

SUPPLEMENTARY INFORMATION

Copper (II)-Human Amylin Complex Protects Pancreatic Cells from Amylin Toxicity

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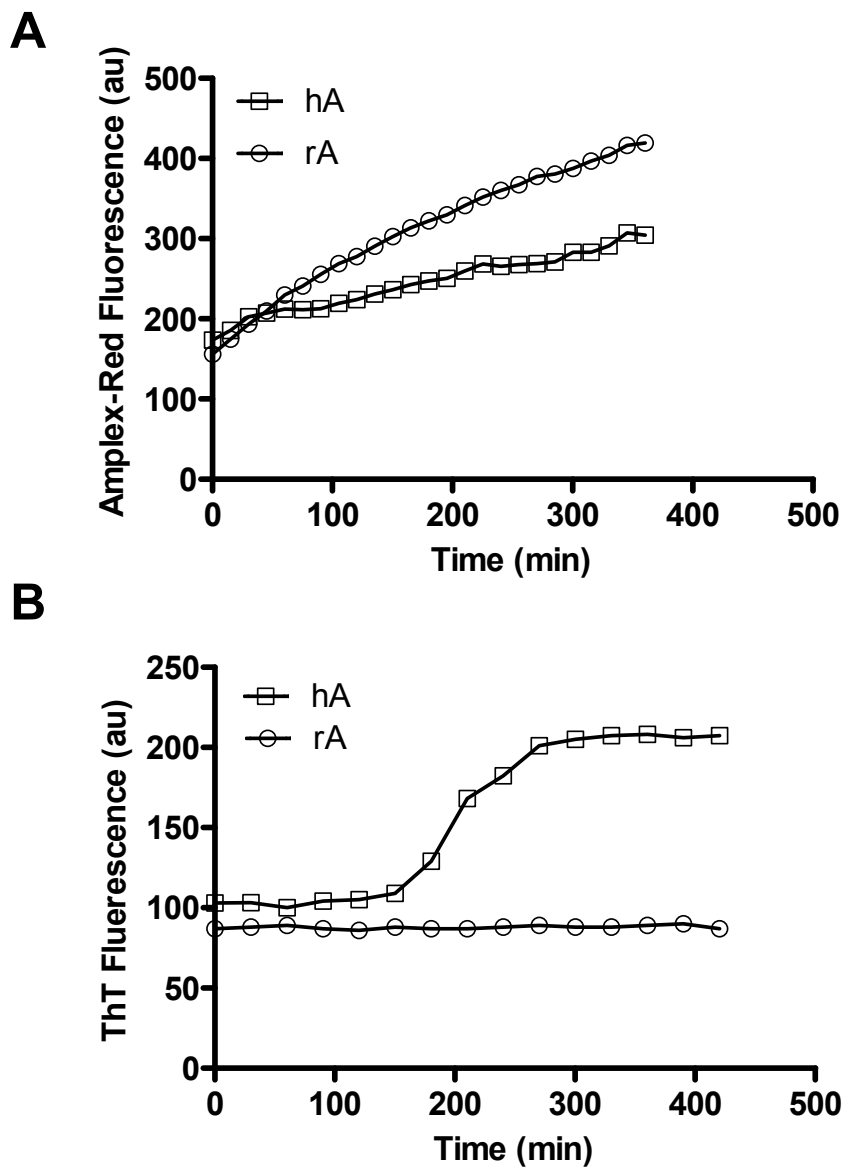


Figure S1. Amylin displays dissimilar aggregation and peroxide-forming kinetic rates. The human and rat amylin-induced peroxide formation (A) and aggregation rates (B) were investigated in real time by Amplex-Red and Th-T fluorescent assays, respectively. **(A)** Human and rat amylin (20 μ M) generated comparable H_2O_2 levels over time. **(B)** Human but not rat amylin slowly aggregated in solution.

