Helical Peptides

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Induction of Structure and Function in a Designed Peptide upon Adsorption on a Silica Nanoparticle**

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The ability to regulate biological processes at the molecular level is often achieved by controlling the function of proteins. Efficient use of proteins and peptides in industrial processes and diagnostic tools also requires means to induce and maintain their functional conformations. Interestingly, the current discussion[1] on the origin of life has highlighted the fact that reactions such as vesicle creation[2] and peptide formation[3,4] are promoted by clay particles. Also in this context, it is interesting to investigate whether silica (which is a main constituent of most clay surfaces) could have a role in inducing functional conformations of peptides. Herein, we present a novel approach in which adsorption on silica nanoparticles is used to induce a well-defined structure in a designed peptide. The ability to generate stable structures on surfaces opens up the possibility of creating closely regulated

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systems with a variety of potential functionalities, which we demonstrate by introduction of a catalytic site.

The introduction of functionality into designed peptides was achieved earlier by Benner and co-workers,[5] who constructed an efficient oxaloacetate decarboxylase, and by Ghadiri and co-workers,[6,7] who constructed a self-replicating peptide and an efficient catalytic synthetic peptide ligase. Chmielewski and co-workers designed a replicating peptide system in which the reactivity is under the control of pH or salt.[8,9] DNA recognition modules, such as the basic region leucine zipper, are unfolded in solution but adopt a stable, ordered conformation upon interaction with DNA.[10–12] DeGrado and co-workers[13] analyzed the leucine zipper proteins, and found fundamental principles for the design of peptides that adopt a helical structure upon interaction with recognition sites in DNA. One important factor for induction of a helical structure is charge–charge interaction between basic residues and negative charges (phosphate groups) on DNA. Thus, it should be possible to induce a helical structure in a designed peptide that contains a motif of basic residues by providing a surface with appropriately spaced negative charges.

From these considerations, we designed a peptide (peptide **1**; Figure 1) that would be unstructured in solution but "forced" to adopt a well-defined helical structure upon adsorption onto silica nanoparticles. The design of peptide **1** was further developed in peptide **2** to include precisely placed amino acids, intended to form a catalytic site[14] upon folding of the helix. To ensure that the induction of structure by interaction with silica nanoparticles will have switchlike properties, it is essential to incorporate a combination of positive and negative design elements; that is, the design

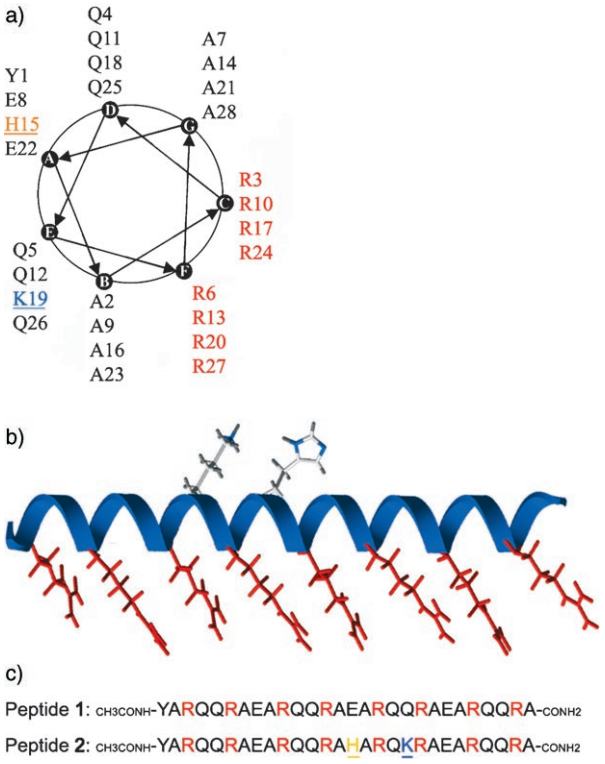

Figure 1. Peptide **2** displayed as a) a helical wheel and b) a helix viewed from the side showing the side chains of Arg and the catalytic His–Lys pair. c) The amino acid sequences of the two peptides.

should strongly favor the formation of helical structure upon binding to the nanoparticles and strongly disfavor an ordered structure in solution. For this reason, we decided to use electrostatic interactions as the structure-determining factor. Herein, we focus on the interactions between a silica nanoparticle and a peptide (peptide **2**) with two rows of positively charged side chains and residues that can form a reactive site (Figure 1).

