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Nanoparticles

Oligoprolines as Scaffolds for the Formation of Silver Nanoparticles in Defined Sizes: Correlating Molecular and Nanoscopic Dimensions**

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Silver nanoparticles (AgNPs) are important for a broad range of applications, which include imaging, catalysis, and the development of antimicrobial coatings.[1,2] The properties of AgNPs depend on their size and shape. Thus, preparing stable AgNPs with defined dimensions is important and remains one of the largest challenges in the field.[1,3] AgNPs are typically obtained by the chemical reduction of Ag^+ salts in the presence of additives.[1] Polymers and surfactants are commonly used additives, and peptides are also becoming more and more important as additives, as they are biocompatible and allow the formation of AgNPs in water.[4–6] The additive is crucial for the initial nucleation, the growth, and the stability of the AgNP. Many strategies to control the size of AgNPs involve variations in the nature and the concentration of the additive.[1,3–6] These variations are in large parts driven empirically, as the correlation between the molecular structure of the additive and the nanoscopic dimensions of the resulting AgNPs is only poorly understood. As a result, many peptidic additives have been identified by using combinatorial screening methods,[7,8] whereas the rational design of additives to control the formation of AgNPs in defined sizes is still at an early stage.[9]

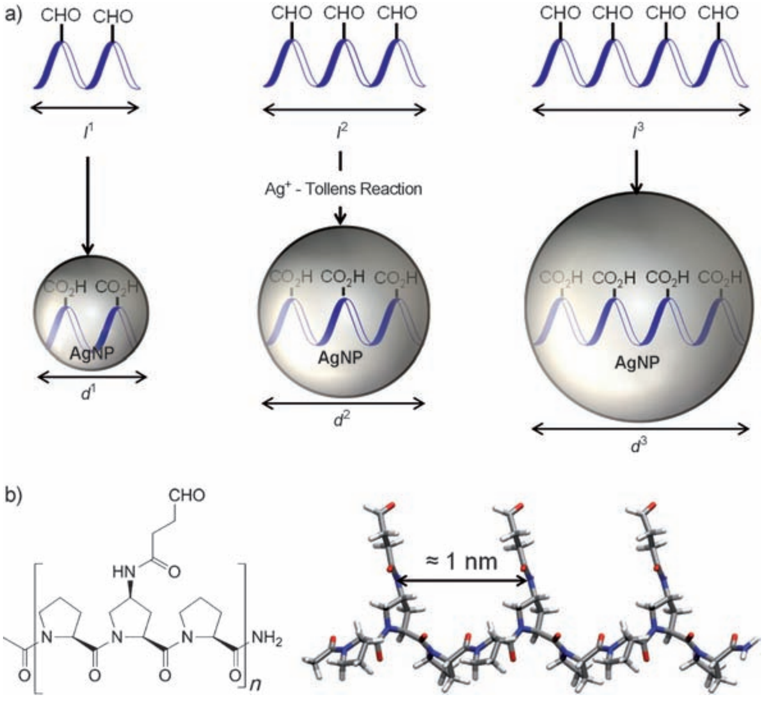

Figure 1. a) Metallization of aldehyde-functionalized oligoproline helices. b) General structure and model of oligoprolines functionalized with aldehydes in every third position.

We have recently introduced oligoprolines that contain azidoproline (Azp) as conformationally well-defined molecular scaffolds that can be functionalized with different moieties in defined spatial arrangements.[10] In an aqueous environment, these oligomers adopt the highly symmetric polyproline II (PPII) helix, in which every third residue is stacked on top of each other at a distance of approximately 1 nm (Figure 1 b).[10,11] This conformationally well-defined helix is adopted at chain lengths as short as six proline residues. The length and the pattern of functionalization of this molecular scaffold can be easily fine-tuned by chemical synthesis.[10] We envisioned oligoprolines of different lengths with functional groups that allow the generation of AgNPs as attractive additives to probe whether the nanoscopic size of AgNPs can be controlled by the molecular size of the additive (Figure 1 a).

