characterization. The nanorods are made of repeating units but in most cases do not form bulk crystals, preventing the use of crystallography methods. The core nanorod is beyond the resolving power of most microscopy methods, and the nanostructures are typically insufficient in bulk for spectroscopy measurements. Atomic Force Microscopy (AFM) can confirm the existence of the nanorod assembly and image the superstructure of deposits, while small angle scattering methods can provide the same information for suspensions.[25] Both SANS (small angle neutron scattering) and SAXS (small angle X-ray scattering) can be used to obtain the morphological envelope of molecules but with marked differences. In SAXS the X-ray beam interacts with electron densities of heavier elements, hence more sensitive to revealing core structure. In SANS, the neutron beam interacts with nuclear forces, providing an envelope including hydrogen atoms, that is, a more accurate representation of the actually thickness of the structures that correlates better to AFM. For this reason SANS is also more accurate to measure small oligomeric structures. Thus, SANS was used for these studies.

Synchrotron-based vibration spectroscopy (Fourier transformed infrared spectroscopy, FT-IR) is perhaps the only method that can glean structural information from such low dimensional materials due to the brilliance of the synchrotron.[18, 19] FT-IR is regularly used for the characterization of the secondary structures of peptides and proteins.[26-31] The extensive knowledge base created around peptide FT-IR vibrations can be applied with ease to these substituted oligoamides due to their overarching similarities to natural peptides.[26, 32] The most common bands seen in peptide FT-IR are vibrations modes of the amide groups of the peptide backbone.[30, 31, 33] The amide I and II bands are common diagnostic tools for determining the secondary structures of peptides as both bands strongly absorb, making them easily identifiable.[26, 29-34] The amide I band (predominantly C=O stretch) occurs between 1600 and 1700 cm^{-1} and is highly sensitive to

its local environment, and hence overall secondary structure of the molecule, however its contribution can be influenced by the vibrational bands from water.[26, 29-31, 34] The amide II band (N-H stretch and C-N bend) found between 1510 and 1580 cm^{-1} is free from water contribution but is less sensitive to changes in secondary structure.[31, 33] Amide bands appearing from 1200 to 1350 cm^{-1} are known as the amide III bands and can serve as a particularly sensitive diagnostic tool for secondary structure identification and are not impacted by any residual water bands.[31, 35] However, due to their low intensities they are often undetectable, and as such much less utilised than the amide I and II bands.[31, 33, 35].

A recent study on β^3 amino acid based substituted oligoamide systems reported the amide I and II peaks at 1645 cm^{-1} and 1548 cm^{-1}, respectively, in 14-helix secondary structures, whereas in random unfolded state the amide I and II bands occur at 1657 and 1502 cm^{-1}, respectively.[26] Further diagnostic features include the N-H bands commonly found around 3450 cm^{-1} that shift down to 3285 cm^{-1} when participating in the hydrogen bonding motif of the 14-helix, and the terminal carboxyl group that was identified at the vibrational frequency of 1720 cm^{-1} when in the 14-helix secondary structure.[26, 28, 30, 32] Amide III band locations have not been reported for the 14-helix secondary structures.

FT-IR studies have also investigated the effect of peptide-metal coordination on the amide vibration modes where backbone amide groups were directly involved in coordination.[36-38] For transition metals such as Cu(II) or Ni(II), there are two main coordination structures in short peptides: a charge solvation structure where binding occurs through the O groups of the amides, and amide-iminol rearrangement, where the metal coordinates to the nitrogen atoms of the amide through deprotonation.[36] In the latter motif, the deprotonation of the amide group is identifiable from the absence of the amide II band in the FTIR spectra.[36-38] Consistently, FT-IR is sufficient on its own for structural characterization of metal coordinated oligoamides.

In this paper we show the effect of Cu(II) on self-assembled nanorods of β^3 hexaamides, i.e. helical units of two 14-helix loops. Amine containing sidechains tryptophan and lysine were used to coordinate to Cu(II). AFM, SANS and FTIR measurements confirmed that under the right conditions the coordination occurred at the side chains while the 14-helix secondary structure was maintained.

Materials and Methods

Materials

Two substituted oligoamides were studied (as shown in figure 1) that will be denoted EE and KE. The oligoamides were synthesized using standard solid phase synthesis technique as described previously.[23] The oligoamide sidechains are analogous to proteinogenic amino acids, and as such will be discussed by using standard peptide nomenclature (tryptophan-W, lysine-K, leucine-L, and glutamic acid-E). All amino acids had L stereochemistry. [Figure 1 near here] All solutions were prepared in pure (unbuffered) solvents, to prevent any interactions between buffers and the samples affecting the self-assembly process.

