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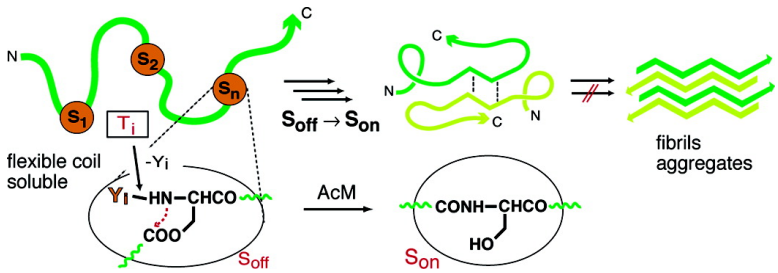

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Switch-Peptides: Controlling Self-Assembly of Amyloid β-Derived Peptides in vitro by Consecutive Triggering of Acyl Migrations

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The onset of conformational transitions as the origin of peptide self-assembly is considered as a fundamental molecular event in early processes relevant in degenerative diseases.[1,2] A detailed investigation of these processes is hampered by intrinsic problems, such as the high tendency of the involved peptides for β-sheet formation and spontaneous aggregation, limiting their experimental accessibility.[3] We have recently developed a new generation of switch-peptides,[4] allowing for the induction of conformational transitions using intramolecular O- → N-acyl migrations[5−8] in situ.

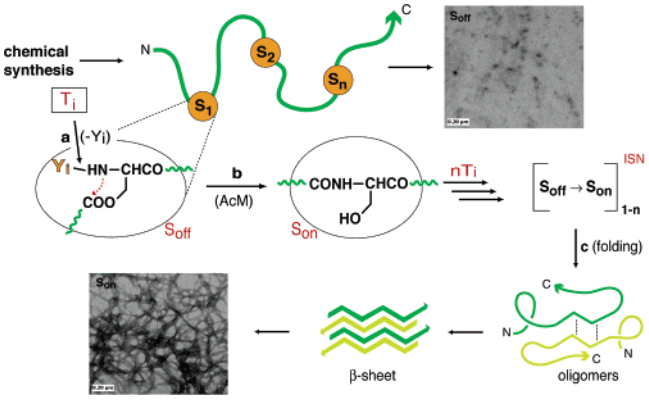
For potential applications in vitro and in vivo, we explore the sequential triggering of O- → N-acyl migrations in amyloid β (Aβ)-derived switch-peptides as a tool for studying onset and inhibition in polypeptide folding, self-assembly, and aggregation. As shown in Scheme 1, N(**Y**)-protected O-acyl isopeptides ("switch (**S**)-peptides") serve as stable, self-contained folding precursors, in which folding and self-assembly is blocked by the presence of the Ser-, Thr-, or Cys-derived switch (**S**)-elements dissecting the regular peptide backbone by an ester and a flexible C−C bond ($\mathbf{S}_{off}$).

Here, we focus on the design and chemical synthesis of **S**-peptides (Scheme 2) and investigate the specific cleavage of the N-protecting groups, **Y**, using chemical or enzymatic triggers, **T** (step a, Scheme 1), the spontaneous intramolecular O- → N-acyl migration (b) and the induction of folding events (c) such as self-assembly, β-sheet and fibril formation in statu nascendi (ISN) of the molecule. The amphipathic **S**-peptide **I**[9] serves as a model for the onset of β-sheets, applying variable triggering systems (Scheme 2). Orthogonal triggering is exemplified for Aβ-derived **S**-peptides **II**, taking the fibril nucleating segment Aβ (14−24)[10] for the in situ induction of helical structures (**IIa**) and as a guest sequence in a β-sheet promoting host peptide (**IIb**).[4] **S**-peptide **IIc** serves as a prototype for the consecutive switching on of folding processes in total Aβ (1−42). For the selective removal of $\mathbf{Y}_i$ by a trigger $\mathbf{T}_i$, the use of exoproteases with "non-native" specificities, such as pyroglutamate aminopeptidase (pGAP) and D-amino acid peptidase (Dap), or with unique cleavage sites, such as dipeptidyl peptidase IV (DPPIV, specific for N-terminal Axx-Pro), is examined.

Solid-phase synthesis of peptides **I** and **II** was achieved by applying Fmoc/*t*Bu-based chemistry.[4,12] Most notably, the presence of one (**I**) or two (**II**) **S**-elements results in highly soluble compounds (folding precursors), facilitating HPLC purification and structural characterization. As shown by CD, the conformational decoupling of the **S**-spaced peptide blocks results in flexible random coil (rc) conformations (CD curves $\mathbf{S}_{off}$, Figure 1). Even after 24 h at physiological pH, no changes in the HPLC and CD spectra are observed for the $\mathbf{S}_{off}$ state of the **S**-peptides, pointing to high chemical and conformational stability.

