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Switch-Peptides: From Conformational Studies to Alzheimer's Disease

Lydiane Saucède[§a], Sonia Dos Santos[a], Arunan Chandravarkar[a], Bhubaneswar Mandal[a], Richard Mimna[a], Karine Murat[a], Marie-Stéphanie Camus[a], Jérémy Bérard[a], Eric Grouzmann[b], Marc Adrian[c], Jacques Dubochet[c], John Lopez[a], Hilal Lashuel[d], Gabriele Tuchscherer[a], and Manfred Mutter*[a]
[§]METTLER TOLEDO Award Winner (Oral Presentation)

Abstract: Studies on designed peptides that exhibit high tendencies for medium-induced conformational transitions have recently attracted much attention because structural changes are considered as molecular key processes in degenerative diseases. The experimental access to these events has been limited so far mainly due to the intrinsic tendency of the involved polypeptides for self-association and aggregation, *e.g.* amyloid β plaque formation, thought to be at the origin of Alzheimer's disease. We have developed a new concept termed 'switch-peptides' which allows the controlled onset of polypeptide folding and misfolding *in vitro* and *in vivo*, starting from a soluble, non-toxic precursor molecule. As a major feature, the folding process is initiated by enzyme-triggered N,O-acyl migrations restoring the native peptide backbone *in situ*. As the folding is set off in the moment of creating the bioactive molecule ('*in statu nascendi*', ISN), our concept allows for the first time the investigation of the early steps of protein misfolding as relevant in degenerative diseases, opening new perspectives for the rational design of therapeutically relevant compounds.

Keywords: Degenerative diseases · Induction of biological function · Protein misfolding · Rational drug design · Switch-peptides

Introduction

Unraveling the relationship between peptide sequence, conformational, and physicochemical properties as well as the impact on synthesis and biological function has been a major focus of our research for many years [1][2]. In this context, the design and investigation of model peptides exhibiting a high propensity for secondary and tertiary structure formation proved to be ideal targets for elucidating the structural and dynamic parameters governing protein folding and function [3]. Interestingly, the design of amphiphilic oligopeptides undergoing medium-induced conformational transitions as a first generation of 'switch-peptides' in the early nineties [4] was considered primarily as a contribution to our understanding of the complex mechanism of peptide self-assembly and folding, whereas its importance in fundamental molecular processes in degenerative diseases has been recognized only more recently [5]. For example, growing evidence suggests that conformational transitions of soluble amyloid β (Aβ) precursor molecules into aggregated β-sheet type forms play a key role in the deposition of amyloid plaques characteristic in Alzheimer's disease (AD) [6]. Similarly, α-helix to β-sheet transitions are considered to be at the molecular origin of the transformation of the physiological PrPc form of the prion protein into pathologic PrPsc [7]. The characteristic pathology of AD patients is associated with extracellular neuritic plaque deposits as well as intracellular neurofibrillary tangles in brain tissues. The plaques are composed of aggregates of the amyloid β peptide (Aβ), a 39–43 amino acid proteolytic fragment of a larger transmembrane protein termed amyloid precursor protein (APP). Although the pathological role of Aβ is unknown, this soluble peptide is present in all individuals at picomolar concentrations [7][8] and the transition from soluble Aβ to the non-native conformation and subsequent aggregation into fibrils appears intimately associated with disease progression. Accordingly, efforts to understand the structural nature of amyloid fibrils, to elucidate the self-assembly as well as the intermediates along the folding pathway, and to determine the factors involved in self-assembly are crucial for therapeutic intervention.

