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Peptide Structures

Disruption of Amyloid-Derived Peptide Assemblies through the Controlled Induction of a β-Sheet to α-Helix Transformation: Application of the Switch Concept**

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β-Sheet-based assemblies have attracted a considerable amount of attention from researchers of various disciplines because of their association with a variety of diseases and their emerging potential in material sciences and biotechnology.[1] Protein misfolding and self-assembly into highly ordered β-sheet-rich fibrillar assemblies that are known as amyloid fibrils are common features of a growing class of systemic and neurodegenerative diseases, which include Alzheimer's, Parkinson's, and Huntington's diseases, senile systemic amyloidoses, as well as type II diabetes.[2] Although there is strong evidence that implicates amyloid formation in the pathogenesis of these diseases, the precise mechanisms of amyloid formation and clearance in vivo, as well as the structural basis of amyloid toxicity, remain unknown. The lack of tools to monitor and/or control the initial structural transitions associated with protein misfolding, amyloid formation, and/or dissociation is the main cause of this gap in knowledge. Significant efforts have been devoted to study proteins and small peptides that self-assemble into amyloid-like fibrillar structures as model systems to investigate amyloid formation or to generate materials with interesting physical properties. However, knowledge of the mechanical and structural dynamics within β-sheet assemblies such as amyloid fibrils remains limited. Early assumptions that β-sheet assemblies, which include amyloid fibrils, occupy a global minimum of free energy that is lower than that of the native state[3] led to a greater emphasis on the understanding and inhibition of amyloid formation, rather than that of amyloid dissociation and clearance. Despite the stability of β-sheet-rich amyloid fibrils against proteases, acids, and chemical denaturants, increasing evidence from human[4] and in vitro studies indicates that a dynamic structure exists within amyloid fibrils and suggests that the process of amyloid formation is reversible.[5a,b] These findings, along with the fact that strategies aimed at the destabilization of amyloid fibrils and/or the acceleration of their clearance seem to reverse the disease phenotype,[6–7] suggest that a detailed understanding of the stability and dynamic behavior of amyloid fibrils is of critical importance to the development of therapeutic strategies for amyloid diseases.

Our research group has previously shown[8–10] that the incorporation of molecular switches into polypeptides, based on an in situ intramolecular O→N acyl group migration,[11] allows for the controlled induction or reversal of secondary structural transitions[12a,b,c] and self-assembly of small peptide chains. Herein we describe a switch peptide that is designed to disrupt amyloid-like β-sheet assemblies through the controlled induced transition from a β-sheet to an α-helix structure (Figure 1). The experimental results illustrate the potential of switch peptides as a tool to investigate the structural dynamics of amyloid fibrils, to provide an insight into the structural basis of amyloid-associated toxicity, to control the peptide self-assembly, and to facilitate the development of novel peptide biomaterials that have potential applications in medicine and biotechnology.

To investigate whether the amyloid formation could be disrupted or reversed through an induced transformation of a β-sheet to an α-helix structure, we designed a switch peptide that contains the fibril-forming sequence HQKLVFFAEDVG, which corresponds to Aβ(14–25), connected to a helix nucleating Ncap[13] (**σ**) through a serine-derived switch element (**S**) (peptide **I**, Figure 1). The Aβ(14–25) peptide contains the amino acid residues 16–20 that are essential for formation of fibrils in the native amyloid β peptide and readily forms fibrils in vitro.[14] We chose the cyclic pentapeptide sequence Ac-(*cyclo*-1–5)-KARAD, which contains a lactam bridge formed between the Lys and Asp residues at positions i and $i+4$, respectively, to serve as the Ncap.[13] This type of lactam bridge effectively constrains the residues into an α-helical turn and, when incorporated into a larger peptide, can greatly increase the overall helicity.[15]

To examine the solution properties of peptide **I** in the S_{off} and S_{on} states (Figure 1), the secondary structure and aggregation state of the peptide was probed by circular

