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Synthetic α -Helix Mimetics as Agonists and Antagonists of IAPP Amyloid Formation**

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The development of small molecules that can modulate the damaging effects of protein aggregation processes remains a high priority goal in contemporary medicinal chemistry.[1] An important class of these aggregates, called amyloids, has been implicated in numerous degenerative diseases including Alzheimer's, type II diabetes, senile systemic amyloidosis (SSA), prion diseases and rheumatoid arthritis. The attribute shared by these symptomatically unrelated diseases is that a normally soluble protein undergoes a conformational change resulting in self-assembly into cytotoxic forms culminating in a β -sheet rich fibrillar structure. Islet amyloid polypeptide (IAPP), or amylin, is one such protein that has been implicated in amyloidogenesis in type II diabetes.[2] IAPP is cosecreted with insulin by the β -cells of the islets of Langerhans and an aggregated form of IAPP is believed to play a role in β -cell toxicity in the pathology of type II diabetes.[3]

Amyloid forming processes proceed via a nucleation dependent reaction mechanism. The structural and energetic basis for nucleation is, however, poorly understood.[5] For IAPP, Miranker and coworkers[6] have proposed a possible mechanism where nucleation is initiated by binding of IAPP to cell membranes via contacts mediated by residues 1-20

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(Figure 1a).[4] The region of IAPP comprising residues 5-19 clearly shows helical structure, with positive charges predominant on one face, and likely forms multi helical aggregates upon interaction with the membrane surface.[7,8] The formation of these α helical intermediates accelerates the assembly of the amyloid structure that is rich in β -sheets.[9] Recent findings have further suggested that these helical oligomeric intermediates may be the relevant cytotoxic form of IAPP.[8,10] An interesting, and previously unexplored, potential therapeutic approach for mitigating the cytotoxic effects of IAPP would be to design molecules that interfere with the helix assembly process (Figure 1b).

Inhibition of IAPP aggregation by small molecules based on a rhodanine scaffold,[11] phenol red[12] and phenolsulfonphthalein[13] has been reported. Similar disruption of amyloid assembly in other protein aggregation diseases by aromatic dyes is known.[14,15] A plausible general mechanism for such inhibition involves π -stacking of the dye with the aromatic amino acid rich core of the developing amyloid.[12,15] In this work, we propose an alternative mechanism of amyloid inhibition where we target the transient α -helical intermediates in IAPP aggregation.

We have previously reported synthetic structures that mimic the residues along one face of an α -helix and successfully disrupt important protein-protein interactions.[16] In particular, the oligopyridylamide scaffold (**1**) uses intramolecular hydrogen bonding to rigidify the backbone and projects functionality on one face of the molecule in direct analogy to an α -helix.[17-19]

An inspection of the N-terminal region of human IAPP (hIAPP) reveals four positive charges in close spatial proximity: Arg11 and His18 (which is likely protonated at the membrane surface) in the helical domain as well as Lys1 and the N-terminus. A potential size and charge complementarity with this region might be achieved by a tetrameric or pentameric form of the oligopyridylamide scaffold containing four or five carboxyl terminated side chains, respectively. To study systematically the effect of an increasing number of negative charges on interaction with IAPP, the monomeric through pentameric pyridylamides **1a-1e** were synthesized. Furthermore, to probe the efficacy of the hydrogen bonding preorganization effect in these molecules, the corresponding oligobenzamide series, **2a-2e** was synthesized,[18,20,21] in which the pyridine rings were replaced by benzene so that bifurcated hydrogen bonding is no longer possible. These molecules have greater conformational flexibility about the aryl-C(=O) bonds and permit an adaptability of structure on binding, albeit at an entropic cost.

The molecules were synthesized using linear solution-phase iterative coupling as reported earlier for the shorter homologues.[18,19] Briefly, chain elongation was achieved using successive amide coupling and nitro group reduction steps (see supporting information). The acid groups, which were protected as *t*-butyl esters, were cleaved in the last step to give the target compounds.

The dimers (**1b**, **2b**) and trimers (**1c**, **2c**) have previously been shown to adopt an elongated rod-like conformation in the solid state with a more curved backbone in the oligopyridylamides than the oligobenzamides.[18] This effect continues in tetrameric **1d** and pentameric **1e**, as seen from their crystal structures (Figure 2), and the side chains project from one face of the molecule forming a recognition surface for potential binding to a complementary face of an α -helix. Due to some positional disorder, only four of the five side chains in the crystal structure of **1e** could be modelled. However, the location of the oxygen atom of the side chains from the electron density map allows us to conclude that **1e**, like its shorter homologs, adopts an extended curved conformation with the side chains projected on one face. Molecular modelling studies with **2d** and **2e** suggest that they adopt a similar extended conformation, with less certainty over the position of the side chains.[20]

IAPP binds lipid membranes and its fibrillization is strongly accelerated in the presence of liposomes containing mixtures of anionic dioleoylphosphatidylglycerol (DOPG) and zwitterionic dioleoylphosphatidylcholine (DOPC).[6] The kinetic profile is characterized by a lag phase followed by a cooperative transition to the aggregated state. The rate of fibrillogenesis of IAPP under lipid-catalyzed conditions, in the presence of the helix mimetics, was determined using an exogenous fluorescent dye, thioflavin-T (ThT). ThT binds specifically to amyloid fibrils without significantly disturbing the IAPP fiber formation pathway[6] and the relative fluorescence intensity is an indicator of the transition to the amyloid state. Since compounds that interact with IAPP and affect fiber formation can interfere with ThT binding, our analysis of changes in amyloid formation kinetics was limited to the midpoint of the transition, t_{50} , rather than the absolute ThT signal.

