Journal of Peptide Science
J. Pept. Sci. 2007; **13**: 709–716
Published online 24 September 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.831

JOURNAL OF PEPTIDE SCIENCE

Backbone-modified amylin derivatives: implications for amyloid inhibitor design and as template for self-assembling bionanomaterials‡

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Received 30 November 2006; Accepted 3 December 2006

Abstract: This report reviews our approach to the design, synthesis and structural/morphological analysis of backbone-modified amylin(20–29) derivatives. Depending on the position in the peptide backbone and the type of amide bond isostere/modification, the amylin(20–29) peptides behave either as inhibitors of amyloid fibril formation, which are able to retard amyloid formation of native amylin(20–29), or as templates for the formation of self-assembled supramolecular structures. Molecular fine-tuning of the hydrogen-bond accepting/donating properties allows the control over the morphology of the supramolecular aggregation motifs such as helical ribbons and tapes, ribbons progressing to closed peptide nanotubes, (twisted) lamellar sheets or amyloid fibrils.

Keywords: amyloid; bionanomaterials; helical structures; peptides and peptidomimetics; protein modifications; self-assembly; β-sheet breaker peptides; supramolecular assemblies

INTRODUCTION

Amyloid formation is the general hallmark of several diseases such as Alzheimer's disease, Parkinson's disease, transmissible spongiform encephalopathies (scrapie, BSE and Creutzfeldt–Jakob disease) and diabetes type II [1]. A general feature of these diseases is the uncontrolled aggregation of proteins or polypeptides leading to the formation of amyloid fibrils [2,3]. The proteins or polypeptides that are involved in these diseases differ in their primary sequence and native folding, but share a common non-native folding topology: a cross β-sheet ((anti)parallel β-sheet). The (anti)parallel organization of β-pleated sheets leads to reduced solubility of the protein/polypeptide and subsequently to the formation of deposits of amyloid plaques.

A well-accepted approach to interfere with β-sheet formation is the design of soluble β-sheet mimics, as shown in Figure 1. These so-called β-sheet breaker peptides induce an improper hydrogen-bond network to prevent further growth of the β-sheet and are effective inhibitors of amyloid formation as was shown in the seminal papers by Tjernberg *et al.* [4] and Soto *et al.* [5].

Type II diabetes (late onset diabetes) is characterized by fibrillar protein deposits in the pancreatic islets of Langerhans [6]. The peptide that is involved in amyloid formation is the islet amyloid polypeptide (IAPP), also known as amylin [7], which consists of 37 amino acid residues and contains a single disulfide bridge, as shown in Figure 2. Amylin is coproduced and cosecreted with insulin by the islet β-cells. In states of insulin resistance, a characteristic feature of type II diabetes, amylin is overproduced, which promotes its aggregation into amyloid fibrils, and amyloid formation is thought to be a main factor responsible for β-cell failure, which ultimately leads to type II diabetes [8].

Structure–activity studies on amylin have shown that the amino acid sequence 20 to 29, H-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser-NH_2, is crucial for amyloid formation [9]. Moreover, a proline scan of this decamer [10] has demonstrated that substitution of the serine residue at position 28 by a proline efficiently reduces the capacity for amyloid fibril formation. In order to interfere with amyloid formation and to come up with β-sheet breaker peptides based on the amylin(20–29) sequence, a series of derivatives has been designed with a single [11] or a triple [12] modification of the peptide backbone (Table 1 and Figure 3).

Although amyloid formation is a highly unwanted process in living organisms, the intrinsic tendency for aggregation can also be exploited in the design of self-assembled bionanomaterials [13,14]. As a model peptide for these studies, the amylin (20–29) sequence has also been used. Variations of the molecular structure of amylin (20–29) exist, in which subtle

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‡ Journal of Peptide Science Travel Award Gdansk 2006. This Article, which has been peer-reviewed in the usual way, is based on a Poster presented at the 29th EPS Symposium in Gdansk which was identified by the EPS as outstanding, meriting an Award, and suitable in principle for publication in the Journal of Peptide Science. The full list of winners will be published at the 30th EPS Symposium in Helsinki.

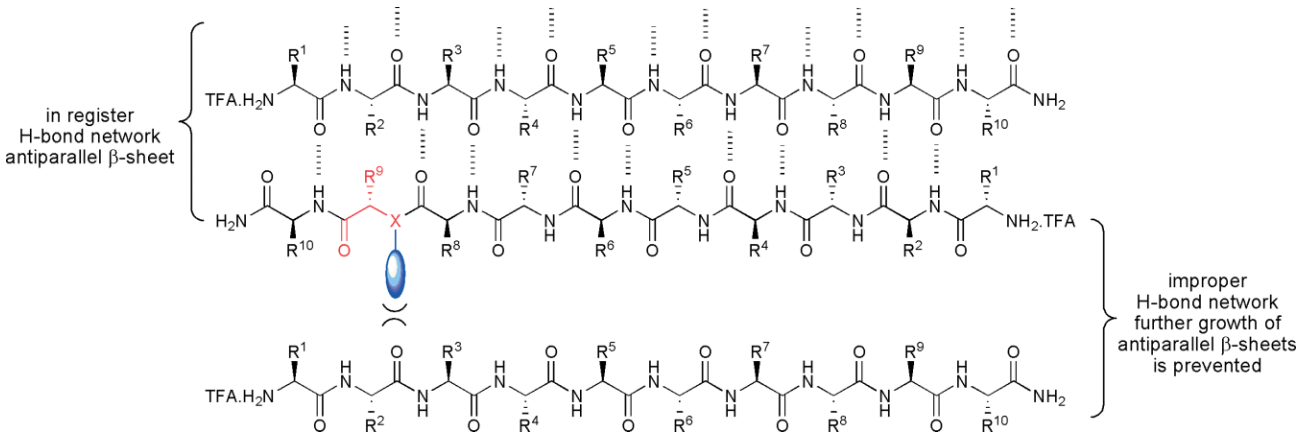

Figure 1 Rationale for the design of β-sheet-breaker peptides based on the amylin (20–29) sequence. Incorporation of steric hindrance or removal of essential hydrogen-bond donors will disrupt the hydrogen-bond network of the (anti)parallel β-sheet and arrest further growth of the β-sheet.

