

Morin hydrate inhibits amyloid formation by islet amyloid polypeptide and disaggregates amyloid fibers

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Abstract: The polypeptide hormone Islet Amyloid Polypeptide (IAPP, amylin) is responsible for islet amyloid formation in type-2 diabetes and in islet cell transplants, where it may contribute to graft failure. Human IAPP is extremely amyloidogenic and fewer inhibitors of IAPP amyloid formation have been reported than for the Alzheimer's A β peptide or for α -synuclein. The ability of a set of hydroxyflavones to inhibit IAPP amyloid formation was tested. Fluorescence detected thioflavin-T-binding assays are the most popular methods for measuring the kinetics of amyloid formation and for screening potential inhibitors; however, we show that they can lead to false positives with hydroxyflavones. Several of the compounds inhibit thioflavin-T fluorescence, but not amyloid formation; a result which highlights the hazards of relying solely on thioflavin-T assays to screen potential inhibitors. Transmission electron microscopy (TEM) and right-angle light scattering show that Morin hydrate (2',3,4',5,7-Pentahydroxyflavone) inhibits amyloid formation by human IAPP and disaggregates preformed IAPP amyloid fibers. In contrast, Myricetin, Kaempferol, and Quercetin, which differ only in hydroxyl groups on the B-ring, are not effective inhibitors. Morin hydrate represents a new type of IAPP amyloid inhibitor and the results with the other compounds highlight the importance of the substitution pattern on the B-ring.

Keywords: amylin; amyloid inhibitors; islet amyloid polypeptide; Morin hydrate; hydroxyflavone, thioflavin-T

Introduction

Amyloid formation plays an important role in a broad range of different human diseases including Alzheimer's disease, Parkinson's disease, and type-2 diabetes.^{1–3} The search for inhibitors of amyloid fiber formation is an active area of research and par-

ticular attention has been paid to flavanoids.^{4–27} Here, we examine the ability of a set of hydroxyflavones to inhibit amyloid formation by (islet amyloid polypeptide (IAPP, Amylin)).

IAPP is a member of the calcitonin-like family of peptides and is found in all mammalian species examined.^{28–31} The 37-residue polypeptide hormone is packaged in the insulin secretory granule and normally acts as an endocrine partner to insulin.^{30,32,33} IAPP aggregates to form islet amyloid in type-2 diabetes; a process which is widely believed to contribute to β -cell death in the disease.^{23,28,29,32,34–40} Recent studies also highlight a potentially important role for IAPP amyloid formation in the failure of islet cell grafts.^{41–43}

There is widespread interest in developing inhibitors of amyloid formation and compounds which can disaggregate preformed amyloid. Much of this work has focused on the A β peptide of

Abbreviations: DDI, distilled and deionized; Fmoc, 9-fluorenylmethoxycarbonyl; IAPP, islet amyloid polypeptide; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; PAL-PEG, 5-(4'-Fmoc-aminomethyl-3',5-dimethoxyphenyl) valeric acid; TEM, transmission electron microscopy; TFA, trifluoroacetic acid.

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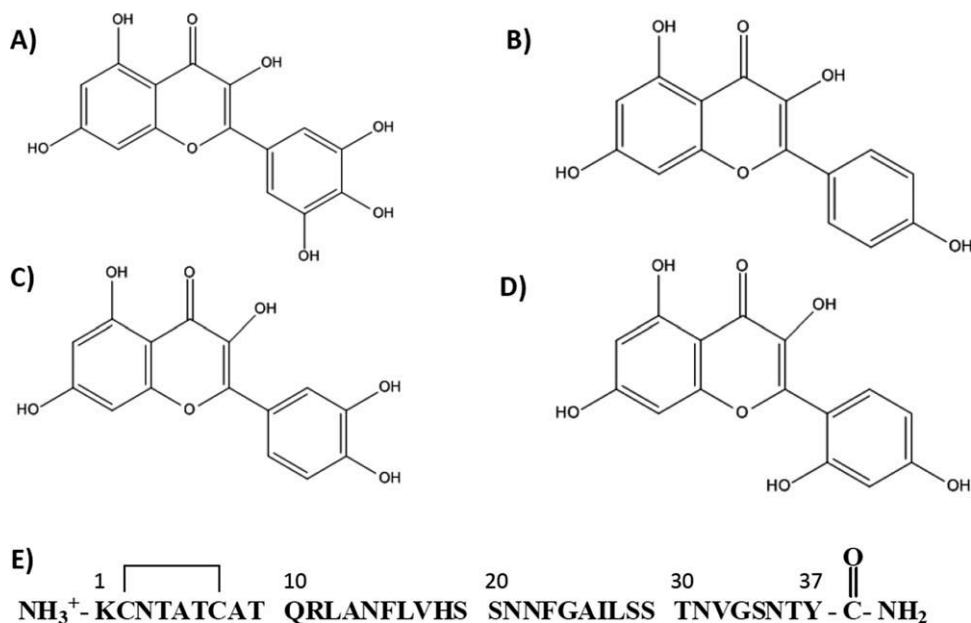


Figure 1. Structures of the compounds studied together with the primary sequence of IAPP. (A) Myricetin, (B) Kaempferol, (C) Quercetin, (D) Morin hydrate, and (E) primary structure of human IAPP. The peptide has an amidated C-terminus and a disulfide bond involving Cys-2 and Cys-7.

Alzheimer's disease or on α -synuclein, the protein which plays a central role in Parkinson's disease, but fewer inhibitors of IAPP amyloid formation have been reported.⁴⁻²⁷ IAPP is extremely amyloidogenic and is thus a challenging target for inhibition studies. Certain hydroxyflavones have been reported to be effective inhibitors of amyloid formation by the A β polypeptide although there are conflicting reports about their effectiveness.⁴⁴⁻⁴⁶ Their ability to inhibit amyloid formation by IAPP has not yet been examined. Here, we test the ability of a set of four related hydroxyflavones, Morin hydrate (2',3,4',5,7-Pentahydroxyflavone), Myricetin (3,3',4',5,5',7-Hexahydroxyflavone), Kaempferol (3,4',5,7-Tetrahydroxyflavone), and Quercetin (3,3',4',5,6-Pentahydroxyflavone), to inhibit amyloid formation by IAPP and to disaggregate already assembled IAPP amyloid fibers. Morin hydrate is shown to be an effective inhibitor, whereas the other compounds are not as effective. We also show that these compounds can compromise standard fluorescence assays of amyloid formation and, in some cases, can lead to false positives in inhibitor screens.

