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Reversal of Aggregation Using β-Breaker Dipeptide Containing Peptides: Application to $A\beta_{(1-40)}$ Self-Assembly and Its Inhibition

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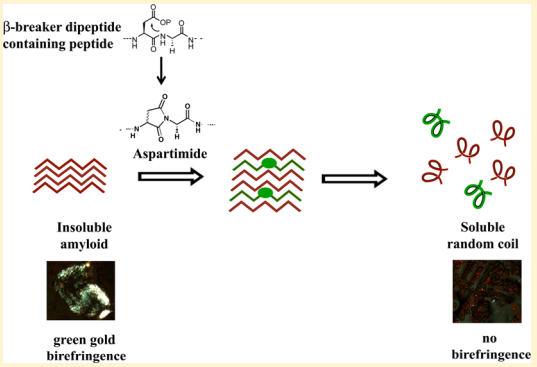
Supporting Information

ABSTRACT: Reversion of protein or peptide aggregation is a formidable task, important in various domains of research at the interface of chemistry, medicine, and nanoscience. A novel class of dipeptides, termed as β-breaker dipeptides (BBDPs), is identified, which can be incorporated into the self-recognizing sequences to generate a novel class of conformational switch which forms β-sheet at an initial stage and then converts in a controlled manner to random coil at specific conditions. Incorporation of BBDPs in a well designed amyloidogenic peptides generates a special class of β-sheet breaker peptides those undergo a chemical change at physiological condition generating a breaker element in situ. These β-breaker peptides are shown to first incorporate into the amyloid and then disrupt it. Such conformational switches may be used to study agrregation/disaggregation process and may find many biomedical applications relevant to aggregation related disorders. Such strategy for reversion of peptide aggregation using chemical tricks may find application in material chemistry as well.



KEYWORDS: *Alzheimer's disease, self-assembly, β-sheet, $A\beta_{(1-40)}$ peptide*

Aggregation and disaggregation of proteins and peptides are at the center of interest in various subjects spanning from nanosciences, membrane technology, gelation, drug delivery to drug discovery, and medicine. Amyloidoses, such as Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, diabetes type II, prion disorders, and so forth, are characterized by the aggregation of peptides or proteins of variable length and amino acid composition.[1] Growing evidence suggests that the self-assembly of amyloid β (Aβ), a 39−42-residue peptide, into amyloid fibrils is the main cause of Alzheimer's disease.[2] Aβ peptide, which usually exists in soluble random coil or helix conformation in its native state, undergoes misfolding to form insoluble composites rich in ordered aggregates of stacked β-sheet structures, which is most stable. Low molecular weight aggregates, which are soluble and thus able to migrate, are believed to be more toxic than insoluble aggregates.[3] Use of β-breaker peptides, first introduced by Soto et al., is a landmark in the direction of fibrillization inhibitory peptide based drug design.[4] Soto et al. prepared β-breaker peptides containing proline as the β-breaker element attached with a recognition sequence. Another promising approach is the N-methylation of certain residues of the Aβ recognizing sequence, which interferes with the hydrogen bonding networks, thus preventing β-sheet stacking.[5] α-Aminoisobutyric acid (Aib) is also known to be more efficient than proline as β-sheet destabilizing element.[6] Aib as a β-breaker element was used to inhibit the aggregation of human IAPP, a 37 residue peptide responsible for type II diabetes.[7] It is reported that these β-sheet breaker peptides can disrupt existing amyloid also.[4b] But adequate literature on the mechanism of disruption is unavailable.[8] Many developments are observed for improving solubility and stability of such peptides,[9] but the basic structure of such peptides was always kept unaltered.

Close analysis of the structure of the existing β sheet breaker peptides reveals that all of them mainly consist of a breaking element (for example, a kink) and a recognition element (the rest of the peptide), where the breaking element was preinstalled. Recognition or binding affinity is governed by the number of H-bonding possibilities, hydrophobic interactions, and structural fit into the planar β-sheet topology. On the contrary, breaking effect is achieved by reducing the number of H-bonds, reducing the hydrophobic nature and introducing a group which can not fit structurally in the planar topology of β-sheet. Thus, "recognition" and "breaking" is in contrast to each other. Insertion of chemical entity in a breaker peptide to improve the disruption phenomenon results in reduction of recognition efficiency and vice versa. Thus, the preinstalled disrupting element, for example, kink (for proline and Aib) or N-methylated amino acids, may lessen the chance of proper alignment and recognition to the aggregating peptide precluding the possibility of the β-breaking effect. Therefore, design of β-sheet breaker peptides which can bind to either