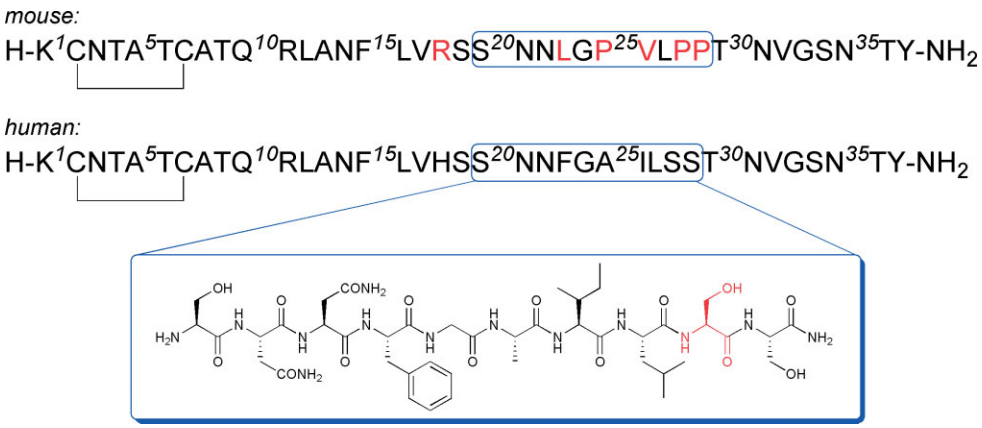

Figure 2 Amino acid sequence (one letter code) of the mouse and human amylin 1–37 peptide IAPP. Mouse amylin has five amino acid residues different from human amylin (given in red), and is, in contrast to human amylin, nonamyloidogenic. The chemical structure of the highly amyloidogenic sequence 20–29 of human amylin is shown; this sequence is used in this paper as the basis for inhibitor design or as template for the supramolecular self-assembly.

Table 1 Peptide Sequence of the Amylin (20–29) Derivatives and the Morphology of their Supramolecular Assemblies

Peptide sequence	Morphology
H-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser-NH_2 (**1**)	Amyloid fibrils
H-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-**Gly**-Ser-NH_2 (**2**)	Amyloid fibrils
H-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-**Pro**-Ser-NH_2 (**3**)	No aggregates/fibrils visible
H-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-***N*Nle**-Ser-NH_2 (**4**)	No aggregates/fibrils visible
H-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-**Glyc**-Ser-NH_2 (**5**)	Some amyloid fibrils visible
H-Ser-Asn-Asn-Phe-**Glyc**-Ala-**Ilec**-Leu-**Glyc**-Ser-NH_2 (**6**)	Helical ribbons progressing into closed tubes
H-Ser-Asn-Asn-Phe-***N*nle**-Ala-***N*Nle**-Leu-***N*Nle**-Ser-NH_2 (**7**)	No aggregates/fibrils visible
H-Ser-Asn-Asn-Phe-Gly-Ala-Ile-**βLeu**-Ser-Ser-NH_2 (**8**)	Lamellar sheets
H-Ser-Asn-Asn-Phe-Gly-Ala-Ile-**Leuψ[CH_2SO_2]**-Ser-Ser-NH_2 (**9**)	Helical ribbons progressing into closed tubes
H-Ser-Asn-Asn-Phe-Gly-Ala-Ile-**Leuψ[CH_2SO_2]*N*Bzl**-Ser-Ser-NH_2 (**10**)	Twisted lamellar sheets

modifications of the hydrogen-bonding network and precisely oriented $\pi-\pi$ stacking interactions have been designed to direct the self-assembly of these derivatives into helical ribbons and peptide nanotubes rather than amyloid fibrils [12,15]. These types of supramolecular structures can be used as designed bionanomaterials, such as insulated wires, drug delivery devices or nanochannels and nanoreactors [16].