Results

Hydroxyflavones can interfere with standard fluorescence assays of amyloid formation

The structures of the compounds examined in this study are shown in Figure 1 together with the primary structure of IAPP. The compounds share the same core structure; they are polyhydroxylated and consist of a 12-membered heterocyclic ring linked to the 6-membered aromatic B-ring (Fig. 1).

Amyloid formation by IAPP displays a lag phase during which no detectable amyloid fibrils are formed followed by a growth phase which leads to a steady state in which amyloid fibrils are in equilibrium with soluble peptide [Fig. 2(A)]. The rate of amyloid formation is typically measured using fluorescence-detected thioflavin-T-binding assays. Thioflavin-T is believed to bind to the surface of amyloid fibers in grooves formed by the alignment of side chains in the cross β -sheet structure. Binding to an amyloid fiber increases the fluorescence quantum yield of the dye by restricting the rotation of the benzamidine and benzothiazole rings and thus reducing self-quenching.⁴⁷ Thioflavin-T assays are simple and convenient, but problems may arise in the studies of inhibitors because compounds which appear to inhibit amyloid formation could just be inhibitors of thioflavin-T fluorescence. This may occur because they absorb strongly which leads to inner filter effects, or because they quench the bound thioflavin-T fluorescence, or because they inhibit thioflavin-T binding to amyloid.^{48,49}

The compounds under study here have the potential to interfere with thioflavin-T assays. Figure 2 shows the results of a thioflavin-T-monitored kinetic experiment in the presence of Myricetin. In the absence of the compound, a typical IAPP kinetic curve is observed [Fig. 2(A), solid circles]. Very different behavior is observed in its presence. There is only a very small change in fluorescence over the entire time course of the experiment [Fig. 2(A), open square]. Taken alone, these results could imply that Myricetin is a potent amyloid inhibitor; however, transmission electron microscopy (TEM) images

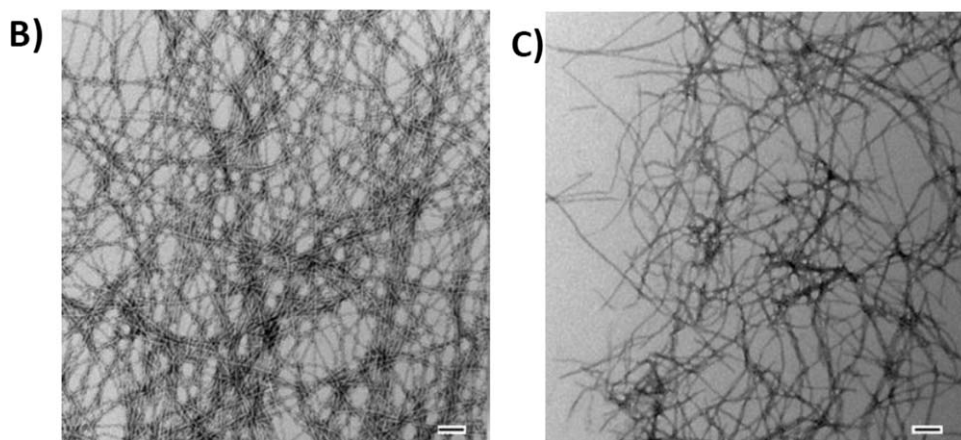
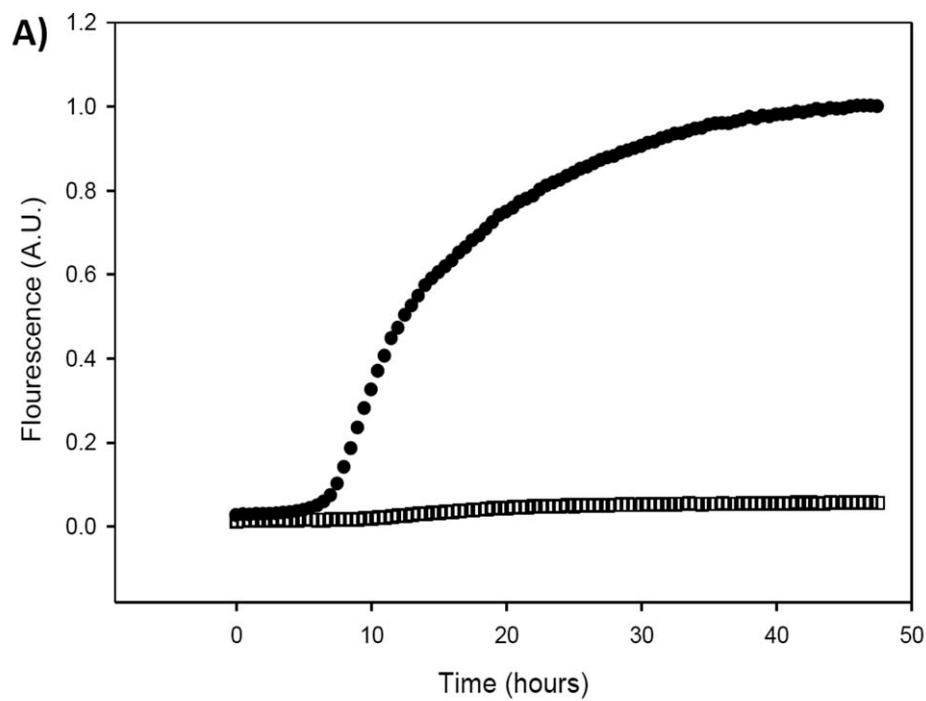


Figure 2. Hydroxyflavones interfere with thioflavin-T-based assays. (A) Thioflavin-T-monitored kinetic experiments. Black circles, IAPP alone. Black open squares, IAPP plus an equimolar amount of Myricetin. (B) TEM image collected at the end of the reaction for the IAPP sample. (C) TEM image collected at the end of the reaction for the 1:1 mixture of IAPP and Myricetin. Scale bars represent 100 nm. Experiments were conducted at 25°C, pH 7.4, 20 mM Tris-HCl, 32 μ M IAPP, 0.25% DMSO (v/v).

collected from samples removed at the end of the reaction revealed the presence of amyloid fibers in the IAPP control sample [Fig. 2(B)] and in the 1:1 mixture of Myricetin and IAPP [Fig. 2(C)]. Thus, Myricetin is actually not an effective IAPP amyloid inhibitor. Similar results were obtained with Kaempferol and Quercetin (Supporting Information). We suspect that these sorts of effects might account for some of the discrepancy in the literature on the potency of this class of compounds in inhibiting other amyloids.^{44–46} The compounds can also interfere with disaggregation studies. Experiments in which Myricetin is added to a sample, which contains IAPP amyloid fibers and thioflavin-T, show that Myricetin interferes with thioflavin-T-based disaggregation

assays. When the compound is added, an immediate drop in fluorescence is observed, but TEM confirms that fibers are still present (Supporting Information).