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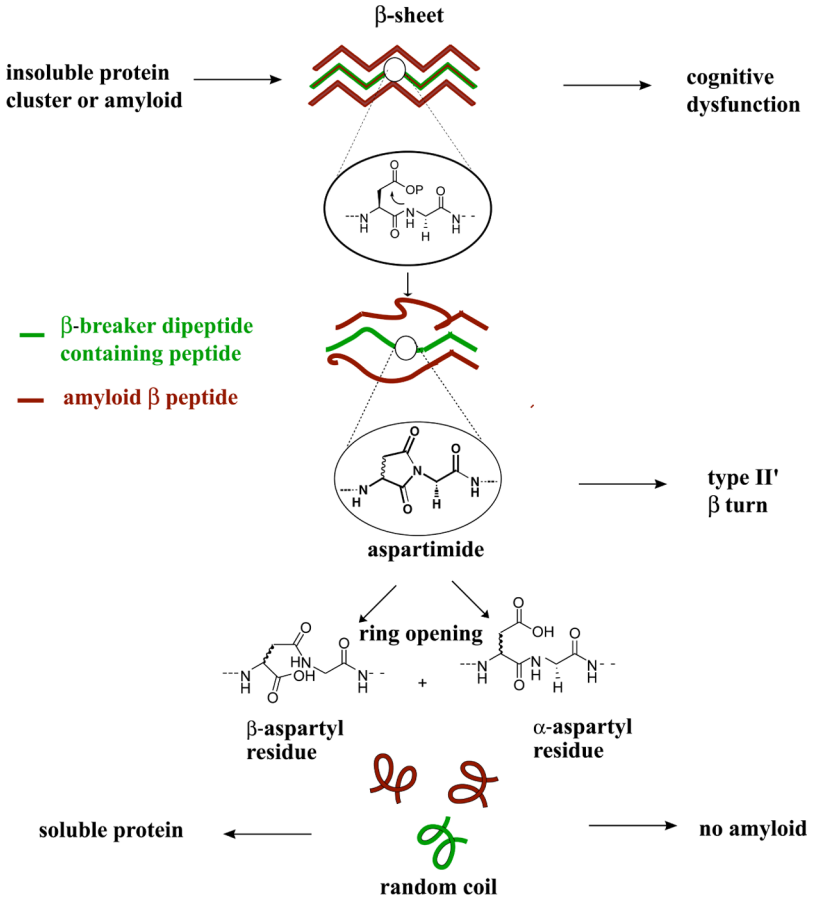

Figure 1. Concept of β-sheet disruption using β-breaker-dipeptide containing β-sheet breaker peptides.

soluble or insoluble aggregates and exert breaking effect by in situ generated breaking element is highly desirable.

To achieve the mentioned target, we have developed a novel concept termed as "the concept of β-breaker dipeptides" (BBDPs). A specially designed novel class of dipeptides is at the center of the concept. When such a dipeptide is incorporated at the middle of an aggregating peptide, it generates a novel class of smart β-sheet breaker peptides, which first forms β-sheet and changes back to random coil conformation on prolonged treatment at basic pH. Kinetics of the conformational change can be controlled by either controlling the pH or altering the chemical structure. This concept may have application across the domain. For example, in the context of peptide based drug design against amyloidoses, a new classs of β-sheet breaker peptides can be designed for which the breaker element may be generated in situ and problems of preinstalled breaker element can be bypassed (Figure 1).

A dipeptide of the general formula [Asp(OP)-Axx] can act as a β-breaker dipeptide unit. "P" stands for a suitable side chain protecting group of an aspartic acid residue, and Axx can be any amino acid. Conceptually, "Asp" can also be replaced by "Glu" or any artificial amino acid bearing a carboxylic acid group on a suitable position of the side chain. A novel class of BBDP-containing β-sheet breaker peptides can be generated by incorporating a suitable β-breaker dipeptide inside the self-recognizing key sequence of the Aβ peptide (e.g., Aβ_{17-21}), which would be effective for disruption of Aβ aggregation. Similar BBDP-containing β-sheet breaker peptides can also be designed for other relevant aggregating peptides. The unique feature of the BBDP concept is that a BBDP-containing β-sheet breaker peptide readily and properly aligns with the Aβ peptide (or any other aggregating peptide) if the sequence homology is maintained. As the breaker element is not formed at the initial stage, the problem of recognition between the breaker peptide and the aggregating peptide is ruled out. When the pH of the medium is adjusted to near physiological conditions, the BBDP unit of the BBDP-containing β-sheet breaker peptide undergoes a chemical transformation. That chemical transformation generates the breaker element in situ, resulting in a kink into the peptide backbone. At this stage, it can disrupt β-sheet or fibril accumulation of itself as well as of the neighboring aggregating peptide(s) when mixed (Figure 1).

Our concept is based on the chemistry of aspartimide formation, a well-known side reaction frequently observed in peptide chemistry.[10] A BBDP forms aspartimide at neutral to basic pH and around 37 °C temperature. Aspartimide induces a type II′ β turn when incorporated into a peptide, which generates a kink and disturbs the β-sheet topology of the peptide.[11] Moreover, ring-opening of the aspartimide residue generates α- and β-aspartyl residues (in 1:3 ratio) at the mentioned pH and temperature. The β-aspartyl derivative introduces conformational flexibility in the system and breaks down the hydrogen bonding network between the BBDP-containing β-sheet breaker peptide and the aggregating peptide. Furthermore, racemization of the Asp residue during the process of aspartimide formation also contributes to the disruption of the H-bonding network of the β-sheet.[12] 12.5% of the total breaker peptide may convert back to the α aspartyl peptide bearing -(L)Asp-Axx-, which may reinforce the effect of

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other in situ generated breaker peptides due to decrement of the hydrophobic nature of the parent peptide via generation of the negative charge on the side chain of Asp. Thus, complete breakdown of the β-sheet architecture and amyloid may be achieved using such a technique.

RESULTS AND DISCUSSION

Peptides Used for the Studies. To test our hypothesis, we first designed four β-breaker peptides (peptides 1−4 in Table 1) in which the [Asp(OBzl)-Axx] unit was incorporated into the middle of the sequence. Additionally, peptide 5, popularly known as Soto's breaker peptide, was also synthesized and used as a control. The BBDP unit was composed of -D(OBzl)S- in peptides 1 and 2, and -D(OBzl)F- in peptides 3 and 4. In all the peptides, "P" was chosen to be "-Bzl".

