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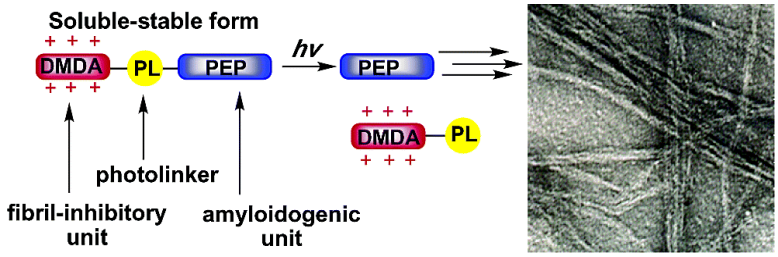

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Photolytic Control of Peptide Self-Assembly

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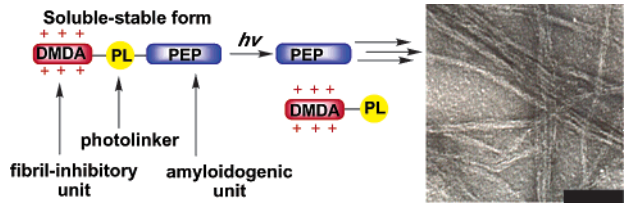
The self-assembly of peptides and proteins into fibrils is one of the major events leading to the development of many neurodegenerative diseases.[1−3] This natural process has served as an inspiration to the field of biomaterials as the independent organization of biomolecules into supramolecular structures provides a powerful approach for the generation of new materials with potentially interesting properties.[4−7] Unfortunately, in most systems, the spontaneous self-assembly process can be very difficult to control. In addition, the uncontrolled polymerization can represent a significant obstacle to the preparation and purification of the system for study. Hence, temporal control of peptide self-assembly is desired, and, ideally, it would be advantageous if a peptide could be synthesized and chemically modified in a soluble and stable form until the fibrillization is triggered, without the need for additional manipulations. Herein, we report a method for the temporal control of peptide self-assembly into fibrils using an amyloidogenic peptide derived from the human prion protein (PrP) fragment 174-195.[8,9] The design (Scheme 1) implements a synthetic photolabile linker and a "fibril-inhibitory unit" that stabilizes the peptide as a soluble species for at least 12 h. Upon photolysis, the fibril-inhibitory unit and photolinker are detached from the amyloidogenic peptide, which is then capable of self-assembly. Previously, other methods including divalent cation release utilizing stimuli-responsive liposomes have been implemented to trigger peptide self-assembly.[10]

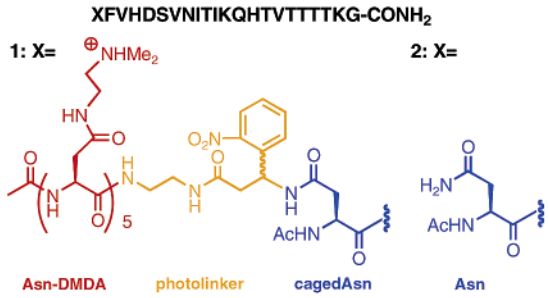
Photolabile linkers have been used in many applications including solid-phase peptide synthesis, for the facile cleavage of peptides from the solid support,[11] as well as for the controlled release of metal ions[12] and bioactive molecules such as peptides and nucleic acids.[13] Caged peptides provide an efficient approach for the controlled exposure of active epitopes upon activation with UV irradiation. Unfortunately, the commercially available photolinkers for peptide applications are only compatible with introduction at the C-terminus of a peptide.[11] If placed at the N-terminus or in the side chain of an amino acid, the photodissociation step releases a non-native species with part of the photoproduct still attached to the N-terminus or the side chain of the peptide, which may not be desirable for further studies. An example of such a situation is in studies on the PrP 174-195 fragment, wherein fibrillization of the peptide is very sensitive to the electrostatic environment near the N-terminus. Therefore, to transiently stabilize the amyloidogenic peptide in a soluble form, the photolinker and a fibril-inhibitory unit should be placed near the N-terminus. To achieve this, the commercially available photolabile linker 3-amino-3-(2-nitrophenyl)-propionic acid (ANP) was reconfigured to allow the attachment via an amide linkage to the aspartic acid side chain of the N-terminal residue of the amyloidogenic peptide (Chart 1). This mode of attachment allows the photolytic release of the intact native peptide **2** (without photolysis side products attached) and leaves unmodified asparagine (Asn 174) at the N-terminus (Chart 1). *N*,*N*-Dimethylethylenediamine (DMDA) groups were employed as the fibril-inhibitory units. DMDA groups have been previously used to stabilize amyloidogenic peptides in solution due to the positive charge of these groups at neutral pH (DMDA pK_a 10−11).[5] Additionally, the low reactivity of tertiary amines is important in photolysis.[14] Thus, the DMDA unit is an excellent candidate for the solubilization of the amyloidogenic peptide. Therefore, an asparagine analogue incorporating the DMDA functionality in the side chain was synthesized for facile incorporation of the DMDA groups into the peptide during SPPS. The photolinker and the Asn-DMDA monomers were easily attached to the side chain of the N-terminal residue to generate peptide **1**.

Scheme 1. Modular Design of the System for Studying Photolytic Control of Peptide Self-Assembly[a]



[a] Scale bar = 200 nm.