Atomic Force Microscopy (AFM)

Oligoamide solutions were prepared by dissolving the oligoamides in deionized water (purified using Sartorius Stedim Arium® pro VF system to 18.2 MΩcm) or HPLC-grade isopropanol (Sigma Aldrich Pty. LTD. Castle Hill NSW 2154 Australia) at 1 mg/ml concentration. Samples were deposited on freshly cleaved mica and dried overnight at room temperature. For the solvent phase Cu(II) samples, $CuCl_2$ was added to the oligoamide solution to make up the concentration of 1 mmol Cu(II) and aged for 1 day before deposition. For the addition of Cu(II) after deposition (KE only), 1µl of 1 mmol $CuCl_2$ solution was added to the to the already dried oligoamide deposit, and dried overnight at room

temperature. A blank of 1mmol $CuCl_2$ was also prepared by depositing 1µl of 1 mmol $CuCl_2$ solution on a freshly cleaved mica disc and dried overnight at room temperature.

Imaging was performed in semi-contact mode using silicon probes with force constants in the range of 3-37 N/m, corresponding to 140-390 kHz resonance frequencies. The Ntegra AFM platform software was used (NT-MDT, Russia) for imaging, while Gwyddion software (Czech Metrology Institute, www.gwyddion.net) was used for all data processing.

Small Angle Neutron Scattering (SANS)

All measurements and sample solution preparation was performed at the Australian Nuclear Science and Technology Organisation (ANSTO) on the QUOKKA SANS instrument.[39]

All samples were prepared in deuterated water as solvent, to the highest concentration achievable to each oligoamide. For this reason the oligoamide EE was made to 3 mg/ml concentration, while KE was made to 2 mg/ml concentration. The difference is not significant in terms of the self-assembly of these oligoamides as the self-assembly equilibrium is strongly skewed to the oligomeric state.[1, 19, 23, 25] Analysis is conducted on the basis that in this dilute limit the scattering is dominated by the shape of scattering structures, i.e. form factor scattering.[40] Prior to SANS measurement samples were left at $40^{o}C$ overnight to fully dissolve and then placed in 2 mm pathlength cylindrical Hellma cells and scattering profile measured to 7200 counts. $CuCl_2$ was then added to the samples to the concentration of 1 mmol, and measurements were repeated as above.

Isotropic scattering patterns were collected with neutrons of wavelength, λ, 5.0 Å $(\Delta\lambda/\lambda = 10\%)$ at two sample to detector distances, 2 and 14 m. The count times for each configuration were 10 minutes and 2 hours, respectively. The raw data was reduced to the $I(q)$ form, where $q = 4\pi \sin(\theta)/\lambda$ and 2θ is the scattering angle, using IgorPro6 (Wavemetrics,

Oswego, USA) and SANS macros adapted by ANSTO[41], using a cell filled with D_2O as an empty cell value and a blocked beam measurement as the background.

Model fitting was used to approximate dimensions of the morphological envelope of the oligoamides in solution using Sasview fitting software. Elliptical cylinder modelling[42] was selected based on the common morphology seen in AFM data, and has the following equation.

$$I(q) = (\frac{scale}{V_{cyl}}) \int d\psi \int d\phi \int p(\theta, \phi, \psi) F^2(\boldsymbol{q}, \alpha, \psi) \sin\theta d\theta + background$$

Where:

$$F(q, \alpha, \psi) = 2\frac{J_1(a)}{a} \cdot \frac{sin(b)}{b}$$

$$a = q.\sin(\alpha) \left[r_{major}^2 sin^2(\psi) + r_{minor}^2 \cos(\psi) \right]^{\frac{1}{2}}$$

$$b = q\frac{L}{2}(\alpha)$$

The model assumes the geometry of the sample suspension as a cylinder with length (L), minor radius (a) and major/minor radius ratio (v), where va = major radius (Figure 2). [Figure 2 near here] The two lateral dimensions available through the elliptical cylinder model give better estimations to the irregularity of the structure compared to a simple cylinder. During fitting, the minor ratio and ratio axis were left as variable parameters, while all other parameters were fixed or constrained to determine changes in lateral dimension upon addition of Cu(II). The parameters for scale were confined to $2 \times 10^{-6} - 7 \times 10^{-6}$ and background was fixed at 0.094 cm^{-1}. The cylinder length parameter was constrained to 190-210 nm, based on fibre sizes seen in AFM data. The scattering length densities of the solvent (D_2O), KE, and EE were 6.334×10^{-6} Å^{-2}, 1.515×10^{-6} Å^{-2} and 1.619×10^{-6} Å^{-2}, respectively, calculated using Igor Pro software with SAS Irena macros.[43]

