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Unraveling the early steps of IAPP-mediated membrane damage: insights from All Atom and Coarse Grained Molecular Dynamics simulations

IAPP-Mediated Membrane Damage

Amyloidoses are a class of diseases, including Type II Diabetes Mellitus (T2DM), Alzheimer's (AD) and Parkinson's Diseases (PD), characterized by the conversion of peptides or proteins from their soluble functional states into fibrillar aggregates showing a cross-beta super-secondary structure termed "amyloid" (1). Investigations looking to explain the observed correlation of amyloid plaques and disease have led to what is known as the "amyloid hypothesis". Nonetheless, more recently it has become evident that the amyloid hypothesis must be amended. This adjustment stems from indications that amyloid fibers themselves are not causal to disease pathology. For example, the correlation of the extent of the Amyloid β peptide (Aβ) deposits with dementia in AD patients is poor (2). Similarly, amyloid deposition in type II diabetes is evident in >90% of patients (3,4). However, this fails to explain the fact that the remaining 10% of patients do not present significant amyloid deposition (5). Such observations suggest that intermediate structures of amyloid formation could be more relevant to pathology. To date, the mechanism by which these amyloid intermediates cause cytotoxicity and disease is not clarified. One of the major hypotheses is that amyloid peptides cause membrane perturbation through changes in membrane fluidity, amyloid peptide-induced channel formation, or free radical production and concomitant lipid peroxidation (6-8). They may affect the plasma membrane as well as internal membranes such as the mitochondrial membranes. Many amyloid-forming peptides are generally amphipathic structures. As such, they may have the capacity to insert into membranes where they may eventually generate protein-stabilized pores (poration), lay on one leaflet of membranes (carpeting), or remove lipid components from the bilayer by a detergent-like mechanism (9). There is as yet no consensus on which perturbations are relevant to disease.

Islet Amyloid Polypeptide (IAPP) is the primary component of the islet amyloid deposits observed in the pancreas of type 2 diabetics (10). It is a 37 amino acid residue peptide and is produced in the islet β-cells and co-secreted with insulin. Like other amyloid-forming peptides, IAPP has been observed to permeabilize lipid membranes (11-15). The non-amyloidogenic rat derivative (rIAPP) and mature IAPP fibrils, however, do not significantly affect the lipid bilayers . Many attempts have been made to determine the nature of the IAPP-induced defects in membrane integrity. Ion channels produced in planar lipid bilayer conductance experiments upon addition of IAPP, had a clearly detectable conductance but pores were poorly selective. This suggests, in accordance to time-lapse AFM experiments, that the IAPP-induced transmembrane ion permeability may not be mediated by the formation of channels

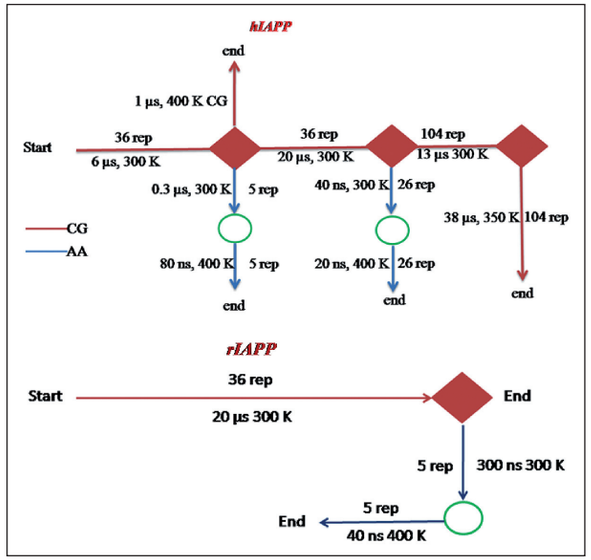

Fig. 1. – Simulations' strategy used in the current work and schematized in the upper part for the hIAPP and in the lower part for rIAPP- Both peptides were embedded in a POPC bilayer and simulated at different temperatures (300, 350 and 400 K). the red arrows indicate the coarse grained (CG) representation and the blue arrows the atomistic one (AA).

with a defined molecular structure and that the instability of the lipid bilayer cannot be explained simply by the formation of discrete isolated pores. On the other hand, some studies point to a critical role of hIAPP monomers in membrane interactions suggesting that the nature of the very initial steps of IAPP-membrane interactions should be found in a cascade of self-assembly mechanisms of membrane-bound IAPP monomers. To date, early and intermediate lipid membrane interaction events of monomeric IAPP, are difficult to access either experimentally or computationally. Experimentally, the major challenge lies in the preparation of structurally homogeneous, monomeric IAPP samples in solution or in lipid membranes. This difficulty stems from the peptide's propensity to spontaneously form mesoscopic oligomers. Computationally, only the NMR-structure of IAPP in micelles, a lipid membrane-mimicking system, is currently available for in silico studies (16).

