

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

Sonia Dos Santos, Arunan Chandravarkar, Bhubaneswar Mandal, Richard Mimna, Karine Murat, Lydiane Saucède, Patricia Tella, Gabriele Tuchscherer, and Manfred Mutter*

Ecole Polytechnique Fédérale de Lausanne (EPFL), Institute of Chemical Sciences and Engineering, CH-1015 Lausanne, Switzerland

Received April 1, 2005; E-mail: Manfred.mutter@epfl.ch

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- \rightarrow N-acyl migrations⁵⁻⁸ in situ.

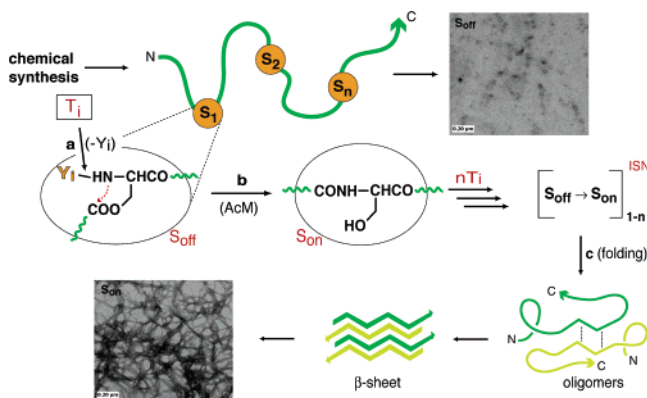
For potential applications in vitro and in vivo, we explore the sequential triggering of O- \rightarrow N-acyl migrations in amyloid β ($A\beta$)-derived switch-peptides as a tool for studying onset and inhibition in polypeptide folding, self-assembly, and aggregation. As shown in Scheme 1, N(Y)-protected 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 (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- \rightarrow 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 $A\beta$ -derived S-peptides **II**, taking the fibril nucleating segment $A\beta$ (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 $A\beta$ (1–42). For the selective removal of Y_i by a trigger 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/tBu-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 S_{off} , Figure 1). Even after 24 h at physiological pH, no changes in the HPLC and CD spectra are observed for the 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 (S_{off}) for the Onset (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	Y_i	T_i
I: Ac-(SL) ₂ -S ₂ -(LS) ₂ LG-NH ₂		
1	H ⁺	OH ⁻
2	Nvoc	hv
IIa: Ac-KARADA-S ₁ -[HQKLVFF-S ₂ -EDV]G-NH ₂		
3	ArgPro	DPPIV
IIb: Ac-SL-S ₁ -L[HQKLVFFAEDV]-S ₂ -LG-NH ₂		
4	pGlu	pGap
5	Arg	Trypsin
IIc: A β [1-25]-S ₁ -[27-36]-S ₂ -[38-42]		
6	D-Ala	Dap

^a $A\beta$ sequences in square brackets. S = (Y₁₋₆)Ser/Thr; S₁/S₂ = (Y₂/Y₁)Ser (**IIa**); (Y₃/Y₄)Ser (**IIb**); (Y₁/Y₃)Ser (**IIc**). Nomenclature desipeptides, see ref 11.

physical properties (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 S_{off} (peak 1), as well as the onset of a new peak (2, 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 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 $S_{\text{off}} \rightarrow S_{\text{on}}$ 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 \leq Ser \ll 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 S₂ 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-