The densities of KE and EE (required to calculate the SLD) were found by editing the 14-helical crystal structure of the only N-acylated β^3 hexaamide that was obtained before.[23] This crystal structure was modified by replacing the side chains to provide an estimated crystal structure of both KE and EE. This is a reasonable approximation given the robustness of the 14-helix fold.[1] Subsequently, the volumes of KE and EE were found with Crysol[44] software using these estimated structures, and were found to be 1.20g /cm^3 and 1.22 g/cm^3, respectively.

Fourier Transformed Infrared Spectroscopy (FT-IR)

All spectra presented here were collected using the IRIS beamline set-up of the BESSYII electron storage ring at Helmholtz Zentrum Berlin (HZB). Two main modes of data collection were used depending on the specifics of a particular sample.

ATR measurements

Humidity controlled attenuated total reflectance (ATR) measurements were performed in single-reflection mode by using Bruker Vertex 80v spectrometer with a liquid nitrogen-cooled MCT detector set-up. A custom built environmental cell allowed for the change of relative humidity conditions inside the vacuumed spectrometer. Custom made large surface area germanium and zinc selenide ATR crystals were used in the experiments; the reported spectral range is well within the accessible wave number range for both crystals. The spectra were processed using the Bruker^{TM} OPUS 7 spectroscopy software.

To perform ATR measurements, the oligoamides were dissolved in a small amount of isopropanol and deposited on the surface of an ATR crystal. It was demonstrated previously that the N-acylated β^3 oligoamides self-assemble well in isopropanol as well as other alcohols; the change of solvent only affects the bundling of the core nanorods. [1, 17, 19, 23]

The isopropanol was evaporated, and the sample cell subjected to humid conditions (RHD=80%). This set-up was designed to emulate oligoamide fibre-packing in water (i.e. the state before metal coordination) in such a way as to be measurable by FT-IR; the high humidity provides sufficient water to mobilise the oligoamide nanorods, without saturating the sample spectra. FT-IR measurements of the oligoamide were collected as 250 sample scans with $4cm^{-1}$ resolution. For background, scans of the same crystal before sample addition were used, taken under the same conditions.

Once the neat oligoamide spectra under humid conditions were obtained, $CuCl_2$ salt was sprinkled over the neat KE oligoamide deposit under ambient conditions and humid conditions were maintained for ten hours, during which regular FT-IR measurements were recorded in 5 minute intervals until spectral changes ceased to occur. A background taken of the clean ATR crystal before oligoamide addition was used throughout.

Spatially resolved measurements of KE structures

A gas-purged Nicolet Continuμm Infrared Microscope (Thermo Scientific) with a liquid nitrogen-cooled MCT detector coupled to a Nexus 870 FT-IR spectrometer was employed to perform transmission measurements. Cassegrain infrared reflective objectives were used. The light from the synchrotron source was illuminating an area of 10 µm x10 µm on the sample by using a knife-edge aperture. The spectra and maps were collected by using the OMNIC Atlμs™ software.

KE dissolved in a small amount of isopropanol was deposited onto a 0.5 mm thick ZnSe disc and dried under ambient conditions. A droplet of 10 mmol $CuCl_2$ solution was then added on top of the oligoamide deposit and allowed to dry. FT-IR spectra were recorded in transmission mode under standard conditions through the sample. 25 spectra were collected and averaged, using background consisting of 8 averaged files taken in a nearby clean area of

the disc.