Silica nanoparticles were chosen as the solid material for two main reasons: their high negative surface charge density and their size, which allows structural investigations of the peptide/nanoparticle complex in solution with standard spectroscopic techniques[15,16] and analytical ultracentrifugation.[17] Arginine residues were incorporated in the design rather than lysine because of their guanidinium group, which has a delocalized positive charge, high pK_a value, and low reactivity. The arginine residues were positioned so that two neighboring rows (Figure 1) would give rise to a large interaction area when a helix was formed upon contact with the negatively charged nanoparticle surface. The positive charge density of the helix interaction surface is of the same magnitude as the negative charge density of the silica nanoparticles at the ion concentration used in this study.[18] The majority of the remaining amino acids in the peptide were selected for their high helix-forming propensities.[19] The requirement for the peptide to be unstructured in solution was also considered in the design. The introduction of arginine side chains in rows C and F should efficiently prevent helix formation in solution, because the electrostatic repulsion between them tends to elongate the peptide. His 15 and Lys 19 (Figure 1) were also included, to introduce a reactive site for ester hydrolysis upon induction of the helical structure.[14]

Sedimentation equilibrium experiments were conducted to determine the aggregation state of the free peptide in solution and its binding to the nanoparticles. The data in Figure 2b show that a mixture of peptide **2** and particles sediments between 2500 and 8000 rpm, that is, all peptide molecules bind to the particles. The nanoparticles used in this study have a particle weight of ≈425 kDa and sediment completely at 8000 rpm as previously shown.[17] In the absence of particles the peptide does not sediment at these rotor speeds, that is, the peptide does not form large aggregates in solution (Figure 2a), and experiments at higher rotor speeds indicated that the peptide behaves as a monomer in solution (data not shown). Similar results were observed for peptide **1** (data not shown).

To determine the extent of formation of the secondary structure, samples of free peptide and peptide bound to the nanoparticles were analyzed by far-UV CD. The small diameter (9 nm) of the silica nanoparticles allows UV light to penetrate the sample without scattering, and therefore conventional light spectroscopy can be used to characterize these systems.[20–22] As illustrated in Figure 3a, the CD signals indicate that the free peptide in solution has no defined secondary structure, irrespective of the pH used in this study (pH 7.9–9.8). Calculations carried out with ContinLL[23] indicate ≈9% α helix for free peptide **1** and ≈8% α helix for free peptide **2**. However, addition of nanoparticles to the

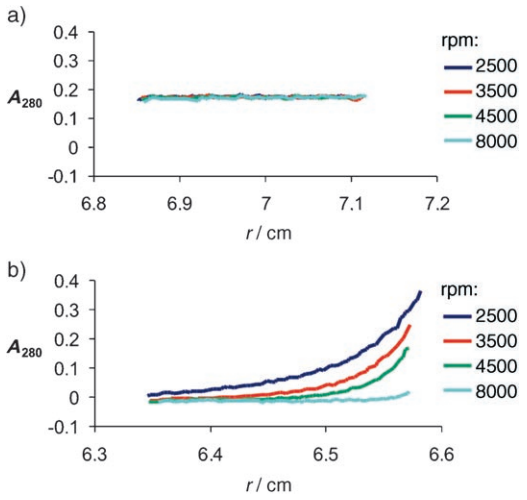

Figure 2. Sedimentation equilibrium analysis of peptide **2** by ultracentrifugation monitored at A_{280} a) without and b) with 9-nm silica particles at pH 9 and 22 °C. The colors represent different rotor speeds (rpm).

solution gave rise to a CD spectrum that is typical for the helical conformation; that is, the nanoparticles induced formation of a helical structure upon interaction with the peptide. Figure 3a also shows that the deletion of the His–Lys pair results in an increase of adopted α helix upon binding of the peptide to the nanoparticle, and the calculations indicate that peptide **1** has 39 % α helix compared with 29 % for peptide **2**. There is a clear correlation between the amount of induced secondary structure in the peptide and the pH of the solution, as demonstrated for peptide **2** in Figure 3b. The calculated amount of α helix is 25, 29, and 35 % at pH 7.9, 9.0, and 9.8, respectively. The nanoparticles induce more secondary structure at higher pH values, probably because the density of negative charges on the surface is increased by raising the pH.[18] The results of melting experiments (Figure 3c) clearly suggest that the helical structure content decreases gradually (noncooperatively) upon increasing the temperature from 25 to 85 °C. The data also reveal that the temperature-dependent structural change is fully reversible, that is, when the sample is cooled to 25 °C the original amount of helix is observed in the sample (Figure 3c, inset).