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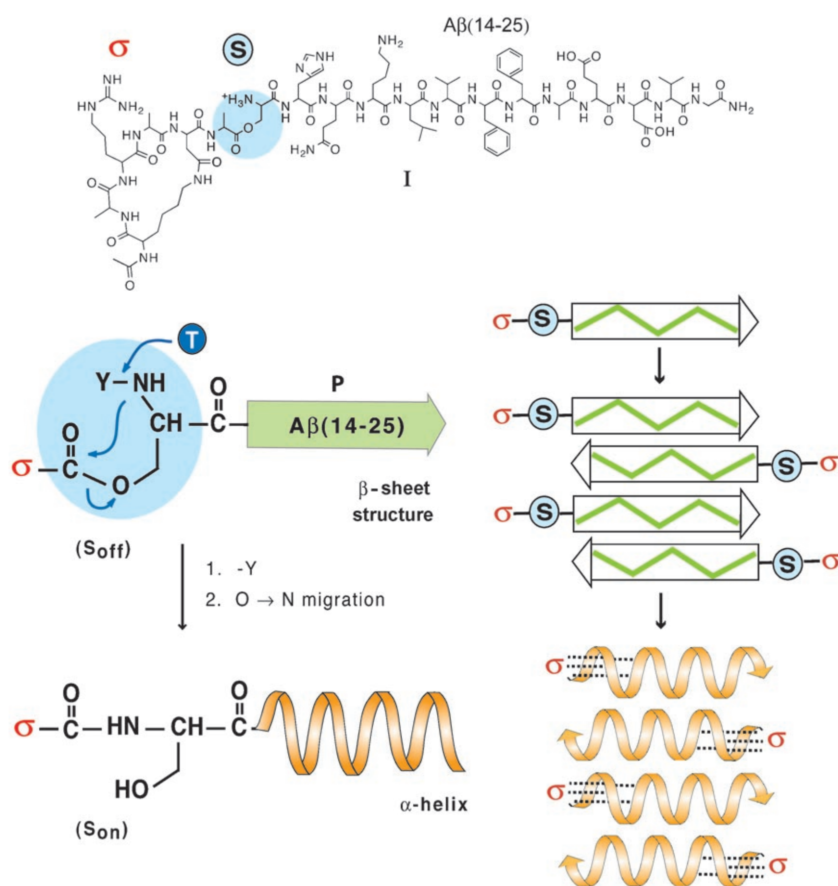

Figure 1. Switch peptide **I**: Aβ(14–25) is linked to a helix-inducing template **σ** (Ncap = Ac-[*cyclo*-1–5]-KARAD) through a serine-derived switch element **S** (O-acyl isopeptide, S_{off} state). On removal of the N-protecting group Y, spontaneous O→N acyl group migration occurs (S_{on} state) resulting in the activation of **σ** and induction of a helix structure in peptide **I** (left). Under the right conditions, the helix-inducing effect of **σ** (right) is strong enough to induce a transformation from β-sheet (step 1) to α-helix (step 2), along with disruption of the preformed Aβ(14–25)-derived β-sheet-rich assemblies within amyloid fibrils.

dichroism (CD) and electron microscopy (EM). In the S_{off} state (pH 4.5, 50 mM acetate, 150 mM NaCl) and at concentrations between 10–100 μM, the CD spectrum of peptide **I** displays the Cotton effect which is typical of a β-sheet structure (curve 1 in Figure 2A). Activation of the helix-inducing Ncap (S_{on}) through a pH-induced O→N acyl group migration [9,11] is not sufficiently strong to overcome the intrinsic β-sheet propensity of Aβ(14–25), thus resulting in identical CD spectra. However, when the Ncap was activated in the presence of 25% 2,2,2-trifluoroethanol (TFE) as a co-solvent to promote the α-helix, we observed an unprecedented transition from a predominantly β-sheet structure in the S_{off} state (curve 1 and 2) to a predominantly α-helix structure in the S_{on} state (curve 3) that was complete in less than five minutes at room temperature ($t_{1/2}$ = 150 s, inset in Figure 2A). It is noteworthy that addition of TFE (20–100%) to peptide **I** in the S_{off} state does not cause the induction of α-helix formation, instead the peptide forms predominately β-sheet-rich fibrillar aggregates (see the Supporting Information).

Electron microscopy revealed that the transition from the β-sheet to the α-helix structure is accompanied by a dramatic change in the morphology and subsequent dissociation of the fibrils. Figure 3 shows negatively stained micrographs of peptide **I** before (S_{off}, A) and after (S_{on}, B and C) triggering the switch element in the presence of 25% TFE. In the S_{off} state, peptide **I** self-assembles into long (> 2 μm) unbranched fibrils with an average diameter of 3.3 nm (inset a, Figure 3A). Lateral association of these thin fibrils results in the formation of twisted amyloid-like fibrils with an average diameter of 7.7 nm, 11 nm, and 20 nm, consistent with the 3.3 nm being the subunit protofilament of the wider fibrils (Figure 3A). These fibrillar morphologies bind to the amyloid-specific dyes such as Congro Red (CR) and Thioflavin T (ThT) (see the Supporting Information). Interestingly, on triggering the O→N acyl group migration (S_{on} state), short ribbon-like structures (Figure 3B) were observed, but these structures quickly disappeared and gave rise to predominantly soluble α-helix structures of peptide **I**. Filtration of a solution of peptide **I** (S_{off} state) through a 0.22 μm membrane resulted in the loss of more than 80% of the peptide along with disappearance of the β-sheet structure (see the Supporting Information). In the S_{on} state, greater than 80% of peptide **I** remains in solution after filtration through a 0.22 μm membrane, consistent with the transition from aggregated β-sheet to soluble α-helix structures (CD analysis) as was evident from the disappearance of the fibrillar assemblies (Figure 3C) and the loss of ThT fluorescence upon activation of the O→N acyl group migration (see the Supporting Information).