Results and Discussion

AFM studies

The two oligoamides were imaged using AFM to determine changes in morphology after Cu(II) addition. When deposited from H_2O the neat oligoamides already show significant differences. The KE sample forms large, fibrous structures that appear as bundles of small fibrils (figure 3A), while in the EE oligoamide deposit there are only a few fibrous structures, for most of the deposit fibres cannot be discerned (figure 3B). These structures alter substantially when the oligoamides are deposited from a solution of 1 mmol $CuCl_2$. The KE oligoamide shows a shift to smaller, regularly aligned fibrous structures of around 4-6 nm diameter (figure 3C). The regularity of the structure suggests that the core nanorods are crosslinked in a controlled, regular way, consistent with metal coordination to a specific moiety. In contrast, the EE oligoamide transformed into larger geometric structures upon Cu(II) addition (figure 3D). The irregular nature of the EE-Cu(II) structures and their overall larger size (7-9 nm diameter) suggests charge driven aggregation rather than coordination or cross-linking. Comparing the two oligoamides it is apparent that the lysine plays an essential role in metal coordination. [Figure 3 near here]

A third type of structure was also seen in the KE oligoamide, when a droplet of $CuCl_2$ solution was dried on top of an oligoamide deposit. After drying, large crystalline structures were observed (Figure 3E & F) that had a markedly different appearance compared to nanocrystals formed from the $CuCl_2$ solution deposited the same way (Figure 3G &H). Thus in the presence of the oligoamide the crystallization was templated by the fibrous structures.

SANS studies

SANS provides information on the geometric dimensions of the oligoamide structures

formed in solution. According to AFM, the assemblies appear as slightly distorted, very long cylinders; therefore elliptical cylindrical model was fitted to the scattering curves, focusing on the major and minor radii of the structures (Figure 4).[42]

Model fitting of the scattering curves showed no significant differences between KE and EE in pure D_2O (18.92 ± 1.07 nm X 48.44 ± 11.18 nm and 18.77 ± 1.04 X 46.88 ± 10.42 nm, respectively). After Cu(II) addition, in the suspension of EE oligoamide there was no significant change (18.21 ± 0.69 nm X 48.34 ± 4.43 nm), while the KE oligoamide structures increased in both major and minor radii (29.15 ± 0.62 nm X 67.24 ± 5.32), showing strong Cu(II)-KE interactions. These trends are consistent with the AFM studies, where KE incubated with Cu(II) yield distinct crosslinked structures. [Figure 4 near here]

Model fitting can provide highly accurate information on the geometry of a suspended particle, however caution must be exercised when interpreting the values found this way if the sample is not monodisperse. SANS records superimposed scattering profiles of all populations in the solution; therefore, when used on polydisperse populations such as those presented here, the fit yields essentially average values of the whole of the population. For this reason, and since the propensity of N-acylated β^3 oligoamides to self-assemble into nanorods under virtually any conditions and already at low concentrations [1, 17-19, 22-25, 45], here the focus was on the change of dimensions of each oligoamide after addition of Cu(II), rather than directly comparing the two samples. Additionally, model fitting requires accurate values for SLD parameters; as SLD is highly dependent on the folding of the molecule, it is expected to change as the oligoamides self-assemble, and as such the polydisperse population measured here likely contains multiple SLD values. However, while some uncertainty lies over the direct accuracy of the cylindrical dimensions calculated here, the trends seen within the four measurements are a good indication of changes that occur in solution phase upon Cu(II) addition.

FT-IR studies

FT-IR studies were conducted on the oligoamide KE to further characterise the structural aspects of Cu(II) coordination. Time resolved monitoring of the coordination process over 10hrs, focusing on the wavenumber range containing the main amide peaks and the observed changes is shown in Figure 5. [Figure 5 near here] The spectra reveal a distinct change, where two peaks at 1139 and 1202 cm^{-1} disappear, and a group of peaks appear at 858, 905 and 961 cm^{-1} wavenumbers (Figure 5). Crucially, the amide bands I and II do not shift throughout this process, although a decrease in intensity during coordination suggests a change in the local chemical environment, such as a shift in the material packing, consistent with coordination crosslinking.

Figure 6 shows more detailed spectra of the neat and coordinated oligoamide KE (after 10 hrs), along with the spectra of EE for comparison, focusing on the amide, amine and carboxylate modes in the range of 800-1800 cm^{-1} and 2700-3600 cm^{-1}. The amide bands I and II seen at 1644-1645 cm^{-1} and 1547-1549 cm^{1} are consistent with those found in 14 helix secondary structures. [26, 32] The EE spectra shows a split in the amide I band, indicating that a fraction of amide moieties are lacking hydrogen bonding partners. This is consistent with AFM imaging that revealed that a significant population is monomeric or small oligomeric. The band at 3282-3288 cm^{-1} is assigned to the backbone amide NH stretch; the peak position is between typical values of amide A and amide B modes. From the broad appearance of the band it is likely an overlap of these two modes, broadened by the hydrogen bonding in the 14-helix secondary structure.[26, 32] The band is slightly different for EE and KE, consistent with the difference in the amide I band.