In contrast, the controlled removal of **Y** in the individual **S**-elements provokes spontaneous intramolecular O, N-acyl migration, resulting in dramatic changes of the conformational and

Scheme 1. Switch-Peptides as Folding Precursors: Consecutive Triggering of O, N-Acyl Migrations (AcM) in Switch-Peptides ($\mathbf{S}_{off}$) for the Onset ($\mathbf{S}_{on}$) of Peptide Folding and Self-Assembly in statu nascendi (ISN) of the Native Molecule



Scheme 2. Investigated Switch-Peptides and Triggering Systems (see Scheme 1)[a]

I: Ac-(SL)$_2$-**S**-(LS)$_2$LG-NH$_2$

IIa: Ac-KARADA-**S$_1$**-[HQKLVFF-**S$_2$**-EDV]G-NH$_2$

IIb: Ac-SL-**S$_1$**-L[HQKLVFFAEDV]-**S$_2$**-LG-NH$_2$

IIc: Aβ[1-25]-**S$_1$**-[27-36]-**S$_2$**-[38-42]

i	Y_i	T_i
1	H^+	OH^-
2	Nvoc	hν
3	ArgPro	DPPIV
4	pGlu	pGap
5	Arg	Trypsin
6	D-Ala	Dap

[a] Aβ sequences in square brackets. **S** = ($\mathbf{Y}_{1-6}$)Ser/Thr; $\mathbf{S}_1/\mathbf{S}_2$ = ($\mathbf{Y}_2/\mathbf{Y}_1$)Ser (**IIa**); ($\mathbf{Y}_3/\mathbf{Y}_4$)Ser (**IIb**); ($\mathbf{Y}_1/\mathbf{Y}_3$)Ser (**IIc**). Nomenclature depsipeptides, see ref 11.

physical properties ($\mathbf{S}_{on}$ state). For example, after adding enzyme DPPIV to **S**-peptide **I**, the evolution of the cleaved dipeptide Arg-Pro (Figure 1A, HPLC peak 3), the gradual disappearance of the $\mathbf{S}_{off}$ (peak 1), as well as the onset of a new peak (2, $\mathbf{S}_{on}$) reflect the overall time course for steps a and b, respectively (Scheme 1). As a general observation, the evolution and subsequent degradation of the $\mathbf{S}_{on}$ peak points to fast aggregation originating from rc to β-sheet transitions (CD, Figure 1A). As studied on **I**, the time course for the process $\mathbf{S}_{off} \rightarrow \mathbf{S}_{on}$ strongly depends on the triggering system (minutes up to hours in the rate-limiting step a in trigger systems $i = 3-6$, Scheme 2), whereas the intramolecular O, N-acyl transfer reaction proceeds generally fast (absence of intermediates) at physiological pH (Thr ≤ Ser ≪ Cys).

The consecutive "switching on" of **S**-elements according to Scheme 1 provides an experimental tool for evaluating the impact of individual peptide segments upon folding and self-assembly. For example, the pH-induced acyl migration at $\mathbf{S}_2$ in **IIa** (HPLC, Figure 1B) does not result in a significant effect upon the CD spectra (predominant rc structure), whereas the switching on of the helix-

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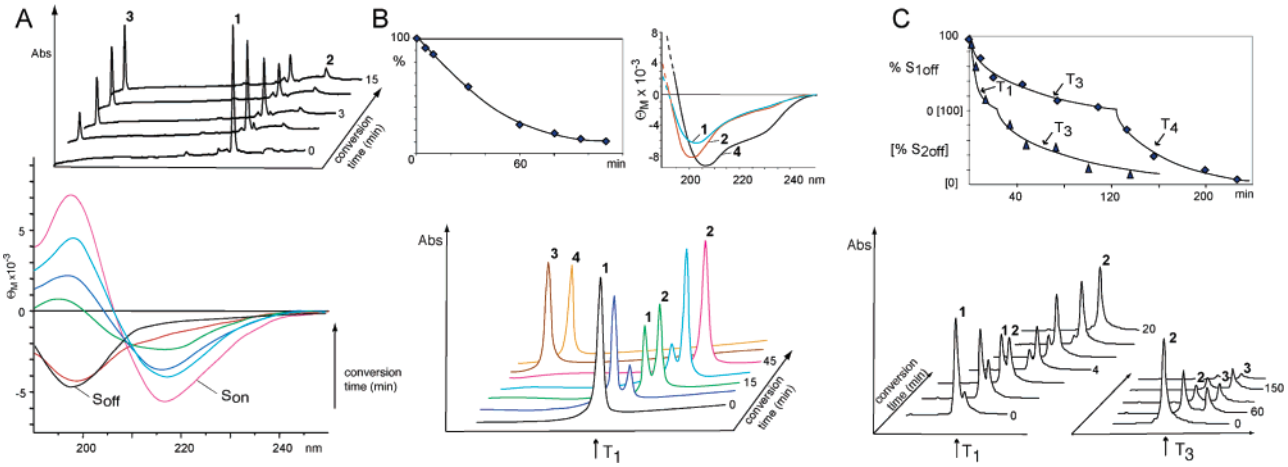