Chart 1. Structures of Peptides **1** and **2**



To determine if irradiation of peptide **1** would afford the desired photoproducts, and also to establish the time frame of the reaction, the peptide was dissolved in water (70 μM peptide, 1 mM DTT) and exposed to UV−vis irradiation from a transilluminator (365 nm, 7.3 mW/cm^2).[15] The reaction was monitored at different irradiation times by analyzing aliquots from the solution by reverse phase HPLC (Figure 1A). The generation of the expected photoproduct (peptide **2**) was confirmed by ESMS. After 15 min of irradiation, complete disappearance of the starting material was observed with no further increase in peptide **2**.

To test the design and the concept that the fibril-inhibitory unit could provide the desired stabilization of the peptide until photolytic cleavage, fibrillization kinetics for the photolyzed and unphotolyzed peptide **1** and the independently synthesized peptide **2** were compared. The peptides (120 μM) were dissolved in water with 1 mM DTT. Peptide **1** was either kept in the dark or exposed to UV light from a transilluminator for 15 min. All samples were then

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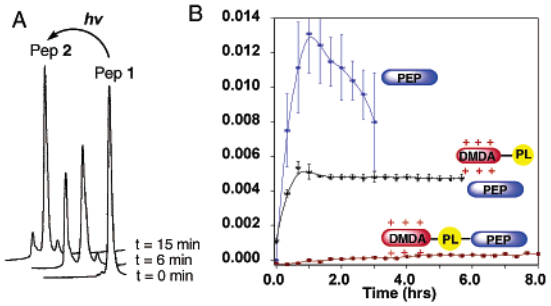

Figure 1. (A) HPLC traces for the photolysis of peptide **1** to produce peptide **2**. (B) Aggregation kinetics for 60 μM photolyzed (black) and unphotolyzed (red) peptide **1** and peptide **2** (blue) at pH 7.5.

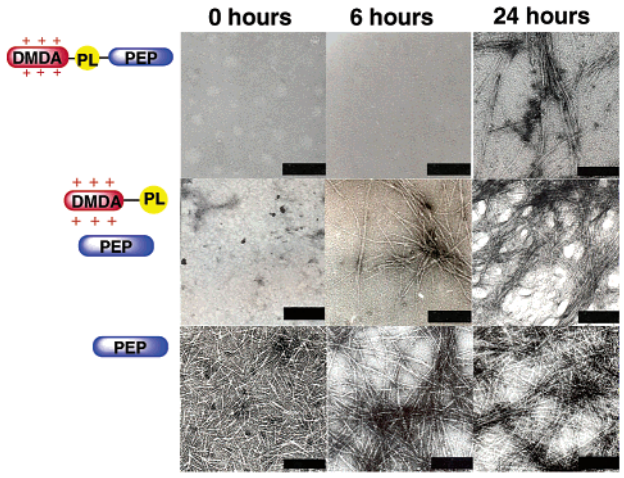

Figure 2. Time-dependent EM analysis for 60 μM peptides incubated at pH 7.5 with schematic representation of the species present in the solutions. Peptide **1** (top); photolyzed peptide **1** (middle); peptide **2** (bottom). Bar size = 200 nm.

rapidly diluted to one-half of the concentration using 5 mM phosphate buffer (pH 7.5), and the aggregation kinetics were monitored using a light-scattering assay.[16] As illustrated in Figure 1B, peptide **2** forms fibrils "aggressively" and immediately without a significant lag time. In contrast, the fibril-inhibitory unit is capable of stabilizing peptide **1** in solution for more than 12 h. However, when the solubility unit is photodissociated from peptide **1**, the photoproduct (peptide **2**) displays rapid aggregation with comparable kinetics to the control peptide. The density of fibrils in the photodissociated sample is somewhat lower, which may be an effect of the detached fibril-inhibitory unit. The stabilization provided by the fibril-inhibitory unit was also reduced when the peptide was incubated at pH 10 (near the pK_a of DMDA) (Supporting Information). These results support the hypothesis that the positive charges on the DMDA groups are responsible for the inhibitory behavior. At pH 10, a significant fraction of the tertiary amines should be neutral and therefore no longer able to affect solubility.

To correlate the aggregation kinetics with the supramolecular arrangement of the peptides, the solutions were also analyzed by transmission electron microscopy (EM). The peptides were placed on EM grids immediately after mixing with phosphate buffer, and at 6 and 24 h after photolysis (Figure 2). The EM images show that fibrillization of peptide **1** is significantly delayed and only a small density of fibrils is observed after 24 h of incubation. In contrast, for the photolyzed peptide sample, fibrils are observed in the EM analysis performed at 6 h. The control peptide **2** showed small filaments at time zero, and extended fibrils were observed by 6 h. These results are consistent with the aggregation kinetics and show the photolysis-dependent fibrillization of the amyloidogenic construct **1**.

Herein, we have presented a methodology that allows for the temporal control of fibrillization of amyloidogenic peptides. This general approach should be of value in a variety of studies where spatial and temporal control of supramolecular association processes is desired. In this study, we have used the electrostatic properties of the DMDA group as the key parameter to stabilize the soluble form of the amyloidogenic peptide until dissociated by photolysis. The linker unit and the general methodology allow the facile incorporation of any "inhibitory unit" desired, depending on the properties of the specific amyloidogenic peptide of interest. For example, sterically encumbering organic moieties or hydrophilic species such as carbohydrates can be used for the modification to inhibit the fibril formation.[17] When fibrillization is desired, the peptide can be exposed to UV radiation and submitted to conditions that promote self-assembly.

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Supporting Information Available: Synthesis and characterization of the photolabile linker, asparagine-DMDA, and peptide. Aggregation kinetics of peptide **1** at different pH and control experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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