The presence of the characteristic amide bands, especially the N-H stretch in both neat oligoamides and coordinated KE gives compelling evidence that the backbone amides are not deprotonated upon exposure to the Cu(II); from the lack of any shifts in the peak

positions it is also apparent that the 14-helix structure is also maintained. [Figure 6 near here]Amide III modes cannot be assigned with any clarity, and it is possible that these bands have too low intensity to detect. The bands at 1139 cm^{-1} 1202 cm^{-1} cannot be amide III bands, as these disappear upon coordination in KE while amide I and II modes show no changes. The band at 1202 cm^{-1}, attributed to the secondary amine of the tryptophan sides chain is apparent in both KE and EE spectra, while the band at 1139 cm^{-1}, attributed to the primary amine of lysine, is only seen in KE, confirming the assignment.[27, 28, 31, 46, 47] The disappearance of these bands suggests that the Cu(II) coordination involves these moieties, likely via partial deprotonation of the primary amine of the lysine side chain and potentially of the secondary amine in the tryptophan. Metal-N modes normally appear at much lower wavenumbers,[48, 49] thus the new peaks are likely the substantially shifted C-N modes of the amine moieties.

The band at 1714-1715 cm^{-1} is assigned to the carboxyl groups from both the oligoamide C-terminus and the glutamic acid side chains. Consistently this band is stronger in EE. The samples are unbuffered, and thus pH is unknown, however the positioning of the this band indicates the carboxyl groups are in a protonated state.[27, 31, 50, 51] While carboxyl moieties are likely candidates for the Cu(II) coordination, the very minor changes in this peak upon addition of the metal confirms that these groups do not have a direct role in the coordination. The KE spectra displays a broader carboxyl band compared to the neat EE spectra. This is likely caused by interactions between lysine on the glutamic acid, creating a larger difference in the chemical environments of the different carboxyl moieties. In the coordinated KE spectra the band narrows, consistent with the lysine-glutamic acid interactions being replaced with lysine-Cu(II) interactions.

Due to the inhomogeneous nature of the crystalline structures formed by drying down Cu(II) solution on a fibre deposit, for this sample measurements were conducted using an IR

microscope. Results are shown in Figure 6 D&H. The spectra reveal persistent, but slightly shifted amide bands (1651 and 1545 cm^{-1}) and the NH band at 3296 cm^{-1}. The shifts may reflect the differences in interaction of the oligoamide with the germanium and ZnSe substrates. The difference could also indicate a change in lateral interactions between fibres, such as those needed to form the large dendritic structures seen *via* AFM (figure 3 E&F). However, while slightly shifted, these bands are still well defined, indicating that the amide moieties are not deprotonated, and the 14-helix motif is also maintained in this structure. Like the coordinated structure, the side chain bands at 1139 cm^{-1} and 1202 cm^{-1} disappear after Cu(II) addition, however the band at 858 cm^{-1} was not observed. This can be explained with the deprotonation of the amine moieties that however did not proceed to coordination in the high salt concentration environment of the drying solvent; instead the deprotonated moieties form an ionic compound with CuCl$_2$, templating crystallization as observed with AFM imaging.

Discussion

KE and EE oligoamides were selected for this study due to their overall similarity, both having three distinct faces: an aromatic face of tryptophan residues, a polar face of glutamic acid/lysine residues, and an aliphatic face of leucine residues. The replacement of glutamic acid with lysine in the 2nd residue of the oligoamide KE provides a point of difference between the two samples, where the presence of lysine and glutamic acid moieties in KE can lead to salt bridge formation across the face of the helix, a characteristic that is linked to helical stability in α-peptides. [2, 10, 52] Consistently, the monomeric population of the EE sample seen through both AFM imaging and the splitting of the amide peak in FT-IR is likely the result of the alignment of the carboxylic side chains on the same face of the 14 helix.

The central question of this work was whether the 14-helical structure can remain

intact in the presence of Cu^{2+} ions, and whether the steric protection provided by the helical fold and nanorod assembly is sufficient to restrict metal coordination to the sidechains. These questions were answered in the affirmative by the evidence of fibrous assembly after metal addition provided by AFM imaging, the evidence of increased diameter from SANS and the persistence of the amide modes in the FT-IR measurements. Thus, the supramolecular assembly of the 14-helical units is robust enough to maintain structure in the presence of Cu^{2+} even if the salt is crystallized onto the fibres.