Morin hydrate, but not Myricetin, Kaempferol, or Quercetin is an effective inhibitor of IAPP amyloid fiber formation

The experiments described in the previous section clearly show that thioflavin-T-based inhibitor assays are not reliable for these hydroxyflavones. Thus, we tested their ability to inhibit IAPP amyloid formation using TEM. Compounds were incubated with IAPP for 48 h and TEM images were recorded. The incubation period is longer than that required for

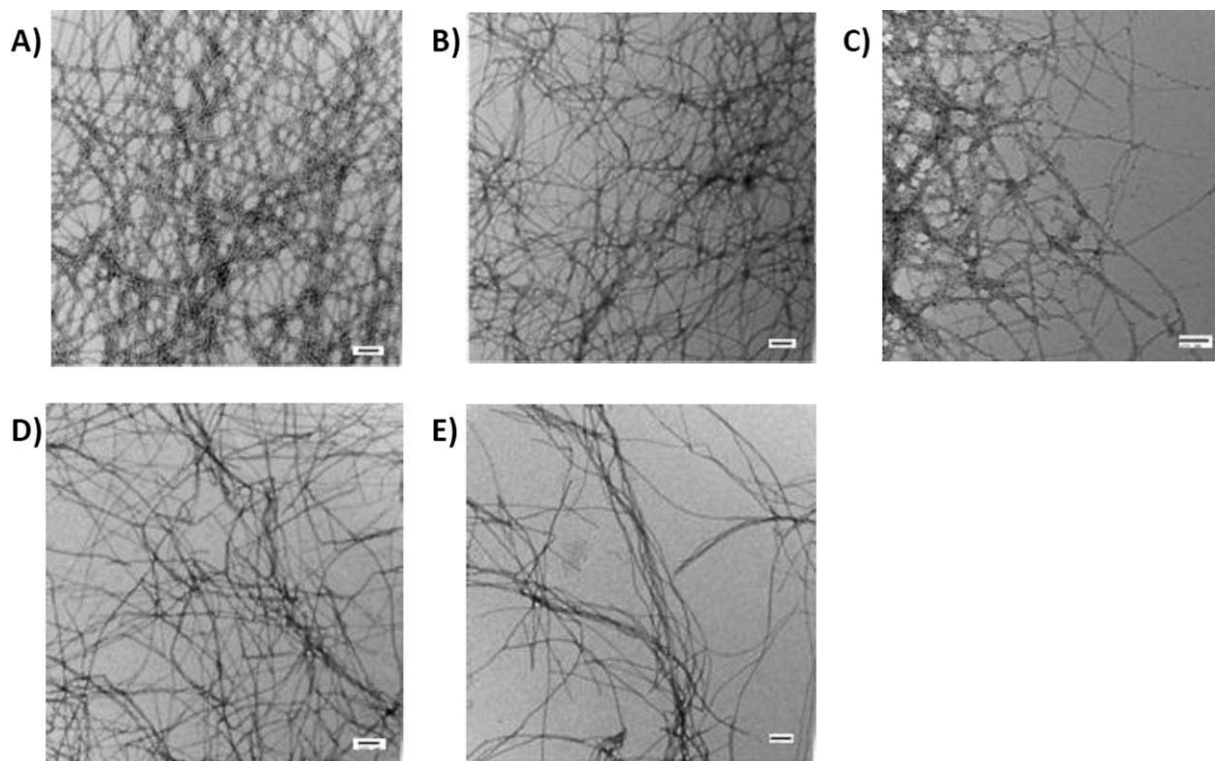


Figure 3. Morin Hydrate, but not Myricetin, Kaempferol, or Quercetin, inhibits amyloid formation by IAPP. TEM images shown are of the samples collected at 48 h after the initiation of amyloid formation. (A) IAPP alone, (B) IAPP:Myricetin = 1:1, (C) IAPP:Kaempferol = 1:1, (D) IAPP:Quercetin = 1:1, and (E) IAPP:Morin hydrate = 1:1. Experiments were conducted at 25°C, pH 7.4, 20 mM Tris-HCl, 32 μ M IAPP, 0.25% DMSO (v/v). Inhibitors, when present, were at 32 μ M. Scale bars represent 100 nm.

IAPP to form amyloid under these conditions [Fig. 2(A), black circles] and dense collections of amyloid fibers are observed in a control sample of IAPP without compound [Fig. 3(A)]. Some aggregated material is observed in the 1:1 mixtures of IAPP with all four of the compounds [Fig. 3(B–E)]. Amyloid fibers are visible in the TEM images of Kaempferol, Quercetin, and Myricetin [Fig. 3(B–D)]; however, the objects detected in the presence of Morin hydrate are thinner and they appeared to be less abundant [Fig. 3(E)]. These studies encouraged us to examine the effect of different concentrations of Morin hydrate on amyloid formation by IAPP.

The TEM micrographs shown in Figure 4 compare the results of 1:1, 1:2, 1:5, and 1:10 mixtures of IAPP and Morin hydrate. In all cases, Morin hydrate was added at time equals zero and the samples were incubated for 48 h. Dense mats of fibers are observed in the control sample of IAPP without Morin hydrate [Fig. 4(A)]. Adding Morin hydrate in a twofold excess leads to more pronounced effects than was observed with the 1:1 mixture [compare Fig. 4(B,C)]. The objects which are detected are even thinner and less abundant in the twofold excess sample relative to the 1:1 sample. Addition of a five or tenfold excess of Morin hydrate led to even more pronounced changes. Very short objects together

with some amorphous aggregates are detected on the grids [Fig. 4(D,E)]. We also examined the other compounds at higher ratios, but they still failed to inhibit amyloid formation by IAPP (Supporting Information).

