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A foldamer approach to targeting membrane bound helical states of islet amyloid polypeptide

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Abstract

A small molecule, protein mimetic based approach is shown to specifically inhibit lipid catalysed self-assembly of islet amyloid polypeptide (IAPP). The lipid-bound oligomerization of this peptide is implicated in cellular dysfunction of insulin secreting β -cells in type II diabetes.

Expression, function, and degradation of proteins in cells is a highly regulated process overseen by a variety of quality-control mechanisms.^[1] In a number of important diseases, this proteostatic oversight is overwhelmed, leading to gains of toxic function associated with protein aggregation. One sub-class of such aggregates are amyloids. These are characterized by self assembly into filamentous networks rich in cross- β secondary structure. The deposition of protein as amyloid has been implicated as causal in many important diseases including Alzheimer's, Parkinson's, and type 2 diabetes.^[2, 3]

Islet amyloid polypeptide (IAPP) is a 37-residue peptide hormone cosecreted with insulin by the pancreatic β -cells.^[4] The conversion of IAPP from a predominantly random coil structure to β -sheet fibers has been implicated in the pathology of both type I and type II diabetes. Recently, the toxic gains of function in many amyloids, including IAPP, have been associated with oligomeric intermediate states that precede fiber formation. In addition, these oligomeric species are membrane active with the capacity to induce leakage on timescales that precede formation of amyloid fibers.

In cell culture, we have demonstrated that gains of toxic function by IAPP do not require the formation amyloid structure. Instead, a heterogeneous set of α -helical, membrane bound pre-amyloid states mediate cell penetration, facilitating intracellular access that is followed by mitochondrial dysfunction and cell death.^[5]

The region of IAPP that is structured and α -helical on membranes has been established spectroscopically as residues 1–22.^[6, 7] A peptide corresponding only to this region shows membrane activity in *in vitro* and cellular studies.^[8] Most recently, we have been able to identify the topology of a dimeric unit of membrane bound IAPP. The complex is mediated by anti-parallel helix:helix interactions with the cationic residues of the peptides residing on the same side of the structure and likely forming the interface to the membrane.^[9]

We hypothesize, therefore, that synthetic chemical approaches that attenuate oligomerization of these α -helical intermediate states of IAPP can facilitate elucidation of the structural mechanism of IAPP's cellular gains of function.^[10]

In collaboration with the laboratory of Andrew Hamilton, we achieved the inhibition of lipid catalysed IAPP aggregation using a peptidomimetic approach based on oligopyridine.^[10,11]

Central to that investigation was a discovery that IAPP oligomerization could be affected using a structured pentacarboxylate.^[11] In the current study, we assess a new scaffold based on polyquinoline.^[12]

Specifically, we compare pentacarboxylates displayed via quinoline (**1**), pyridyl (**3**) and peptoid (**2**) scaffolds (Scheme 1). In this way, pentacarboxylates are displayed in unstructured (**2**), linear (**3**) or helical formats (**1**). These compounds are then assessed for their capacity to affect lipid catalysed IAPP self-assembly.

The synthesis of pentapeptoid (**2**) was achieved using solid phase submonomer approach and purified using HPLC. Pentaquinoline (**1**) was synthesized using linear solution phase iterative amide coupling as reported earlier for the shorter homologues^[12, 13] and pentapyridyl is synthesized using known procedure.^[11] (see Materials and Methods section in ESI[†]).

Lipid catalysed fiber formation by IAPP is unaffected by the presence of an unstructured pentacarboxylate presented by (**2**), slowed in the presence of (**3**) and completely inhibited in the presence of (**1**). An established lipid catalysed fibril formation assay was used to assess the effects of (**1**), (**2**) and (**3**) on membrane interactions by IAPP. Briefly, stock solutions of IAPP were prepared at 500–1000 μM in phosphate buffer (100 mM KCl, 50 mM Na phosphate, pH 7.4). To initiate the reaction, 10 μM IAPP was diluted into buffer containing 20 μM thioflavin T (ThT) and 100 μM of ligand with 630 μM unilamellar liposomes [dioleoylphosphatidylglycerol (DOPG) and dioleoylphosphatidylcholine (DOPC) at 1:1 molar ratio] extruded to 100 nm diameter (for detailed procedure see ESI[†]). Fiber formation kinetics were then monitored by measuring changes to the intensity of ThT fluorescence which increases in response to binding amyloid structures.^[14] As fiber formation kinetics display a nucleation dependent profile, fits to the data were used to extract the time required for 50% conversion of the precursor material (t_{50}) (see Fig. S3 and see Fig S4 in ESI[†]). In the absence of liposomes, the reaction requires 22.4 ± 2.2 hours to reach t_{50} (data not shown). In contrast, the presence of liposomes results in acceleration of fiber formation to a t_{50} of 1.1 ± 0.1 hours. These values are consistent with previously reported effects which were 20.1 ± 0.6 and 0.63 ± 0.01 hours under lipid free and lipid catalysed conditions respectively.^[10]

The presence of 100 μM (**2**) had no effect on the rate of conversion of IAPP (10 μM) to fiber form suggesting it does not interact with IAPP even at 10:1 stoichiometric ratio (Fig. 1). Lipid catalysed fiber formation by IAPP is strongly affected by the presence of structured pentacarboxylates presented by (**1**) and (**3**). In marked contrast to (**2**), the presence of 100 μM (**3**) shifted the t_{50} of our standard assay to 2.9 ± 0.3 hr. Expressed as a relative change in midpoint, this corresponds to a lengthening of the reaction by a factor of 2.7 ± 0.1 which compares well with the inhibition by a factor of 3.7 ± 0.1 (at 150 μM of (**3**)) in our previous work.^[11] Dramatically, 100 μM compound (**1**) wholly eliminates any detectable conversion ($t_{50} > 16$ hours) of IAPP to amyloid under the same conditions (Fig. 2 and see Fig. S2 and Table S2 in ESI[†]).

