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Non-selective Ion Channel Activity of Polymorphic Human Islet Amyloid Polypeptide (Amylin) Channels

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Abstract

Fundamental understanding of ion channel formation by amyloid peptides, which is strongly linked to cell toxicity, is very critical for (pre)clinical treatment of neurodegenerative diseases. Here, we combine atomistic simulations and experiments to demonstrate a broad range of conformational states of hIAPP double channels in lipid membranes. All individual channels display high selectivity of Cl[−] ions over cations, but the co-existence of polymorphic double channels of different conformations and orientations with different populations determines the non-ionic selectivity nature of the channels, which is different from the typical amyloid- β channels that exhibit Ca²⁺ selective ion-permeable characteristics. This work provides a more complete physicochemical mechanism of amyloid-channel-induced toxicity.

Keywords

Amylin; Amyloid channels; Ion selectivity; Ion permeability; Amyloid diseases; Molecular dynamics

1. Introduction

Human islet amyloid polypeptide (hIAPP or amylin) is a 37-residue hormone peptide that is synthesized, stored, and secreted with insulin from the β -cells of pancreatic islets.^{1–3} Increasing evidence suggests that the interactions of hIAPP aggregates, particularly small and soluble oligomers, with cell membranes are at least partially responsible for the dysfunction and death of β -cells in type II diabetes.^{4–7} However, the exact mechanisms of hIAPP-oligomer-induced toxicity remain unclear, for example, how do hIAPP oligomers interact with cell membranes to induce cell toxicity and which types of oligomer conformations are involved in type II diabetes? Characterization of the conformations of hIAPP oligomers and their interactions with cell membranes at the atomic level still presents a daunting challenge.

Human IAPP and other amyloidogenic peptides have been hypothesized to induce membrane disruption by several mechanisms (i.e. receptor-dependent, ion channel, activation of endogenous ion channels, membrane detergent-like, and membrane thinning/curvature models)⁸. Among these models, the ion channel hypothesis has long argued that toxicity is mediated by unregulated channel formation by amyloidogenic proteins, leading to ion dyshomeostasis that triggers amyloid-induced neuronal apoptosis⁹. Unlike receptor-dependent mechanisms that depend on specific amyloid sequences, a number of structurally similar ion channels have been characterized by different amyloid peptides including hIAPP^{9–11}, A β ^{12–17}, and K3^{18, 19} using atomic force microscopy (AFM), electrophysiology, cell calcium imaging, and other techniques, indicating a common amyloid toxicity mechanism likely exists that is independent of the particular sequence. While channels formed from different amyloid peptides show many similarities and important differences. For example, A β channels exhibited Ca²⁺-selective ion-permeable characteristics^{20–23}, while K3¹⁸ and hIAPP^{9, 15, 24} formed relatively nonselective, voltage independent, ion-permeable channels in lipid membranes. Common experimental tools (AFM, CD, FTIR, etc) provide low resolution information about channel structures and their relation to ion permeability and selectivity, while high resolution characterization (NMR and x-ray) of these heterogeneous and dynamic channels in cell membranes has proved to be extremely difficult. More importantly, recent studies have shown that amyloid channels bear striking resemblance in channel structure and activity to other ion channels formed by antimicrobial peptide and toxin proteins²⁵, further suggesting that a general principle may govern channel-induced membrane insertion/disruption by different peptides.

Recent molecular modeling and molecular dynamics (MD) studies of single amyloid channels in cell membranes by our and others have revealed that although all A β ^{12, 13, 26, 27}, K3^{18, 19}, and hIAPP^{10, 28} channel models in lipid bilayers are highly polymorphic in regards to channel²⁹ structures and topologies, they also share some common structural characteristics: (1) the channels have irregular shapes consisting of several loosely contacting mobile subunits with a certain extent of β -sheet structures; (2) the channels have similar inner diameters of 1–2 nm and outer diameters of 8–12 nm, which has been verified by high resolution AFM¹⁵; (3) the channels are assembled by U-shaped-conformational peptides derived from their parent fibrillar structures; (4) the U-shaped peptides adopt the same “turn-to-tail” orientation to form the channels, in which the U-turn region orients towards the upper bilayer leaflet, while the N-/C-terminus orients towards the lower bilayer leaflet. Computational studies provide atomic details for illustrating the different ion conductivity and selectivity of particular amyloid channels, which could be attributed to the variability in the peptide sequence and composition, side-chain packing and backbone conformation, and channel topology. However, considering the rugged energy landscape of amyloid peptides upon interacting with cell membrane, a “one-size-fit-all” of a common amyloid channel formed by amyloid peptides with the same insertion orientation in the membrane may not be sufficient and accurate enough to illustrate the mechanisms of amyloid channel-induced toxicity and structural parameters affecting amyloid channels. It is very likely that amyloid peptides could insert into the membrane with different orientations and conformations to form heterogeneous channels in membranes.