We conducted right-angle light scattering experiments to obtain an independent test of the ability of Morin hydrate to inhibit IAPP amyloid formation and to confirm that these compounds can interfere with thioflavin-T assays. The curve recorded in the absence of inhibitor shows a sigmoidal transition which closely follows the time course of the thioflavin-T studies [Fig. 5(A)]. TEM confirms that amyloid fibers are formed [Fig. 5(B)]. Very different results were obtained when Morin hydrate was present at a fivefold excess relative to IAPP. No change in signal was observed over the course of the experiment, and TEM confirmed the lack of amyloid [Fig. 5(A,C)]. We also used right-angle light scattering to examine the effects of Myricetin on IAPP amyloid formation. The light scattering curve recorded in the presence of Myricetin resembles the curve obtained from the sample of pure IAPP and is very different from the data obtained in the presence of Morin hydrate. TEM confirms that amyloid fibers are formed [Fig. 5(D)]. These experiments further verify that Myricetin is not an effective inhibitor of

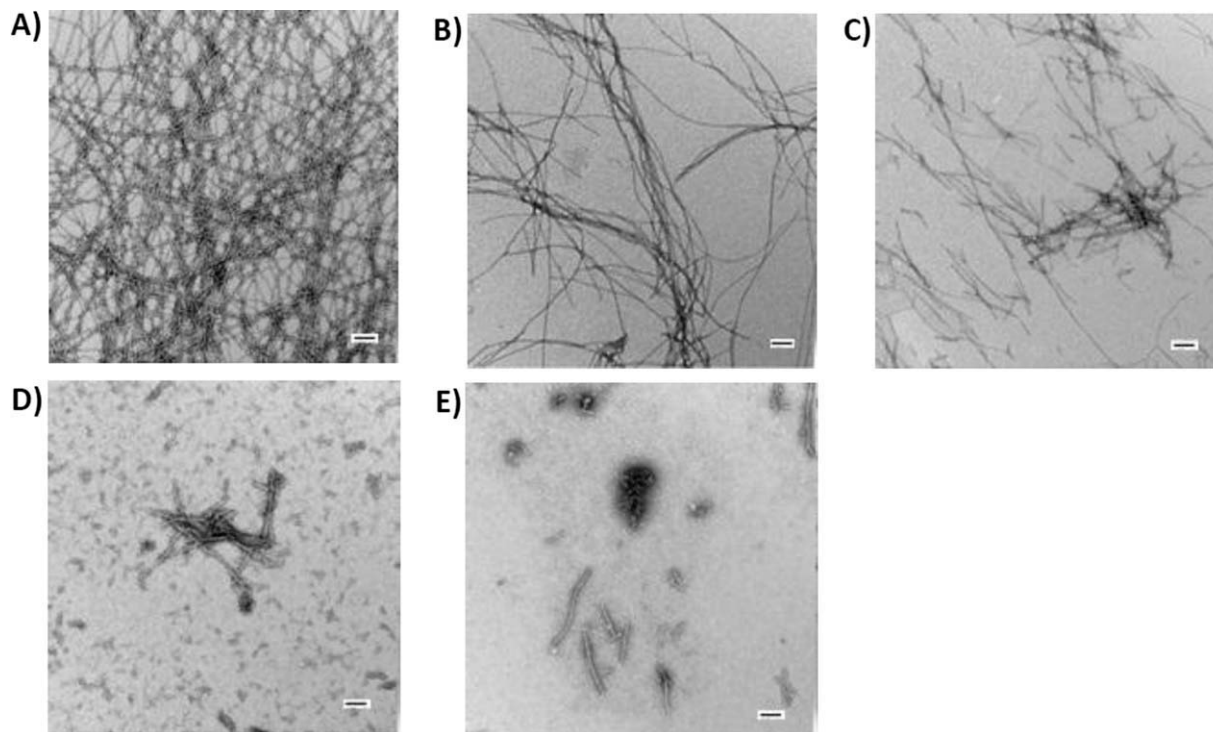


Figure 4. The effects of Morin hydrate are concentration dependent. TEM images shown are of the samples collected at 48 h after the initiation of amyloid formation. (A) IAPP alone, (B) 1:1 mixture of IAPP and Morin hydrate, (C) 1:2 mixture of IAPP and Morin hydrate, (D) 1:5 mixture of IAPP and Morin hydrate, and (E) 1:10 mixture of IAPP and Morin hydrate. Experiments were conducted at 25°C, pH 7.4, 20 mM Tris-HCl, 32 μ M IAPP, 0.25% DMSO (v/v). The concentration of Morin hydrate, when present, ranged from 32 to 320 μ M. Scale bars represent 100 nm.

IAPP amyloid formation and, even more importantly, confirm that Morin hydrate is an inhibitor.

Morin hydrate disaggregates IAPP amyloid fibers

We next examined the ability of Morin hydrate to disaggregate preformed IAPP fibers (Fig. 6). A sample of IAPP was incubated for 45 h and a fivefold excess of Morin hydrate was added [Fig. 6(A), black arrow]. The thioflavin-T curve is included simply to illustrate that the amyloid reaction had reached the plateau region before the compound was added. Thioflavin-T fluorescence cannot be used to follow the kinetics of disassembly because the hydroxyflavones interfere with thioflavin-T assays. TEM images of a sample removed just before the addition of the compound revealed dense mats of amyloid fibers [Fig. 6(B)]. Additional aliquots were removed for TEM analysis starting 1 h after addition of Morin hydrate [Fig. 6(A), colored arrows]. No fibers were detected in the TEM images of these samples (Fig. 6C-F).

Morin hydrate also disaggregates IAPP amyloid fibers when it is added at a ratio of 1:1. In this case, the dense collection of amyloid fibers is disrupted to produce a smaller amount of thinner and shorter fibers (Supporting Information) which bear some resemblance to the species observed when Morin

hydrate is added to an IAPP solution at time equals zero. However, disaggregation does not have to be the reverse of amyloid fiber formation and has been shown to be different for at least one inhibitor.¹⁸

Discussion

The results presented here highlight the difficulty of using thioflavin-T-based assays to test for amyloid inhibitors and for compounds which disaggregate amyloid fibers. They reiterate the importance of conducting experiments which directly test for the presence of amyloid fibers. Here, we used TEM to test for the presence of amyloid and light scattering to monitor the kinetics of aggregation. The data show that Morin hydrate inhibits amyloid formation by IAPP and demonstrate that it can disassemble IAPP amyloid fibers. The compound represents a new class of IAPP amyloid inhibitors. The ability of this class of compounds to inhibit amyloid formation by IAPP appears to be strongly dependent on the substitution pattern in the B-ring because the other compounds tested were much less effective.