DOI: 10.1002/psc

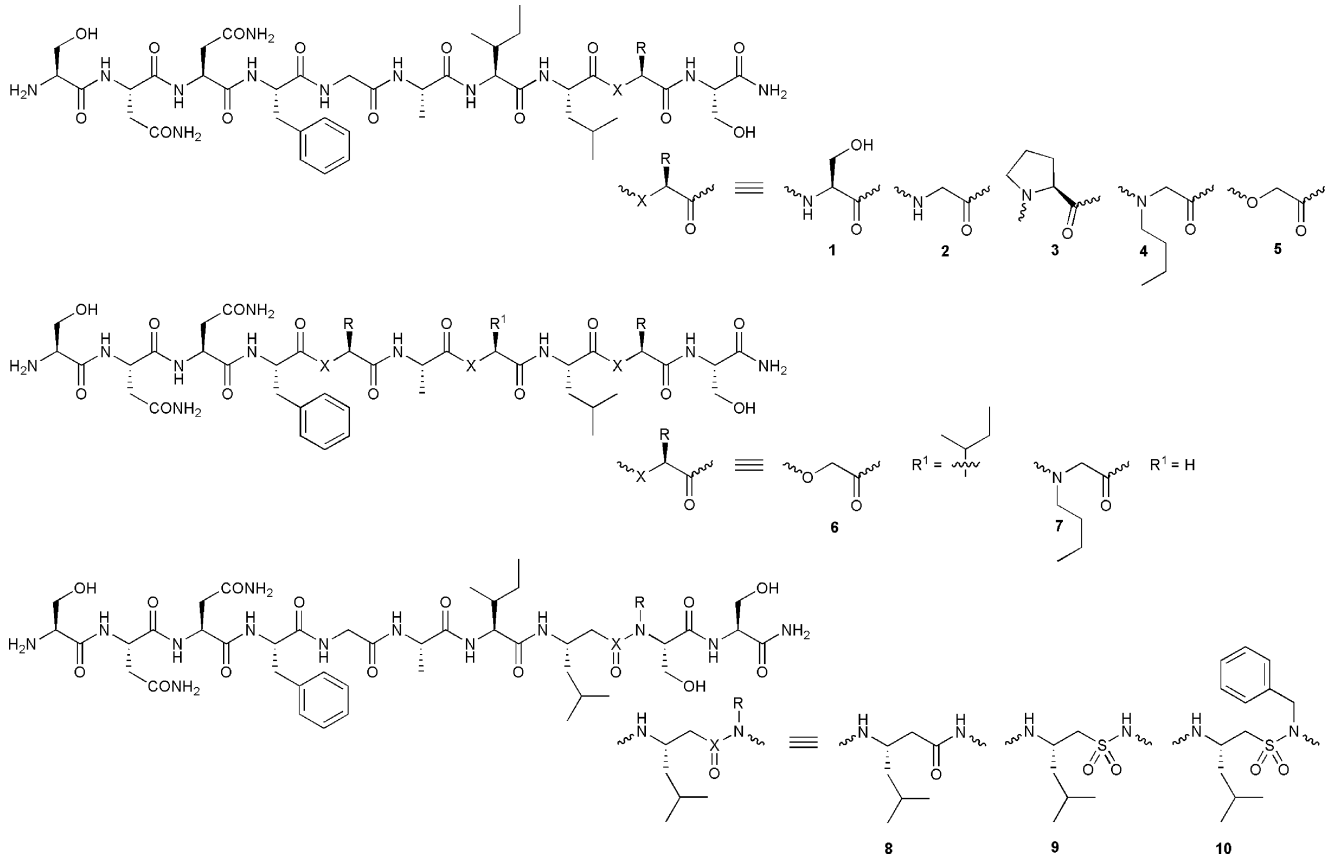

Figure 3 Chemical structures of the amylin (20–29) derivatives described in this paper.

MATERIALS AND METHODS

Peptide Synthesis

Details of the peptide synthesis, their purification by preparative HPLC and their subsequent analysis by analytical HPLC, electrospray mass spectrometry and MALDI-TOF analysis have been described recently by Elgersma *et al.* [12,15]. To summarize, peptides **1**–**3** and **8** were synthesized on an Applied Biosystems 433A peptide synthesizer using the FastMoc protocol [17] on a 0.25 mmol scale on Argogel Fmoc-RinkAmide resin to obtain *C*-terminally amidated peptides [18]. The peptides were detached from the resin and deprotected by treatment with TFA/TIS/H_2O 95 : 2.5 : 2.5 v/v/v for 3 h. The peptides were precipitated with MTBE–hexane (1 : 1 v/v) at −20 °C and finally lyophilized from *tert*-BuOH/H_2O 1 : 1 v/v. The peptoid–peptide hybrids **4** and **7** were synthesized according to the procedure described by Kruijtzer *et al.* [19]. The amino acid coupling to the secondary amine of the peptoid moiety was performed with HATU/HOAt/DIPEA [20] as coupling reagents. For the synthesis of depsipeptide **5**, the glycolic acid moiety was introduced via *O*-trityl glycolic acid as the building block using BOP/DIPEA [21] as the coupling reagent. After removal of the trityl group with dilute TFA, the resulting primary alcohol was coupled with DIC/DMAP to the next Fmoc-protected amino acid and the synthesis was completed using the Fmoc/^{t}Bu SPPS protocol [17]. The α-hydroxy acid analog of isoleucine as present in depsipeptide **6** was synthesized as described by Shin *et al.* [22] and subsequently introduced as its depsidipeptide building block Fmoc-Ala-Ilec-OH. The coupling of Fmoc-Ala-OH to the secondary hydroxyl functionality was performed using DCC/DMAP. After introduction of the second glycolic acid moiety, the synthesis was completed using the Fmoc/^{t}Bu SPPS protocol [17]. For the synthesis of the β-peptidosulfonamide–peptide hybrid **9**, the *N*-β-Fmoc-protected β-aminoethane sulfonyl chloride derivative of leucine [23] was coupled to the α-amino functionality of resin-bound H-Ser(^{t}Bu)-Ser(^{t}Bu)-RinkAmide ArgoGel in the presence of *N*-methylmorpholine in dichloromethane [24,25]. Then, the synthesis proceeded following the Fmoc/^{t}Bu SPPS protocols [17]. Alkylation of the sulfonamide moiety, as is present in the protected and resin-bound derivative of peptide **9**, to obtain the *N*-benzylated β-peptidosulfonamide-peptide hybrid **10** was performed in the solid phase using the Mitsunobu conditions [26] in the presence of DIAD, triphenylphosphine and benzyl alcohol in freshly distilled THF for 16 h at room temperature.

Transmission Electron Microscopy (TEM)

A peptide gel solution (10 mg peptide per ml in 0.1% TFA/H_2O) aged for 3 weeks (10 µl) was placed on a carbon-coated copper grid. After 15 min, any excess of peptide was removed by washing the copper grid on a drop of demi-H_2O (this was repeated four times). Finally, the samples were visualized under a Jeol 1200 EX transmission electron microscope operating at 60 kV. The magnification ranged from 10 000× to 60 000×.