We have recently simulated two types of single β -sheet hIAPP channels in the DOPC bilayer: a CN p NC channel with charged N-terminal β -strands facing a solvated pore and a NC p CN model with C-terminal β -strands facing a solvated pore, with each type of channels with a varying number of hIAPP peptides (12-, 18-, 24-, and 36-mer) all aligned to be parallel to the lipid bilayer via a “turn-to-tail” orientation²⁸. MD simulation results demonstrated that (i) the 18-mer and 24-mer channels of CN p NC and NC p CN presented similar numbers of oligomeric units and similar overall dimensions to the channels observed by AFM¹⁵; (ii) all hIAPP single channels induced directional permeability of multiple ions across the bilayers from the lower to the upper leaflet; (iii) more importantly, potential-mean-force (PMF) profiles showed a similar energy barrier for a single hIAPP peptide to penetrate into the bilayer via either the tail or the turn orientation. More importantly, single hIAPP channel simulations appear not to be consistent with experimental observation that amylin forms nonselective, ion-permeable channels in planar phospholipid bilayer membranes by Mirzabekov et al⁹.

To solve this discrepancy, we hypothesize that the coexistence of “turn-to-tail” and “tail-to-turn” channels in the membrane and the relative populations of these channels may play an important role in determining overall ion conductivity and selectivity, which were not achieved in the early studies. To test this hypothesis, in line with a series of computationally determined β -sheet single channel models of A β ^{12, 13, 26, 27}, K3^{18, 19}, and hIAPP^{10, 28} in lipid bilayers and the limited structural information from experiments¹⁵, we for the first time modeled and simulated a number of hIAPP “double channels” - two separate hIAPP channels - in a DOPC bilayer. The double channels are composed of 18 mers by considering a complete combination of channel conformations (CN p NC and NC p CN) and orientations (parallel and antiparallel) of the form $\uparrow_N\uparrow_N$, $\uparrow_C\uparrow_C$, $\uparrow_N\downarrow_N$, $\uparrow_C\downarrow_C$, $\uparrow_N\downarrow_C$, $\uparrow_C\downarrow_N$, where $\uparrow$ stands for a “turn-to-tail” orientation and $\downarrow$ for a “tail-to-turn” orientation and N stands for a CN p NC conformation and C for NC p CN (Figure 1). We used the image facility of CHARMM to mimic double channels of $\uparrow_N\uparrow_N$, $\uparrow_C\uparrow_C$ using a single channel of either $\uparrow_N$ or $\uparrow_C$. Complement to single channel models, double channel models allow to directly and simultaneously establish a correlate among ion conductivity, ion flow direction, and net flow of ions. We first demonstrated the feasibility of atomistic modeling of double hIAPP ion channels and provided atomic explanation of non-ionic selectivity of hIAPP channels, due to the co-existence of “turn-to-tail” and “tail-to-turn” channels in the membrane. This work provides a more complete picture of membrane permeability and disruption by amyloid peptides.

2. Materials and Method

hIAPP channel models

Initial hIAPP₁₋₃₇ monomer was extracted and averaged from 10 solid-state NMR-derived structures from Tycko's lab.³⁰ The monomeric hIAPP₁₋₃₇ had a U-bend structure with two antiparallel β -strands connected by a loop, i.e. β -strand (Lys1-Val17)-loop(His18-Leu27)- β -strand(Ser28-Tyr37)³¹⁻³³. An intra-molecular disulfide bond was present between Cys2 and Cys7 at the N-termini. The N- and C-termini were blocked by NH₃⁺ and COO⁻ groups, respectively. Our previous MD simulations of hIAPP channels in the DOPC bilayer have

demonstrated that 18-mer and 24-mer channels presented similar numbers of oligomeric units and overall dimensions to the channels observed by AFM. The bilayer did not support too small 12-mer channels or too large 36-mer hIAPP single channels²⁸. In this work, we proposed to use the single 18-mer hIAPP channel extracted from previous 60-ns MD simulations to construct double 18-mer channels by considering different combinations of (CNpNC and NCpCN) and orientations (parallel and antiparallel). The initial separation distance between two individual channels in the same lipid bilayer was set to at least 35 Å to ensure no interactions between them. Such setup of simulation systems help to clearly distinguish different ion behaviors caused by different channel topologies and to determine the preferred pore sizes, orientations, and organizations that can consistently explain ion conductivity and selectivity by experiments. The channel structures were then minimized with harmonic position constraints for structural relaxation and optimization with the surrounding water molecules and lipids. The image facility in CHARMM is used to replicate the original simulation system throughout space to form an “infinite” system with any defined size and shape, i.e. to generate periodic boundary conditions (PBCs) for any finite simulation system. In our channel-lipid systems, slab PBCs were applied to all x and y directions, allowing to mimic a planar lipid bilayer at a macroscopic level. For ↑N↑N or ↑C↑C system, a single channel of either ↑N or ↑C was imaged and replicated to mimic double channels with the same channel orientation.

MD simulations

The resulting energy-minimized channel systems were subject to equilibrium and production runs by MD simulations. In the equilibrium stage, each channel system was gradually relaxed by performing a series of dynamic cycles, in which the harmonic restraints on peptides in the channels were gradually removed to optimize the peptide-lipid and peptide-water interactions for 10 ns. In the production stage, all simulations were performed using the NPAT (constant number of atoms, pressure, surface area, and temperature) ensemble at 300 K. The surface area in the xy plane was kept constant while allowing a volume change in the z direction. The van der Waals (vdW) interactions were calculated using a switch function with a twin-range cutoff at 12 and 14 Å. Long-range electrostatic interactions were calculated using the Particle Mesh Ewald (PME) method. The equations of motion were integrated using the velocity Verlet integrator with a step of 2 fs. Each hIAPP-lipid system was repeated twice using the same channel configuration, different lipid conformations randomly selected from the lipid library, different initial velocities for all atoms. All MD simulations were performed using the NAMD software³⁴ with CHARMM27 force field³⁵. All MD simulations ran for 80 ns and MD trajectories were saved by every 2 ps for analysis. A summary of all simulation systems was listed in Table 1.