To probe the influence of the molecular scaffold on the formation of AgNPs, we sought a redox reaction that would reduce Ag^+ ions but avoid an additional reducing agent that could complicate the analysis of the correlation between the additive and the resulting AgNPs. With this in mind, we decided to use the Tollens reaction to reduce Ag^+ with aldehydes that are attached to the molecular scaffold.[12] The aldehyde moieties are oxidized to carboxylic acids that are known to coordinate to AgNPs.[13] Thus, the reducing agent and the additive to control the nucleation as well as the growth of the AgNPs are within the same molecule. Herein, we present aldehyde-functionalized oligoprolines of different lengths as additives for the generation of AgNPs with a narrow size distribution. Moreover, we show that the

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diameter of the AgNPs correlates linearly with the length of the peptidic additives.

We started our investigations by preparing oligoprolines of different lengths bearing aldehyde moieties in every third position. Based on the stoichiometry of the Tollens reaction, two Ag^+ ions are reduced per aldehyde moiety. Thus, this design should lead to the formation of Ag atoms at approximately 1 nm intervals along the oligopeptide, which was envisioned to be short enough to allow the formation of stable Ag clusters.[12,14] Eight aldehyde-functionalized oligoprolines with lengths ranging from approximately 2 nm to 8 nm were prepared (Figure 2a). The synthesis commenced with the preparation of oligoprolines bearing azidoproline residues in the appropriate positions by standard solid-phase peptide synthesis. Subsequently, the azido groups were reduced under Staudinger conditions and the resulting amines were coupled with 3-(1,3-dioxolan-2-yl)propanoic acid (**1**, see the Supporting Information for details). Simultaneous removal of the acetal protecting groups and cleavage of the peptides from the solid support by treatment with trifluoroacetic acid (TFA) yielded peptides **2**–**8** (Figure 2a).

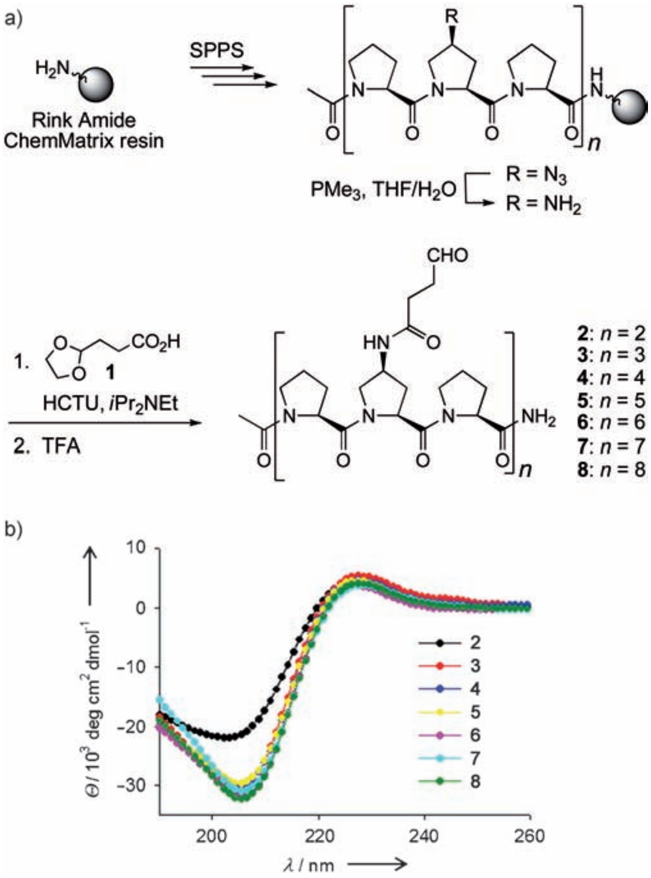

Figure 2. a) Synthesis and b) CD spectra of oligoprolines **2**–**8**.

Conformational analysis of **2**–**8** by CD spectroscopy showed that all of the functionalized oligoprolines adopt PPII conformations in aqueous environments, as indicated by the minima and maxima at wavelengths of 206 nm and 228 nm, respectively (Figure 2b).[10,11] This finding demonstrates that the aldehyde moieties do not disturb the conformational preferences of the oligoprolines. As expected, the PPII helicity is the least pronounced for peptide **2**, which consists of only six proline residues.