DOI: 10.1002/psc

Gelation Behavior and Structural Analysis of the Supramolecular Assemblies

A peptide sample (10 mg) was dissolved in 0.1% TFA/H_2O (1 ml) at 25 °C. The aggregation state was determined by eye at regular time intervals by tilting the test tube and checking if the solution still flowed. If no flow was observed, gelation was said to have taken place.

A peptide gel solution (10 mg peptide per ml in 0.1% TFA/H_2O) aged for 3 weeks (100 µl) was lyophilized and subsequently resuspended in D_2O (150 µl) and lyophilized. This treatment was repeated twice. A peptide sample was mixed with KBr and pressed into a pellet. The optical chamber was flushed with dry nitrogen for 5 min before data collection started. The interferograms from 1000 scans with a resolution of 2 cm^{-1} were averaged and corrected for H_2O and KBr. The experimental FTIR spectra were correlated with data from the literature [27].

The CD spectra were measured at 1.0 nm intervals in the range 195–260 nm as the average of 20 runs using a spectral bandwidth of 2.0 nm in 0.5 mm cuvettes thermostatted at 20 °C with the optical chamber continually flushed with dry N_2 gas. The spectra were measured in 0.1% TFA/H_2O. The concentrations (1 mg/ml) were determined on the basis of the calculated molecular mass of the purified lyophilized peptides as the TFA salt. A peptide sample was dissolved in 0.1% TFA/H_2O and stored for 4 days at 4 °C prior to analysis.

RESULTS AND DISCUSSION

Peptide **1**, which represents amino acid residues 20 to 29 of the highly amyloidogenic fragment of amylin, was used as a control peptide for fibril formation and gelation behavior. At a concentration of 10 mg/ml in aqueous TFA (0.1% TFA/H_2O), this peptide formed a gel rapidly (within 10 min), and amyloid fibrils were visible by TEM (Figure 5(A)). The presence of amyloid fibrils was independently confirmed by FTIR and CD experiments, which showed the typical type I amide absorption band (~1631 cm^{-1}), and a minimum and a maximum at λ 225 and 205 nm, respectively (data not shown, see Ref. 15).

Peptide **2** (Ser28 → Gly) was synthesized to study the influence of the side chain on the tendency of amyloid fibril formation in relation to peptide **1** and the backbone-modified peptides **4** and **5**. It was found that amyloid formation of peptide **2** was comparable to that of the control peptide **1** (data not shown, see Ref. 11). However, peptide **3** (Ser28 → Pro) did not form amyloid fibrils and adopted a random coil conformation as judged by FTIR and CD analyses [11]. These results were in line with the data published earlier by Moriarty and Raleigh [10], who performed a proline scan of the amylin (20–29) sequence and found that the Ser28 → Pro substitution was the most effective way to inhibit amyloid formation.

These data made us decide to synthesize the peptoid–peptide hybrid **4** and depsipeptide **5** to study the influence of the absence of a single hydrogen-bond donor (**5**) or the presence of a tertiary amide (**4**) on amyloid formation. The *N*-norleucine peptoid building block can be seen as an 'open' proline analog in which the tertiary amide cannot form any hydrogen bonds. Depsipeptide **5** gelled the solution (10 mg peptide dissolved in 1 ml 0.1% TFA/H_2O) after 24 h, in contrast to a solution of the peptoid–peptide hybrid **4**, which remained fluidly for more than 3 months. TEM of the gel formed by derivative **5** evidenced the ability of the amide-to-ester replacement to retard amyloid formation due to the absence of a single hydrogen-bond donor. However, the backbone conformation in the depsipeptide, in terms of φ and ψ dihedral angles, remained virtually unaffected and the *trans* ester conformation, as is the case in native amide bonds, was still strongly preferred [28]. Possibly, as a result of this *trans* conformation, depsipeptide **5** still retained a low tendency to aggregate into amyloid fibrils [11]. However, the peptoid–peptide hybrid **4** did not form amyloid fibrils as was evidenced by TEM, FTIR and CD. Removing a single hydrogen bond but also inducing a ciscoid conformation of the peptide backbone by the *N*-alkyl glycine (peptoid) moiety resulted in the complete absence of any amyloidogenic aggregates [11].

Although a single Ser28 → Pro or Ser28 → *N*Nle mutation was able to inhibit amyloid formation of native amylin (20–29), these peptides **3** and **4** were not able to inhibit/retard fibril formation of full-length amylin (1–37) or to resolubilize preformed amylin fibrils [29]. Therefore, amylin derivatives, **6** and **7** were designed and synthesized, in which amide bonds at alternate positions have been modified by ester- and *N*-alkyl glycine moieties, respectively [12]. This concept has been used successfully by Meredith *et al.* with the Alzheimer peptide fragment Aβ(16–22) in order to obtain aggregation inhibitors [30].

A solution of depsipeptide **6** (10 mg/ml in 0.1% TFA/H_2O) formed a gel rapidly, which was rather unexpected since a single amide bond replacement (as in **5**) significantly postponed gel formation. Typical amyloid fibrils were not observed by TEM (confirmed by FTIR and CD, data not shown; see Ref. 12); however, large helical ribbons, some of them progressing even into a tube-like supramolecular nanostructure, were formed, as shown in Figure 5(B). Although neither described nor studied in depsipeptides, this morphology of helical ribbons has been observed previously as intermediate nanostructures in the self-assembly of amyloid fibrils or as final-stage folding assemblies in designed β-sheets [31,32].