Population analysis

To quantitatively obtain the relative populations of the variant hIAPP channels, we extracted the channel structures from the last 20-ns MD simulations. A total of 3000 conformations (500 conformations for each double hIAPP channel) were used to construct the free energy landscapes of the channel conformers and to evaluate the conformer probabilities using in-house Monte Carlo (MC) simulations³⁶. Specifically, two conformers of i and j were randomly selected, and then compared using $e^{-E_j - E_i / KT}$, where E_i and E_j are the

conformational energies of the respective conformers of *i* and *j*, *K* is the Boltzmann constant, and *T* is the absolute temperature of 300 K used here. If the Boltzmann factor value is larger than a random number, the move from conformer *i* to conformer *j* is allowed. After 1 million steps, the conformers visited for each channel system were counted. Finally, the relative probability of a channel system (*n*) was evaluated as: $P_n = N_n / N_{\text{total}}$, where P_n is the population of the channel *n*, N_n is the total number of conformations visited for the channel *n*, and N_{total} is the total number of steps. Considering the complex structural polymorphism of hIAPP ion channels, the populations of various channels are only indicative.

Materials

hIAPP and rIAPP (97% purity) was purchased from Anaspec (Fremont, CA). 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine sodium salt (POPS) were purchased from Avanti Polar lipids Inc. (Alabaster, AL). 6-carboxyfluorescein was purchased from Sigma-Aldrich (St. Louis, MO). Lucigenin was purchased from Invitrogen (Eugene, OR).

Preparation of model membrane

Large unilamellar vesicles (LUV) of POPC/POPS 7/3, were prepared from a chloroform solution of lipids in the desired ratio. The solution was gently dried under nitrogen flow and then placed under a high vacuum overnight to further evaporate any residual solvent. The dry lipid film was hydrated with a buffer solution containing 1 mM Lucigenin (10 mM phosphate buffer, 100 mM KNO₃, pH 7.4) to a final concentration of 10 mg/ml. The resulting solutions were extruded 23 times through a 100 nm polycarbonate Nucleopore membrane filter (Whatman) mounted on a mini-extruder in order to obtain LUVs with an average diameter of 100 nm. Removal of any non-encapsulated carboxyfluorescein or lucigenin was performed by running the extruded LUV solution through a Sephadex G50 gel exclusion column (Sigma-Aldrich) and collecting the first band detectable under UV light which contained the separated dye-containing vesicles. The final lipid concentrations were measured using the Stewart assay. All solutions were freshly prepared before each experiment.

Lucigenin assay

The presence of membrane disruption was detected by measuring decreasing in the fluorescence at 520 nm upon binding of Cl⁻ to encapsulated lucigenin or through the concentration dependent increase in carboxyfluorescein fluorescence from its release from LUVs upon membrane disruption. Samples were prepared by first diluting the dye-filled vesicles solution with buffer solution (10 mM phosphate buffer solution, 100 mM NaCl, pH 7.4) to a final concentration of 250 μM. Then, hIAPP was added to a final concentration of 5 μM. Experiments were carried out in Corning 96 well non-binding surface plates. Time traces were recorded using Biotek Synergy 2 plate reader using a 2 nm bandpass filters at 440 nm excitation and 520 nm emission at 25°C, shaking samples for 10 seconds before each read. The fraction of membrane disrupted was calculated by: $1 - [(I - I_{100}) / (I_0 - I_{100})]$, where *I* is the emission intensity of the sample, *I*₀ is the emission intensity obtained in the

absence of peptide (baseline control) and I_{100} is the emission intensity obtained after adding 1 μ l of Triton X-100, a detergent which acted as a positive control to give 100% leakage.

3. Results and Discussion

Figure 2 shows the top-view of channel topologies by projecting the C α atoms of hIAPP peptides on the bilayer surface averaged from the last 10-ns simulations. It can be seen that all hIAPP double channels lost their initial perfect circular shapes and gradually formed 3–5 subunits, which loosely contacted with each other to retain the pore-like structures. The channels kept open during the simulations with average inner/outer diameters of 1.8/8.0 Å, which allow water and some ions to be readily diffuse through the channels. The two channels were separated by at least 35 Å, indicating that the two channels did not interact with each other to induce conformational changes. Such hIAPP double channels exhibited similar sizes as other single amyloid channels consisting of several loosely contacting mobile subunits with a certain degree of β -sheet structure^{15, 24, 37–39} and exhibited similar irregularly-shaped morphologies. Visual inspection of MD trajectories also showed that once the hIAPP channels were formed in the DOPC bilayer, they remained open pore-like structures without disaggregation of individual channels or exchange of hIAPP between the two channels during the simulations. Our hIAPP channels containing 18–24 mers have similar channel sizes to other β -sheet amyloid channels of A β , k3, and PG-1 containing 16–24 mers^{40–45}. This suggests that similar size of β -structure-based amyloid pores may indicate the common pore-assembling structures independent of the sequences.