Table 1. Sequences of the Peptides Synthesized and Their Role in the Current Study

peptide no.	sequence	role of the peptide
1	SLS**D(OBzl)**SLS-GNH_2	breaker
2	LS**D(OBzl)**SLS-GNH_2	breaker
3	Ac-SLSLHQKL**D(OBzl)**FFAEDVSL-GNH_2	breaker
4	Ac-L**D(OBzl)**FFD-NH_2	breaker
5	Ac-LPFFD-NH_2	control
6	SLSL(**H**$^+$)SLS-GNH_2	aggregating
7	Ac-SLSLHQKLVFF(**H**$^+$)SEDVSL-GNH_2	aggregating
8	SLS**P**SLSGNH_2	control
9	SLS**D**SLSGNH_2	control

The sequences of these peptides were designed following the concept of Hecht et al. of alternating hydrophilic and hydrophobic amino acids[13] (for peptides 1 and 2), and picking the hydrophobic region of Aβ peptide (for peptides 3 and 4), so that they form β-sheet and fibrils readily at the initial stage. Once pH is altered from acidic to basic range, the BBDP unit generates aspartimide and subsequently aspartyl derivatives of the peptides, which results in disruption of self-aggregation.

Peptides 6 and 7 were used as model aggregating peptide or functional mimic of Aβ peptide. Peptides 8 and 9 were used as control peptide. In addition to that, commercially available $A\beta_{(1-40)}$ peptide was also used. Sequence homology was maintained among peptides 1, 2, 6, 8, and 9 for proper comparison. Similarly, sequence homology was maintained between peptides 3 and 7, as well as between peptides 4 and 5.

Reversion of Self-Aggregation of BBDP Containing Model Peptides. The chemical conversion of the BBDP unit of the breaker peptides to aspartimide and further ring-opening to aspartyl residues (α:β = 1:3) was monitored by liquid chromatography−mass spectrometry (LC−MS) in a time dependent manner at pH 7.0 for peptides 1−3 and pH 7.4 for peptide 4 (see Figure 2a for peptide 1 and Figure 5a for peptide 4; Supporting Information Figure S11 for peptide 2 and Figure S12 for peptide 3). In general, conversion to aspartimide took 24 h and conversion of that to α- and β-aspartyl peptides took 48 h. Stability of the BBDP unit of peptide 1 in sodium acetate buffer (pH 4.0) was also examined, and no acyl migration was noticed even after 1 week (Figure 2b). Next, we investigated the ability of the BBDP-containing β-sheet breaker peptides to inhibit/disrupt self-aggregation.

Conformational changes of all the breaker peptides were monitored by Fourier transform infrared (FT-IR) spectroscopy and circular dichroism (CD). In FT-IR spectroscopy, we noticed the presence of a strong band at 1625 cm^{-1} for peptide 1 and 1626 cm^{-1} for peptide 2 incubated in sodium acetate buffer for 4 days, indicating the amide I band of the peptide in β-sheet conformation. On the other hand, after incubation for 4 days in PBS at pH 7.0, the same peptides revealed bands at 1645 and 1640 cm^{-1}, respectively, which were assigned to the amide I band of the peptide in random coil conformation (Figure 2c).

The β-breaker peptides exhibited characteristic signals corresponding to β-sheets when incubated in sodium acetate buffer pH 4.0 for 4 days in their CD profiles. On the other hand, when pH was regulated to neutral range, these peptides underwent chemical modification and converted to the random coil conformation (Supporting Information Figures S14 and S15). When the solid sample of peptide 1 was dissolved in PBS (pH 7) and measured, the CD profile exhibited a maximum at 195 nm and minimum at 220 nm, characteristic of β-sheet conformation (Figure 2d) at the initial stage. When the same sample was checked next day, the characteristic curve of a β-turn was observed, that was converted to a characteristic curve of a random coil after two more days. The identity of the peptide, whether it is in α-aspartyl form, aspartimide form, or β-aspartyl form, was confirmed by ESI-MS at specific time points. The pH at which chemical conversion takes place depends on the side chain protection of Asp and the following amino acid (Axx) of the BBDP unit.[11] For example, peptide 1 was observed (both via ESI-MS and CD) to stay in α-aspartyl form (β-sheet) up to 7 days in pH 4 (acetate buffer), whereas it was in α-aspartyl form (β-sheet) at pH 7 (PBS) up to 12 h, which then converted to aspartimide (β-turn) and aspartyl derivatives (random coil). Thus, kinetics of the generation of the breaking element in the newly proposed β-sheet breaker peptides can be controlled by altering the BBDP unit. The amyloidogenic nature of these peptides was studied using thioflavin T fluorescence, transmission electron microscopy (TEM), and Congo red birefringence. BBDP-containing peptides formed amyloid-like fibrils in acidic buffer (Figure 2e, left panel), and no such fibrillar aggregates were observed when incubated in PBS (Figure 2e, right panel) and viewed under TEM after 4 days of incubation.

Also a characteristic green-gold birefringence was noticed when peptide 1 was incubated in sodium acetate buffer for 4 days, stained with Congo red and observed under polarized light (Figure 2f, left panel), indicating the presence of amyloid. No birefringence was noticed when the same peptide was incubated in PBS pH 7.0 for same time period of incubation (Figure 3f, right panel). These results support our hypothesis that O to N acyl migration at pH 7.0 leads to the formation of aspartimide, and subsequent ring-opening of the aspartimide generates α- and β-aspartyl derivatives of the BBDP-containing β-sheet breaker peptide that completely inhibit the self-assembly of the amyloidogenic peptides.