The Role of Aβ in Alzheimer's Disease

The insoluble amyloid fibrils and deposits have long been thought to be neurotoxic species [9]. However, increasing evidence now suggests a clear correlation between dementia and soluble Aβ oligomers. Oligomeric species have appeared as common entities associated with fibril formation of various peptides, including Aβ, α-synuclein, polyglutamine tracts and the larger prion proteins [10][11]. The ability to aggregate and to form fibrils are properties of many peptides and proteins associated with disease states or mutations in the primary sequence. Dobson and co-workers

*Correspondence: Prof. Dr. M. Mutter[a]
Tel.: + 41 21 693 94 11
Fax: + 41 21 693 94 15
E-Mail: manfred.mutter@epfl.ch
[a]Ecole Polytechnique Fédérale de Lausanne (EPFL)
Institute of Chemical Sciences and Engineering
CH-1015 Lausanne
[b]Centre Hospitalier Universitaire Vaudois (CHUV)
Division of Clinical Pharmacology and Toxicology,
CH-1011 Lausanne
[c]Université de Lausanne (UNIL)
Laboratoire d' Analyse Ultrastructurelle,
CH-1015 Lausanne
[d]Ecole Polytechnique Fédérale Lausanne (EPFL)
Laboratory of Molecular Neurobiology and
Neuroproteomics, CH-1015 Lausanne

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have recently shown that under conditions which destabilize the native state, proteins are prone to form amyloid with formation of regular, highly organized protein aggregates [12]. In general, the underlying structure of amyloid fibrils is a cross-β structure comprising β-strands of the peptide/protein arranged perpendicular to, and ribbon-like β-sheets parallel to the axis of the fibril [6a].

The particular structural form that the peptide adopts when exerting a toxic effect is not clear. In an *in vitro* investigation of the toxicity associated with fibrillar and non-fibrillar (amorphous) forms of Aβ, it was found that fibrillar forms of the peptide were more toxic. In contrast, Dahlgren *et al.* have reported that soluble oligomeric preparations of Aβ are 10-fold more toxic than preparations largely comprising fibrils, and 40-fold more toxic than non-aggregated peptide solutions [13].

Currently, there are no known cures for AD, and although there are some drugs that have been approved for the treatment of the disease, the efficacy relies on their ability to delay the progression of the disease, not to halt the dementia. Neurological damage in AD is complex and has been shown to involve several different biochemical pathways, each of which providing a possible mechanism for therapeutic intervention. In view of these data, the experimental elucidation of early molecular processes in Aβ folding, notably of β-sheet formation, appears to be of utmost concern.