To confirm the existence of open pores during the early phase of aggregation, we measured the influx of negatively charged molecules into POPC/POPS (7/3) Large Unilamellar Vesicles (LUVs) after incubation with hIAPP (Figure 3) by using a lucigenin fluorescence assays along with a comparison to previous results⁴¹ with the larger negatively charged dye carboxyfluorescein. Briefly, we prepared POPC/POPS LUV samples filled with either the Cl[−] sensitive dye Lucigenin or the concentration sensitive dye carboxyfluorescein. Pore formation by hIAPP allows Cl[−] to enter the previously inaccessible interior of the vesicle, where it can bind the dye and change its fluorescence. A significantly smaller increase is seen for carboxyfluorescein. The results are in agreement with the several aspects of the simulations. First, the fluorescence of lucigenin increases immediately after the addition of hIAPP. The fast response indicates Cl[−] ions can penetrate into the LUVs almost immediately after the addition of hIAPP, well before amyloid formation begins after several hours⁴⁶. Immediate membrane disruption is consistent with the early phase of the two-step model of membrane disruption^{43, 44}, in which membrane defects form before the membrane is totally disrupted by fiber growth on the membrane surface^{46–48}. Although we cannot confirm the existence of discrete pores as opposed to other types of non-specific membrane disruption, the results are consistent with pore formation preceding fiber formation, as has been shown to occur with A β_{1-40} ¹⁷. Second, the higher rate of permeability for the smaller Cl[−] ion (stokes radius = 0.2 nm)¹⁶ compared to the larger carboxyfluorescein molecule (stokes radius = 0.7 nm)¹⁰ suggests the pore may be relatively narrow in some places, consistent with previous simulations.²⁸

To quantitatively examine whether the membrane environment supports channel structures and activity, the interaction of channel peptides with lipids and water molecules were respectively calculated by averaging over time and the number of peptides in the channels (Figure 4). Overall, all double channels had favorable interactions (negative value) with both lipids and water molecules, suggesting that the environment supports channel conformation. Specifically, the total interaction energies between peptides and lipids for all channel systems were similar to each other (-130 to -122 kcal/mol), but the conformational energy of the CNpNC channels was relatively lower than that of the NCpCN channels, because the CNpNC channels had more favorable hydrophobic interactions between the hydrophobic C-terminal and the hydrophobic lipid tails. Different channel conformations (CNpNC vs. NCpCN) and orientations (parallel vs. antiparallel) also induced different contributions from the decomposed electrostatic and VDW energy. For the double channels with the same channel conformation, $\uparrow_N\uparrow_N$ and $\uparrow_N\downarrow_N$ channels had comparable VDW and electrostatic interaction energies ($\sim 50\%$), while $\uparrow_C\uparrow_C$ and $\uparrow_C\downarrow_C$ channels had more favorable electrostatic interaction energies ($\sim 62\%$) because the hydrophilic N-terminal residues (including positively charged Lys1 and Arg11) were packed against the zwitterionic heads of the lipids to enhance electrostatic interactions. In the heterogeneous double channels of $\uparrow_N\downarrow_C$ and $\uparrow_N\uparrow_C$ was in between $\uparrow_N\uparrow_N/\uparrow_N\downarrow_N$ systems and $\uparrow_C\uparrow_C/\uparrow_C\downarrow_C$ systems.

For peptide-water interactions, visual inspection of MD trajectories showed that all hIAPP double channels were well hydrated, including a water pore and a hydrated cavity inside the U-turns. Peptide-water interactions were much stronger than peptide-lipid interactions, suggesting that unbalanced interactions could be attributed to the channel breaking into several subunits. Further, the deuterium order parameter S_{CD} was used to quantify the perturbation effects of hIAPP channels on the lipid orderings. S_{CD} values of lipids for all channel systems were similar to each other, but slightly smaller than those in a pure DOPC bilayer (Figure 5), suggesting that the membrane-embedded hIAPP channels induce only minor local deformation and ordering of neighboring lipids. The minor local deformation of the membrane seen in the simulations is similar to experimental results^{49, 50} obtained on an hIAPP variant (hIAPP₁₋₁₉) believed to form pores⁵¹, and is in contrast to the larger membrane disordering occurring during nonspecific membrane disruption by hIAPP^{11, 52}. Taken together, the simulation results indicate that different combinations of CNpNC and/or NCpCN double channels in a parallel or antiparallel packing can coexist in the lipid environment and neither conformation or orientation is strongly preferred.

