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Influence of a Curcumin Derivative on hIAPP Aggregation in the Absence and Presence of Lipid Membranes

Amit S. Pithadia^a, Anirban Bhunia^{a,b,d}, Rajendran Sribalan^c, Vediappen Padmini^{a,c,d}, Carol A. Fierke^{a,d}, and Ayyalusamy Ramamoorthy^{a,d}

^aDepartment of Chemistry, University of Michigan, Ann Arbor, MI 48109

^bDepartment of Biophysics, Bose Institute, Kolkata 700 054, India

^cDepartment of Organic Chemistry, School of Chemistry, Madurai Kamraj University, Madurai 21, India

^dBiophysics Program, University of Michigan, Ann Arbor, MI 48109

Abstract

The deposition of aggregates of human islet amyloid polypeptide (hIAPP) has been correlated with the death of β -cells in type II diabetes mellitus. The actual molecular mechanism of cell death remains largely unknown; however, it has been postulated that the process of aggregation from monomeric hIAPP is closely involved. A possible cause of cellular toxicity may be through the disruption of structural integrity of the cell membrane by IAPP. Herein, a water-soluble curcumin derivative, CurDAC, is used to investigate the mitigation of hIAPP aggregation in the absence and presence of lipid membrane.

Graphical abstract

Human islet amyloid polypeptide (hIAPP) or amylin, is a 37 residue peptide hormone secreted from β -cells within the islet of Langerhans in the pancreas. hIAPP has received much attention due to its possible involvement in the pathology of diabetes mellitus, or type-II diabetes.¹ The protein found in islet cell deposits was characterized as IAPP and further

confirmed the deposits as amyloid fibers,² a particular form of misfolded proteins which adopt a cross- β sheet structure with each monomer in the fibril adopting a β -sheet structure. More careful analysis indicated that β -cell mass is reduced strongly in islets containing IAPP deposits suggesting a possible toxic effect of IAPP on β -cells due to intermediary species (*e.g.*, oligomers) generated during amyloid fibril assembly. A well-studied mechanism of toxicity by IAPP is the disruption of cellular (plasma and organelle) membranes.³ These disruption events are suggested to happen through pore-like and fragmentation mechanisms which compromise the integrity of the lipid bilayer.⁴

A method to probe the inhibition of aggregation as well as rescue membrane integrity has been through the employment of small molecules.⁵ These chemical modulators could stabilize the monomer thus blocking the formation of toxic oligomers, divert the monomeric peptide to off-pathway non-toxic intermediates, prevent the primary nucleation process by destabilizing oligomers, or destabilize fibrils to form monomers or non-toxic oligomers. Specifically, natural products make a promising class of viable candidates as small molecule inhibitors of amyloids.^{6–8} Curcumin, a natural product found abundantly in turmeric that is used in most south Asian spices, has been widely categorized as having therapeutic properties due to its antioxidant, anti-cancer, antibiotic and anti-amyloidogenic properties.⁹ It has been shown to non-specifically bind to amyloid- β monomers and fibrils and modify the protein aggregation pathway;^{9,10} but less is known about the interactions between curcumin and hIAPP. Curcumin has demonstrated inhibitory properties against hIAPP; however, its mechanism of action still remains elusive.^{11,12} Many recent studies have focused on the anti-amyloidogenic, properties, but limited utility of curcumin, thus leading to the generation of rationally designed curcumin analogues and derivatives which have demonstrated to have increased stability and solubility compared to curcumin while showing very promising activity against amyloid proteins both *in vitro* and *in vivo*.^{13,14}

Herein, we present a curcumin derivative, curcumin diacetate (**CurDAc**) (Fig. 1) which exhibits increased stability and solubility in aqueous conditions, as an ideal small molecule candidate to study against IAPP aggregation and membrane stability. Our results demonstrate that this derivative has the propensity to modulate amyloid aggregation through an inhibition mechanism in the presence and absence of biological membrane mimics, unlike the results seen for this molecule with A β , which did not have a substantial influence on A β aggregation.¹³ **CurDAc** serves as a template to modify curcuminoids which can help toward developing therapeutic compounds for modulating hIAPP aggregation and rescuing membrane integrity thus greatly reducing IAPP-induced toxicity. Biophysical characterization has helped us evaluate the effects of the curcumin derivative, **CurDAc** on an inhibitory mechanism of mature IAPP fibril formation.¹⁵