Increase in fluorescence intensity upon binding to thioflavin T is considered as a characteristic property of fibrils and therefore kinetics of fibril formation was assessed using this technique.[14] Strong enhancement of the thioflavin T fluorescence emission intensity in presence of BBDP-containing β-sheet breaker peptides at the initial stages of the experiment (up to 24 h) followed by the gradual decrease of the fluorescence over time revealed that the BBDP-containing

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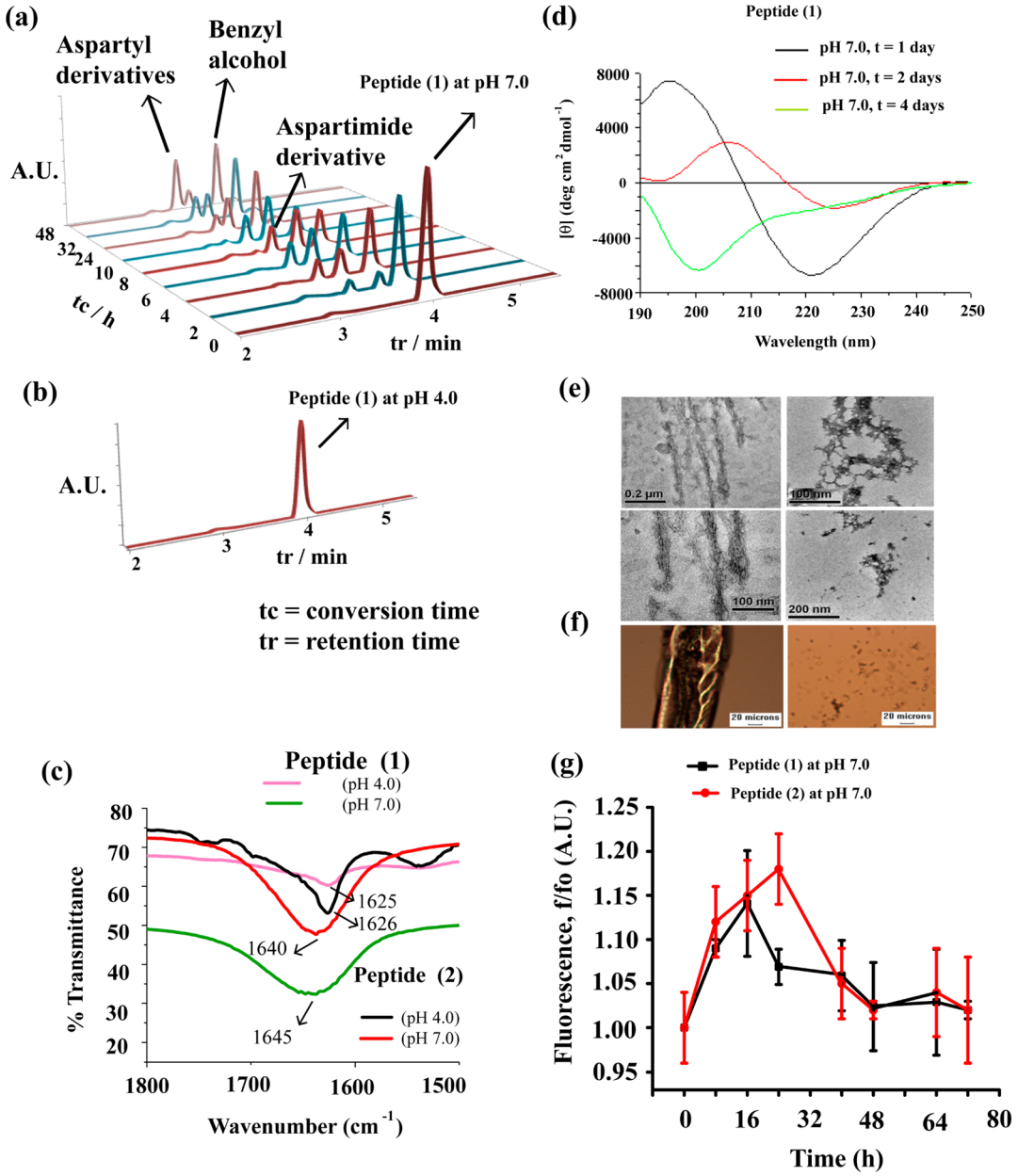

Figure 2. (a) LC−MS monitoring of aspartimide formation and subsequent hydrolysis of BBDP-containing peptide 1. (b) LC−MS profile of peptide 1 incubated in sodium acetate buffer after 1 week, indicating stability. (c) FT-IR spectra of peptide 1 and 2 incubated in pH 4 and PBS pH 7.0. (d) CD spectra of peptide 1 in PBS (pH 7.0). (e) TEM images of peptide 1 incubated in sodium acetate buffer (left panel) and PBS pH 7.0 (right panel), showing the presence and absence of fibrils, respectively, after 4 days of incubation. (f) Congo red birefringence image of peptide 1 incubated in acidic (left panel) and basic buffer (right panel), indicating the presence and absence of amyloid, respectively, after 4 days of incubation. (g) Time dependent studies of fibril evolution using thioflavin T fluorescence experiment at pH 7.0 (black curve for peptide 1 and red curve for peptide 2).

peptides first aggregate to form amyloid-like fibrils and then slowly disrupt that (Figure 2g). Kinetics of O to N acyl migration revealed by LC−MS studies (Figure 2a) also demonstrates that peptide 1 takes about 24 h for complete conversion to either aspartimide or the aspartyl derivatives. Such concordance of the kinetics of rearrangement, aggregation, and disruption was noted for all other BBDP containing peptides as well, which indicates that fibril disruption was caused by in situ aspartimide formation and subsequent ring-opening. Results mentioned above demonstrates that BBDP containing peptides first forms β sheet and fibril then self-aggregation is disrupted slowly by specific chemical conversion at specific pH range. To the best of our knowledge, this is the first report of a model aggregating peptide, which first aggregates to form fibril and then disintegrates by itself via a chemical reaction in a controlled manner. Such system can be useful for studies of the process of aggregation and disaggregation.