To better understand the structural properties of peptide **I**, we probed its stability in the S_{on} and S_{off} states towards heat-induced dissociation or denaturation. To this end, the peptide solutions were heated gradually and the conformation of the peptide was monitored by CD (Figure 2B). Heating peptide **I** in the S_{off} state (pH 4.5, 25 °C, curve 1) to 95 °C resulted in a transition from the β-sheet to a predominantly unordered structure (curve 2). Interestingly, when the sample was cooled to 25 °C, the peptide did not return to its original β-sheet structure but rather formed an α-helical structure (curve 3). This observation suggests that at higher temperatures, the helix-inducing effect of the Ncap comes into force as a result of the temperature-induced O→N acyl group migration and the activation of the Ncap. Most notably, once the β-sheet is destabilized at high temperature, the helix-inducing effect of the Ncap is strong enough to overcome the intrinsic β-sheet predisposition of peptide **I**, that is, an Ncap-

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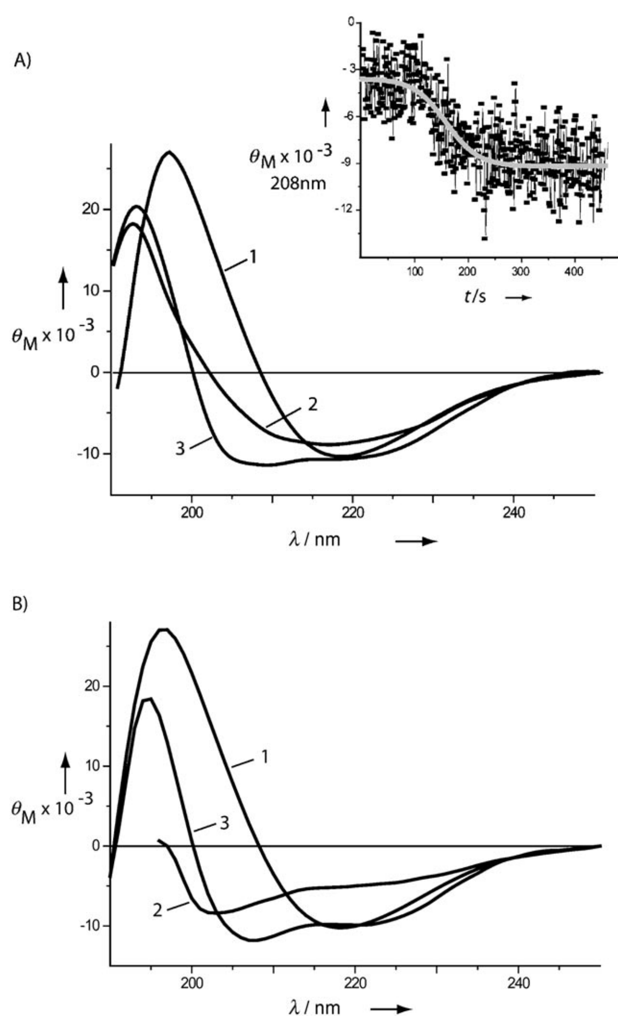

Figure 2. Conformational transitions of peptide **I** (see Figure 1) in the CD spectrum ($c = 5 \times 10^{-5}$ M, $T = 25$ °C): A) curve 1: pH-4.5 buffer (S_{off}); curve 2: pH-4.5 buffer/TFE 75:25 (S_{off}); curve 3: pH 7.0 buffer/TFE 75:25) (S_{on}). B) pH-4.5 buffer, curve 1: $T = 25$ °C (S_{off}); curve 2: $T = 95$ °C (S_{on}); curve 3: after cooling to T = 25 °C (S_{on}). Inset: the kinetics of the transformation from β-sheet (S_{off}) to α-helix (S_{on}) structures as monitored by changes in the CD signal.