There are compounds that inhibit amyloid formation by both IAPP and A β .^{4,13,18} In contrast, some of the hydroxyflavones studies here have different effects on the A β peptide. In particular, Myricetin has been reported to inhibit A β amyloid

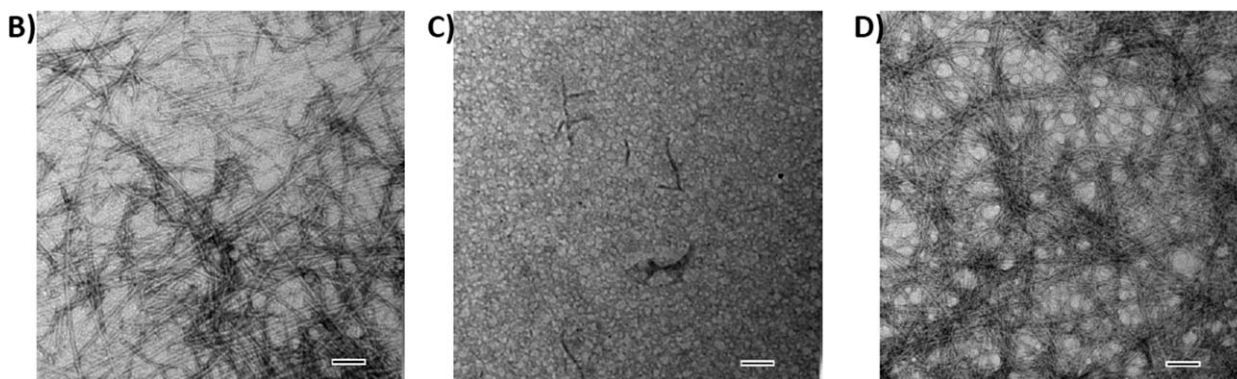
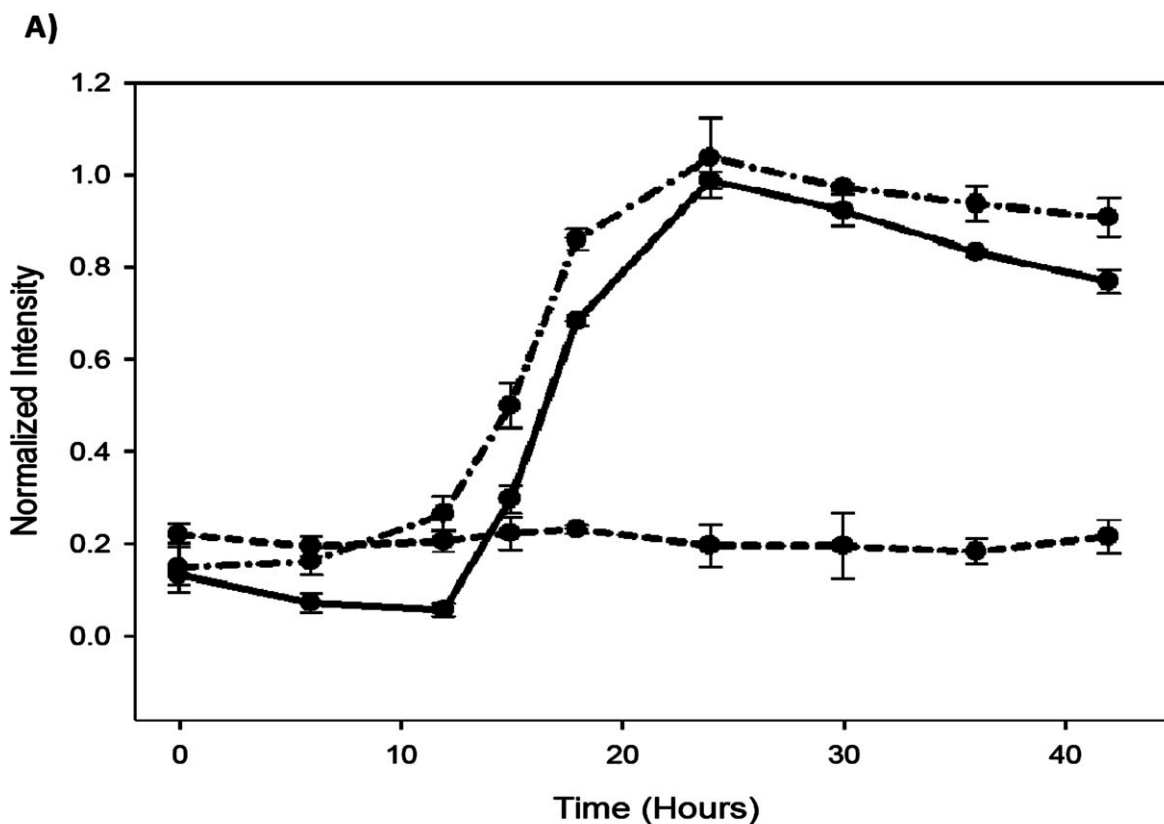


Figure 5. Right-angle light scattering confirms that Morin hydrate, but not Myricetin, inhibits IAPP amyloid formation. (A) Light scattering: solid line (—) IAPP alone, dashed line (---) IAPP plus Morin hydrate, and dashed-dotted line (-.-.-) IAPP plus Myricetin. (B) TEM image of the sample of IAPP without compound. Sample was removed at 42 h. (C) TEM image of a sample from the experiment conducted in the presence of Morin hydrate in fivefold excess. Sample was removed at 42 h. (D) TEM image of a sample from the experiment conducted in the presence of Myricetin in fivefold excess. Sample was removed at 42 h. All samples contained 32 μM IAPP, 0.25% DMSO (v/v) 20 mM Tris-HCl, pH 7.4. The signal was monitored at 500 nm. Experiments were conducted at 25°C. Scale bars in TEM images represent 100 nm. The light scattering data are the result of three separate measurements for each sample. The error bars represent the standard deviation of each data point.

formation,^{44–46} but the data presented here show that it is not effective against IAPP. Several hydroxyflavones, in addition to Myricetin, have been tested for their ability to inhibit amyloid formation by the A β peptide and there are differing reports of their effectiveness.^{44–46} The difficulty of quantitatively interpreting thioflavin-T fluorescence intensities in the presence of the hydroxyflavones may contribute to the apparent differences. These compounds interfere with thioflavin-T assays of amyloid formation by

IAPP, thus perhaps they could also interfere or partially interfere with the assays of A β amyloid formation.