Oligoprolines **2**–**8** were then treated with Tollens solution, which resulted in an immediate yellow coloration of each of the solutions. The UV/Vis spectra of solutions of **2**–**8** have plasmon absorption at wavelengths between 400–430 nm, which is characteristic of AgNPs. The absorption intensified within 5 h to a maximum that remained constant thereafter (Figure 3).[15]

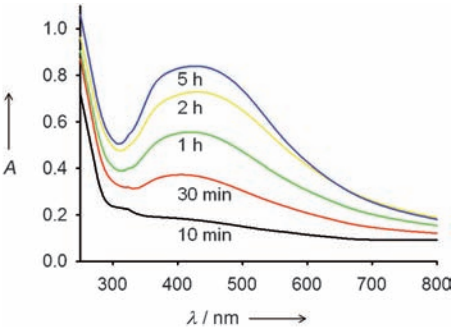

Figure 3. UV/Vis spectra of a solution of oligoproline **3** after the addition of Tollens solution.

The AgNPs were then analyzed by TEM. The images were obtained without further purification of the AgNPs by, for example, ultracentrifugation, which is often used to remove larger aggregates. Remarkably, the TEM images of each of the samples revealed a high degree of homogeneity in the size of the AgNPs. However, the size of the AgNPs was dependent on the length of the oligoproline additive (Figure 4a–c). The largest AgNPs had an average diameter of 9 nm and were formed in the presence of the longest oligoproline **8**, which has an approximate length of 8 nm, whereas the smallest AgNPs had an average diameter of 2 nm and were formed in the presence of oligoproline **2**, which has an approximate length of 2 nm. Most remarkably, a plot of the approximate lengths of the functionalized oligoprolines against the average diameter of the AgNPs that were generated in the presence of each peptide revealed a linear correlation (Figure 4d). These results demonstrate that the molecular dimensions of the templating peptidic scaffolds is reflected in the size of the resulting AgNPs.

Understanding the mechanism of how the functionalized oligoprolines control the size of the resulting AgNPs is not trivial. The finding that the diameters of the AgNPs are comparable to the lengths of the molecular scaffolds suggests that the oligoprolines serve as “molecular rulers” that initiate the nucleation of small Ag clusters, which then grow into stable particles. These particles are then limited in their size by the length of the scaffold on which the initial nuclei were formed. The importance of the rigidity of the template is supported by control experiments in which peptides **9**–**12** were used for the generation of AgNPs (Figure 5). Peptides **9**–**12** bear the same number of aldehyde moieties as their

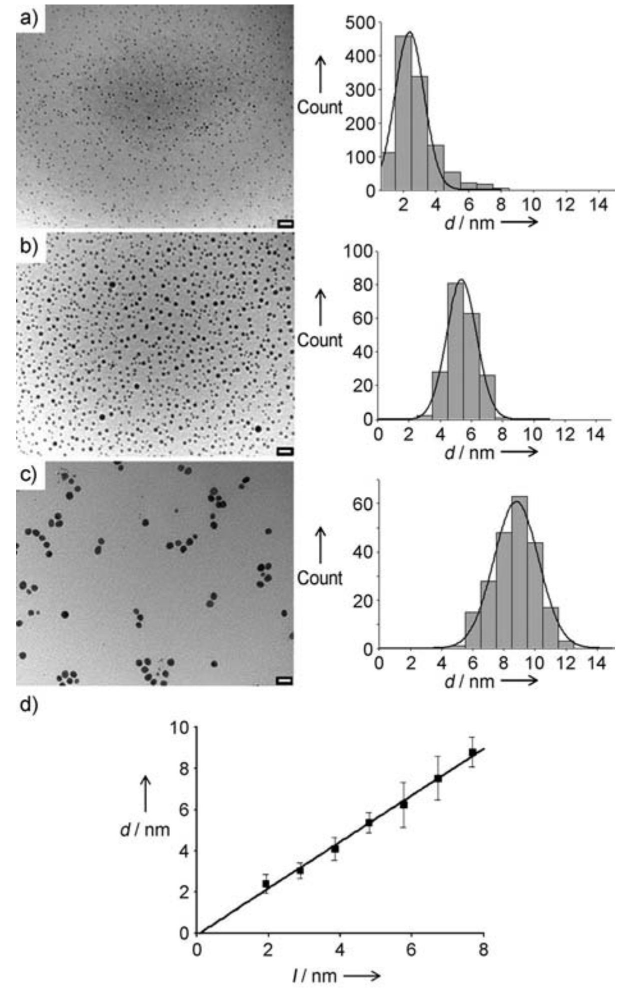

Figure 4. a–c) TEM images and size distribution of AgNPs that were formed in the presence of oligoprolines **2** (a), **5** (b), and **8** (c, scale bars = 20 nm). d) Correlation of the lengths of oligoprolines **2**–**8** with the average diameter of the corresponding AgNPs.