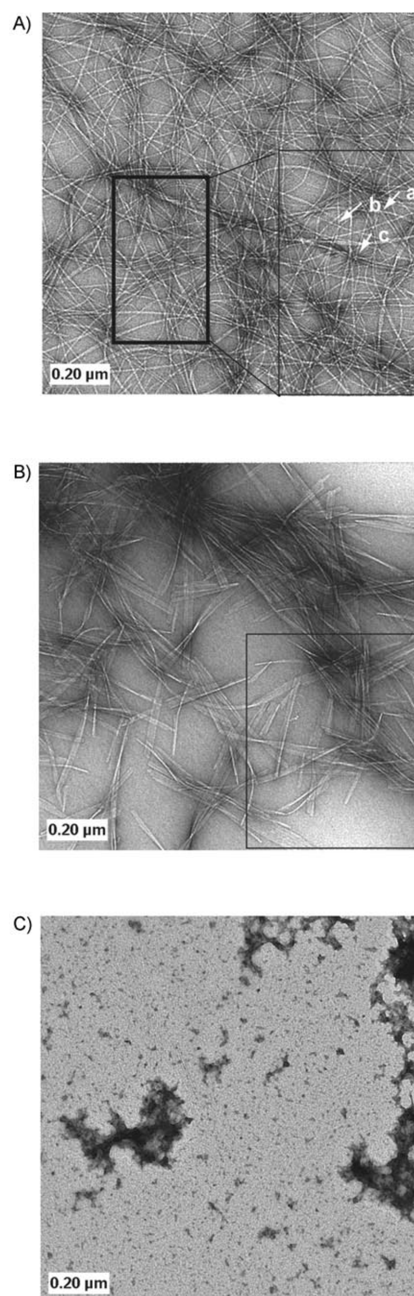

Figure 3. Negative electron micrographs of peptide **I** ($c = 5 \times 10^{-5}$ M) in the S_{off} state (A) and S_{on} state 5 min (B) and 12 h (C) after activation of the intramolecular O→N acyl group migration.

induced transition from the random coil (at 95 °C) to an α-helix structure (at 25 °C) is observed.

Using peptide **I**, we were able to investigate the feasibility of disrupting amyloid formation through the controlled induced transformation from β-sheet to α-helix structures of the self-assembled peptides within the amyloid structure. The experimental data offer new insights into the stability and structural properties of amyloid fibrils. Our studies support the hypothesis of a dynamic structure for amyloid[5] and demonstrate that significant structural rearrangements can take place within the β-sheet structure of amyloid fibrils. Furthermore, these studies demonstrate for the first time that structural changes in polypeptide regions[12c] that are not involved in amyloid formation could have a significant influence on the stability and structural dynamics of amyloid fibrils.

The development of tools to disrupt and/or reverse β-sheet-based self-assembly has important implications, not only for understanding the mechanisms of protein aggregation and clearance in vivo, but also in the development of therapeutic strategies aimed at the prevention or reversal of amyloid formation. We are currently aiming to extend the utility of this switch system to investigate the stability, structural dynamics, and toxic properties of fibrils, as well as of the early intermediates (protofibrils) on the amyloid pathway of diseases associated with amyloid-forming peptides. The ability to control the structure and morphology of the aggregates formed by peptide **I** suggests that by engineer-

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ing of specific switch elements and/or structure-templating motifs within amyloid-forming proteins and peptides could provide the necessary means to correlate the structural differences between the different amyloid morphologies with their toxicity. Furthermore, flexibility in the incorporation of multiple, orthogonally protected switch elements, which can be triggered in a selective and specific manner by using chemical, enzymatic, and/or photolytic procedures,[8–10] could open new possibilities in protein engineering and the design of "smart" materials with tunable structural, functional, and physiochemical properties.

Experimental Section

Peptide **I**: The fibril-forming peptide Aβ(14–25), HQKLVFFAEDVG, was assembled on Rink amide resin (0.60 mmol g^{-1}, 0.30 g, 0.18 mmol), by using standard 9-fluorenylmethyloxycarbonyl (Fmoc)-based solid-phase techniques.[16] The switch element was introduced by manually coupling the protected depsidipeptide Fmoc-Ala-(Boc)Ser-OH (Boc = *tert*-butoxycarbonyl)[17] as described by Coin et al.[11b] Next, the Ncap template was introduced as the protected building block Fmoc-(*cyclo*-1–5)-KAR(Pbf)AD-OH (Pbf = 2,2,4,6,7-pentamethyldihydrobenzofurane-5-sulfonyl).[12]
Cleavage from the resin and purification by semipreparative reverse phase HPLC afforded peptide **I**, Ac-[(*cyclo*-1–5)-KARAD]A-$\mathbf{S_1}$-HQKLVFFAEDVG-NH_2 ($\mathbf{S_1}$ = ($^+$H)Ser),[17] as a white powder and was characterized by analytical HPLC (> 95% purity) and ESIMS (m/z 1056.86 $[M+2H/2]^+$, 704.81 $[M+3H/3]^+$, 529.31 $[M+4H/4]^+$).

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