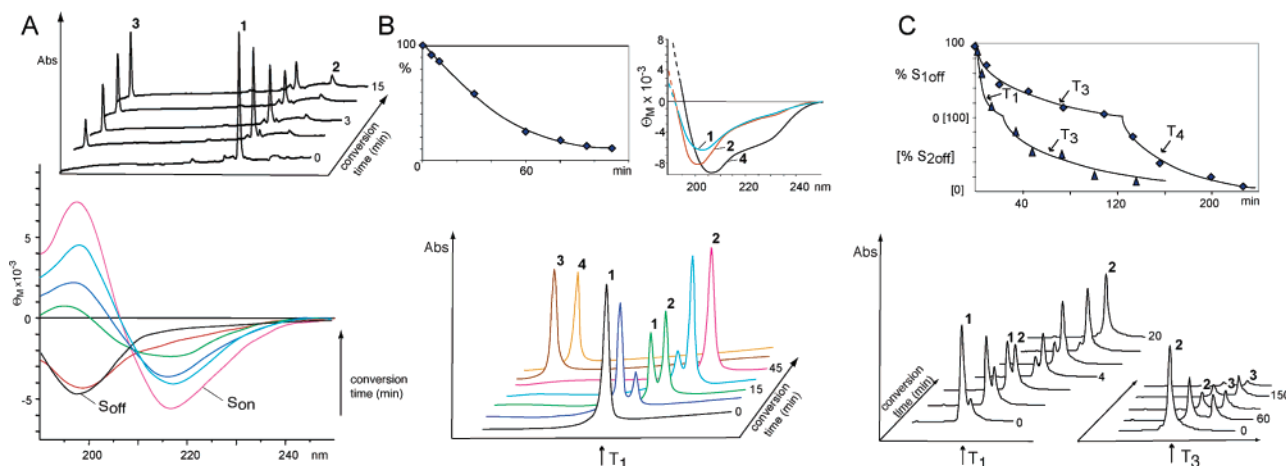


Figure 1. (A) CD of enzyme-triggered (T_3) conformational transition of **I** monitored over 60 min ($t = 0$ (black), 30 min (pink)). Inset: HPLC of time course; 1, S_{off} ; 2, S_{on} ; 3, ArgPro. (B) HPLC of the sequential T_1/T_2 -triggered acyl migration of **IIa**: 1, $S_{1/2\text{off}}$; 2, $S_{1\text{off}/2\text{on}}$; 3, intermediate 2 after cleavage of **Y2**; 4, $S_{1/2\text{on}}$; inset, time course of $h\nu$ cleavage (left) and CD (right) in H_2O/TFE (83/17). (C) HPLC of the sequential T_1/T_3 -triggered acyl migration of **IIc**: 1, $S_{1/2\text{off}}$; 2, $S_{1\text{on}/2\text{off}}$; 3, $S_{1/2\text{on}}$; inset, kinetics of acyl migrations for peptide **IIb** (T_3 , T_4) and **IIc** (T_1 , T_3).

nucleating system¹³ by photolytic cleavage at 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, S_{off} , Figure 1B; inset (left): time course) and step b (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 T_3 and 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 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 SerLeuGlyNH₂ (applying T_4), thus providing interesting clues for the onset of β -sheets.

Finally, the consecutive switching on of peptide segments is exemplified for [Ser³⁷]A β (1–42) containing a chemical (S_1) and enzymatic cleaving (S_2) site (**IIc**). Here, the pH-induced acyl migration at S_1 proceeds very fast ($t_{1/2} = 5$ min, inset, Figure 1C) restoring native A β (1–36) (HPLC, peak 2, Figure 1C). Interestingly, by the subsequent enzymatic switching on (T_3 , inset Figure 1C) of the C-terminal segment (37–42), the characteristic phenomena observed for native A β (1–42),³ that is, β -sheet and fibril formation, are initiated, accompanied by self-association and aggregation (disappearance of 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 A β (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.

Acknowledgment. This work was supported by the Swiss National Science Foundation. DPPIV was a generous gift from PD Dr. Eric Grouzmann, CHUV, Lausanne, Switzerland.

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

References

- (1) *Self-Assembling Peptide-Systems in Biology, Medicine and Engineering*; Aggeli, A.; Boden, N.; Zhang, S., Eds.; Kluwer Academic Publishing: Dordrecht, The Netherlands, 2001; references therein.
- (2) Soto, C. *Nat. Rev. Neurosci.* **2003**, *4*, 49–60.
- (3) Gorman, P. M.; Chakrabarty, A. *Biopolymers* **2001**, *60*, 381–394.
- (4) Mutter, M.; Chandravarkar, A.; Boyat, C.; Lopez, J.; Dos Santos, S.; Mandal, B.; Mimna, R.; Murat, K.; Patiny, L.; Saucède, L.; Tuchscherer, G. *Angew. Chem., Int. Ed.* **2004**, *43*, 4172–4178.
- (5) Coltart, D. M. *Tetrahedron* **2000**, *56*, 3449–3491.
- (6) Hamada, Y.; Matsumoto, H.; Yamaguchi, S.; Kimura, T.; Hayashi, Y.; Kiso, Y. *Bioorg. Med. Chem.* **2002**, *10*, 4155–4167.
- (7) Carpino, L. A.; Krause, E.; Sferdean, C. D.; Schühmann, M.; Fabian, H.; Bienert, M.; Beyermann, M. *Tetrahedron Lett.* **2004**, *45*, 7519–7523.
- (8) Sohma, Y.; Hayashi, Y.; Kimura, M.; Chiyomori, Y.; Taniguchi, A.; Sasaki, M.; Kimura, T.; Kiso, Y. *J. Pept. Sci.* **2005**, *11*, 441–451.
- (9) Mutter, M.; Gassmann, R.; Buttke, U.; Altmann, K.-H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1514–1516.
- (10) Tjernberg, L. O.; Callaway, D. J. E.; Tjernberg, A.; Hahne, S.; Lillihöök, C.; Terenius, L.; Thyberg, J.; Nordstedt, C. *J. Biol. Chem.* **1999**, *274*, 12619–12625.
- (11) Filip, S. V.; Cavelier, F. *J. Pept. Sci.* **2004**, *10*, 115–118.
- (12) *Houben-Weyl, Methods of Organic Chemistry*; Goodman, M., Felix, A., Moroder, L., Toniolo, C., Eds.; Thieme: Stuttgart, 2003; Vol. E 22d.
- (13) Shepard, N. E.; Abbenante, G.; Fairlie, D. P. *Angew. Chem., Int. Ed.* **2004**, *43*, 2687–2690.
- (14) Dos Santos, S.; Chandravarkar, A.; Adrian, M.; Dubochet, J.; Camus, M.-S.; Schmid, A.; Lashuel, H.; Tuchscherer, G.; Mutter, M. Manuscript in preparation.

JA052083V