Materials and Methods

Peptide synthesis

IAPP was synthesized using a CEM Liberty Automatic Microwave Peptide Synthesizer and 9-fluorenylmethoxycarbonyl (Fmoc) solid-phase peptide

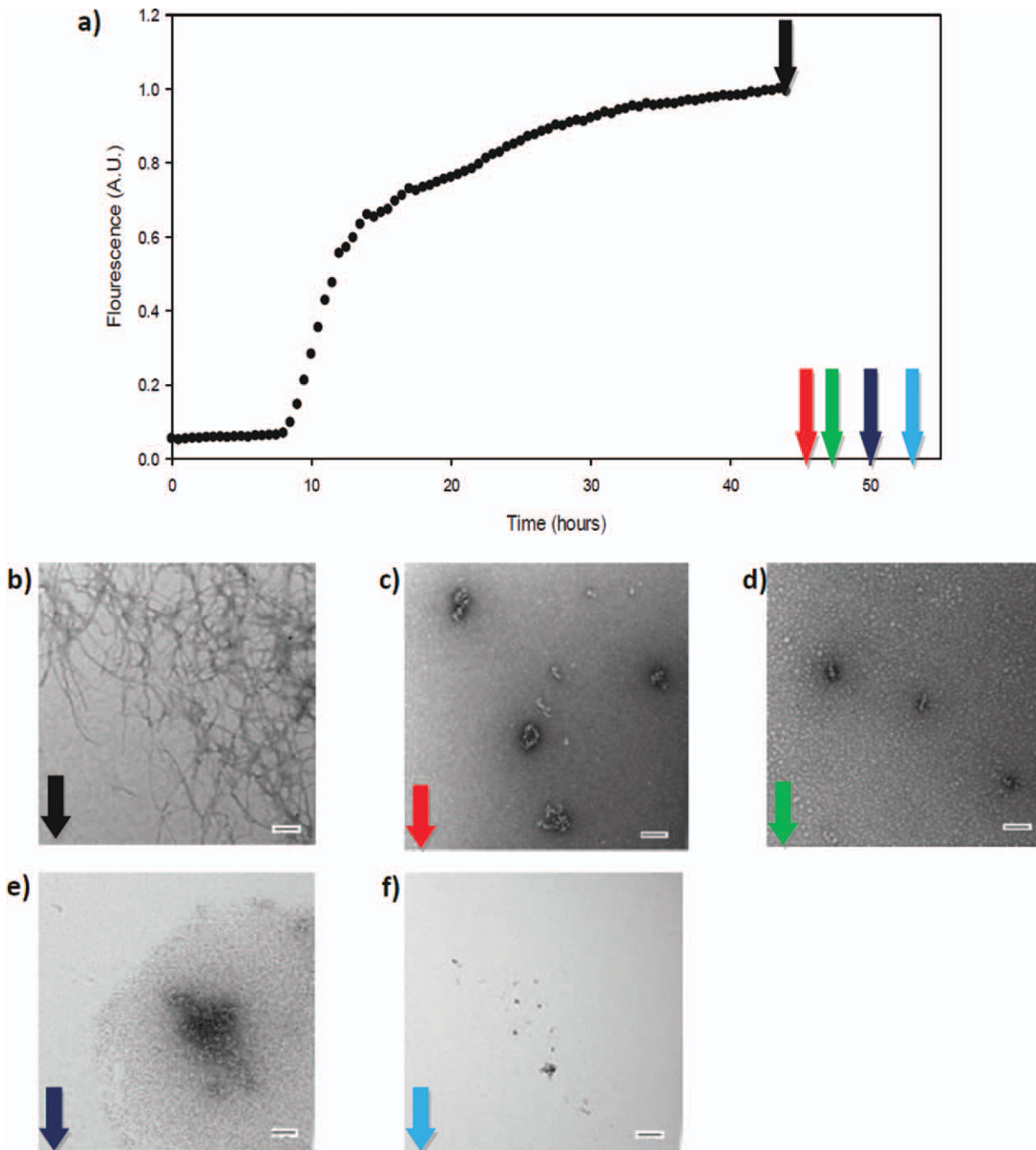


Figure 6. Morin hydrate disaggregates human IAPP amyloid fibers. (A) A thioflavin-T-monitored kinetic experiment in the absence of Morin hydrate. Morin hydrate was added in fivefold excess at the time point indicated by the black arrow. (B) TEM image of a sample removed just prior to the addition of Morin hydrate (black arrow). (C) TEM image 1 h after addition of Morin hydrate (red arrow). (D) TEM image 2 h after addition of Morin hydrate (green arrow). (E) TEM image 4 h after addition of Morin hydrate (dark blue arrow). (F) TEM image 6 h after addition of Morin hydrate (light blue arrow). Experiments were conducted at 25°C, pH 7.4, 20 mM Tris-HCl, 32 μ M IAPP, 0.5% DMSO (v/v). The concentration of Morin hydrate was 160 μ M. Scale bars represent 100 nm.

chemistry. Fmoc-PAL-Polyethylene-Glycol-Polystyrene (Fmoc-PAL-PEG-PS) resin was used and standard Fmoc reaction cycles were employed. Pseudoproline were used to assist the synthesis as described previously.⁵⁰ The peptide was cleaved from the resin using standard trifluoroacetic acid (TFA) methods and was then lyophilized for 24–48 h. To

increase the solubility of the peptides for later stages of purification, the crude material was dissolved in 20% acetic acid for 2–3 h and lyophilized. The crude peptide was oxidized in dimethyl sulfoxide (DMSO).⁵¹ Peptides were purified using reversed-phase high-performance liquid chromatography (HPLC) using a Vydac C18 preparative column with a 2-buffer system. Buffer A

contained 0.045% HCl in distilled and deionized (DDI) water and buffer B contained 0.045% HCl in 80% acetonitrile (ACN) and 20% DDI water. The identity of the pure peptide was confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). IAPP expected, 3903.3 Da; observed, 3903.9 Da.

Myricetin, Quercetin, Morin hydrate, and Kaempferol were obtained from Sigma-Aldrich and dissolved in DMSO at a concentration of 15.8 mM for the experiments in which the compound was added at a ratio of 1:1 and 130 mM for experiments in which it was added at a higher ratio. The small molecules were dissolved in DMSO on the day of the experiment.

Thioflavin-T fluorescence assays

Fluorescence experiments were performed using a Beckman model D880 plate reader. The thioflavin-T studies used excitation and emission filters of 430 and 485 nm, respectively. The experiments were performed in 96-well plates at 25°C.

IAPP was dissolved in hexafluoroisopropanol (HFIP) at a final concentration of 1.58 mM. The stock solution of IAPP in HFIP was filtered using a 0.45- μ m syringe filter. Aliquots of filtered stock solution were pipetted into Eppendorf tubes and lyophilized. All of the thioflavin-T assays were prepared by dissolving the dried samples of IAPP in Tris-HCl buffer (20 mM, pH = 7.4) immediately before measurements. The final peptide concentration was 32 μ M in 0.25% DMSO, 20 mM Tris-HCl buffer at pH 7.4, and 32 μ M thioflavin-T with or without inhibitors. For experiments with the inhibitor, concentrated stock solutions of compounds in DMSO were diluted into the thioflavin-T IAPP solution. The data were analyzed using Sigma-Plot v11.