In the presence of the helix mimetics, the rate of acceleration of lipid-catalyzed IAPP fiber formation was significantly diminished. A representative kinetic data plot for **1e** is shown in Figure 3a. Molecule **1e** was an effective inhibitor of lipid-catalyzed IAPP fiber formation with a relative t_{50} that is four-fold higher than the control reaction. This inhibition was dose-dependent and an IC_{50} of 8 μ M was obtained (Figure 4a) under the conditions of the assay. Electron microscopy images obtained after 1h in the presence of **1e** under lipid-catalyzed conditions confirmed the much slower growth rate of fibers (Figure 4b,c). NMR binding experiments with rat IAPP, which does not form fibers, showed a discrete binding interaction between **1e** and IAPP with an approximate K_d of about 40 μ M (see supporting information).

For both series of compounds, a comparison of the relative t_{50} values for lipid-catalyzed aggregation showed a gradual increase in antagonistic activity from the dimer to the longer oligomers while the monomer had virtually no effect on the kinetics (Figure 5a). The activities of the tetramers and the pentamers were comparable although the pentamers were slightly less active in both cases.

To probe the mechanism of inhibition of aggregation, kinetic experiments were performed in the absence of the lipid. Under these conditions, the molecules acted as agonists of amyloid formation and compound **1e** showed two to three fold acceleration in aggregation kinetics (Figure 3b). As with the lipid-catalyzed conditions, the rate of acceleration was proportional to the length and charge of the compounds and the pentamers in both the series were the most effective accelerants (Figure 5b). However, under lipid-catalyzed conditions, higher bulk concentrations of the compounds were required to strongly affect fiber formation (Fig 5a vs. 5b) presumably because under these conditions the compounds do not effectively partition into the membrane while IAPP is bound at the membrane surface.

Diethylene triamine pentaacetic acid (DTPA), **3**, a control molecule bearing five carboxylic acid groups in a less well-defined orientation, had very little effect on the kinetics of

aggregation (Figure 5). While the lipid-catalyzed kinetic data were virtually indistinguishable from the control, it accelerated the *de novo* fiber formation kinetics somewhat, perhaps due to non-specific charge neutralization effects. A control molecule based on **1c**[22] with positively charged side chains or oligomers where the acid groups were protected as esters (**1c_{ester}** and **1e_{ester}**) did not have a similar effect on the kinetics (see supporting information). Lipid-free kinetics for **1c_{ester}** and **1e_{ester}**, however, were not directly comparable presumably due to their hydrophobicity and highly aggregating nature. Taken together these data suggest that the number, nature and orientation of the charges are crucial to the activity of the molecules indicating a specific interaction most likely with the complementary α -helical region of IAPP. A detailed mechanistic study will be reported elsewhere.[23]

In conclusion, two series of compounds based on an oligoamide backbone were designed to provide a complementary surface to interact with the α -helical domain on IAPP. These molecules project their anionic substituents at the right distance and orientation and under lipid-free conditions accelerate the aggregation of IAPP. Under lipid-catalyzed conditions, however, they retard the formation of amyloid deposits. While both series of compounds follow the same general trend, the oligopyridylamide series shows a slight but consistently higher effect potentially due to a reduced entropic penalty on binding (see supporting information). This study validates the targeting of discrete amyloidogenic intermediates as an alternative therapeutic approach to amyloid diseases and paves the way for research into novel type II diabetes drugs with particular focus on inhibiting lipid-catalyzed acceleration of IAPP aggregation.

Supplementary Material

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Figure 1.

a) Model for IAPP amyloid formation with α -helical intermediate states,[4] b) Schematic representation of α -helix mimetics of varying length interacting with the helical intermediates in IAPP fibril formation pathway.

Figure 2.

a) X-ray crystal structure of the *t*-butyl ester of **1d** (*t*-Bu ester groups and non-NH hydrogen atoms have been omitted for clarity) b) Crystal structure of the backbone of **1e** (Y= NH₂), the side chains could not be modelled due to positional disorder and are shown as red balls. red O, blue N, green C, white H.

Figure 3.

Relative rate of hIAPP (10 μM) aggregation in the presence of **1e** (100 μM) compared to the control reaction showing a) inhibition under lipid-catalyzed and b) acceleration under lipid-free conditions.

Figure 4.

a) Dose response curve for lipid-catalyzed IAPP amyloid inhibition with **1e**. Electron microscopy pictures showing b) the presence in the control reaction and c) absence of fiber formation with **1e** under lipid catalyzed conditions after 1h.

Figure 5.

Bar charts showing comparison of the relative rates of fiber formation with the designed molecules; [hIAPP]=10 μ M; a) inhibition under lipid-catalyzed conditions DOPG:DOPC=1:1(500 μ g/mL); [compound]=150 μ M and b) acceleration under lipid-free conditions [compound]=10 μ M.