CurDAc was designed as a water-soluble derivative of curcumin that requires organic solvents for solubilization and use in aqueous buffered systems (Fig. 1).¹⁶ This was achieved through the insertion of an acetate moiety that introduces two negative charges on the framework at pH > 5 (sodium salt form).¹⁴ A degradation mechanism through the autoxidation of curcumin that occurs through the phenolic moieties has been proposed.¹⁷ Therefore, by capping these sites, more stable derivatives can be formed, as in the case of **CurDAc** that appends acetate functional groups to diminish this oxidation event. These

negative charges may also provide a molecular basis for interaction with hIAPP through electrostatic as well as hydrogen bonding. The design of a curcumin derivative was of particular interest due to the instability of curcumin in aqueous conditions, which has made it increasingly difficult to study the activity of the parent structure with amyloid proteins (Fig. S1 in ESI[†]).¹⁴

Both the excitation and emission profiles for **CurDac** do not interfere with thioflavin-T (ThT), thus making it possible to study aggregation through fluorescence.¹¹ In our experimental conditions, hIAPP displayed a lag phase of ~75 min and fully mature fibrils at ~200 min (Fig. 1A). In the presence of 1 equiv. **CurDac**, a complete inhibition of hIAPP aggregation was seen, which is attributed to the stabilization of monomers[†], or low molecular weight (LMW) oligomers that do not consequently form β -sheet rich fibrillar species in the presence of a small molecule. To verify this inhibition and at the same time to rule out false positives results by fluorescence experiments, ¹H STD NMR experiment was employed (Fig. 2B). This technique is commonly used to understand ligand-receptor interactions by measuring the magnetization transfer between the receptor (hIAPP), which is irradiated at a specific on-resonance frequency, and the ligand (**CurDac**).¹⁸ The top spectrum is a standard ¹H NMR spectrum of **CurDac** displaying only the aromatic region of the ligand. The ¹H STD spectrum displayed no signal when **CurDac** was co-incubated with freshly prepared monomers, indicating that the ligand, **CurDac**, and hIAPP monomers tumble fast in solution due to their low molecular weight. Remarkably, no signal was observed even when the experiment was continued over 18 h (**CurDac** + monomer), suggesting that hIAPP did not exist in its fibrillar form and may be stabilized as LMW species through an inhibition mechanism. In contrast, when hIAPP fibril was added to the ligand, efficient magnetization transfer from the large-size hIAPP fibrils to **CurDac** resulted in a strong STD effect, mainly in the aromatic region of the ligand revealing interaction of aromatic rings with hIAPP (Fig. 2B, **CurDac** + fibril). These results are also in good agreement with microscopy images (Fig. S2). Under the conditions of ThT assay, **CurDac** induced small, but more amorphous, hIAPP species that may not propagate toward toxic higher-order fibrils.

As shown in Fig. 3A, circular dichroism (CD) experiments revealed the expected random coil to β -sheet conformation transition of hIAPP after 24 h. However, in the presence of **CurDac**, a more pronounced helical conformation was observed, indicating selective IAPP intermediate species stabilized through interactions with the ligand. This may occur through charge-charge interaction between **CurDac** and hIAPP, which may force the peptide to a specific conformation and thus altering its aggregation properties rather than disrupting the helix to force IAPP aggregation as reported previously.¹¹ Investigations are on going to decipher if **CurDac** is reordering the monomer to a helix, or if the small molecule binds to a helical conformation, similar to the interactions seen between curcumin and the amyloid protein PrP.¹⁹ This observation is unique to this derivative as studies on curcumin showed the instability of the helical intermediate upon incubation.¹² Dynamic light scattering (DLS) experiments further confirms the formation of a LMW hIAPP species in solution with

[†]Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

CurDac present. (Fig. S3). Filtered samples of **CurDac** and hIAPP at 4 h showed relatively mono disperse intensity peaks ~10 nm in hydrodynamic radius (Fig. S3B), different from peptide alone sample that has a heterogeneous dispersity of large oligomers and protofibrils between 50 and 100 nm (Fig. S3B). This conformational change could be responsible for the inhibitory properties of **CurDac**, which does not allow for specific interactions between hIAPP strands that are responsible for the formation and growth of fibrils.²⁰

Inhibitors have the connotation that they mitigate fibril formation from freshly prepared monomers in solution; however, identifying some of these compounds as chemical modulators, instead of inhibitors is more accurate. The disassembly of hIAPP in solution was also monitored using the ThT assay by adding **CurDac** at ~200 min, when a high population of mature β -sheet containing fibrils was present (Fig. S4). Upon addition of **CurDac**, a gradual loss in the ThT fluorescence signal was seen, inferring that the population of mature fibrils was diminishing in solution through the disaggregation of hIAPP. A depolymerisation event of α -synuclein has also been seen with curcumin-pyrazole derivatives,¹⁰ alluding to the possible broader scope of **CurDac** for disassembling fibrils of other amyloids. The intensity did not equilibrate to the baseline, which may suggest that the small molecule does not disaggregate hIAPP fibers to monomers.⁷ Taken together, these results suggest that **CurDac** has both the ability to halt fibril formation from fresh monomers as well as depolymerize preformed fibrils in solution.