To characterize the ion binding selectivity of the channels, the PMF of each channel was calculated along the z axis of the channel interior to represent the relative free energy profile for each ion across the bilayer via pore permeation, using $G_{PMF} = -k_B T \ln(\rho_z/\rho_{bulk})$ where k_B is the Boltzman constant, ρ_z is the ion density at position z along the channel axis, and ρ_{bulk} is the ion density in the bulk phase (Figure 6). The channel systems contained three cations of Ca^{2+} , Na^+ , K^+ at the same concentration (~ 35 mM) and an anion of Cl^- . A negative (positive) PMF value indicates favorable (unfavorable) interactions of ions with the surrounding environment, which suggests a high (low) probability for ion binding to the bilayers. In all channels, the PMF curves of Cl^- displayed a similar asymmetrical and continuous S-shape along the channels. The horizontal S-shape PMF curves clearly

presented an energy barrier of 1–2 kcal/mol for Cl^- ion transport through the channels in a preferential direction from the turn (Ser19 to Asn22) to the tail region (Lys1 and Tyr37), almost independent of channel orientations and conformations. For the CNpNC channels, Lys1 (–1.7 nm) and Arg11 (–0.7 nm) near the N-terminal tail formed a circular cluster which provides a strong attractive force to drive Cl^- into the interior of the solvated channels, while on the opposite side of the channels hydrophobic Phe15 and Val17 clusters (~1.8 nm) near the turn region created an energy barrier of 1~2 kJ/mol for preventing Cl^- from entry into the channels from this direction. Both effects would cause Cl^- to preferentially enter the CNpNC channels from the tail region. This trend remained in the NCpCN channels but was less pronounced. In the NCpCN channels, the amphiphilic C-terminal β -strands, rather than the positively charged N-terminal β -strands, formed the solvated pore, resulting in a decrease of attractive electrostatic interactions between Cl^- and Lys1/Arg11 residues. Accordingly, the minima around ± 1 nm observed in the PMF curves of CNpNC channels are either greatly decreased or entirely absent in the PMF curves of the NCpCN channels (Figure 6). So, the coexistence of the CNpNC and NCpCN channels in a parallel or antiparallel orientation would enhance (reduce) the direction permeability of Cl^- ions. The three cations had distinctly different PMF profiles. The energy barriers for Ca^{2+} binding were lower than those of K^+ and Na^+ . Ca^{2+} ions were dominantly bound to both ends of the channels, as evidenced by two negative minima at $\sim \pm 2.7$ nm. This fact indicates that Ca^{2+} had favorable interactions with the zwitterionic phosphate heads of the lipids, as observed experimentally⁵³. Unlike Ca^{2+} , which exhibits a high binding probability to bilayers, all channels possessed strong repulsive forces against the entry and binding of Na^+ and K^+ to the channels. Moreover, the preferential and dominant binding of Ca^{2+} to the DOPC bilayer also reduces the possibility for Na^+ and K^+ to bind to and cross the bilayer due to a Guoy-Chapman effect.

To investigate the net ion conductivity of hIAPP double channels, Figure 7 shows the number of ions across each of double channels as a function of time, where a positive value indicates ion transport from the tail to the turn, and a negative value indicates ion transport from the turn to the tail. Because of the low energy barrier for Cl^- transportation, all channels were highly selective and conductive for Cl^- over other cations. Cl^- conductivity curves, combined with visual inspection of MD trajectories, showed that the entering Cl^- ions were hopping concertedly inside the channels in a pulse-like motion. Such hop-jump motion of Cl^- ions in a confined space can be described by the continuous-time random walk model⁵⁴. More importantly, regardless of channel conformations (NCpCN or CNpNC), Cl^- conductivity displayed directional permeability in a preferential direction, i.e. Cl^- tended to conduct through the channels from the tail side to the turn side, as shown by positive conductivity values in the $\uparrow$ channel orientation and negative values in a $\downarrow$ orientation, consistent with the PMF results. Specifically, for $\uparrow_{\text{N}}\downarrow_{\text{N}}$ or $\uparrow_{\text{C}}\downarrow_{\text{C}}$ systems, Cl^- exhibited opposite ion conductance in the two conformationally the same, but orientational opposite, channels although all Cl^- ions transported from tail side to turn side. The same amount of Cl^- ions permeated through the bilayer in each direction for the $\uparrow_{\text{N}}/\downarrow_{\text{N}}$ double channel (+12/–12), while only a slight excess of Cl^- permeated to the turn side (+10/–13) for the $\uparrow_{\text{C}}/\downarrow_{\text{C}}$ channels, leading to almost “zero” selectivity and conductivity of Cl^- for both types of homogenous double channels. For heterogeneous double channels of $\uparrow_{\text{N}}\uparrow_{\text{C}}$ and

$\uparrow_N\downarrow_C$, directional permeability of Cl^- was also observed. In the $\uparrow_N\uparrow_C$ system, Cl^- exhibited positive conductance for both single channels, although the net conductance was small. In the $\uparrow_N\downarrow_C$ system, opposite Cl^- conductance through $\uparrow_N$ and $\downarrow_C$ channels was observed. Considering the relatively higher energy barrier for cation permeation observed in the PMF data, most of the double channels exhibited weak or negligible cation conductivities. Nevertheless, there still exists small possibility for the cations to across the channels via thermodynamic diffusion. This is particularly true for Ca^{2+} , since a large amount of Ca^{2+} ions were bound to the zwitterionic lipid head region, thus increasing the channel conductance probability for this cation. Taken together, all double channels with an antiparallel packing ($\downarrow_N\uparrow_C$, $\uparrow_N\downarrow_N$, $\uparrow_C\downarrow_C$) directly exhibited non-selective ion conductivity, while the other parallel-packing double channels ($\uparrow_N\uparrow_N$, $\uparrow_C\uparrow_C$, $\uparrow_N\uparrow_C$) showed directional but weak ion conductivity, in agreement with our previous simulations of single hIAPP channels.