The two oligoamides were also chosen for their ability to coordinate metals in their side chains. Both amine and carboxyl moieties can act as ligands for metal coordination[53, 54], therefore one of the central questions of the work was whether Cu(II) has a preference to one of these moieties over the other. The FT-IR data provided decisive evidence that only the amine moieties, that of the lysine and the tryptophan side chains, are affected by the addition of the metal, that is, the Cu(II) coordinates to these moieties from adjacent nanorods, forming bundles with metal coordination sites between each oligoamide unit. This behaviour is specific to the supramolecular assembly of the oligoamide units, based on the steric shielding of the amide moieties both around the sides and termini of the helix. The assembly realizes a framework material, involving organic molecules and metal coordination; however a clear distinction has to be made compared to metal-organic frameworks where the organic linker is only connecting the metal centres. Here the framework is provided by a metallosupramolecular assembly; therefore it may be called a metallosupramolecular framework (MSF), that is, a novel class of nanostructured materials.

Conclusions

Results from the AFM, SANS and FTIR measurements confirmed that under specific

conditions, Cu(II) can coordinate to substituted oligoamide side chains with the core 14-helix and supramolecular head to tail motif remaining intact. The coordination was attributed to amine groups of the tryptophan and lysine side chains of adjacent KE chains. Hence, a new class of materials: metallosupramolecular frameworks was established.

Acknowledgments

References

1.   Gopalan RD, Del Borgo MP, Mechler AI, Perlmutter P, Aguilar MI. Geometrically Precise Building Blocks: the Self-Assembly of beta-Peptides. Chem Biol. 2015;22(11):1417-23.
2.   Seebach D, Matthews JL. beta-peptides: a surprise at every turn. Chem Commun. 1997(21):2015-22.
3.   Cheng RP, Gellman SH, DeGrado WF. beta-peptides: From structure to function. Chem Rev. 2001;101(10):3219-32.
4.   Gellman SH. Foldamers: A manifesto. Accounts Chem Res. 1998;31(4):173-80.
5.   DeGrado WF, Schneider JP, Hamuro Y. The twists and turns of beta-peptides. J Pept Res. 1999;54(3):206-17.
6.   Frackenpohl J, Arvidsson PI, Schreiber JV, Seebach D. The outstanding biological stability of beta- and gamma-peptides toward proteolytic enzymes: An in vitro investigation with fifteen peptidases. Chembiochem. 2001;2(6):445-55.
7.   Wu YD, Wang DP. Theoretical studies of beta-peptide models. J Am Chem Soc. 1998;120(51):13485-93.
8.   Appella DH, Christianson LA, Karle IL, Powell DR, Gellman SH. beta-peptide foldamers: Robust Helix formation in a new family of beta-amino acid oligomers. J Am Chem Soc. 1996;118(51):13071-2.
9.   Wang PSP, Nguyen JB, Schepartz A. Design and High-Resolution Structure of a beta(3)-Peptide Bundle Catalyst. J Am Chem Soc. 2014;136(19):6810-3.
10.   Craig CJ, Goodman JL, Schepartz A. Enhancing beta(3)-Peptide Bundle Stability by Design. Chembiochem. 2011;12(7):1035-8.
11.   Horne WS, Gellman SH. Foldamers with Heterogeneous Backbones. Accounts Chem Res. 2008;41(10):1399-408.
12.   Goodman JL, Petersson EJ, Daniels DS, Qiu JX, Schepartz A. Biophysical and structural characterization of a robust octameric beta-peptide bundle. J Am Chem Soc. 2007;129(47):14746-51.
13.   Martinek TA, Fulop F. Side-chain control of beta-peptide secondary structures - Design principles. Eur J Biochem. 2003;270(18):3657-66.
14.   Raguse TL, Lai JR, LePlae PR, Gellman SH. Toward beta-peptide tertiary structure: Self-association of an amphiphilic 14-helix in aqueous solution. Org Lett. 2001;3(24):3963-6.
15.   Seebach D, Gademann K, Schreiber JV, Matthews JL, Hintermann T, Jaun B, et al. 'Mixed' beta-peptides: A unique helical secondary structure in solution. Helv Chim Acta. 1997;80(7):2033-8.
16.   Krauthauser S, Christianson LA, Powell DR, Gellman SH. Antiparallel sheet formation in beta-peptide foldamers: Effects of beta-amino acid substitution on conformational preference. J Am Chem Soc. 1997;119(48):11719-20.
17.   Seoudi RS, Hinds MG, Wilson DJD, Adda CG, Del Borgo M, Aguilar M-I, et al. Self-assembled nanomaterials based on beta (beta(3)) tetrapeptides. Nanotechnology. 2016;27(13).
18.   Seoudi RS, Dowd A, Smith BJ, Mechler A. Structural analysis of bioinspired nano materials with synchrotron far IR spectroscopy. Phys Chem Chem Phys. 2016;18(16):11467-73.
19.   Seoudi RS, Dowd A, Del Borgo M, Kulkarni K, Perlmutter P, Aguilar MI, et al. Amino acid sequence controls the self-assembled superstructure morphology of N-acetylated tri-beta(3)-peptides. Pure Appl Chem. 2015;87(9-10):1021-8.
20.   Czajlik A, Beke T, Bottoni A, Perczelt A. Structure and stability of short beta-peptide nanotubes: A non-natural representative of collagen? J Phys Chem B. 2008;112(26):7956-66.