Right-angle light scattering

Experiments were conducted using an Applied Phototechnology Fluorescence Spectrophotometer at 500 nm. This wavelength was chosen because none of the compounds has any absorbance in this region. Experiments were conducted at 25°C, pH 7.4, 20 mM Tris-HCl, 32 μ M IAPP, 0.25% DMSO (v/v).

TEM

Aliquots were removed from the fluorescence assays and were analyzed by TEM. Samples were prepared by pipetting 15 μ L of the solution onto carbon-coated Formvar 300 mesh copper grids. The grids were stained using saturated uranyl acetate for 1 min. An FEI Technai12 BioTwinG² Transmission Electron Microscope was used and the grids were viewed at a magnification of 98,000 \times (high voltage, 80 kV).

Amyloid disaggregation experiments

Normal procedures for forming fibers were performed as described above. Morin hydrate was added at approximately 45 h after the initiation of fiber formation. Aliquots of the assay were periodically removed from the solution for TEM analysis.

References

1. Sipe JD (1994) Amyloidosis. *Crit Rev Clin Lab Sci* 31: 325–354.
2. Selkoe DJ (2004) Cell biology of protein misfolding: the examples of Alzheimer's and Parkinson's diseases. *Nat Cell Biol* 6:1054–1061.
3. Chiti F, Dobson CM (2006) Protein misfolding, functional amyloid, and human disease. *Annu Rev Biochem* 75:333–366.
4. Yan LM, Tatarek-Nossol M, Velkova A, Kazantzis A, Kapurniotu A (2006) Design of a mimic of nonamyloidogenic and bioactive human islet amyloid polypeptide (IAPP) as nanomolar affinity inhibitor of IAPP cytotoxic fibrillogenesis. *Proc Natl Acad Sci USA* 103: 2046–2051.
5. Feng BY, Toyama BH, Wille H, Colby DW, Collins SR, May BCH, Prusiner SB, Weissman J, Shoichet BK (2008) Small-molecule aggregates inhibit amyloid polymerization. *Nat Chem Biol* 4:197–199.
6. Blazer LL, Neubig RR (2009) Small molecule protein-protein interaction inhibitors as CNS therapeutic agents: current progress and future hurdles. *Neuropsychopharmacology* 34:126–141.
7. Takahashi T, Mihara H (2008) Peptide and protein mimetics inhibiting amyloid beta-peptide aggregation. *Acc Chem Res* 41:1309–1318.
8. Abedini A, Meng F, Raleigh DP (2007) A single-point mutation converts the highly amyloidogenic human islet amyloid polypeptide into a potent fibrillization inhibitor. *J Am Chem Soc* 129:11300–11301.
9. Scrocchi LA, Chen Y, Wang F, Han K, Ha K, Wu L, Fraser PE (2003) Inhibitors of islet amyloid polypeptide fibrillogenesis, and the treatment of type-2 diabetes. *Lett Pept Sci* 10:545–551.
10. Gazit E (2002) A possible role for pi-stacking in the self-assembly of amyloid fibrils. *FASEB J* 16:77–83.
11. Mishra R, Bulic B, Sellin D, Jha S, Waldmann H, Winter R (2008) Small-molecule inhibitors of islet amyloid polypeptide fibril formation. *Angew Chem Int Ed Engl* 47:4679–4682.
12. Porat Y, Abramowitz A, Gazit E (2006) Inhibition of amyloid fibril formation by polyphenols: structural similarity and aromatic interactions as a common inhibition mechanism. *Chem Biol Drug Des* 67:27–37.
13. Meng F, Abedini A, Raleigh DP (2010) Combination of kinetically selected inhibitors in trans leads to highly effective inhibition of amyloid formation. *J Am Chem Soc* 132:14340–14342.
14. Ehrnhoefer DE, Bieschke J, Boeddrich A, Herbst M, Masino L, Lurz R, Engemann S, Pastore A, Wanker EE (2008) EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers. *Nat Struct Mol Biol* 15:558–566.
15. Tatzelt J, Rambold AS, Miesbauer M, Olschewski D, Seidel R, Riemer C, Smale L, Brumm L, Levy M, Gazit E, Oesterhelt D, Baier M, Becker CFW, Engelhard M, Winklhofer KF (2008) Green tea extracts interfere with the stress-protective activity of PrP(C) and the formation of PrP(Sc). *J Neurochem* 107: 218–229.

