Disassembling peptide-based fibres by switching the hydrophobic–hydrophilic balance†

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Amyloid-like model peptides, modified on the N-terminus with an alkyl tail and on the C-terminus with a PEG chain, yielded fibres that were susceptible to triggered disassembly by removal of the alkyl chain, which affected the hydrophobic–hydrophilic balance.

Alkylated peptides represent a versatile class of self-assembling building blocks, due to the subtle interplay between directional hydrogen-bonds and hydrophobic interactions.1–3 This versatility has recently been explored in a variety of applications. For example, Stupp et al. have demonstrated the usefulness of peptide amphiphile assemblies for the construction of bioactive materials.4–6 Furthermore, well defined peptide aggregates have also been used to direct and organize assemblies of synthetic polymers to enhance material properties.7–9 One of the interesting aspects of peptide assembly is that it can be affected by changing the balance between the non-covalent forces that govern the supramolecular architecture. Previously, we have shown that introduction of a hydrophobic tail to a fibril-forming peptide increases the stability of the fibrils and renders the assemblies amenable to manipulation.10–12 Induction of peptide assembly has in this respect already been shown using light,12–14 chemical switches15,16 and enzymes.17,18 In all of these cases, subtle changes in hydrophobicity lead to an increased affinity for aggregation.

To our knowledge, only examples in which peptides have been manipulated to promote self-assembly have been described in the literature so far. The other possibility, disassembly of peptide aggregates by changing non-covalent interactions in situ, has not been exploited yet. This concept is worthwhile pursuing, since it could lead to new release mechanisms and controlled degradation processes for e.g. biomedical applications.19 In this communication we report on a convenient methodology to disassemble peptide fibre assemblies by manipulating the hydrophobic-hydrophilic balance (Fig. 1). For this purpose, peptides were modified with both a hydrophilic and a cleavable hydrophobic moiety, for which the overall balance of non-covalent interactions was still in favour of peptide assembly. After cleaving off the hydrophobic tail, the peptide fibres spontaneously disassembled.

In previous reports we discussed the stabilization of assemblies of the amyloid-forming hexapeptide Ac-Lys-Thr-Val-Ile-Ile-Glu-NH2. It was shown that the introduction of a hydrophobic moiety led to a complete absence of fibre disassembly, even at elevated temperatures (up to 90 °C), whereas the unmodified peptide could be disassembled upon heating.10,20 We then wanted to investigate whether the opposite effect could be achieved by introduction of a hydrophilic group. Therefore a PEG chain was introduced C-terminally in the peptide sequence using a resin with an immobilized PEG moiety (Fig. 2).

Peptide 1 was synthesized on a resin with an immobilized PEG moiety of 3000 g mol⁻¹ molecular weight by solid-phase peptide synthesis following a standard Fmoc-protection protocol.21 Next,

Fig. 1 Control of peptide fibril stability. Stabilization of PEGylated peptide fibrils by introduction of a hydrophobic tail (top). Destabilization of peptide fibrils by cleaving off a hydrophobic tail in situ (bottom).

Fig. 2 PEGylated hexapeptide 1; amphiphilic peptides 2a–f (a: n = 1; b: n = 2; c: n = 3; d: n = 4; e: n = 5; f: n = 6); and cleavable amphiphilic peptide 3.
To study the balance between hydrophilic destabilization and hydrophobic stabilization of fibres, a hydrophobic tail was introduced to the N-terminus of the peptide (Fig. 2). C16-functionalized peptide 2f showed a spectrum with signals at 220 nm and at 200 nm, identical to the spectra obtained for the previously examined self-assembling amphiphilic peptides (Fig. 3A).10 On heating the solution to 90 °C the CD spectrum did not change, indicating a stable aggregate of the peptide. TEM confirmed that fibrils were present in the sample (Fig. S2-1/2†). Next, a series of peptides was prepared with varying hydrophobicity. Solutions of the peptides with alkyl tails of C8, C10, C12 (2a–c respectively) showed the same CD spectrum as peptide 1, indicating the absence of fibrillar aggregates. In contrast, solutions of peptides with C12 (2d) and C14 (2e) tails showed a CD spectrum similar to the fibril-forming peptide 2f. Interestingly, for both samples (2d and 2e) the CD signal did not change on heating the sample to 90 °C. This suggests that once a critical hydrophobicity is acquired, strong stabilization of the assemblies is established.

PEGylation of the fibril-forming hexapeptide KTVIIE seems thus to prevent aggregation, and introduction of a hydrophobic n-alkyl group longer than C12 overcomes this solubilizing effect and the peptide amphiphiles form fibres in aqueous solution. Removing the stabilizing group in situ should now allow us to trigger the disassembly of the fibrils. In order to investigate this possibility, peptide 3 was synthesized, containing both a hydrophilic PEG moiety and an alkyl chain. In this case, however, the alkyl chain was connected to the peptide by a UV-sensitive nitrobenzyl moiety.23–25 Upon irradiation, this group was cleaved, thereby removing the hydrophobic tail from the peptide.

Peptide 3 containing the UV-cleavable linker formed the same fibril structures as the amphiphilic peptide 2f, corroborated by CD spectroscopy and TEM. The CD spectrum of cleavable peptide 3 only differed at 200 nm compared to C16-functionalized peptide 2f (Fig. 3A). This is attributed to the presence of the UV-cleavable linker, which also absorbs in this region.

Irradiation of assemblies of 3 with a strong UV source resulted in clear changes to the CD spectrum after 3 minutes of exposure (Fig. 3B). The ratio of the peaks at 200 and 220 nm increased, characteristic for a change from β-sheet to random coil. Prolonged exposure changed the CD spectrum completely to a spectrum typical for the dissolved state, showing the destruction of the fibrils (Fig. 3A and Fig. S1-1) which was substantiated by TEM, confirming the absence of fibrillar structures. Moreover, the cleavage was established by TLC (Fig. S3-1). In a control experiment, fibres of peptide 2f without the labile linker were exposed to the same UV source for 1 hour (Fig. S1-2). This exposure did not cause any change in the assembly of the fibrils as determined by CD spectroscopy.

In conclusion, the introduction of a hydrophilic group to a fibril-forming peptide can inhibit the formation of structured assemblies of this peptide. More interestingly, upon increasing the amphiphilic character of the peptide by introducing an additional hydrophobic group, the well-structured aggregates return. This effect on assembly led us to introduce a moiety that enables us to selectively control the presence of aggregates. We are exploring this strategy to further manipulate self-assembled peptide amphiphile architectures.

Notes and references