21.   Rueping M, Schreiber JV, Lelais G, Jaun B, Seebach D. Mixed beta(2)/beta(3)-hexapeptides and beta(2)/beta(3)-nonapeptides folding to (P)-helices with alternating twelve- and ten-membered hydrogen-bonded rings. Helv Chim Acta. 2002;85(9):2577-93.

22.   Del Borgo MP, Kulkarni K, Tonta MA, Ratcliffe JL, Seoudi R, Mechler AI, et al. beta(3)-tripeptides act as sticky ends to self-assemble into a bioscaffold. APL Bioeng. 2018;2(2):9.

23.   Del Borgo MP, Mechler AI, Traore D, Forsyth C, Wilce JA, Wilce MCJ, et al. Supramolecular Self-Assembly of N-Acetyl-Capped beta-Peptides Leads to Nano- to Macroscale Fiber Formation. Angew Chem-Int Edit. 2013;52(32):8266-70.

24.   Seoudi RS, Del Borgo MP, Kulkarni K, Perlmutter P, Aguilar MI, Mechler A. Supramolecular self-assembly of 14-helical nanorods with tunable linear and dendritic hierarchical morphologies. New J Chem. 2015;39(5):3280-7.

25.   Buchanan C, Garvey CJ, Perlmutter P, Mechler A. Co-assembly of helical beta(3)-peptides: a self-assembled analogue of a statistical copolymer. Pure Appl Chem. 2017;89(12):1809-16.

26.   Vasantha B, Yamanappa H, Raghothama S, Balaram P. Conformational properties and aggregation of homo-oligomeric beta(3)(R)-valine peptides in organic solvents. Biopolymers. 2017;108(3).

27.   Wolpert M, Hellwig P. Infrared spectra and molar absorption coefficients of the 20 alpha amino acids in aqueous solutions in the spectral range from 1800 to 500 cm(-1). Spectrochim Acta A. 2006;64(4):987-1001.

28.   Barth A. The infrared absorption of amino acid side chains. Prog Biophys Mol Bio. 2000;74(3-5):141-73.

29.   Jansen TL, Dijkstra AG, Watson TM, Hirst JD, Knoester J. Modeling the amide I bands of small peptides. J Chem Phys. 2006;125(4):9.

30.   Barth A, Zscherp C. What vibrations tell us about proteins. Q Rev Biophys. 2002;35(4):369-430.

31.   Barth A. Infrared spectroscopy of proteins. Biochim Biophys Acta, Bioenerg. 2007;1767(9):1073-101.

32.   Dutot L, Gaucher A, Elkassimi K, Drapeau J, Wakselman M, Mazaleyrat J-P, et al. Synthesis and characterisation of helical beta-peptide architectures that contain (S)-beta(3)-HDOPA (crown ether) derivatives. Chem: Eur J. 2008;14(10):3154-63.

33.   Kong J, Yu S. Fourier transform infrared spectroscopic analysis of protein secondary structures. Acta Biochim Biophys Sin. 2007;39(8):549-59.

34.   Haris PI, Chapman D. THE CONFORMATIONAL-ANALYSIS OF PEPTIDES USING FOURIER-TRANSFORM IR SPECTROSCOPY. Biopolymers. 1995;37(4):251-63.

35.   Xie MX, Liu Y. Studies on amide III infrared bands for the secondary structure determination of proteins. Chem J Chin Univ-Chin. 2003;24(2):226-31.

36.   Dunbar RC, Martens J, Berden G, Oomens J. Complexes of Ni(II) and Cu(II) with small peptides: deciding whether to deprotonate. Phys Chem Chem Phys. 2016;18(38):26923-32.