Considering the complex structural polymorphism of hIAPP ion channels, our proposed double-channel models and single-channel models are likely to represent only a very small percentage among the channel ensemble, however they cover the most likely membrane-supported channel organizations. We estimated the overall population for (1) six different double channels and (2) two individual channels with different orientations and conformations using an in-house Monte Carlo simulation to calculate conformational energies of the corresponding channels (3000 conformations), as reported in our previous work³⁶. For the single-channels, the $\uparrow_N$ model (71.5%) was much more populated than the $\uparrow_C$ model (28.5%) (Figure 8). $\uparrow_N$ had a more favorable hydrophobic match between hydrophobic C-terminal residues and interior lipids, while $\uparrow_C$ had the mismatch of cationic N-terminal residues with interior lipids. When individual channels assemble into double channels, the population distribution becomes much wider. Based on the populations and the relative stabilities, these double channel models can be classified into three categories: $\uparrow_N\downarrow_N$ occupied the highest population of 30.2%; $\uparrow_N\uparrow_N$ (13.1%), $\uparrow_N\uparrow_C$ (17.6%), $\uparrow_N\downarrow_C$ (19.4%), and $\uparrow_C\downarrow_C$ (15.8%) had comparable populations; $\uparrow_C\uparrow_C$ only represented 3.8% of the ensemble, but it does exist in varied polymorphic channels. Interestingly, the three double channels with opposite channel orientations ($\uparrow_N\downarrow_C$, $\uparrow_N\downarrow_N$, $\uparrow_C\downarrow_C$) accounted for 66.4% of the total and were more populated than the other three double channels with the same orientation (Figure 8). The coexistence of “turn-to-tail” and “tail-to-turn” channels in the membrane, with the dominant channel populations with an opposite orientation, helps to interpret overall non-ionic conductivity and selectivity.

4. Conclusions

To conclude, we for the first time investigate the structure and activity of double hIAPP channels in the DOPC bilayer using MD simulations and experiments. Six different double hIAPP channels are modeled by considering different channel conformations (NCpCN and CNpNC) and channel orientations. The computational results support that hIAPP peptides can penetrate the lipid bilayer via the turn region or the N/C-terminal region with further self-assembly into “turn-to-tail” and “tail-to-turn” channels. The resultant channels fragment into three to five oligomeric units that are loosely associated, but remain open, channel-like structures in the bilayer, similar to other amyloid channels by A β and K3. The hIAPP

channels exhibit relatively high ion binding selectivity and ion conductivity of Cl^- over cations. Although all cations exhibit relatively weak ion fluxes, poor ion conductivity of Na^+ and K^+ is due to the bare adsorption on the lipid bilayer while that of Ca^{2+} is due to strong binding affinity to phosphate groups in the lipid heads. A broad range of conformational states of hIAPP channels with comparable populations of these double channels result in non-ionic-selective channel conductance, consistent with experimental data. Considering the polymorphic nature of amyloid aggregates, our hIAPP β -sheet pore models do not necessarily exclude other possible pore structures (e.g. β -barrel⁵⁵, helical bundle pore, mixed helical/ β -sheet pore). As such we expect a range of amyloid pore structures in the rugged energy landscape. We hope that tying together with single amyloid channel models, our β -sheet double channel models could provide a more complete picture for the activity of amyloid channels associated with amyloid toxicity.

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References

1. Westerma P, Grimeliu L. Acta Pathologica Et Microbiologica Scandinavica Section a-Pathology. 1973; A 81:291–300.
2. Cooper GJS, Willis AC, Clark A, Turner RC, Sim RB, Reid KBM. Proceedings of the National Academy of Sciences of the United States of America. 1987; 84:8628–8632. [PubMed: 3317417]
3. Kanatsuka A, Makino H, Ohsawa H, Tokuyama Y, Yamaguchi T, Yoshida S, Adachi M. FEBS Letters. 1989; 259:199–201. [PubMed: 2689229]
4. Haataja L, Gurlo T, Huang CJ, Butler PC. Endocrine Reviews. 2008; 29:303–316. [PubMed: 18314421]
5. Schneider HM, Storkel S, Will W. Dtsch Med Wochenschr. 1980; 105:1143–1147. [PubMed: 7004825]
6. Clark A, Lewis CE, Willis AC, Cooper GJS, Morris JF, Reid KBM, Turner RC. Lancet. 1987; 2:231–234. [PubMed: 2441214]
7. Dekoning EJP, Morris ER, Hofhuis FMA, Posthuma G, Hoppener JWM, Morris JF, Capel PJA, Clark A, Verbeek JS. Proceedings of the National Academy of Sciences of the United States of America. 1994; 91:8467–8471. [PubMed: 8078905]
8. Brender JR, Salamekh S, Ramamoorthy A. Acc Chem Res. 2012; 45:454–462. [PubMed: 21942864]
9. Mirzabekov TA, Lin M-c, Kagan BL. Journal of Biological Chemistry. 1996; 271:1988–1992. [PubMed: 8567648]
10. Xu W, Wei G, Su H, Nordenskiöld L, Mu Y. Physical Review E. 2011; 84:051922.
11. Ping J, Weixin X, Yuguang M. PLoS Computational Biology. 2009;5.
12. Jang H, Zheng J, Nussinov R. Biophys J. 2007; 93:1938–1949. [PubMed: 17526580]
13. Jang H, Zheng J, Lal R, Nussinov R. Trends Biochem Sci. 2008; 33:91–100. [PubMed: 18182298]
14. Jang H, Arce FT, Ramachandran S, Capone R, Azimova R, Kagan BL, Nussinov R, Lal R. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:6538–6543. [PubMed: 20308552]
15. Quist A, Doudevski I, Lin H, Azimova R, Ng D, Frangione B, Kagan B, Ghiso J, Lal R. PNAS. 2005; 102:10427–10432. [PubMed: 16020533]