As expected, the peptoid–peptide hybrid **7** rapidly dissolved in 0.1% TFA/H_2O to give a clear solution and gel formation was absent. Additionally, amyloid formation and self-assembly into supramolecular structures was absent as judged by TEM, FTIR or CD spectroscopy, which was in line with the peptoid–peptide hybrid **4**. Unfortunately, however, this amylin (20–29)

DOI: 10.1002/psc

derivative was also unable to inhibit fibril formation of full-length amylin (1–37), and preformed fibrils were also unaffected by the addition of **7**. Apparently, the highly amyloidogenic amylin (20–29) sequence is not the optimal sequence for efficient molecular recognition of amylin (1–37) or fibrils thereof, in line with the recently described results of Gazit *et al.* [33], who found that amino acid residues at the *N*-terminus are important for binding to full-length amylin.

Subtle modifications of the position and character of the hydrogen-bond donor/acceptor system will dramatically alter the tendency to form amyloid fibrils or supramolecular folding assemblies such as helical ribbons and tube-like nanostructures. Previously we reported that the incorporation of a single β-peptidosulfonamide moiety in a short helical β-peptide hexamer completely abrogated its helical structure; thus the β-peptidosulfonamide moiety acts as a helix-breaking entity [25]. This helix-breaking property of the β-peptidosulfonamide moiety inspired us to study the incorporation of β-peptidosulfonamides into amyloid peptides with a high tendency to form (anti)parallel β-sheets. Incorporation of a single β-aminoethane sulfonyl amide moiety in an all α-amino acid peptide sequence introduces an extra methylene group, thereby inducing an out-of-register hydrogen-bonding network compared to an antiparallel β-sheet, as shown in Figure 4. Moreover, the sulfonamide *O*-atoms are poor hydrogen-bond acceptors but the increased acidity of the sulfonamide N*H* makes it a better hydrogen-bond donor compared to a peptide amide moiety. This dichotomy of hydrogen-bond acceptor/donor properties can be used for the design of soluble β-sheet mimics or to direct the supramolecular folding tendency. As stated above, the increased acidity of the sulfonamide N*H* is particularly useful for the chemoselective and regiospecific alkylation of the peptide backbone [34,35]. This alkylation will result in two novel properties of these β-peptidosulfonamide–α-peptide molecular constructs: first, removal of a hydrogen-bond donor to disrupt amyloid formation, and second, the introduction of an alkyl chain to tune hydrophobicity and thus to optimize their supramolecular folding properties for material design based on self-assembly.

The solubility of peptide **8** in 0.1% TFA/H_2O was significantly lower than that of the native amylin (20–29) peptide **1**. The highly turbid solution/suspension formed a viscous gel rapidly. However, this peptide did not form amyloid fibrils since the characteristic type I amide absorption at 1631 cm^{-1} was absent as judged by FTIR, and the CD spectrum was completely different (a minimum at λ 217 nm and a maximum at λ 195 nm) compared to an (anti)parallel β-sheet as was the case with the amylin derivative **1**. Instead of fibrils, long lamellar sheets were observed with TEM (Figure 5(C)). Apparently, an extra methylene moiety in the peptide backbone inhibited amyloid formation owing to an out-of-register hydrogen-bonding network (Figure 4). In this peptide (with X = C, Figure 4), the formation of hydrogen bonds was still possible, and a combination of hydrophobic and side-chain-to-side-chain interactions together with the intrinsic chirality of the peptide backbone resulted in the supramolecular folding into lamellar sheets.

Substitution of β-leucine ((*S*)-3-amino-5-methylhexanoic acid) in peptide **8** by the β-aminoethane sulfonyl derivative of leucine to give amylin(20–29)-derived peptide **9** affects the chemical and physical properties of the amine of the serine residue at position 28 (Figures 3 and 4 (X = S)). This peptide formed a turbid solution/gel (10 mg/ml in 0.1% TFA/H_2O). On the basis of FTIR and CD measurements, it was concluded that this amylin derivative did not form amyloid fibrils. However, this β-peptidosulfonamide–peptide hybrid aggregated

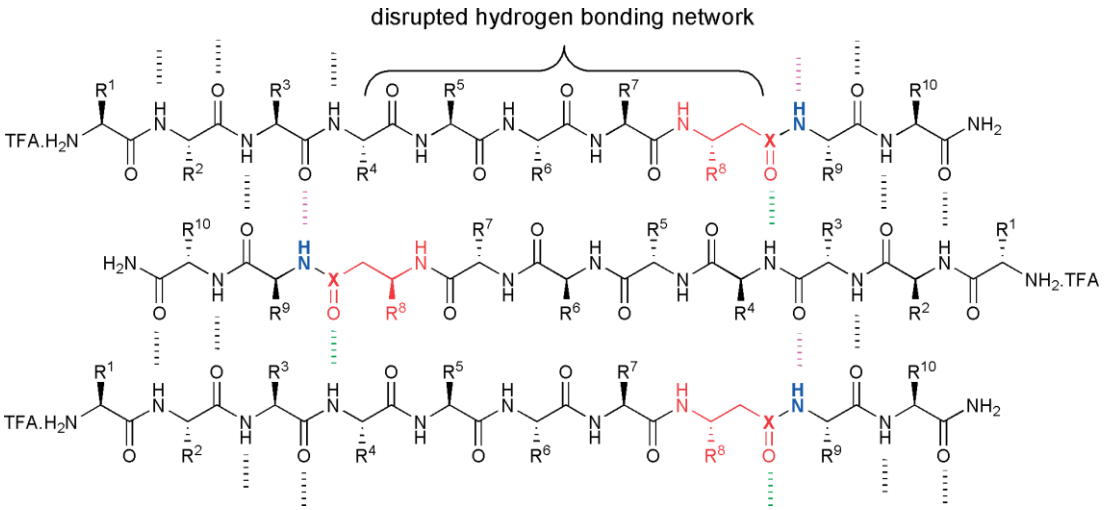

Figure 4 Structural representation and the corresponding hydrogen-bond network of an antiparallel β-sheet in which a single α-amino acid is replaced by a β-amino acid, a β-aminoethane sulfonyl amide or an *N*-alkylated sulfonamide moiety.