Given the promising results, we wanted to further investigate how **CurDac** behaves in the presence of lipid membrane to measure its amyloid inhibitory role towards lipid membrane protection and stabilization. Studies in the presence of lipid membrane have demonstrated that curcumin can interact with the lipid bilayer through two modes: (i) a surface interaction through electrostatic interaction with positively charged lipid head groups by H-bonding or (ii) a transmembrane insertion that segmentally orders lipid bilayers due to its rigidity.²¹

We investigated the influence of **CurDac** on hIAPP aggregation in the presence of model membranes such as bicelles and lipid vesicles. The kinetics of hIAPP aggregation are altered in the presence of lipid bilayers due to possible surface level interactions that help nucleate monomers and LMW oligomers into fibril formation more quickly (Fig. 4).²² Though the exact mechanism for the interaction between **CurDac** and membrane has not been worked out yet, surface adsorption of the small molecule could coat the membrane to disable fibrillation at the surface.

Experimental results shown in Fig. 4A and 4B suggest that the fibrillation of hIAPP was negligible when **CurDac** was incubated with DMPC/DHPC (2:1) bicelles and hIAPP or POPC/POPG (7:3) large unilamellar vesicles (LUVs) and hIAPP. These results shed insights into molecular frameworks that could be used as membrane-protective agents against amyloid-induced cellular toxicity.²³ A surface level or membrane insertion mechanism has been considered and further investigated; but a membrane protection occurs with **CurDac** as seen from dye leakage studies. POPC/POPG vesicles encapsulated with 6-carboxyfluorescein are traditionally used to measure membrane permeation through pore-

like or detergent-like mechanisms.²⁴ In this case, we see direct membrane fragmentation of the vesicles by hIAPP (Fig. S5)²⁵ which are inhibited by the addition of **CurDAc**.

In summary, our study demonstrated a curcumin derivative, **CurDAc**, that can be used in stoichiometric amounts to suppress hIAPP fibril formation in solution both in the absence and presence of lipid membrane. The adoption of a helical intermediate may be a feature to exploit for further atomic-level structural investigations due to its stability over time. Furthermore, the ability of **CurDAc** to protect lipid membrane may make it a useful scaffold for potential therapeutic applications. This small molecule possesses advantageous characteristics compared to its natural product counterpart, and further studies toward understanding its high-resolution interaction with hIAPP and lipid bilayer should be fruitful.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Chemical structures of curcumin ((1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) and **CurDAc** (sodium 2,2'-((((1*E*,6*E*)-3,5-dioxohepta-1,6-diene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))diacetate). The hydroxyl moieties are modified to acetates.

Fig. 2.

Inhibition of hIAPP by **CurDAc**. A) Thioflavin-T (ThT) fluorescence assay to measure the aggregation of hIAPP in the absence (10 μ M, black) and presence of 1 equiv. **CurDAc** (10 μ M, grey). B) ^1H NMR spectra of **CurDAc** (250 μ M) alone (top, blue), ^1H STD NMR spectra of **CurDAc** + hIAPP (25 μ M) fibril (middle) and **CurDAc** + hIAPP (25 μ M) monomer (bottom). At a stoichiometric ratio of **CurDAc**, a full mitigation of fibrillation was observed. The absence of a STD signal in (B) indicates lack of large, slow-tumbling species or aggregates.

Fig. 3.

CD measurements indicating a change in peptide secondary structure after 24 h. CD spectra of 20 μ M hIAPP solution without (A) and with (B) 20 μ M **CurDac**. A transition from random coil to β -sheet in the absence of **CurDac** (A) and random coil to α -helix in the presence of 10 μ M **CurDac** (B, grey) was observed after 24 h. **CurDac** may stabilize a specific monomer conformation or small oligomer assemblies of hIAPP.

Fig. 4. Thioflavin-T assays of 10 μ M hIAPP (A, black) incubated with 2:1 DMPC:DHPC bicelles (A) and with **CurDAc** added (A, grey). B) Aggregation of 10 μ M hIAPP in 7:3 POPC:POPG large unilamellar vesicles (black) and with the addition of **CurDAc** (grey). Fibrillation is mitigated in both model membrane systems showing full inhibition of fibrillation as discussed in the text.