16. Hasegawa M, Masuda M, Suzuki N, Taniguchi S, Oikawa T, Nonaka T, Iwatsubo T, Hisanaga S, Goedert M (2006) Small molecule inhibitors of alpha-synuclein filament assembly. *Biochemistry* 45:6085–6094.
17. Ehrnhoefer DE, Duennwald M, Markovic P, Wacker JL, Engemann S, Roark M, Legleiter J, Marsh JL, Thompson LM, Lindquist S, Muchowski PJ, Wanker EE (2006) Green tea (–)-epigallocatechin-gallate modulates early events in huntingtin misfolding and reduces toxicity in Huntington's disease models. *Hum Mol Gen* 15:2743–2751.
18. Meng F, Abedini A, Plesner A, Verchere CB, Raleigh DP (2010) The Flavanol (–)-Epigallocatechin 3-Gallate inhibits amyloid formation by islet amyloid polypeptide, disaggregates amyloid fibrils, and protects cultured cells against IAPP-induced toxicity. *Biochemistry* 49:8127–8133.
19. Chandrashekar IR, Adda CG, MacRaid CA, Anders RF, Norton RS (2011) EGCG disaggregates amyloid-like fibrils formed by *Plasmodium falciparum* merozoite surface protein 2. *Arch Biochem Biophys* 513:153–157.
20. Bieschke J, Russ J, Friedrich RP, Ehrnhoefer DE, Wobst H, Neugebauer K, Wanker EE (2010) EGCG remodels mature alpha-synuclein and amyloid-beta fibrils and reduces cellular toxicity. *Proc Natl Acad Sci USA* 107:7710–7715.
21. Hauber I, Hohenberg H, Holstermann B, Hunstein W, Hauber J (2009) The main green tea polyphenol epigallocatechin-3-gallate counteracts semen-mediated enhancement of HIV infection. *Proc Natl Acad Sci USA* 106:9033–9038.
22. Hasegawa M, Masuda M, Nonaka T, Oikawa T, Yonetani M, Yamaguchi Y, Kato K, Hisanaga S, Goedert M (2009) Inhibition of alpha-synuclein fibril assembly by small molecules: analysis using epitope-specific antibodies. *FEBS Lett* 583:787–791.
23. Aitken J, Loomes K, Konarkowska B, Cooper GJS (2003) Suppression by polycyclic compounds of the conversion of human amylin into insoluble amyloid. *Biochem J* 374:779–784.
24. Lansbury PT, Lashuel HA (2006) A century-old debate on protein aggregation and neurodegeneration enters the clinic. *Nat Cell Biol* 443:774–779.
25. Ray S, Nowak R, Brown R, Lansbury P (2005) Small-molecule-mediated stabilization of familial amyotrophic lateral sclerosis-linked superoxide dismutase mutants against unfolding and aggregation. *Proc Natl Acad Sci USA* 102:3639–3644.
26. Chen J, Armstrong AH, Koehler AN, Hecht MH (2010) Small molecule microarrays enable the discovery of compounds that bind the Alzheimer's A β peptide and reduce its cytotoxicity. *J Am Chem Soc* 132:17015–17022.
27. Meng F, Abedini A, Plesner A, Middleton CT, Potter KJ, Zanni MT, Verchere CB, Raleigh DP (2010) The sulfated triphenyl methane derivative acid fuchsin is a potent inhibitor of amyloid formation by human islet amyloid polypeptide and protects against the toxic effects of amyloid formation. *J Mol Biol* 400:555–566.
28. Westermark P, Wernstedt C, Wilander E, Hayden DW, O'Brien TD, Johnson KH (1987) Amyloid fibrils in human insulinoma and islets of langerhans of the diabetic cat are derived from a neuropeptide-like protein also present in normal islet cells. *Proc Natl Acad Sci USA* 84:3881–3885.
29. Cooper GJS, Willis AC, Clark A, Turner RC, Sim RB, Reid KBM (1987) Purification and characterization of a peptide from amyloid-rich pancreases of type-2 diabetic-patients. *Proc Natl Acad Sci USA* 84:8628–8632.
30. Cooper GJS (1994) Amylin compared with calcitonin-gene-related peptide—structure, biology, and relevance to metabolic disease. *Endocrine Rev* 15:163–201.
31. Hay DL, Christopoulos G, Christopoulos A, Sexton PM (2004) Amylin receptors: molecular composition and pharmacology. *Biochem Soc Trans* 32:865–867.
32. Nishi M, Sanke T, Nagamatsu S, Bell GI, Steiner DF (1990) Islet amyloid polypeptide—a new beta-cell secretory product related to islet amyloid deposits. *J Biol Chem* 265:4173–4176.
33. Hutton JC (1989) The insulin secretory granule. *Diabetologia* 32:271–281.
34. Kahn SE, Andrikopoulos S, Verchere CB (1999) Islet amyloid: a long-recognized but underappreciated pathological feature of type 2 diabetes. *Diabetes* 48:241–253.
35. Clark A, Lewis CE, Willis AC, Cooper GJS, Morris JF, Reid KBM, Turner RC (1987) Islet amyloid formed from diabetes-associated peptide may be pathogenic in type-2 diabetes. *Lancet* 2:231–234.
36. Kahn SE, Hull RL, Westermark GT, Westermark P (2004) Islet amyloid: a critical entity in the pathogenesis of type 2 diabetes. *J Clin Endocrinol Metab* 89:3629–3643.
37. Marzban L, Park K, Verchere CB (2003) Islet amyloid polypeptide and type 2 diabetes. *Exp Geront* 38:347–351.
38. Lorenzo A, Razzaboni B, Weir GC, Yankner BA (1994) Pancreatic-islet cell toxicity of amylin associated with type-2 diabetes-mellitus. *Nature* 368:756–760.
39. Clark A, Wells CA, Buley ID, Cruickshank JK, Vanhegan RI, Matthews DR, Cooper GJS, Holman RR, Turner RC (1988) Islet amyloid, increased alpha-cells, reduced beta-cells and exocrine fibrosis—quantitative changes in the pancreas in type-2 diabetes. *Diabetes Res Clin Pract* 9:151–159.
40. Butler PC, Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA (2003) Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102–110.
41. Westermark GT, Westermark P, Berne C, Korsgren O (2008) Widespread amyloid deposition in transplanted human pancreatic islets. *N Engl J Med* 359:977–979.
42. Teplow DB, Kirkitadze MD, Bitan G (2002) Paradigm shifts in Alzheimer's disease and other neuro degenerative disorders: the emerging role of oligomeric assemblies. *J Neurosci Res* 69:567–577.
43. Potter KJ, Abedini A, Marek P, Klimek AM, Butterworth S, Driscoll M, Baker R, Nilsson MR, Warnock GL, Oberholzer J, Bertera S, Trucco M, Korbitt GS, Fraser PE, Raleigh DP, Verchere CB (2010) Islet amyloid deposition limits the viability of human islet grafts but not porcine islet grafts. *Proc Natl Acad Sci USA* 107:4305–4310.
44. Akaishi T, Morimoto T, Shibao M, Watanabe S, Sakai-Kato K, Utsunomiya-Tate N, Abe K (2008) Structural requirements for the flavonoid fisetin in inhibiting fibril formation of amyloid β protein. *Neurosci Lett* 444:280–285.
45. Ono K, Yoshiike Y, Takashima A, Hasegawa K, Naiki H, Yamada M (2003) Potent anti-amyloidogenic and fibril-destabilizing effects of polyphenols in vitro: implications for the prevention and therapeutics of Alzheimer's disease. *J Neurochem* 87:172–181.
46. Kim H, Park B, Lee K, Choi C, Jang S, Kim Y, Lee S (2005) Effects of naturally occurring compounds on fibril formation and oxidative stress of β -amyloid. *J Agric Food Chem* 53:8537–8541.

47. Levine H (1995) Thioflavine-T interaction with amyloid beta-sheet structures. *Amyloid* 2:1–6.
48. Meng F, Marek P, Potter KJ, Verchere CB, Raleigh DP (2008) Rifampicin does not prevent amyloid fibril formation by human islet amyloid polypeptide but does inhibit fibril thioflavin-T interactions: implications for mechanistic studies beta-cell death. *Biochemistry* 47:6016–6024.
49. Hudson SA, Ecroyd H, Kee TW, Carver JA (2009) The thioflavin T fluorescence assay for amyloid fibril detection can be biased by the presence of exogenous compounds. *FEBS Lett* 276:5960–5972.
50. Abedini A, Raleigh D (2005) Incorporation of pseudo-proline derivatives allows the facile synthesis of human IAPP, a highly amyloidogenic and aggregation-prone polypeptide. *Org Lett* 7:693–696.
51. Abedini A, Singh G, Raleigh DP (2006) Recovery and purification of highly aggregation-prone disulfide-containing peptides: application to islet amyloid polypeptide. *Anal Biochem* 351:181–186.