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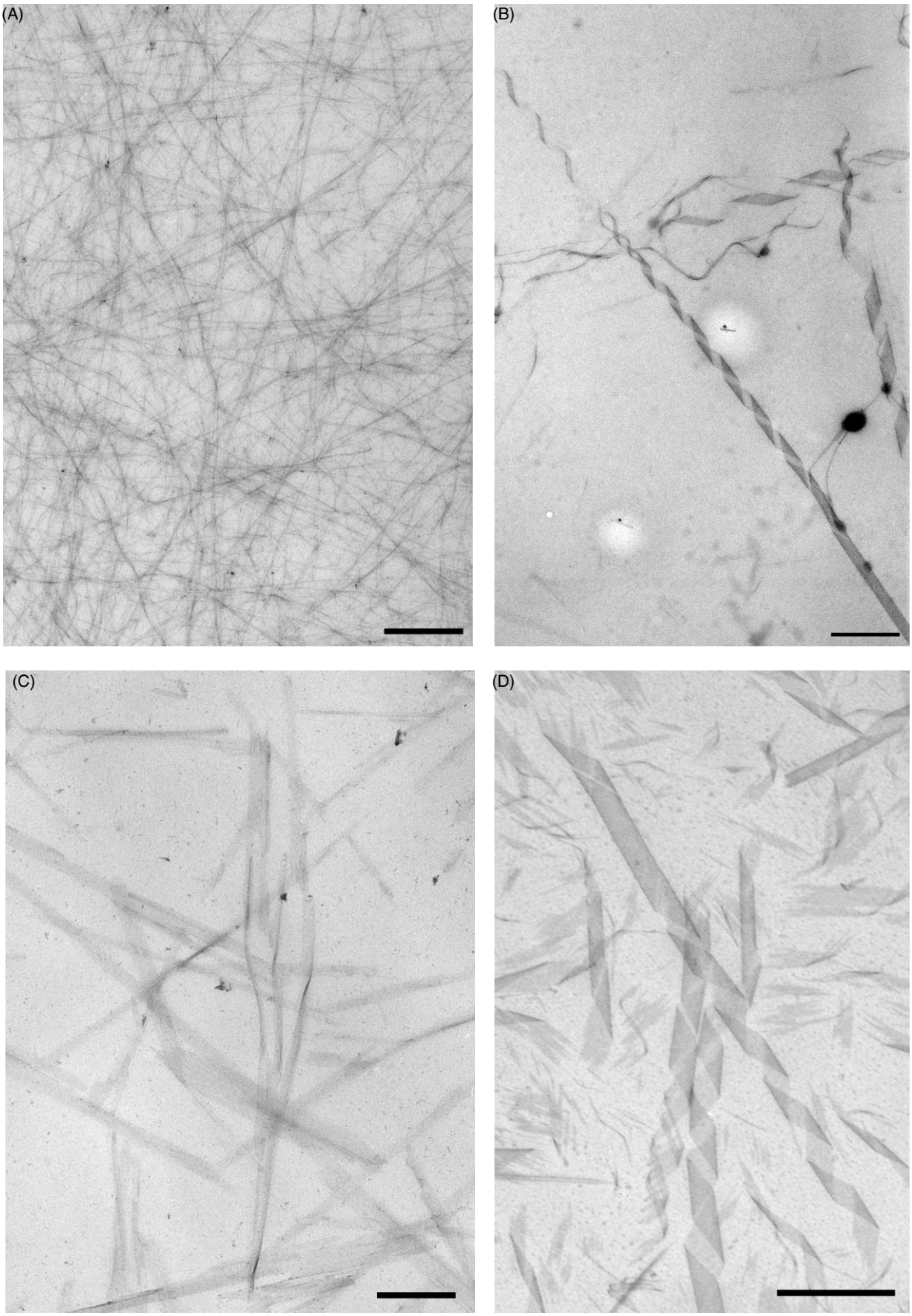

Figure 5 (A) TEM picture of the amylin (20–29) derivative **1**. Scale bar represents 500 nm. (B) TEM picture of the amylin (20–29) derivative **6**. Scale bar represents 1 μm. (C) TEM picture of the amylin (20–29) derivative **8**. Scale bar represents 500 nm. (D) TEM picture of the amylin (20–29) derivative **9**. Scale bar represents 500 nm. (E) TEM picture of the amylin (20–29) derivative **10**. Scale bar represents 500 nm.

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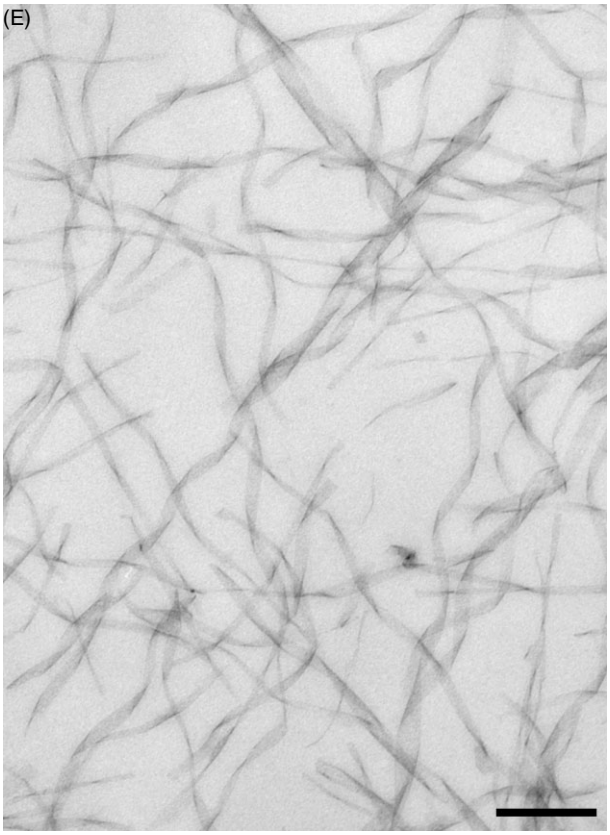

Figure 5 (*Continued*).

into helical ribbons and some of these ribbons progressed into closed peptide nanotubes of up to 12 μm in length (Figure 5(D)). As was the case in helical peptides, the β-peptidosulfonamide was also able to disrupt the secondary structure of an (anti)parallel β-sheet. These supramolecular helical ribbons were formed by a subtle interplay between hydrogen bonds, $\pi-\pi$ stacking interactions between the phenylalanine residues and the intrinsic chirality of the peptide backbone.

