Evolutionary Origins of Amyloidogenicity - Reconstruction and Analysis of Ancestral IAPP Sequences

Ivan Filippov[1], Aria Baghai[1], Alexander Zhyvoloup[1], Lauren Chisholm[2], Michael J. Harms[2], Daniel P. Raleigh[1,3]

[1]Institute of Structural and Molecular Biology, University College London; [2]Institute of Molecular Biology, University of Oregon; [3]Department of Chemistry, Stony Brook University

Introduction

IAPP is a 37-residue pancreatic signalling peptide that forms amyloid fibrils in type 2 diabetes (1). Human IAPP (hIAPP) is among the most amyloidogenic peptides known. Some IAPP variants, such as rat IAPP (rIAPP), and a close, similar paralogue calcitonin gene-related peptide (CGRP) are non-amyloidogenic (Figure 1) (2). Evolutionary origins of IAPP amyloidogenicity are unknown. Here we use phylogenetic, computational and experimental methods to reconstruct and evaluate ancestral IAPP sequences.
Ancestral sequence reconstruction is difficult to perform on highly conserved, usually resulting in low-confidence results. We use a method involving bootstrapopoing the tree to perform reconstruction of IAPP.

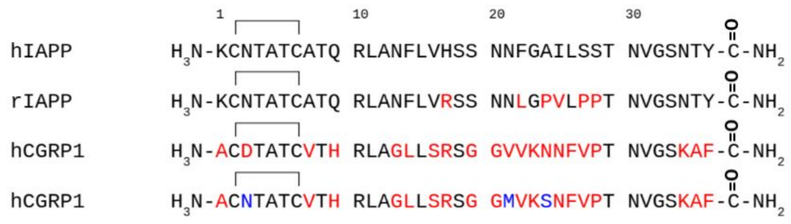


Figure 1. Comparison of hIAPP, rIAPP hCGRP1 and hCGRP2 sequences. Differences from hIAPP are shown in red, differences between hCGRP1 and hCGRP2 are shown in blue. All four polypeptides contain a disulfide bond between residues 2 and 7 and an amidated C-terminus.

Methods

IAPP sequences were gathered using NCBI BLAST with hIAPP as query sequence. Resulting sequences were confirmed by annotation, cleavage signal presence and Gly32 presence (required for amidation). Resulting 308 sequences were aligned using muscle and filtered for duplicates to reduce bias, and the remaining 143 sequences were used to generate a tree using RaxML. Tree was evaluated with GeneRax and ancestors were reconstructed on bootstrapped tree with RaxML. This analysis was performed on the University of Oregon cluster Talapas. Sequences of interest were evaluated using computational amyloidogenicity prediction methods.

Samples were prepared by solid-phase Fmoc synthesis, purified by reverse-phase high-performanmce liquid chromatography and product identity was confirmed with time-of-flight mass spectrometry. Amyloid formation rate was measured with a Thioflavin-T assay.

Alignment analysis

The first alignment of 308 species was evaluated, confirming high conservation oif IAPP (Figure 2). Highest conservatiuon was observed in the 1-12 and 30-34 regions, with no substitutions of cysteine at positions 2 and 7 forming the disulfide bridge. C-terminal tyrosine only has 2 histidine ssubstitutions across all species. Most variable region is 22-29, which includes the hIAPP FGAIL region, previously reported as having an effect on amyloidogenicity and is involved in forming a transient β-sheet during oligomer formation in the lag phase of amyloid fibril formation (3). Clades with most duplicate sequencews were birds and rodents.

Figure 2. Sequence logo of IAPP alignment including 308 species.