oligoproline counterparts, **2**, **4**, **6**, and **8**, respectively, but lack a defined secondary structure because of the flexible aminohexanoic acid spacers that link the aldehyde-functionalized proline residues. The AgNPs that were generated in the presence of these conformationally flexible peptides under otherwise identical conditions are polydisperse and there is no correlation between the number of aldehyde moieties and the average size of the AgNPs. These results demonstrate that not only the number of aldehyde moieties, but also their arrangement on the rigid scaffold is responsible for the correlation between the length of the functionalized oligoproline and the diameter of the AgNPs.

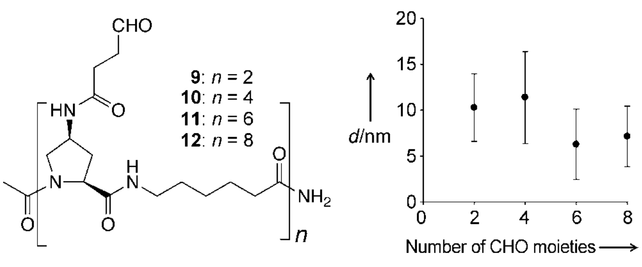

Figure 5. Plot of the size of the AgNPs that were synthesized in the presence of conformationally flexible peptides **9**–**12** versus the number of aldehydes moieties contained in the peptides **9**–**12**.

The high stability of the AgNPs that have no tendency to aggregate suggests that the peptides coordinate to the AgNPs. As carboxylic acid moieties are known to coordinate to AgNPs,[13] it is plausible that the coordination is mainly driven by the carboxylic acids that form on the oligoprolines during the Tollens reaction by oxidation of the aldehyde moieties. To examine the coordination of the peptides to the AgNPs, oligoprolines that have carboxylic acids in place of the aldehyde moieties, for example oligoproline **5a** (Figure 6), were examined by CD spectroscopy in the presence and absence of the AgNPs. All of the spectra that were obtained are typical for PPII helices, however, the maxima and minima that are observed in the presence of AgNPs are significantly more pronounced relative to those of the oligoprolines alone (Figure 6 and the Supporting Information). This result is strong support for the coordination of the oligoprolines to the AgNPs through the carboxylic acid moieties.[16] Further studies to gain a deeper understanding of the mechanisms by which the oligoprolines control the size of the AgNPs are ongoing.[17]

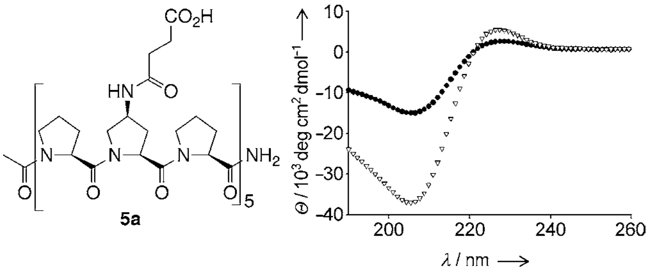

Figure 6. CD spectra of the carboxylic acid functionalized oligoproline **5a** in the presence (triangles) and absence of AgNPs (circles).

In conclusion, we have shown that the size of AgNPs can be controlled by the length of functionalized, rigid, oligoproline-based additives. The diameter of the AgNPs correlates linearly with the length of the peptidic scaffold. Thus, the molecular dimensions of the additive are directly reflected in the nanoscopic dimensions of the metal nanoparticle that is formed. These insights will allow the rational design of not only further peptide-based additives, but also other additives that can control the dimensions of metal nanoparticles. Moreover, the narrow size distribution of the AgNPs combined with the mild conditions that were used for their synthesis render these AgNPs attractive for applications where biocompatibility is required.

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[17] Initial experiments to probe the effect of temperature, concentration of Ag ions, and other parameters on the formation of the AgNPs suggest that the linear correlation between the molecular dimensions of the oligoprolines and the nanoscopic dimensions of the AgNPs remains.