On the basis of these results, it was decided to synthesize the *N*-benzylated β-peptidosulfonamide-amylin (20–29) peptide hybrid **10**, to study the simultaneous effect of the absence of the hydrogen-bond donor and the presence of an extra benzyl moiety to form aromatic $\pi-\pi$ stacking interactions on the supramolecular morphology. Peptide **10** rapidly gelled a solution (10 mg/ml in 0.1% TFA/H_2O), and this amylin derivative aggregated into twisted lamellar sheets, and in some cases they were visible as closed peptide nanotubes (Figure 5(E)). The driving force of the supramolecular aggregation was not based on (anti)parallel β-sheet formation, since both FTIR and CD data clearly demonstrated the absence of any secondary structures in solution. As mentioned earlier in the literature [36], aromatic moieties, e.g. benzyl groups (the side chain of phenylalanine: $\pi-\pi$ stacking interactions), were found to be important determinants of supramolecular folding either into amyloid fibrils [37] or peptide nanotubes [38], which was nicely demonstrated in case of peptide **10**.

CONCLUSIONS

We have found that by modifying the peptide amide linkage of the amylin (20–29) sequence it is possible to alter dramatically the aggregation behavior of this amyloidogenic peptide by subtle variations in the hydrogen-bonding network. The self-assembly of the described amylin (20–29) derivatives into structures other than amyloid fibrils makes them of high value for the design of hydrogelators and novel peptide-based nanomaterials such as insulated wires, nanocontainers as drug delivery devices and nanochannels and nanoreactors.

Acknowledgement

These investigations were supported by the Council for Chemical Sciences of the Netherlands Organization of Scientific Research (CW-NWO).

REFERENCES

1. Rochet J-C, Lansbury PT Jr. Amyloid fibrillogenesis: themes and variations. *Curr. Opin. Struct. Biol.* 2000; **10**: 60–68.
2. Glenner GG, Wong CW. Alzheimer's disease and down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. *Biochem. Biophys. Res. Commun.* 1984; **122**: 1131–1135.
3. Koo EH, Lansbury PT Jr, Kelly JW. Amyloid diseases: abnormal protein aggregation in neurodegeneration. *Proc. Natl. Acad. Sci. U.S.A.* 1999; **96**: 9989–9990.
4. Tjernberg LO, Näslund J, Lindqvist F, Johansson J, Karlström AR, Thyberg J, Terenius L, Nordstedt C. Arrest of β-amyloid fibril formation by a pentapeptide ligand. *J. Biol. Chem.* 1996; **271**: 8545–8548.
5. Soto C, Sigurdsson EM, Morelli L, Kumar RA, Castaño EM, Frangione B. β-Sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: implications for alzheimer's therapy. *Nat. Med.* 1998; **4**: 822–826.
6. Höppener JWM, Ahrén B, Lips CJM. Islet amyloid type 2 diabetes mellitus. *N. Engl. J. Med.* 2000; **343**: 411–419.
7. Kapurniotu A. Amyloidogenicity and cytotoxicity of islet amyloid polypeptide. *Biopolymers (Pept. Sci.)* 2001; **60**: 438–459.
8. Lorenzo A, Razzaboni B, Weir GC, Yankner BA. Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. *Nature* 1994; **368**: 756–760.
9. Westermark P, Engström U, Johnson KH, Westermark GT, Betsholtz C. Islet amyloid polypeptide: pinpointing amino acid residues linked to amyloid fibril formation. *Proc. Natl. Acad. Sci. U.S.A.* 1990; **87**: 5036–5040.
10. Moriarty DF, Raleigh DP. Effects of sequential proline substitutions on amyloid formation by human amylin$_{20-29}$. *Biochemistry* 1999; **38**: 1811–1818.
11. Rijkers DTS, Höppener JWM, Posthuma G, Lips CJM, Liskamp RMJ. Inhibition of amyloid fibril formation of human amylin by *N*-alkylated amino acid and α-hydroxy acid residue containing peptides. *Chem. Eur. J.* 2002; **8**: 4285–4291.
12. Elgersma RC, Meijneke T, Posthuma G, Rijkers DTS, Liskamp RMJ. Self-assembly of amylin(20–29) amide-bond derivatives into helical