Since there is a possibility of coaggregation of such BBDP-containing β-sheet breaker peptides with neighboring aggregating peptides, for example, Aβ peptide when mixed, we hypothesized that a suitably designed BBDP-containing β-breaker peptide can disrupt the Alzheimer's Aβ amyloid when coincubated in an appropriate proportion with Aβ peptide. However, synthesis and handling of the full length Aβ peptide is difficult. Therefore, we thought if we have a system that is

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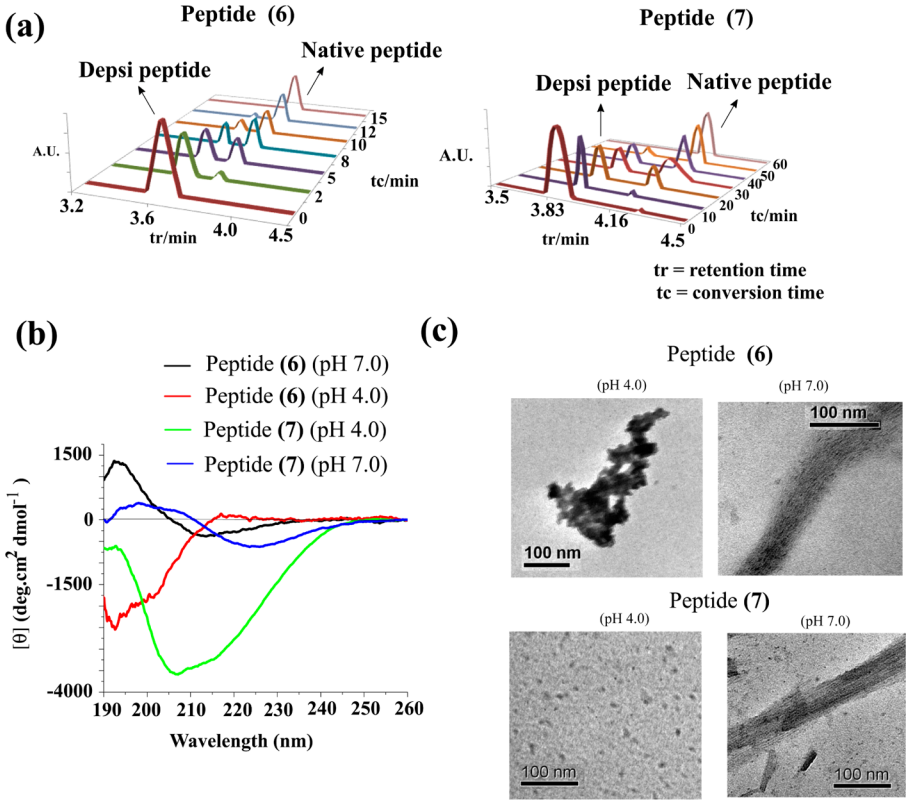

Figure 3. (a) pH induced O to N acyl migration of peptide 6 (left) and 7 (right). (b) CD spectra of peptide 6 and 7 in sodium acetate buffer (pH 4.0) and PBS (pH 7.0) buffer after 4 days of incubation. (c) TEM micrographs of peptide 6 and 7 at various pH after four days of incubation.

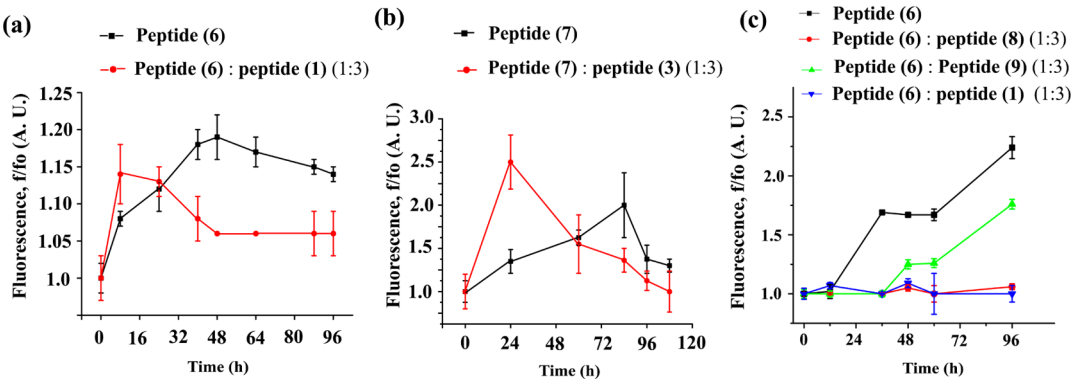

Figure 4. Time dependent thioflavin T fluorescence measurement at pH 7.0 of (a) peptide 6 in the absence (black) and presence (red) of the breaker peptide 1; (b) peptide 7 in the absence (black) and presence (red) of breaker peptide 3; (c) peptide 6 in the absence (black) and presence of the control peptide 8 (red), 9 (green), the breaker peptide 1 (blue).

easily accessible and behaves similar to the full length Aβ peptide (Aβ mimic), it would be easy to test the hypothesis before testing it on the full length Aβ.