16. Xu W, Zhang C, Morozova-Roche L, Zhang JZH, Mu Y. *The Journal of Physical Chemistry B*. 2013; 117:8392–8399. [PubMed: 23786168]
17. Zhao LN, Long HW, Mu Y, Chew LY. *International Journal of Molecular Sciences*. 2012; 13:7303–7327. [PubMed: 22837695]
18. Mustata M, Capone R, Jang H, Arce FT, Ramachandran S, Lal R, Nussinov R. *Journal of the American Chemical Society*. 2009; 131:14938–14945. [PubMed: 19824733]
19. Jang H, Arce FT, Ramachandran S, Capone R, Lal R, Nussinov R. *Journal of Physical Chemistry B*. 2010; 114:9445–9451.
20. Kawahara M, Kuroda Y. *Brain Research Bulletin*. 2000; 53:389–397. [PubMed: 11136994]
21. Demuro A, Mina E, Kayed R, Milton SC, Parker I, Glabe CG. *J Biol Chem*. 2005; 280:17294–17300. [PubMed: 15722360]
22. Arispe N, Pollard HB, Rojas E. *PNAS*. 1993; 90:10573–10577. [PubMed: 7504270]
23. Lin H, Bhatia R, Lal R. *FASEB J*. 2001; 15:2433–2444. [PubMed: 11689468]
24. Hirakura Y, Kagan BL. *Amyloid*. 2001; 8:94–100. [PubMed: 11409039]
25. Butterfield SM, Lashuel HA. *Angew Chem Int Ed*. 2010; 49:5628–5654.
26. Jang H, Arce FT, Capone R, Ramachandran S, Lal R, Nussinov R. *Biophysical Journal*. 2009; 97:3029–3037. [PubMed: 19948133]
27. Jang H, Arce FT, Ramachandran S, Capone R, Lal R, Nussinov R. *Journal of Molecular Biology*. 2010; 404:917–934. [PubMed: 20970427]
28. Zhao J, Luo Y, Jang H, Yu X, Wei G, Nussinov R, Zheng J. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 2012; 1818:3121–3130.
29. U S C. 1994; 42:102–105.
30. Luca S, Yau WM, Leapman R, Tycko R. *Biochemistry*. 2007; 46:13505–13522. [PubMed: 17979302]
31. Zhao J, Yu X, Liang G, Zheng J. *Biomacromolecules*. 2011; 12:1781–1794. [PubMed: 21428404]
32. Liang G, Zhao J, Yu X, Zheng J. *Biochemistry*. 2013; 52:1089–1100. [PubMed: 23331123]
33. Zhao J, Yu X, Liang G, Zheng J. *Biomacromolecules*. 2011; 12:210–220. [PubMed: 21158384]
34. Kale L, Skeel R, Bhandarkar M, Brunner R, Gursoy A, Krawetz N, Phillips J, Shinozaki A, Varadarajan K, Schulten K. *J Comput Phys*. 1999; 151:283–312.
35. MacKerell AD, Bashford D, Bellott M, Dunbrack RL, Evanseck JD, Field MJ, Fischer S, Gao J, Guo H, Ha S, Joseph-McCarthy D, Kuchnir L, Kuczera K, Lau FTK, Mattos C, Michnick S, Ngo T, Nguyen DT, Prodhom B, Reiher WE, Roux B, Schlenkrich M, Smith JC, Stote R, Straub J, Watanabe M, Wiorkiewicz-Kuczera J, Yin D, Karplus M. *J Phys Chem B*. 1998; 102:3586–3616. [PubMed: 24889800]
36. Yu X, Zheng J. *PLoS ONE*. 2011; 6:e20575. [PubMed: 21687730]
37. Petrache HI, Tristram-Nagle S, Gawrisch K, Harries D, Parsegian VA, Nagle JF. *Biophysical Journal*. 2004; 86:1574–1586. [PubMed: 14990484]
38. Lashuel HA, Lansbury PT Jr. *Quarterly Reviews of Biophysics*. 2006; 39:167–201. [PubMed: 16978447]
39. Chang Z, Luo Y, Zhang Y, Wei G. *Journal of Physical Chemistry B*. 115:1165–1174.
40. Jang H, Teran Arce F, Ramachandran S, Capone R, Lal R, Nussinov R. *The Journal of Physical Chemistry B*. 2010; 114:9445–9451. [PubMed: 20608696]
41. Connelly L, Jang H, Teran Arce F, Capone R, Kotler SA, Ramachandran S, Kagan BL, Nussinov R, Lal R. *The Journal of Physical Chemistry B*. 2012; 116:1728–1735. [PubMed: 22217000]
42. Jang H, Teran Arce F, Ramachandran S, Kagan BL, Lal R, Nussinov R. *The Journal of Physical Chemistry B*. 2013; 117:11518–11529. [PubMed: 24000923]
43. Connelly L, Jang H, Teran Arce F, Ramachandran S, Kagan BL, Nussinov R, Lal R. *Biochemistry*. 2012; 51:3031–3038. [PubMed: 22413858]
44. Mustata M, Capone R, Jang H, Arce FT, Ramachandran S, Lal R, Nussinov R. *Journal of the American Chemical Society*. 2009; 131:14938–14945. [PubMed: 19824733]
45. Capone R, Jang H, Kotler SA, Kagan BL, Nussinov R, Lal R. *Biochemistry*. 2012; 51:776–785. [PubMed: 22242635]