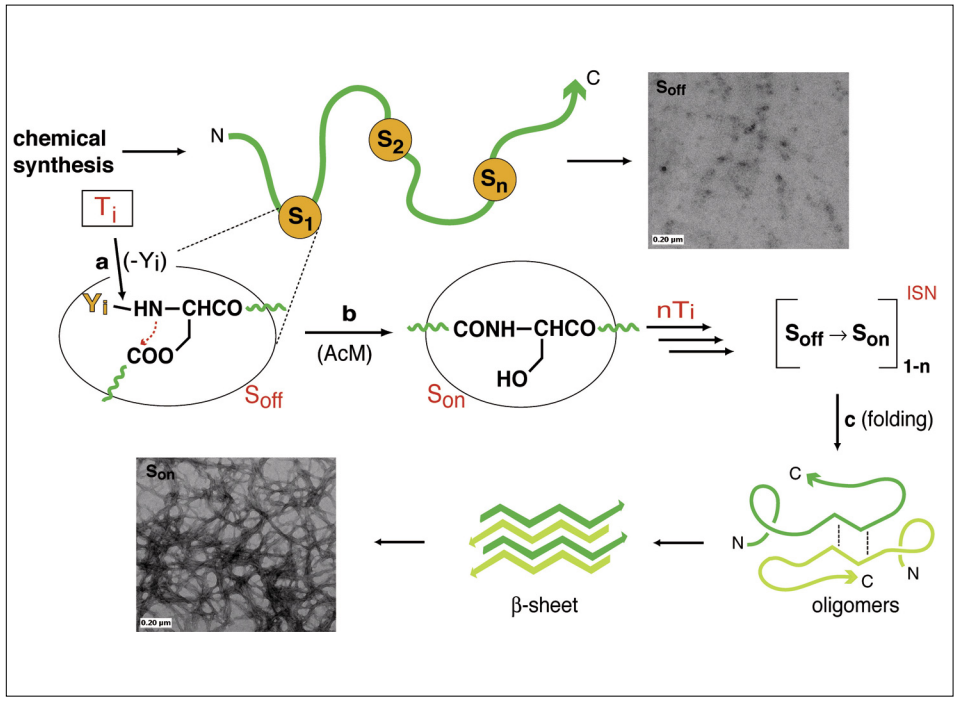

Fig. 1. The concept of switch-peptides for the *in situ* induction of folding processes relevant in degenerative diseases [15]. Switch-peptides contain N(Y)-Ser-, Thr-, or Cys-derived isoacyl residues as switch (S)-elements, disrupting the regular amide backbone (non-folded, flexible state S_{off}, absence of fibrils). Consecutive triggering (chemically or enzymatically, Fig. 2, step a) of O,N-acyl migrations (AcM, step b) restores the native backbone (S_{on}-state), initiating folding events (step c) such as self-assembly, β-sheet and fibril formation *'in statu nascendi'* (ISN) of the native molecule. The experimental access to early molecular events in degenerative diseases represents a starting point for the rational design of inhibitors of protein misfolding.

The Concept of Switch-peptides

As well known from systematic research on β-sheet forming oligopeptides, a detailed investigation of these processes is strongly hampered by the strong tendency of the involved peptides for spontaneous self-assembly and aggregation, limiting their experimental accessibility [1–3][14]. To overcome these intrinsic problems in the preparation and investigation of β-sheet forming peptides, we have recently developed a new generation of switch-peptides for the induction of conformational processes starting from a flexible, unfolded precursor molecule (switch-peptide, S_{off}-state, Fig. 1) *in situ* [15]. For the purpose at hand, we use intramolecular O,N-acyl migration reactions as structural switch (S-elements) from a depsipeptide bond containing, unfolded state (S_{off}) to an all-amide, native state (S_{on}). As a major conceptual feature, chemically or enzymatically [15] triggered acyl migrations allow for the controlled induction of folding events in the process of primary structure evolution, that is '*in statu nascendi*' (ISN) of the native molecule.

So far, acyl transfer reactions have been the subject of extensive mechanistic studies [16], and their role in protein biosynthesis and splicing [17], peptide synthesis and solubilization [18], prodrug design [19] and native chemoselective ligation strategies [20] has attracted much attention. Our focus over the last few years was directed toward the elaboration of the concept of 'switch-peptides' for the study of peptide self-assembly, secondary structure formation and disruption [15][21]. For potential applications *in vitro* and *in vivo*, we developed a broad palette of orthogonal triggering systems (Fig. 2), giving access to a sequential induction of O,N-acyl migrations as exemplified for Aβ-derived switch-peptides.

Triggering the Folding of Amyloid β-Peptides '*in statu nascendi*' (ISN)

The chemical synthesis of switch-peptides follows standard solid-phase techniques [22] with exception of the depsibond formation. Here, the pre-formation of the N(Y)-protected isoacyl dipeptides as building blocks seems to be the method of choice [15][22]. Due to their proteinogenic cleavage sites, triggering systems 3–6 (Fig. 2) are compatible with applications *in vitro* and *in vivo*, for modulating the O,N-acyl migration step at will.