ribbons and peptide nanotubes rather than fibrils. *Chem. Eur. J.* 2006; **12**: 3714–3725.
13. Zhang S. Fabrication of novel biomaterials through molecular self-assembly. *Nat. Biotechnol.* 2003; **21**: 1171–1178.
14. Rajagopal K, Schneider JP. Self-assembling peptides and proteins for nanotechnological applications. *Curr. Opin. Struct. Biol.* 2004; **14**: 480–486.
15. Elgersma RC, Meijneke T, de Jong R, Brouwer AJ, Posthuma G, Rijkers DTS, Liskamp RMJ. Synthesis and structural investigations of *N*-alkylated β-peptidosulfonamide-peptide hybrids of the amyloidogenic amylin (20–29) sequence: implications of supramolecular folding for the design of peptide-based bionanomaterials. *Org. Biomol. Chem.* 2006; **4**: 3587–3597.
16. Gao X, Matsui H. Peptide-based nanotubes and their applications in bionanotechnology. *Adv. Mat.* 2005; **17**: 2037–2050.
17. Fields CG, Lloyd DH, Macdonald RL, Otteson KM, Noble RL. HBTU activation for automated Fmoc solid-phase peptide synthesis. *Pept. Res.* 1991; **4**: 95–101.
18. Rink H. Solid-phase synthesis of protected peptide fragments using a trialkoxy-diphenyl-methylester resin. *Tetrahedron Lett.* 1987; **28**: 3787–3790.
19. Kruijtzer JAW, Hofmeyer LJF, Heerma W, Versluis C, Liskamp RMJ. Solid-phase synthesis of peptoids using Fmoc-protected *N*-substituted glycines: the synthesis of (retro)peptoids of Leu-enkephalin and substance P. *Chem. Eur. J.* 1998; **4**: 1570–1580.
20. Carpino LA, El-Faham A, Minor CA, Albericio F. Advantageous applications of azabenzotriazole (triazolopyridine)-based coupling reagents to solid phase peptide synthesis. *J. Chem. Soc., Chem. Commun.* 1994; 201–203.
21. Castro B, Dormoy JR, Evin G, Selve C. Reactifs de couplage peptidique IV (1)-L'hexafluorophosphate de benzotriazolyl *N*-oxytrisdimethylamino phosphonium (B.O.P.). *Tetrahedron Lett.* 1975; **16**: 1219–1222.
22. Shin I, Lee M-R, Lee J, Jung M, Lee M, Yoon J. Synthesis of optically active phthaloyl D-aminooxy acid from L-amino acids or L-hydroxy acids as building blocks for the preparation of aminooxy peptides. *J. Org. Chem.* 2000; **65**: 7667–7675.
23. Brouwer AJ, Monnee MCF, Liskamp RMJ. An efficient synthesis of *N*-protected β-aminoethanesulfonyl chlorides: versatile building blocks for the synthesis of oligopeptidosulfonamides. *Synthesis* 2000; 1579–1584.
24. Monnee MCF, Marijne MF, Brouwer AJ, Liskamp RMJ. A practical solid phase synthesis of oligopeptidosulfonamide foldamers. *Tetrahedron Lett.* 2000; **41**: 7991–7995.
25. de Jong R, Rijkers DTS, Liskamp RMJ. Automated solid-phase synthesis and structural investigation of β-peptidosulfonamides and β-peptidosulfonamide/β-peptide hybrids: β-peptidosulfonamide and β-peptide foldamers are two of a different kind. *Helv. Chim. Acta* 2002; **85**: 4230–4243.
26. Mitsunobu O. The use of diethyl azadicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis* 1981; 1–28.
27. Jackson M, Mantsch HH. The use and misuse of FTIR spectroscopy in the determination of protein structure. *Crit. Rev. Biochem. Mol. Biol.* 1995; **30**: 95–120.
28. Deechongkit S, Dawson PE, Kelly JW. Toward assessing the position-dependent contributions of backbone hydrogen bonding to β-sheet folding thermodynamics employing amide-to-ester perturbations. *J. Am. Chem. Soc.* 2004; **126**: 16762–16771.
29. Rijkers DTS, Höppener JWM, Posthuma G, Lips CJM, Liskamp RMJ. Inhibition of amyloid formation of human amylin. In *Peptide Revolution: Genomics, Proteomics and Therapeutics: Proceedings of the 18th American Peptide Symposium*, Chorev M, Sawyer TK (eds.). Kluwer Academic Publishers: Dordrecht, 2003; 773–774.
30. Gordon DJ, Meredith SC. Probing the role of backbone hydrogen bonding in β-amyloid fibrils with inhibitor peptide containing ester bonds at alternate positions. *Biochemistry* 2003; **42**: 475–485.
31. Deechongkit S, Powers ET, You S-L, Kelly JW. Controlling the morphology of cross β-sheet assemblies by rational design. *J. Am. Chem. Soc.* 2005; **127**: 8562–8570.
32. Aggeli A, Nyrkova IA, Bell M, Harding R, Carrick L, McLeish TCB, Semenov AN, Boden N. Hierarchical self-assembly of chiral rod-like molecules as a model for peptide β-sheet tapes, ribbons, fibrils, and fibers. *Proc. Natl. Acad. Sci. U.S.A.* 2001; **98**: 11857–11862.
33. Mazor Y, Gilead S, Benhar I, Gazit E. Identification and characterization of a novel molecular-recognition and self-assembly domain within the islet amyloid polypeptide. *J. Mol. Biol.* 2002; **322**: 1013–1024.
34. Miller SC, Scanlan TS. Site-selective *N*-methylation of peptides on solid support. *J. Am. Chem. Soc.* 1997; **119**: 2301–2302.
35. Reichwein JF, Liskamp RMJ. Site-specific *N*-alkylation of peptides on the solid phase. *Tetrahedron Lett.* 1998; **39**: 1243–1246.
36. Makin OS, Atkins E, Sikorski P, Johansson J, Serpell LC. Molecular basis for amyloid fibril formation and stability. *Proc. Natl. Acad. Sci. U.S.A.* 2005; **102**: 315–320.
37. Azriel R, Gazit E. Analysis of the minimal amyloid-forming fragment of the islet amyloid polypeptide. An experimental support for the key role of the phenylalanine residue in amyloid formation. *J. Biol. Chem.* 2001; **276**: 34156–34161.
38. Reches M, Gazit E. Casting metal nanowires within discrete self-assembled peptide nanotubes. *Science* 2003; **300**: 625–627.

DOI: 10.1002/psc