Peptide **2** includes a His–Lys pair that forms a catalytic unit for ester hydrolysis, provided that peptide **2** becomes helical. Thus, strong proof of a successful design would be a significant rate enhancement of ester hydrolysis in samples containing both peptide **2** and nanoparticles. Experiments in which peptide **2** or the nanoparticles were dissolved in buffer showed no detectable rate enhancement over the background reaction in buffer alone. Notably, samples containing the peptide **2**/nanoparticle complex showed a dramatically higher catalytic efficiency (Figure 4). A control experiment with the peptide **1**/nanoparticle complex showed no rate enhancement over the background reaction in buffer alone. Thus, the enhancement of catalysis requires both the His–Lys pair and the formation of structure upon binding to the nanoparticles.

The design of the catalytic His–Lys site was inspired by the study of Lundh et al.,[14] in which the imidazole moiety of a

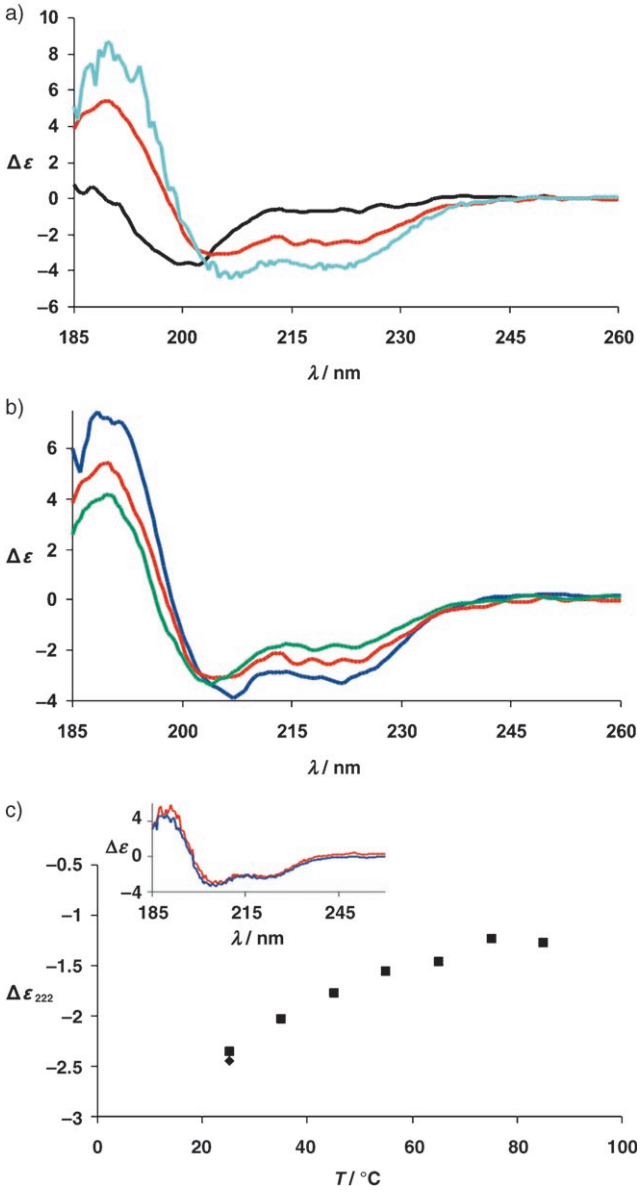

Figure 3. a) CD data for peptides **1** and **2** at pH 9.0 and 22 °C. Black = peptide **2** in solution, blue = peptide **1** with 9-nm silica particles, and red = peptide **2** with 9-nm silica particles. b) CD data for peptide **2** at pH 7.9 (green), 9.0 (red), and 9.8 (blue) with 9-nm silica particles. c) Melting of peptide **2** adsorbed on 9-nm particles (■). The (◆) represents the observed CD value after the sample was cooled to 25 °C. The inset shows the CD spectrum of the sample at 25 °C before heating (red) and after heating to 85 °C and subsequent cooling (blue).

His residue acts as a nucleophile and the positive charge of a nearby ornithine (Orn) residue may stabilize the transition state of the reaction. A comparison of second-order rate constants shows that the peptide **2**/nanoparticle complex ($k_2 = 0.25\ \mathrm{mM^{-1}\,min^{-1}}$) is 54 times more efficient in catalysis than peptide **2** ($k_2 = 0.0046\ \mathrm{mM^{-1}\,min^{-1}}$) alone. Clearly, the nanoparticles can be used as an efficient switch to turn on catalysis. Notably, catalysis with the peptide **2**/nanoparticle complex is 40 times more efficient than the catalysis reported for the peptide that was designed by Lundh et al., and 410 times more efficient than catalysis by imidazole.