Inhibition of IAPP fiber formation by (**1**) is dose dependent and complete. Fiber formation over a series of concentrations of (**1**) was conducted (Fig. 2 and see Fig. S2 and Table S2 in ESI[†]). At a mole ratio of 1, IAPP:ligand (each 10 μM), (**1**) achieves a level of inhibition

[†]Electronic Supplementary Information (ESI) available: [Experimental details, raw data, fits and tables. See DOI: 10.1039/b000000x/

comparable to that observed for (3) at a mole ratio of 10. Importantly, continued assessment at higher mole ratios resulted in complete inhibition preventing the measure of a meaningful IC_{50} . At elevated doses ($\sim 400 \mu M$), inhibition by (3) is instead saturated with a maximum t_{50} that is four times that of the ligand free assays.^[11] This clearly indicates that while (1) and (3) likely have a common charge:charge basis for some of their interactions with IAPP, the mechanism of inhibition by these two compounds are likely to have significant differences.

Pentacarboxylate displayed on polyquinoline does not inhibit elongation processes associated with amyloid formation. The mechanistic steps of amyloid aggregation include the nucleation of new fibers as well as elongation of existing fibers. In order to assess whether (1) acts on one or both facets of fiber formation, a study was conducted in which (1) was added at alternative time points during the growth phase of our standard reaction. Addition of $10 \mu M$ (1) after a $10 \mu M$ IAPP amyloid formation reaction was complete had no effect on the magnitude of signal response from ThT (Fig. 3, black). This indicates that amyloid was not dissociated by (1) and also shows no effect of (1) on the ThT assay itself. In marked contrast, addition of (1) midway through the amyloid conversion wholly arrested further self-assembly (Fig. 3, green). At this time point, half of the protein present has converted to amyloid. The kinetic profile here is dominated by the elongation of already formed fibers. In contrast, such elongation processes were previously shown to be wholly unaffected by (3). Plainly, compound (1) is both more potent, and inhibits via a mechanism that affects both nucleation and elongation components of IAPP's self assembly.

Polyquinoline inhibition of IAPP fiber assembly is unique in the strength of its capacity to strongly affect lipid catalysed processes. Numerous other small molecules including rhodamine,^[15] phenol red,^[16] phenolphthalein,^[17] Epigallocatechin-3-gallate (EGCG) derivatives,^[18] sibnin,^[19] sulphated triphenyl methane derivative acid fuchsin,^[20] resveratrol,^[21] and di-substituted N-methyl derivative of IAPP^[22] efficiently inhibit solution phase fiber formation but are not designed to interact specifically with the helical intermediate states of IAPP. EGCG, for example, is a relatively planar, polyphenol that inhibits fiber formation of IAPP in solution but has no effect on fiber formation under lipid catalysed conditions.^[23] The quinoline derivatives can adopt a helical structure^[12] which is close but not identical to the pitch of a peptide α -helix. We speculate that binding by the polyquinoline design distorts the peptide thereby renders it more effective than polypyridyl on the lipid bound, α -helical intermediates of IAPP.

A new scaffold is presented that efficiently interrupts the kinetics of IAPP fibrillation, likely by interacting with the pre-amyloid oligomeric intermediate states. To our knowledge, the pentaquinoline scaffold is the most effective compound so far discovered for inhibition under lipid catalysed conditions. Given the importance of membrane bound helical intermediate states to other systems such as α -synuclein in Parkinson's and A β in Alzheimer's, we are optimistic that this scaffold will have general utility. As a structure-based perturbant of biological activity, these compounds will lay a foundation for the development of mechanistic insights, identification of novel therapeutic targets and potentially lead therapeutics for a host of important degenerative disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Inhibition of fiber formation by IAPP in the presence of various pentacarboxylates. A standard 10 μ M IAPP reaction was conducted in the presence of 630 μ M liposomes (in phospholipid monomer units, 1:1, DOPG:DOPC) and monitored by a change in fluorescence of the indicator dye, ThT. Reactions additionally contained 100 μ M of (1), (2) or (3). Relative fluorescence changes are renormalized to facilitate direct comparison of the change in assembly timescale (Uncorrected data shown in Fig. S1). Inset: Statistics of t_{50} values of fiber formation under each of these conditions (Representative fits shown in Fig. S3). Note, ND indicates that no fiber formation was detected over the time course of this experiment.

Fig. 2. Dose dependence of (1) on lipid catalysed fiber formation by IAPP. Reactions were performed as described in Fig. 1 with the addition of the indicated concentrations of (1). Fluorescence intensities (apart from 100 μM (1)) are renormalized to facilitate direct comparison of the change in assembly timescale. (Representative fits shown in Fig. S3)

Fig. 3. Time dependent effects of addition of (1) to lipid catalysed fiber formation. Kinetic monitoring of fiber formation was conducted as per Fig. 1 with 10 μ M (1) added at the time points indicated with arrows.

Scheme 1.

Chemical structures of IAPP and ligands used in this study. Positively charged residues (under physiological conditions) are shown in blue, negative charges in red. Note, the C-terminus of wild-type IAPP is amidated and there is a disulphide bond between residues 2 and 7.