Characterization of Aggregation Properties of Model Aggregating Peptides. Mutter et al. reported -(Ser-Leu)$_n$- based switch-peptides that form amyloid-like fibrils rich in β sheet in physiological pH and temperature.[15] In these peptides, one serine switch element was incorporated into a self-aggregating peptide via depsi bond to control the kinetics of self-assembly by adjusting the pH of the system. The switch element present in the sequence helped the model aggregating peptides to adopt a random coil conformation at acidic pH, as the peptide fragments attached to it were shorter for β-sheet formation for those sequences. When pH was adjusted from neutral to basic range, O to N acyl migration occurred, resulting in a regular peptide longer enough to form β-sheet as well as fibrils. Along a similar line, a host−guest switch peptide was also reported where the hydrophobic core sequence of the Aβ peptide was embedded inside the -(Ser-Leu)$_n$- based aggregating sequence containing a serine-based switch at a suitable position.[15b]

We have chosen a -(Ser-Leu)$_n$- based switch peptide bearing the sequence SLSL(**H**$^+$)SLS-GNH$_2$ (6) and the Aβ derived host−guest switch peptide (Ac-SLSLHQKLVFF(**H**$^+$)SEDVSL-GNH$_2$, 7) as the model aggregating peptides for our studies. O to N acyl migration of these peptides was studied using LC−MS (Figure 3a). Their aggregation potential was investigated using various biophysical tools. In the CD spectrum, we observed a band corresponding to β-sheet at pH 7.0 (Figure 3b), and further these peptides produced amyloid-like fibrils

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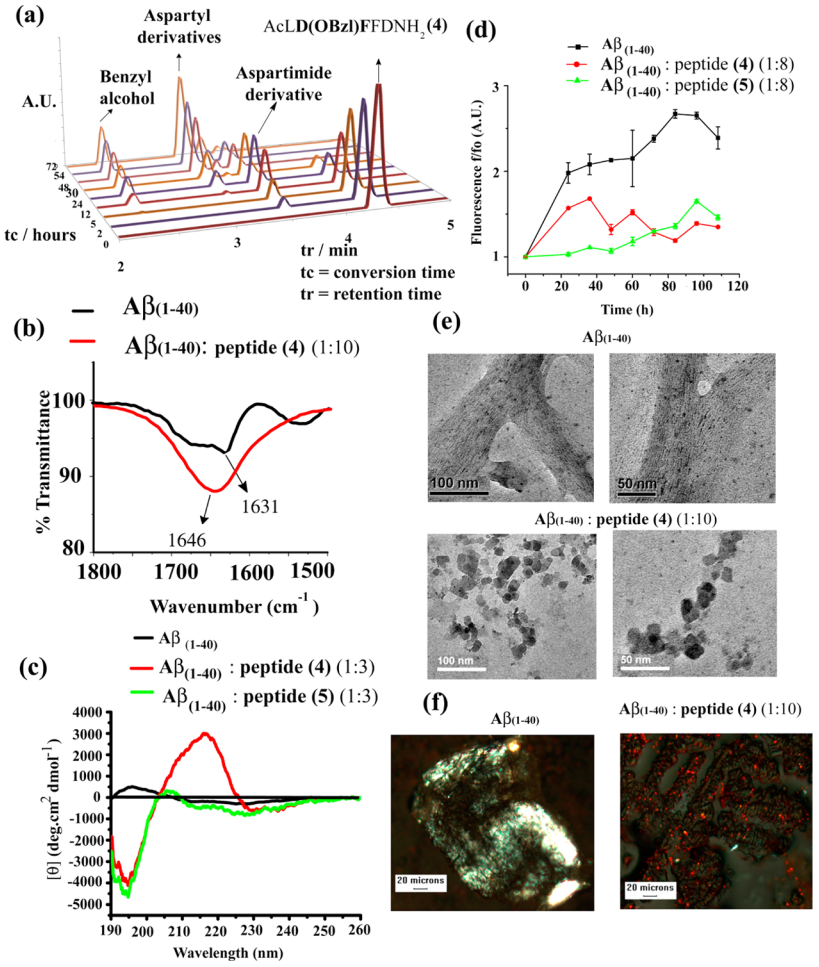

Figure 5. (a) Monitoring the intramolecular cyclization of peptide 4 by LC-MS at pH 7.4. (b) FT-IR spectrum of Aβ_{1-40} in the absence (black) and presence (red) of peptide 4, checked after 4 days of incubation. (c) Mixing experiments of the Aβ peptide and the breaker peptides by CD at pH 7.4, checked after 4 days of incubation. (d) Time dependent thioflavin T fluorescence studies of Aβ_{1-40} in the absence (black) and presence of peptide 4 (green) or 5 (red) at pH 7.4. (e) TEM micrographs of Aβ_{1-40} in the absence (upper panel) and presence (lower panel) of peptide 4, checked after 4 days of incubation. (f) Congo red birefringence image of Aβ_{1-40} in the absence (left) and in the presence (right) of peptide 4, checked after 4 days of incubation. All studies pertaining to A$\beta_{(1-40)}$ were carried out at pH 7.4.

which was confirmed by TEM (Figure 3c). Also, we noticed a characteristic green-gold birefringence from 4 day old peptide solution upon staining with Congo red under polarized light using a polarizable microscope (see the Supporting Information, Figures S37 and S38) confirming amyloid formation.