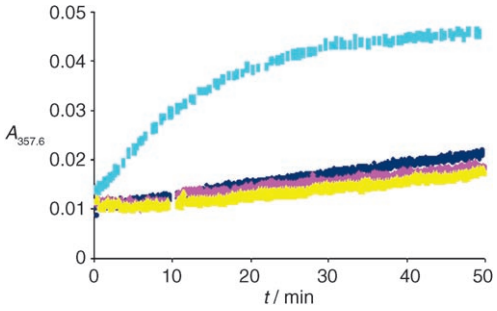

Figure 4. Ester bond hydrolysis by: the peptide **2**/nanoparticle complex (turquoise); peptide **2** (pink); nanoparticles (yellow); and buffer (dark blue). The reaction product *m*-nitrophenol was monitored at $A_{357.6}$.

It is difficult to distinguish between the alternatives that either all peptide molecules adopt a partial helical structure or an equilibrium situation occurs, in which a fraction of peptides is completely helical while another fraction is without helical conformation. The latter alternative would indicate that the temperature dependence of the catalytic activity is directly correlated to the temperature-dependent change in CD signal shown in Figure 3c. However, measurements of ester hydrolysis show that the peptide **2**/nanoparticle complex is catalytically active at 12 °C ($k_2 = 0.17$ mM^{-1} min^{-1}) and 22 °C ($k_2 = 0.25$ mM^{-1} min^{-1}), while there is no observable increase over the background buffer catalysis at 32 and 52 °C. Apparently, the helical conformation at the catalytic site is critically affected in the 22–32 °C temperature range. Thus, the peptides on the silica surface probably all adopt a partial helical conformation, which gradually melts at higher temperatures. Notably, the original esterase activity is completely restored (98 %) after heating to 80 °C and subsequent cooling to 22 °C, which further shows that the catalytic activity is well-correlated to the formation of a helix, that is, the system can be regulated by temperature.

The results of the experiments collectively show that the designed peptides adsorb efficiently and bind strongly to silica nanoparticles, and that they adopt a defined helical structure upon binding. Moreover, the design strategy also successfully incorporated the planned functional properties, as the observed catalysis of ester hydrolysis shows that the formation of a helix leads to a catalytic unit that is oriented away from the surface of the nanoparticles so that catalysis can proceed unhindered.

The described method has potential use in the creation of novel recognition elements and catalysts that can be switched on by the introduction of nanoparticles. By adjusting the design it might also be possible to construct a two-state system that would allow the activity to be switched on and off by small changes in temperature. The ability to create surfaces with well-defined properties (such as reactive groups arranged at predetermined distances on the nanometer scale) would have several important applications in areas such as biocatalysis, biosensing, and nanotechnology. Moreover, it is well-known that interaction with inorganic surfaces tends to induce conformational and functional changes in proteins.[15–17,21,23] Interestingly, it has recently been shown that these rearrangements do not occur at random, but instead bring positively charged side chains closer to the silica surface.[23,24] These observations, together with the results of the present study, point toward the possibility that peptides with a high fraction of positively charged amino acid residues have a high probability of forming well-defined structures on clay. As the amino acid content in peptides can be highly diverse, the adsorption on clay offers rich possibilities that side chains are brought together into functional (catalytic) units. In fact, the possibility for diversity is much larger on peptides than on RNA, which has been suggested to form functional structures on clay.[25] Hence, the results add a new twist to the hypothesis that inorganic clays had an important role in the prebiotic chemistry that led to the origin of life.

Experimental Section

CD spectra were recorded as described by Lundqvist et al.[17] The samples consisted of peptide (≈0.1 mM) with 9-nm silica particles added to give a peptide/nanoparticle ratio of 1:0.25. The helical content was calculated by use of the program ContinLL.[26] Ultracentrifugation experiments were performed and analyzed as described by Lundqvist et al.[17] The samples were the same as those used in the CD analysis. The kinetics of esterase activity were measured at pH 8.2 in Tris buffer (20 mM), with 4-sulfamoyl(benzoylamino)acetic acid 3-nitrophenyl ester (0.04 mM) as substrate. The reaction was monitored at the isosbestic point for *m*-nitrophenol of 357.6 nm. The peptide concentration was 0.1 mM and the concentration of silica nanoparticles was approximately 0.05 mM. Under these conditions, the rate with buffer solution alone was indistinguishable from those measured on samples with nanoparticles or on samples with free peptide in solution. To determine the (very low) rate enhancement from free peptide in solution, experiments with 2 mM peptide were performed under otherwise identical conditions. Catalysis by 10 and 50 mM imidazole was also measured. Second-order rate constants k_2 were calculated after subtraction of the background rate in buffer alone. The peptides were synthesized by standard 9-fluorenylmethoxycarbonyl (fmoc) protection group chemistry.

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