46. Engel MFM, Khemtemourian L, Kleijer CC, Meeldijk HJD, Jacobs J, Verkleij AJ, de Kruijff B, Killian JA, Hoppener JWM. PNAS. 2008; 105:6033–6038. [PubMed: 18408164]
47. Sparr E, Engel MFM, Sakharov DV, Sprong M, Jacobs J, de Kruijff B, Hoppener JWM, Antoinette Killian J. FEBS Letters. 2004; 577:117–120. [PubMed: 15527771]
48. Brender JR, Durr UHN, Heyl D, Budarapu MB, Ramamoorthy A. Biochimica et Biophysica Acta (BBA) - Biomembranes. 2007; 1768:2026–2029.
49. Zhuang, Y. Gujian chungiu. Sichuan kexue jishu; Chnegdu: 1989.
50. Smith PES, Brender JR, Ramamoorthy A. Journal of the American Chemical Society. 2009; 131:4470–4478. [PubMed: 19278224]
51. ECD, Xiao D, CAF, Zhou S, EBF, Cao Z, Li Z, F2A FB, Miao Y, DB, Zhu S, C6A BB, Wang J, FB, Wang Y, AAB, Fang H. 2008 @4E2D@56FD@51FA@7248@901A@53F2, @4E2D@56FD@4E66@7C4D, @5317@4eac.
52. Ye, Y.; Liu, C. Natural gas hydrates experimental techniques and their applications. Springer; Berlin; New York: 2013.
53. Sciacca, Michele FM.; Milardi, D.; Messina, Grazia ML.; Marletta, G.; Brender, Jeffrey R.; Ramamoorthy, A.; La Rosa, C. Biophysical Journal. 2013; 104:173–184. [PubMed: 23332070]
54. Hummer G. Molecular Physics. 2007; 105:201–207.
55. Chang Z, Luo Y, Zhang Y, Wei G. The Journal of Physical Chemistry B. 2010; 115:1165–1174. [PubMed: 21192698]

Figure 1.
Illustration of double hIAPP channels with a “turn-to-tail” and a “tail-to-turn” orientations.

Figure 2.

Schematic presentation and MD snapshots of the six most likely double channels of hIAPP. C α atoms of the channels are projected onto the x - y plane to illustrate overall channel topologies. The probability to find C α atoms is calculated and colored in an increased order of red, orange, yellow, green, blue, and purple to represent subunit organization of the channels. Color codes in the schematic presentation are N-terminal strands (red) and C-terminal strands (blue).

Figure 3.

Influx of chloride and efflux of carboxyfluorescein ions induced by 5 μ M hIAPP 250 μ M POPC/POPS (7/3) LUVs. Freshly dissolved hIAPP was added to each sample at time zero.

Figure 4. Interaction energy of channel peptides with (left) DOPC lipids and (right) surrounding water molecules. All interactions energies are normalized by the number of peptides.

Figure 5.

Deuterium order parameter (SCD) for two unsaturated acyl chains of DOPC lipids for all hIAPP channels.

Figure 6.

Potential of mean force (PMF), GPMF, for Cl^- (black), Na^+ (red), K^+ (green), and Ca^{2+} (blue) as a function of the distance along the center axis of the hIAPP channels. GPMF is calculated using $-kBT \ln(\rho_z/\rho_{\text{bulk}})$, where kB is the Boltzmann constant, T is the simulation temperature, ρ_z is the ion density at the position z along the channel axis, and ρ_{bulk} is the ion density in the bulk region. PMF represents the relative binding probability of each ion with the channels.

Figure 7.

Net ion conductivity of Cl^- (black), Na^+ (red), K^+ (green), and Ca^{2+} (blue) across the hIAPP double channels. A positive value indicates ion transport from the tail to the turn, and a negative value indicates ion transport from the turn to the tail.

Figure 8. Population of polymorphic hIAPP channels. The populations had been estimated via in-house Monte-Carlo simulations.

Table 1

Simulation details of hIAPP double channels in the DOPC bilayer.

Systems	Atom Number	Lipid Number (upper, lower)	Ion Number		Ion Strength (mM)	Time (ns) and runs
			Cl ⁻	Cations		
↑N↑N	82388	133,125	104	17	238.3	80, 2
↑C↑C	80516	128,119	104	17	243.2	80, 2
↑N↑C	162904	257, 248	208	34	241.8	80, 2
↑N↓C	162904	252, 253	208	34	241.8	80, 2
↑N↓N	164776	260, 256	208	34	238.3	80, 2
↑C↓C	161032	249, 245	208	34	243.2	80, 2
DOPC	33712	60, 60	16	12	165.5	40, 2