As a representative example (Fig. 3), a host-guest switch-peptide containing Aβ (14-24) as guest sequence in a β-sheet promoting host peptide (H = $[Leu\text{-}Ser]_n$) was prepared by solid-phase synthesis. In the S_{off}-state, the switch-peptide containing two enzyme-cleavable (DPPIV and pGap) S-elements proved to be highly soluble at physiologic conditions, thus facilitating HPLC purification and structural characterization. As shown by CD (Fig. 3, left), the conformational decoupling of the individual peptide blocks results in a flexible random coil (rc) conformation, showing no tendency for self-association or fibril formation (TEM-studies, Fig. 3, top right) after 24 h. In contrast, after selective triggering of the acyl migrations, dramatic changes in the physicochemical (self-assembly, precipitation) and conformational properties (transition of type rc to β-sheet, typical CD curve $S_{1/2}$ on) paralleled by the onset of Aβ-like fibrils (Fig. 3, bottom right) are observed. Interestingly, in changing the sequential order of adding enzymes DPPIV and pGap, a differential impact of the N- and C-terminal host peptide block upon β-sheet and fibril formation could be revealed.

For example, by switching on S_1, *i.e.* ligating the N-terminal host peptide to Aβ (14-24), the peptide retains its high solubility as monitored by the evolution of HPLC-

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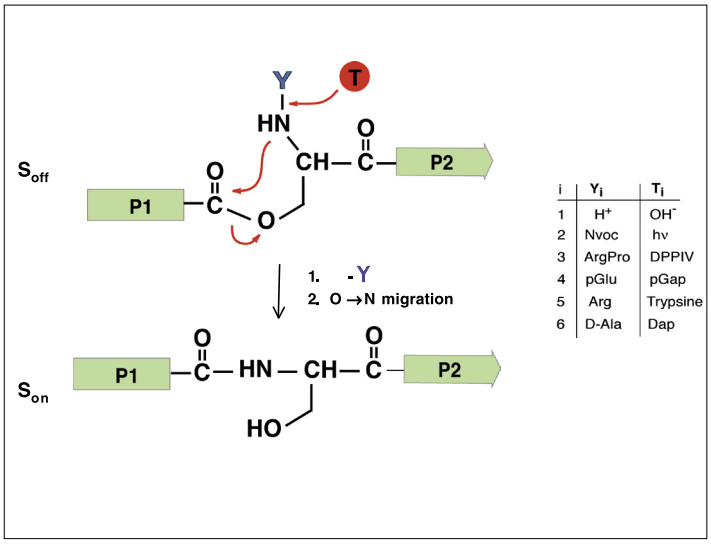

Fig. 2. Orthogonal triggering systems for applications at physiologic conditions: The N(Y)-protected switch-peptide containing an O-isoacyl-serine (S_{off}-state) as S-element is transformed to the native state (S_{on}) by chemical (triggering systems 1,2) or enzymatic (3-6) cleavage of Y *in vitro* and *in vivo*, triggering spontaneous intramolecular acyl migration [15].

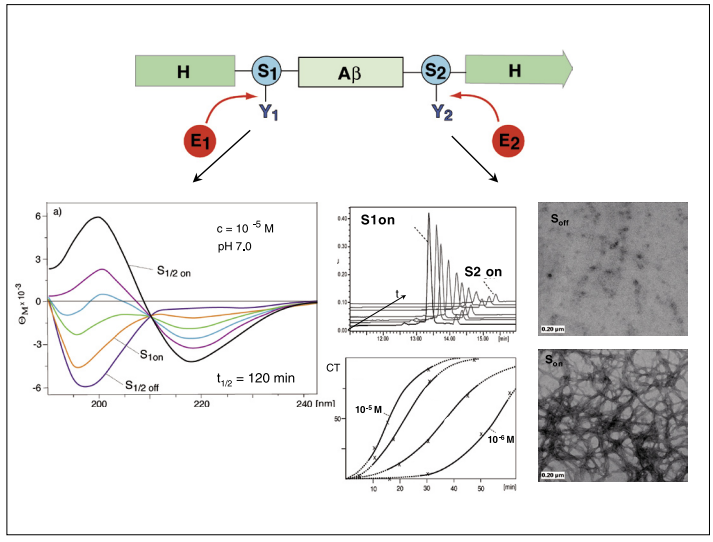

Fig. 3. Consecutive triggering of acyl migrations by enzymes in host-guest peptides for the onset of folding processes [15]. In the S_{off}-state, the switch-peptide adopts a flexible random-coil conformation (typical CD curve, left) and shows no fibril formation (TEM, right) after 24 h. In switching on the N-terminal host peptide (S_1 on) by E1 (DPPIV, see Fig. 2), no significant conformational changes are observed. In contrast, the addition of E2 (pGap) results in a transition to a β-sheet, paralleled by fibril formation and precipitation (disappearance of HPLC peak S_1/S_2 on) (middle, top). The kinetics of the conformational transition from random-coil to β-sheet structure is strongly concentration dependent (middle, bottom).