Disruption of Fibril Formation of Model Amyloid Forming Peptides by BBDP Containing Model Breaker Peptides. We then set out to test the ability of the BBDP-containing peptides to disrupt the aggregation of these model aggregating peptides that were used as an Aβ mimic in this study. Incremental fibril accumulation of the model aggregating peptide 6 was observed up to 48 h in a thioflavin T fluorescence study in the absence of the BBDP-containing breaker peptide 1 at pH 7.0. On the other hand, when peptide 1 was coincubated with peptide 6 under similar experimental conditions, we observed fibril formation up to only 6 h, which was slowly disrupted thereafter (Figure 4a). Similarly, in a parallel experiment, substantial decrease in the fluorescence signal of peptide 7 was observed after 24 h when mixed with breaker peptide 3 in 3-fold molar excess at pH 7.0 (Figure 4b). In concordance with thioflavin T fluorescence, disruption of amyloid was observed from TEM (see the Supporting Information, Figures S33 and S34) and Congo red birefringence studies (see the Supporting Information, Figures S37c and S38c) as well. These findings support our hypothesis that BBDP-containing peptide first aligns and coaggregates with the neighboring aggregating peptide even at pH 7.0 when sequence homology is maintained and as long as the breaker element is not formed. Over time, at that pH, the breaker element is generated in the form of a kink on the backbone due to aspartimide formation. That kink slowly disrupts the self-assembly as well as the aggregation of the neighboring peptide. Ultimately, the ring-opening of the aspartimide, formation of the α- and β-aspartyl peptide, and racemisation of the Asp residue cause total collapse of the β-sheet architecture. In a control experiment (Figure 4c), proline containing peptide SLS**P**SLSGNH$_2$ (8) inhibited the aggregation of peptide 6 from the beginning, but the BBDP containing peptide SLS**D(OBzl)**-SLSGNH$_2$ (1) partially coaggregated and then disrupted. On the other hand, mixing experiment with another control peptide 9 (sequence: SLS**D**SLSGNH$_2$), where D(OBzl) was replaced with an unprotected Asp, shows extended lag time and reduced amount of fibril, indicating it as a weak β sheet breaker.

Disruption of Fibril Formation of A$\beta_{(1-40)}$ by a BBDP Containing Peptide. Finally, we designed and synthesized a five membered BBDP-containing β-sheet breaker peptide (4),

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checked for chemical conversions (Figure 5a), and tested its efficiency for inhibition of aggregation of the full length $A\beta_{1-40}$. It was designed to get a comparison with peptide 5, which is a known β-sheet breaker peptide, and therefore, high sequence homology was maintained with it. $A\beta_{1-40}$ produced a sharp band at 1631 cm^{-1} which was assigned to the amide I band of a peptide in β-sheet conformation in FT-IR studies in the absence of peptide 4. On the contrary, in the presence of peptide 4, a new band emerged at 1646 cm^{-1} corresponding to random coil (Figure 5b).

The influence of peptide 4 on the conformational change of $A\beta_{1-40}$ was also evident from CD studies (Figure 5c). Pure Aβ peptide shows a characteristic curve of mixture of α-helix and β-sheet (black curve). The mixture Aβ and peptide 5 show a mixture of β-sheet and random coil (green curve). Whereas the red curve of Figure 5c, which was obtained from the mixture of Aβ and peptide 4, clearly indicates induction of a turn. Similar results were obtained independent of amount of the breaker peptides present in the solution (for 3-fold molar excess, Figure 5c; 5-fold, Supporting Information Figure S18; and 10-fold, Supporting Information Figure S19).

A notable decrease in the thioflavin T fluorescence signal was observed when $A\beta_{1-40}$ was coincubated with peptide 5 as a control experiment. Further decrement in the fluorescence signal was observed when coincubated with similar excess of peptide 4 (Figure 5d for 8-fold; see Supporting Information Figures S28−S30 for 3-, 5-, and 10-fold molar excess of breaker peptides). It was assumed that peptide 4 will form β-sheet at the initial stage as long as the chemical conversion does not take place. Templating effect of the Aβ peptide also may play its role for coaggregation. The coaggregation was evident from the increase in amount of fibril at the initial stage (until ∼24 h) when Aβ was mixed with peptide 4 (Figure 5d, red curve). Further decrease in the fluorescence signal was due to the breaking effect after the chemical conversion. The chemical conversion or intramolecular cyclization for peptide 4 took 24−72 h at pH 7.4., which is in concordance with the results obtained in fluorescence measurements.

We looked for fibril by TEM after 4 days of incubation. $A\beta_{1-40}$ alone produced fibrillar aggregates. However, amorphous aggregates were observed instead when coincubated with peptide 4 for the same duration (Figure 5e). In agreement with the TEM studies, a characteristic green gold birefringence was noticed under polarized light from the $A\beta_{1-40}$ sample and no such birefringence was observed from the sample coincubated with peptide 4, checked after 4 days (Figure 5f). This confirms complete disruption of Aβ amyloid by BBDP-containing breaker peptide (4). Importantly, there was no evidence of reaggregation of the rearranged BBDP containing peptides.

Some of the results (for example, comparison of the red curve and green curve in Figure 5d and comparison of the red curve and blue curve in Figure 4c) suggest that β-breaking efficiency of BBDP containing peptide (peptide 4) is comparable to that of the preinstalled breaker element containing peptide homologue (peptide 5), if not better. Nevertheless, the problem of binding to the target peptides due to the preinstalled breaker element can be bypassed using such a prodrug type concept. However, it is necessary to modulate the kinetics of mentioned chemical conversion, which may be achieved by varying the side chain protection of Asp of BBDP, so that it takes place at physiological pH but not before it reaches to the target aggregating peptide, soluble oligomers, or amyloid in vivo. If the conversion occurs before the BBDP containing breaker peptide reaching to the target, it may work as known β-breaker peptides.