Figure 1. (A) CD of enzyme-triggered ($\mathbf{T}_3$) conformational transition of **I** monitored over 60 min (t = 0 (black), 30 min (pink)). Inset: HPLC of time course; 1, $\mathbf{S}_{off}$; 2, $\mathbf{S}_{on}$; 3, ArgPro. (B) HPLC of the sequential $\mathbf{T}_1/\mathbf{T}_2$-triggered acyl migration of **IIa**: 1, $\mathbf{S}_{1/2off}$; 2, $\mathbf{S}_{1off/2on}$; 3, intermediate 2 after cleavage of $\mathbf{Y}_2$; 4, $\mathbf{S}_{1/2on}$; inset, time course of $h\nu$ cleavage (left) and CD (right) in H_2O/TFE (83/17). (C) HPLC of the sequential $\mathbf{T}_1/\mathbf{T}_3$-triggered acyl migration of **IIc**: 1, $\mathbf{S}_{1/2off}$, 2, $\mathbf{S}_{1on/2off}$; 3, $\mathbf{S}_{1/2on}$; inset, kinetics of acyl migrations for peptide **IIb** ($\mathbf{T}_3$, $\mathbf{T}_4$) and **IIc** ($\mathbf{T}_1$, $\mathbf{T}_3$).

nucleating system[13] by photolytic cleavage at $\mathbf{S}_1$ and subsequent acyl migration induces helical conformation (inset (right) Figure 1B). Notably, photolytic cleavage at acidic pH allows one to independently monitor step a (peak 3, $\mathbf{S}_{off}$, Figure 1B; inset (left): time course) and step b ($\mathbf{S}_{on}$, peak 4), opening interesting applications for the use of orthogonal switch arrays in organic and aqueous solvents. Selective switching on of the N- and C-terminal host sequence in **IIb** is achieved upon consecutive addition of triggers $\mathbf{T}_3$ and $\mathbf{T}_4$, respectively (inset Figure 1C, time course). Again, the sequential order of triggering acyl migrations proves to be essential; setting off the N-terminal Ac-SerLeu by adding $\mathbf{T}_3$ does not affect the overall properties of the peptide (rc conformation, solubility), whereas a conformational transition of type rc $\rightarrow$ β-sheet, followed by aggregation, is induced upon ligating the C-terminal $SerLeuGlyNH_2$ (applying $\mathbf{T}_4$), thus providing interesting clues for the onset of β-sheets.

Finally, the consecutive switching on of peptide segments is exemplified for [Ser^{37}]Aβ (1−42) containing a chemical ($\mathbf{S}_1$) and enzymatic cleaving ($\mathbf{S}_2$) site (**IIc**). Here, the pH-induced acyl migration at $\mathbf{S}_1$ proceeds very fast ($t_{1/2}$ = 5 min, inset, Figure 1C) restoring native Aβ (1−36) (HPLC, peak 2, Figure 1C). Interestingly, by the subsequent enzymatic switching on ($\mathbf{T}_3$, inset Figure 1C) of the C-terminal segment (37−42), the characteristic phenomena observed for native Aβ (1−42),[3] that is, β-sheet and fibril formation, are initiated, accompanied by self-association and aggregation (disappearance of $\mathbf{S}_{on}$ peak 3, Figure 1C). Though these observations will be the subject of detailed conformational analyses,[14] our preliminary CD and TEM studies point to the central impact of the hydrophobic C-terminus of Aβ (1−42) upon self-association and aggregation. Most notably, consecutive switching on allows for the experimental identification of aggregation "hot spots", setting the stage for a rational design of specific inhibitors.

In summary, we present a novel concept for the controlled, sequential onset of peptide assembly in vitro. In particular, the enzymatic triggering of O, N-acyl migrations allows for novel applications in prodrug design and biosensor technology. In further exploring the immense potential of peptide and protein synthesis, switch-peptides may become a general tool for the study of early steps in polypeptide self-assembly and inhibition as a key process in degenerative diseases.

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Supporting Information Available: Switch-peptide synthesis and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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