Tree building and ancestor reconstruction

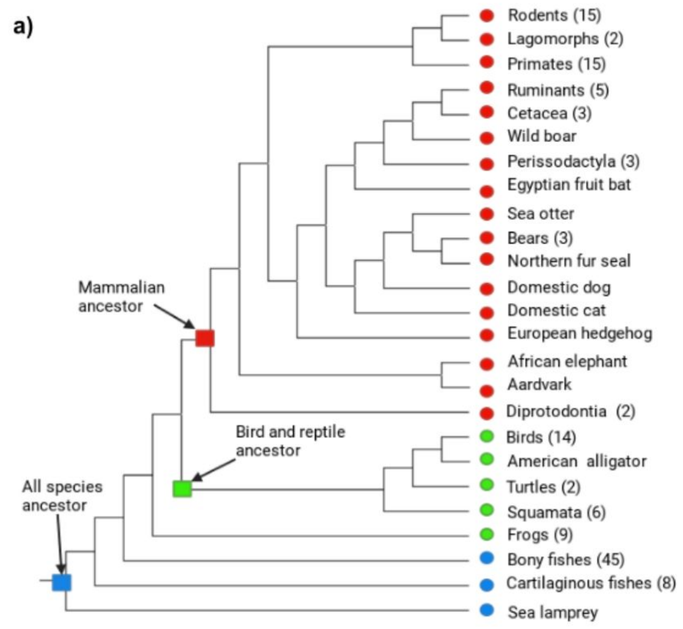
We generated a multiple-likelihood tree from the 143 sequences remaining after duplicate removal and reconciled it with a species tree using GeneRax (figure 3a). Ancestors were reconstructed for each node of the tree, and three ancestral candidates were piucked for further analysis - all-mammal ancestor (mIAPP), birds and reptiles ancestor (bIAPP) and the all-species ancestor (aIAPP). (Figure 3b). All ancestors had a confidence value of >0.99 and any alternative candidate sequences were not of significant probability.

Coincidentally, we found that mIAPP has previously been studied as consensus sequence of mammalian IAPPs, and has been compared to hIAPP in that study, showing that mIAPP has a lower amyloid formation rate than hhIAPP, but does form amyloid (4).

References:

1. Raleigh, D., Zhang, X., Hastoy, B., and Clark, A. (2017) The β-cell assassin: IAPP cytotoxicity. J. Mol. Endocrinol. 59, R121–R140
2. Sekiguchi, T. (2018) The calcitonin/calcitonin gene-related peptide family in invertebrate deuterostomes. Front. Endocrinol. (Lausanne). 9, 695
3. Abedini, A., Plesner, A., Cao, P., Ridgway, Z., Zhang, J., Tu, L. H., Middleton, C. T., Chao, B., Sartori, D. J., Meng, F., Wang, H., Wong, A. G., Zanni, M. T., Verchere, C. B., Raleigh, D. P., and Schmidt, A. M. (2016) Time-resolved studies define the nature of toxic IAPP intermediates, providing insight for anti-amyloidosis therapeutics. Elife. 5, 1–28
4. Noh, D., Bower, R. L., Hay, D. L., Zhyvoloup, A., and Raleigh, D. P. (2020) Analysis of Amylin Consensus Sequences Suggests That Human Amylin Is Not Optimized to Minimize Amyloid Formation and Provides Clues to Factors That Modulate Amyloidogenicity. ACS Chem. Biol. 15, 1408–1416

Contacts:
ivan.filippov.18@ucl.ac.uk
d.raleigh@ucl.ac.uk



b)

	1 10 20 30
Human IAPP	KCNTATCATQ RLANFLVHSS NNFGAILSST NVGSNTY
Rat IAPP	KCNTATCATQ RLANFLVRSS NNLGPVLPPT NVGSNTY
Mammals	KCNTATCATQ RLANFLVRSS NNLGAILSPT NVGSNTY
Birds+Reptiles	KCNTATCVTQ RLADFLVRSS NNIGAIYSPT DVGSNTY
All-species	KCNTATCVTQ RLADFLSRSN NNLGAFYMPT NVGSNTY

Figure 3. a) Condensed phylogenetic tree constructed from 143 IAPP sequences used for ancestral reconstruction. Branches are labelled with species or clade names, clades include number of species in brackets. Branches are colour coded: red for mammals, green for birds and reptiles, blue for fishes. Ancestral nodes of interest are labelled with squares. b) Alignment showing IAPP sequences of human, rat, mammalian ancestor, birds and reptiles ancestor and all- species ancestor. Residues different from hIAPP are shown in red.