37.   Zhao J, Wang JP. Uncovering the Sensitivity of Amide-II Vibration to Peptide-Ion Interactions. J Phys Chem B. 2016;120(36):9590-8.

38.   Marsh BM, Zhou J, Garand E. Vibrational spectroscopy of isolated copper(II) complexes with deprotonated triglycine and tetraglycine peptides. RSC Adv. 2015;5(3):1790-5.

39.   Wood K, Mata J, Garvey CJ, Wu CM, Hamilton WA, Abbeywick P, et al. QUOKKA, the Pinhole Small-angle Neutron Scattering Instrument at the OPAL Research Reactor, Australia: Design, Performance, Operation and Scientific Highlights. J App Crys. 2018;51:294-314.

40.   Svergun DI, Koch MH, Timmins PA, May RP. Small Angle X-Ray and Neutron Scattering from Solutions of Biological Macromolecules: Oxford University Press; 2013.
41.   Kline SR. Reduction and analysis of SANS and USANS data using IGOR Pro. J Appl Crystallogr. 2006;39:895-900.
42.   Feigin LA, Svergun DI. Structure Analysis by Small-Angle X-ray and Neutron scattering. Taylor GW, editor. New York: Plenum; 1987.
43.   Ilavsky J, Jemian PR. Irena: tool suite for modeling and analysis of small-angle scattering. J Appl Crystallogr. 2009;42:347-53.
44.   Svergun D, Barberato C, Koch MHJ. CRYSOL - A program to evaluate x-ray solution scattering of biological macromolecules from atomic coordinates. J Appl Crystallogr. 1995;28:768-73.
45.   Mechler A, Seoudi R, Del Borgo MP, Aguilar M-I, Perlmutter P. Nano-architecture: creating complex surface structures using supramolecular self-assembly of tripeptides. Micro/Nano Materials, Devices, and Systems. 2013;8923.
46.   Hernandez B, Pfluger F, Derbel N, De Coninck J, Ghomi M. Vibrational Analysis of Amino Acids and Short Peptides in Hydrated Media. VI. Amino Acids with Positively Charged Side Chains: L-Lysine and L-Arginine. J Phys Chem B. 2010;114(2):1077-88.
47.   Hernandez B, Pfluger F, Adenier A, Kruglik SG, Ghomi M. Vibrational Analysis of Amino Acids and Short Peptides in Hydrated Media. VIII. Amino Acids with Aromatic Side Chains: L-Phenylalanine, L-Tyrosine, and L-Tryptophan. J Phys Chem B. 2010;114(46):15319-30.
48.   Xerri B, Flament J-P, Petitjean H, Berthomieu C, Berthomieu D. Vibrational Modeling of Copper-Histamine Complexes: Metal-Ligand IR Modes Investigation. J Phys Chem B. 2009;113(45):15119-27.
49.   Ilhan S, Temel H, Kilic A. Synthesis and characterization of new macrocyclic Cu(II) complexes from various diamines, copper(II) nitrate and 1,4-bis(2-formylphenoxy)butane. Chinese J Chem. 2007;25(10):1547-50.
50.   Koleva B, Kolev T, Spiteller M. SPECTROSCOPIC ANALYSIS AND STRUCTURAL ELUCIDATION OF SMALL PEPTIDES - EXPERIMENTAL AND THEORETICAL TOOLS. Taylor JC, editor. Hauppauge: Nova Science Publishers, Inc; 2010. 675-755 p.
51.   Max JJ, Chapados C. Infrared spectroscopy of aqueous carboxylic acids: Comparison between different acids and their salts. J Phys Chem A. 2004;108(16):3324-37.
52.   Hol WGJ, Vanduijnen PT, Berendsen HJC. Alpha-Helix Dipole and Properties of Protiens. Nature. 1978;273(5662):443-6.
53.   Iqbal M, Ali S, Tahir MN. Octahedral copper(II) carboxylate complex: synthesis, structural description, DNA-binding and anti-bacterial studies. J Coord Chem. 2018;71(7):991-1002.
54.   Benhassine A, Boulebd H, Anak B, Bouraiou A, Bouacida S, Bencharif M, et al. Copper(II) and zinc(II) as metal-carboxylate coordination complexes based on (1-methyl-1H-benzo d imidazol-2-yl) methanol derivative: Synthesis, crystal structure, spectroscopy, DFT calculations and antioxidant activity. J Mol Struct. 2018;1160:406-14.