peak S_1on, whereas the ligation of the C-terminal host (S_2on) induces the conformational transition of type rc → β. As β-sheet formation is a concentration dependent process, the kinetics for the onset of a β-sheet structure varies according to Fig. 3.

As a most challenging proof of concept, the full length Aβ (1-42), considered as a 'difficult sequence' for chemical synthesis due to its pronounced tendency for self-aggregation [14][18], was designed as a switch-peptide as shown in Fig. 1.

To this end, Ser^{26} of native Aβ (1-42) was used as chemically (*e.g.* change of pH from 4.0 to 7.0, T_1) inducible triggering site S_1, and native Gly^{37} was replaced by Ser^{37} for the insertion of an enzymatically cleavable S-element (Y = Arg-Pro, T = DPPIV; Fig. 4).

Again, the switch-peptide in the S_{off}-state showed high water solubility and no tendency for self-association or fibril formation as monitored by CD and TEM studies. As before, restoring the native Aβ (1-36) fragment by pH-induced acyl migration ($t_{1/2}$ ~ 5 min, Fig. 4b) at S_1 did not result in significant conformational changes as evidenced by the evolution of HPLC peak 2 (Fig. 4a).

In contrast, by the subsequent enzymatic (T_3) switching on of S_2, the characteristic phenomena observed for native Aβ (1-42), such as β-sheet formation and aggregation followed by fibril formation (disappearance of HPLC peak, precipitation, Fig. 4a, 4c) occurred, pointing to the dominant impact of the hydrophobic C-terminal Aβ (37-42) segment upon self-association and folding.

In extending these studies to Aβ containing multiple switching sites according to Fig. 1, we are presently exploring the role of individual amino acid sequences as nucleation sites ('hot spots') for misfolding in various polypeptides related to degenerative diseases. These studies on the mechanism of self-association, folding and onset of toxicity will set the stage for a rational design of specific inhibitors.

Outlook: Switch-peptides in Drug Design

Finally, beside its importance for the study of protein folding, the present concept represents a novel tool for the ISN-nucleation and disruption of biologically relevant conformations, as schematically shown in Fig. 5a.

We have shown recently that the insertion of a switch-element S between an induction unit σ (*e.g.* Ncap or pseudoproline (ΨPro) [15][21a]) and a peptide ligand P results in a complete conformational decoupling of σ and P in the S_{off}-state, allowing a potentially β-sheet forming ligand P to favorably interact with a β-sheet template ('recognition state', Fig. 5a). In triggering O,N-acyl migration as shown above, *i.e.* in setting off the conformational impact of σ upon peptide ligand P (S_{on}-state), the nucleation of an α-helical structure (typical CD curves, Fig. 5b) or a flexible coil conformation (resulting from a ΨPro-induced *cis*-amide bond ('kink') in the native peptide sequence, Fig. 5c) may lead to a destabilization or even disruption of the template β-sheet. It is noteworthy that the dramatic conformational changes *in situ, e.g.* the reversal of a receptor recognition (S_{off}, 'agonistic') to a disruptive (S_{on}, 'antagonistic') state according to Fig. 5a opens new perspectives in drug design. In particular, the triggering of O,N-acyl migrations *in vitro* and *in vivo* [15] offers new perspectives for the design of bioactive compounds of so far unattained affinity, specificity or activity. First studies on switch-peptides derived from Cyclosporines exhibiting non-immunosuppressive, antiviral properties give encouraging results [23]. The therapeutic implications of these concepts are presently under investigation in our laboratories.