CONCLUSION

We have demonstrated that peptides containing a BBDP unit form a new generation of conformational switch peptide, which forms β-sheet and fibril at the beginning and then breaks the fibrillar nanostructure at a specific condition. It also easily aligns with neighbor aggregating peptides due to the presence of sequence homology and absence of the breaker element at the initial stage easily, which then undergo a chemical conversion to aspartimide-containing peptides at physiological pH and generate a kink in the peptide backbone in situ. Formation of aspartimide, subsequent ring-opening and racemisation of Asp finally disrupt the native backbone of the breaker peptide. Such chemical and structural changes of the BBDP unit forcefully disrupt self-aggregation as well as aggregation of the neighboring peptides upon coincubation, although the aggregated state is thermodynamically most stable. Based on all these findings, we conclude that the BBDP concept is valid for β-sheet inhibition and disruption and may be useful for the development of novel therapeutics against many amyloidoses including Alzheimer's disease. However, modulation of the kinetics of the in situ intramolecular cyclization, reducing toxicity caused by the leaving group, investigation on the presence of the inherent toxicity of the designed molecules, increasing ability to cross blood-brain barrier are some of the remaining problems to be addressed. Nevertheless, many compounds designed based on this concept may be used to study formation and disintegration of aggregation at least in vitro and may find biomedical applications for diverse amyloidoses.

Furthermore, such a concept can also be useful for designing novel material for biomedical application. For example, some switchable hydrogel based drug carrier can be prepared using BBDP concept, which may disrupt its architecture and release the drug under physiological temperature and pH at the site.

METHODS

Peptide Synthesis. Peptides were synthesized by stepwise solid phase peptide synthesis using Fmoc/tBu protection strategy on Rink amide resin (loading 1.1. mmol/g). Resin was swollen in DCM for 1.5 h, and DMF for 0.5 h. Two equivalents of Fmoc amino acids, 2.5 equiv of PyBOP, and 5 equiv of DIPEA were used. Coupling time varied from 45 min to 6 h. Each coupling was monitored by Kaiser test, and in the case of incomplete acylations coupling cycle was repeated followed by capping. Fmoc removal was achieved using 20% piperidine in DMF mixture for 21 min (7 min × 3). After final Fmoc removal, peptide was cleaved from the resin using TFA/DCM/H_2O (8:1.5:0.5) for 3 h. Crude peptide was precipitated by cold diethyl ether and then centrifuged.

Crude peptides were purified on a Waters 600E semipreparative RP-HPLC using a C18-μ Bondapak column at a flow rate of 5 mL/min. The solvent system was composed of solvent A (0.09% TFA in H_2O) and solvent B (0.09% TFA in CH_3CN). A Waters 2489 UV detector was used with an option of dual detection at 214 and 254 nm. Gradient used for purification was 5−20% CH_3CN for 5 min, 20−60% CH_3CN over 5−25 min.

Aβ Sample Preparation.[16] An amount of 0.5 mg of commercially available $A\beta_{(1-40)}$ was dissolved in TFA to obtain homogeneous solution free of aggregates. TFA was evaporated using N_2. To remove TFA completely, HFIP was added and evaporated using N_2 to obtain a filmlike material. This process was repeated twice. A volume of 2.0 mL of PBS (50 mM, pH 7.4) was added to the Eppendorf tube followed by sonication and vortexing to obtain a final concentration of 50 μM.

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Specified volume from this stock solution was used for various experiments.

Fourier Transform Infrared Spectroscopy. A volume of 20 μL of the peptide sample after 4 days of incubation was mixed with KBr, and a pellet was prepared. To obtain final spectra, the background scan was subtracted from the sample scans and text files were plotted using Origin 61 software.

Circular Dichroism. Lyophilized peptide samples alone or mixed with suitable breaker peptides were dissolved in PBS (50 mM, pH 7.4 for A$\beta_{(1-40)}$ and its related sequences and pH 7.0 for other sequences) and sodium acetate buffer (50 mM, pH 4.0) to obtain a final concentration of 150 μM (50 μM in the case of A$\beta_{(1-40)}$ peptide). Then 400 μL of the sample was taken in a cuvette of 1 mm path length using a bandwidth of 1 nm. Spectra were recorded from 190 to 260 nm on a JASCO J-815 instrument. Two measurements were accumulated, and average data is reported.

Thioflavin T Analysis. Thioflavin T (Sigma Aldrich) solution was prepared at a concentration of 50 μM and then stored at 4 °C with proper protection from light to prevent quenching.[14a] Lyophilized peptide samples and when necessary mixed with breaker peptides were dissolved in PBS (50 mM, pH 7.0 and 7.4 for A$\beta_{(1-40)}$ and its related sequences) and sodium acetate buffer (50 mM, pH 4.0) to prepare a stock solution of 150 μM (50 μM in the case of A$\beta_{(1-40)}$ peptide) and incubated at 37 °C over a water bath. At different time intervals, 40 μL of peptide sample was mixed with 200 μL of thioflavin T solution (50 μM), and total volume was made up to 400 μL using suitable buffer. Fluorescence emission was measured at 485 nm and excitation at 435 nm, using a slit of 5 nm on a Fluoromax-4, Horiba instrument.

Stock solution which was prepared for thioflavin T experiment was also used for TEM, FT-IR, and Congo red birefringence analysis.

Transmission Electron Microscopy. Peptide solution (10 μL) after 4 days of incubation was added over the carbon coated grid and allowed to float for 1 min. Next, 2% uranyl acetate solution (10 μL) was added on to the same grid and then was allowed to float for 1 min. Excess solution was removed using blotting paper. After drying, TEM analysis was performed on JEOL instrument at 80 kV.

Congo-Red Birefringence. A volume of 20 μL of the peptide sample after 4 days of incubation was added on a glass slide followed by 40 μL of the saturated Congo red solution. Excess solution was removed using blotting paper, and the sample was allowed to dry at room temperature. On the glass slide, the dried red spot was analyzed under a Leica DM 2500P polarizable microscope.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and copies of spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

K.C.N. and A.P. synthesized the compounds and performed the experiments. K.C.N. and B.M. designed the experiments and wrote the paper. All authors approved the submission.

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Notes

The authors declare no competing financial interest.

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