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

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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...
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.

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, Soff-state, Fig. 1) in situ [15]. For the purpose at hand, we use intramolecular O,N-acetyl migration reactions as structural switch (S-elements) from a depsipeptide bond containing, unfolded state (Soff) to an all-amide, native state (Son). 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 situ 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.

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-Ser]) was prepared by solid-phase synthesis. In the Soff-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-association, precipitation) and conformational properties (transition of type rc to β-sheet, typical CD curve S1/2 on) paralleled by the onset of β-sheet 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 S1, 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-
peak S$_{1on}$, whereas the ligation of the C-terminal host (S$_{on}$) induces the conformational transition of type $rc \rightarrow \beta$. As $\beta$-sheet formation is a concentration dependent process, the kinetics for the onset of a $\beta$-sheet structure varies according to Fig. 3.

As a most challenging proof of concept, the full length A$\beta$ (1-42) was used as chemically (<i>e.g.</i> change of pH from 4.0 to 7.0, T$_1$) inducible triggering site S$_1$, and native Gly$^{37}$ was replaced by Ser$^{17}$ for the insertion of an enzymatically cleavable S-element (Y = Arg-Pro, T = DPPIV, see 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 A$\beta$ (1-36) fragment by pH-induced acyl migration ($t_{1/2} \sim 5$ 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$_2$) switching on of S$_2$, the characteristic phenomena observed for native A$\beta$ (1-42), such as $\beta$-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 A$\beta$ (37-42) segment upon self-association and folding.

In extending these studies to A$\beta$ 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-aggregation, 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 $\sigma$ (<i>e.g.</i> Ncap or pseudoprolines (ΨPro) [15][21a]) and a peptide ligand P results in a complete conformational de-coupling of $\sigma$ and P in the S$_{off}$-state, allowing a potentially $\beta$-sheet forming ligand P to favorably interact with a $\beta$-sheet template (‘recognition state’, Fig. 5a). In triggering O,N-acetyl migration as shown above, <i>i.e.</i> in setting off the conformational impact of $\sigma$ upon peptide ligand P (S$_{on}$-state), the nucleation of an $\alpha$-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 $\beta$-sheet. It is noteworthy that the dramatic conformational changes in <i>situ</i>, <i>e.g.</i> 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-acetyl migrations in <i>vitro</i> and in <i>vivo</i> [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 Cycloporesines 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). S₁ = trigger system 1; S₂ = 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 situ nascent(n) (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, \( \Psi \)-Pro, b) leads to \( \beta \)-sheet destabilization or disruption; c) CD of conformational change from random-coil (S\(_{random}\)) to \( \alpha \)-helix (S\(_{h} \)); inset: pH-induced acyl migration monitored by HPLC; d) Impact of a \( \beta \)-sheet breaking peptide (S\(_{off}\)-state, 1) upon a \( \beta \)-sheet template (2). In triggering acyl migration (S\(_{off}\)), a conformational transition of type \( \beta \)-sheet to random-coil is observed (3).