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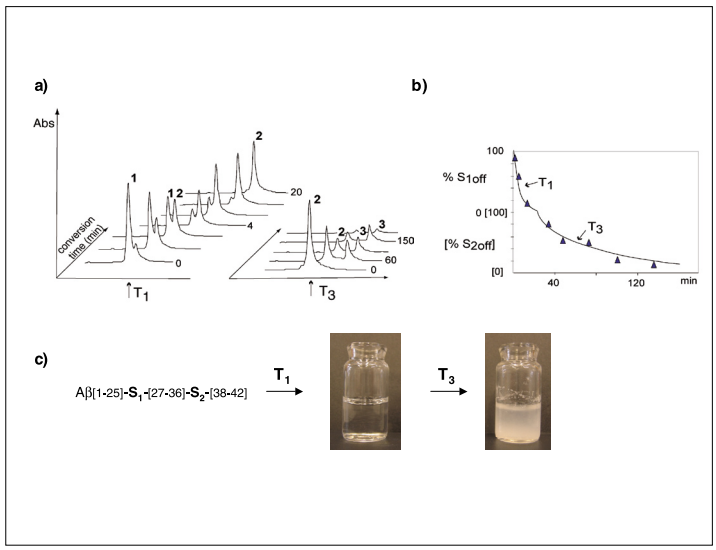

Fig. 4. Self-assembly and folding of Aβ (1-42)-derived switch-peptide Aβ [1-25]-S1-[27-36]-S2-[38-42] (see text). S_1 = trigger system 1; S_2 = 3, see Table in Fig. 2). a) HPLC chromatogram monitoring the acyl transfer reactions upon consecutive pH- and enzyme triggering; b) Kinetics of acyl migrations; c) Consecutive restoring of the Aβ backbone leads to self-association and precipitation.

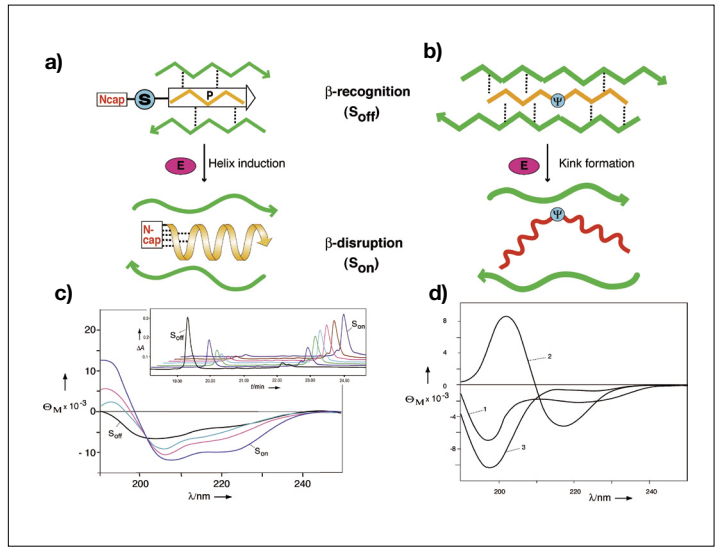

Fig. 5: *In statu nascendi* (ISN)-induction of conformational transitions as a new principle in drug design [15] (see text). Induction of conformational transitions by using templating effects (*e.g.* N-caps, a) and peptidomimetics (*e.g.* Pseudoprolines, ΨPro, b) leads to β-sheet destabilization or disruption; c) CD of conformational change from random-coil (S_{off}) to α-helix (S_{on}); inset: pH-induced acyl migration monitored by HPLC; d) Impact of a β-sheet breaking peptide (S_{off}-state, 1) upon a β-sheet template (2). In triggering acyl migration (S_{on}), a conformational transition of type β-sheet to random-coil is observed (3).

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