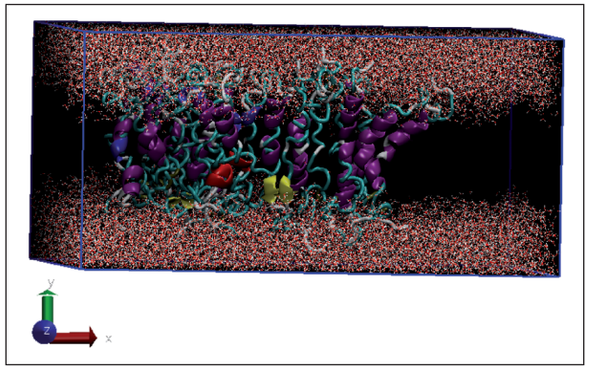

Fig. 2. – Side view of the structure of the semi-toroidal aggregate formed by 26 hIAPP molecules after 20 ns of AA simulation at 300 K.

Furthermore, full atomistic Molecular Dynamics are computationally challenging and existing simulation studies have been limited to a small portion of the bilayer with one peptide and short time duration (5-20 ns). Therefore, it is necessary to adopt computational methods, which can simulate bigger sized bilayers with many peptides for a sufficiently large time period.

In this paper, we use a Coarse-Grained Molecular Dynamics (CG-MD) simulation method to study the self-assembling between IAPP peptides (up to 36 replicas) embedded in a relatively large piece of a palmitoy-oleyl-phosphatidylcoline (POPC) lipid bilayer membrane in equilibrium with an electrolyte-containing box for a very long time period (70 µs, Figure 1). In order to overcome limitations inherent to the poor description of structural details in CG protocols we have periodically performed full-atomistic simulations of reverse-mapped CG frames along all the whole simulation time. The outcome of MD simulations has allowed us to unveil the effects of IAPP-membrane interaction on properties such as different kind of interaction between peptides, internal conformation of peptides and membrane curvature, parameters which are all relevant for the poration/leakage of membranes (Figure 2). Finally, the MD results on the protein aggregation within lipid bilayers have enabled us to develop a general thermodynamic model aimed at describing much larger equilibrium systems lying outside the present available computational tools for what concerns both the number of monomers and the computation time.

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References

(1) G. Merlini and V. Bellotti, Molecular mechanisms of amyloidosis. *N Engl J Med,* **349**, 583-596 (2003).
(2) D.W. Dickson, The pathogenesis of senile plaques. *J. Neuropathol. Exp. Neurol.* **56**, 321-39 (1997).
(3) J.W. Hoppener, B. Ahren and C.J. Lips, Islet amyloid and type 2 diabetes mellitus. *N. Engl. J. Med.* **343**, 411-19 (2000).
(4) S.E. Kahn, S. Andrikopoulos, and C.B. Verchere, Islet amyloid: a long-recognized but underappreciated pathological feature of type 2 diabetes. *Diabetes* **48**, 241-53 (1999).
(5) L. Haataja, T. Gurlo, C.J. Huang and P.C. Butler, Islet amyloid in type 2 diabetes, and the toxic oligomer hypothesis. *Endocr. Rev.* **29**, 303-16 (2008).
(6) J.J. Kremer, M.M. Pallitto, D.J. Sklansky, and R.M. Murphy, Correlation of beta-amyloid aggregate size and hydrophobicity with decreased bilayer fluidity of model membranes. *Biochemistry*, **39**, 10309-10318 (2000).
(7) D.A. Butterfield, A. Castegna, C.M. Lauderback and J. Drake, Evidence that amyloid beta-peptideinduced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death. *Neurobiol. Aging,* **23**, 655-664 (2002).
(8) B.L. Kagan, Y. Hirakura, R. Azimov, R. Azimova and M.C. Lin, The channel hypothesis of Alzheimer's disease: current status. *Peptides*, **23**, 1311-1315 (2002).
(9) Hedba and Miranker, *Annu. Rev. Biophys.* **38**,125-52 (2009).
(10) G.J. Cooper, A.C. Willis, A. Clark, R.C. Turner, R.B. Sim and K.B. Reid, Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. *Proc. Natl. Acad. Sci. USA*, **84**, 8628-8632 (1987).
(11) T.A Harroun, J.P. Bradshaw and R.H. Ashley, Inhibitors can arrest the membrane activity of human islet amyloid polypeptide independently of amyloid formation. *FEBS Letters*, **507**, 200-204 (2001).
(12) Y. Hirakura, W.W. Yiu, A. Yamamoto and B.L. Kagan, Amyloid peptide channels: blockade by zinc and inhibition by Congo red (amyloid channel block). *Amyloid*, **7**, 194-199 (2000).
(13) T.A. Mirzabekov, M.C. Lin and B.L. Kagan, Pore formation by the cytotoxic islet amyloid peptide amylin. *J. Biol. Chem.* **271**, 1988-1992 (1996).
(14) M. Anguiano, R.J. Nowak and P.T. Lansbury Jr, Protofibrillar islet amyloid polypeptide permeabilizes synthetic vesicles by a pore-like mechanism that may be relevant to type II diabetes. *Biochemistry*, **41**, 11338-11343 (2002).
(15) M. Kawahara, Y. Kuroda, N. Arispe and E. Rojas, Alzheimer's beta-amyloid, human islet amylin, and prion protein fragment evoke intracellular free calcium elevations by a common mechanism in a hypothalamic GnRH neuronal cell line. *J. Biol. Chem.* **275**, 14077-14083 (2000).
(16) Structure and membrane orientation of IAPP in its natively amidated form at physiological pH in a membrane environment. *Biochim. Biophys. Acta*, **1808**, 2337-2342 (2011).