Computational predictions of amyloidogenicity

We used multiple amyloid prediction softwares to evaluate hIAPP, rIAPP and ancestral sequences. We first confirmed that hIAPP was scored higher than rIAPP, since this is experimentally confirmed. Figure 4 shows results of ANuPP predictions, showing that all ancestral sequences are predicted lower amyloidogenicity than hIAPP. aIAPP is shown to have the lowest, lower than rIAPP, which has previously been considered as the least amyloidogenic IAPP variant.

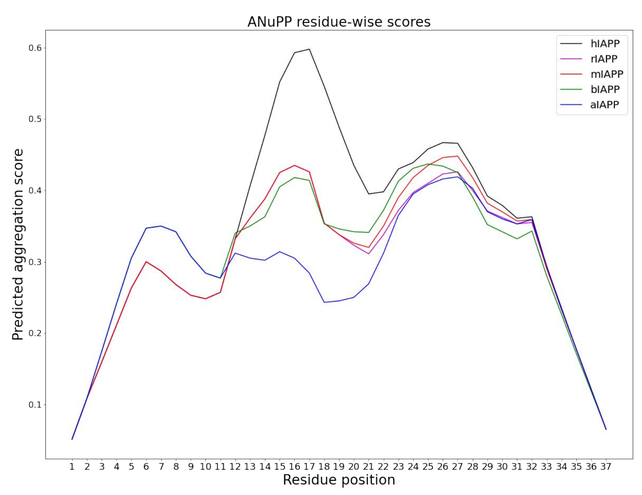


Figure 4. Plots showing residue-wise aggregation probability scores predicted by ANuPP for the 5 IAPP variants studied. X-axis shows predicted aggregation score, Y-axis shows residue position.

Experimental comparison of amyloid formation rate

hIAPP and bIAPP amyloid formation rate was measured using a Thioflavin-T assay. confirming that bIAPP forms amyloid, but at a much lower rate than hIAPP (T_{50} = 3.3 hours for hIAPP; T_{50} = 11.5 hours for bIAPP). Resulting plot is seen in Figure 5.

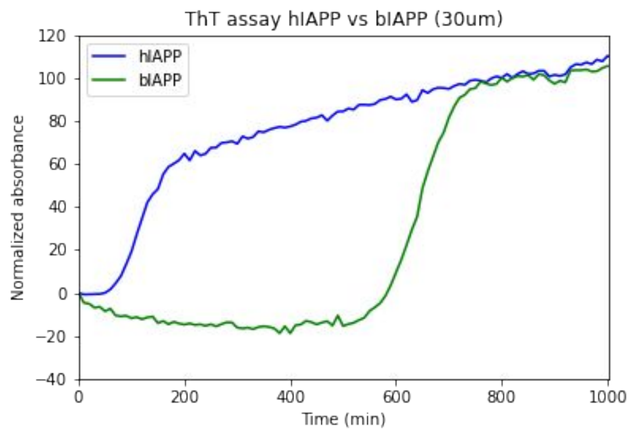


Figure 5. ThT assay plot, showing hIAPP in blue and bIAPP in green.

Discussion

We built the largest rigorously annotated IAPP alignment and performed the first successful ancestral sequence reconstruction of IAPP. Our results indicate that ancestral sequences have lower amyloidogenicity than hIAPP. and indicate that the al-species ancestor is possibly less amyloidogenic than rIAPP. It is necessary to finish experimental evaluation of all ancestors in relation to hIAPP and each other to confirm these results. It is also necessary to perform similar studies within clades to determine if the trend of increasing amyloidogenicity is present at at smaller evolutionary time scale.
We attemnpted reconstruction of CGRP, but confirming sequence identity is challenging. Once CGRP reconstruction is complete, a comparison of IAPP and CGRP ancestors can help determine if both peptides were non-amyloidogenic at the duplication event.