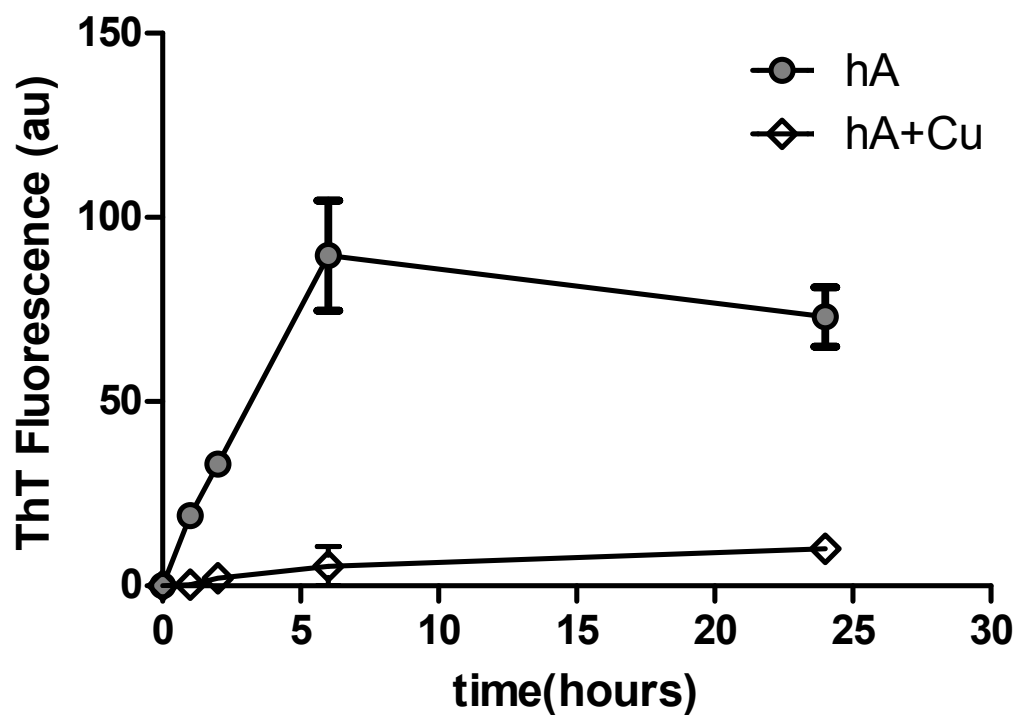


Figure S2. Cu^{2+} inhibits human amylin aggregation. 20 μM of human amylin was incubated in phosphate buffer, in the absence or presence of equimolar CuCl_2 and the extent of amylin-aggregation analyzed during 24h by end-point Th-T fluorescent assay.

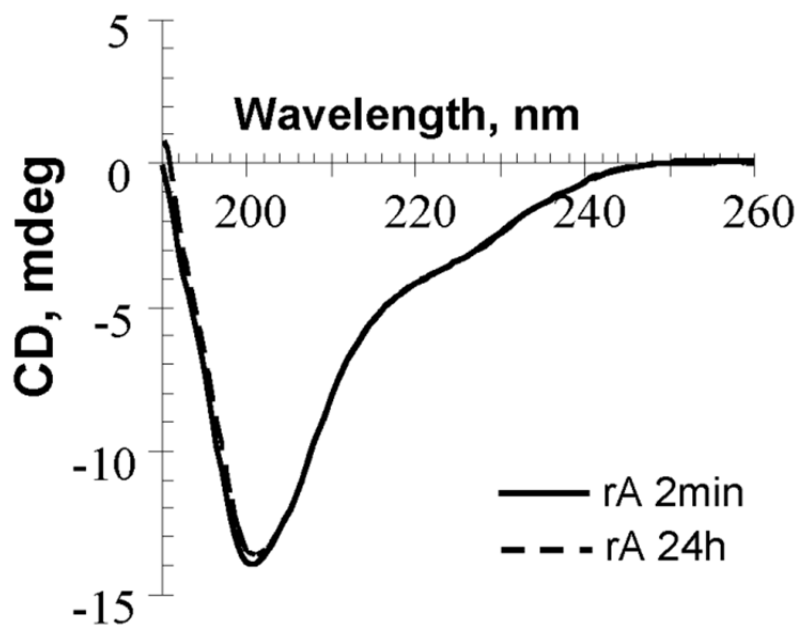


Figure S3. Dynamics of rat amylin secondary conformation in solution examined by CD spectroscopy. Following dissolution, conformation changes of rat amylin (20 μ M) was monitored by CD during 24h period. Rat amylin retained random coil conformation throughout the experiment.

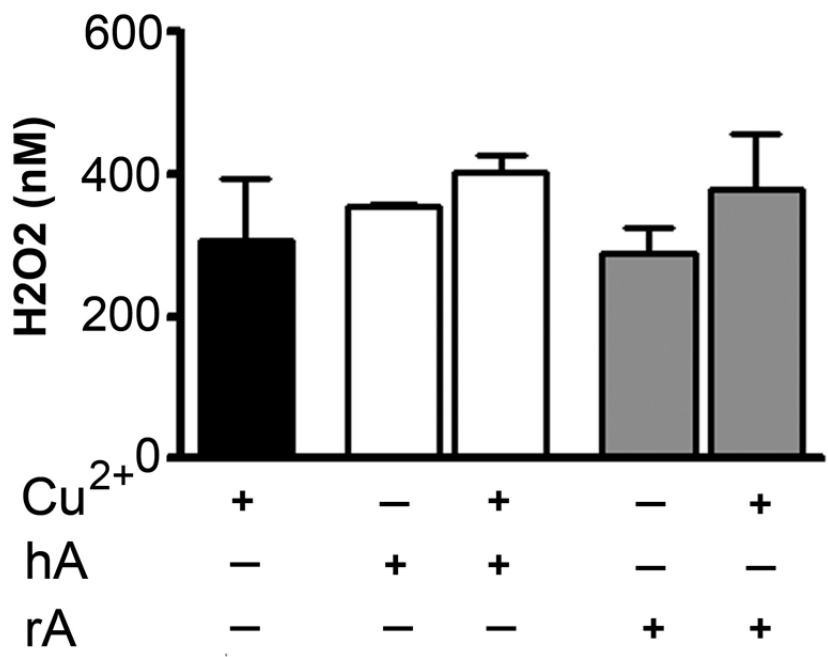


Figure S4. Effect of Cu²⁺ on amylin-evoked peroxide production in vitro. 20 μM of human amylin was added to phosphate buffer that was or was not supplemented with 20 μM CuCl₂ and the extent of H₂O₂ production analyzed by Amplex-Red fluorescent assay. Supplementation of Cu²⁺ had no significant effect on amylin-evoked H₂O₂ production and accumulation in solution.

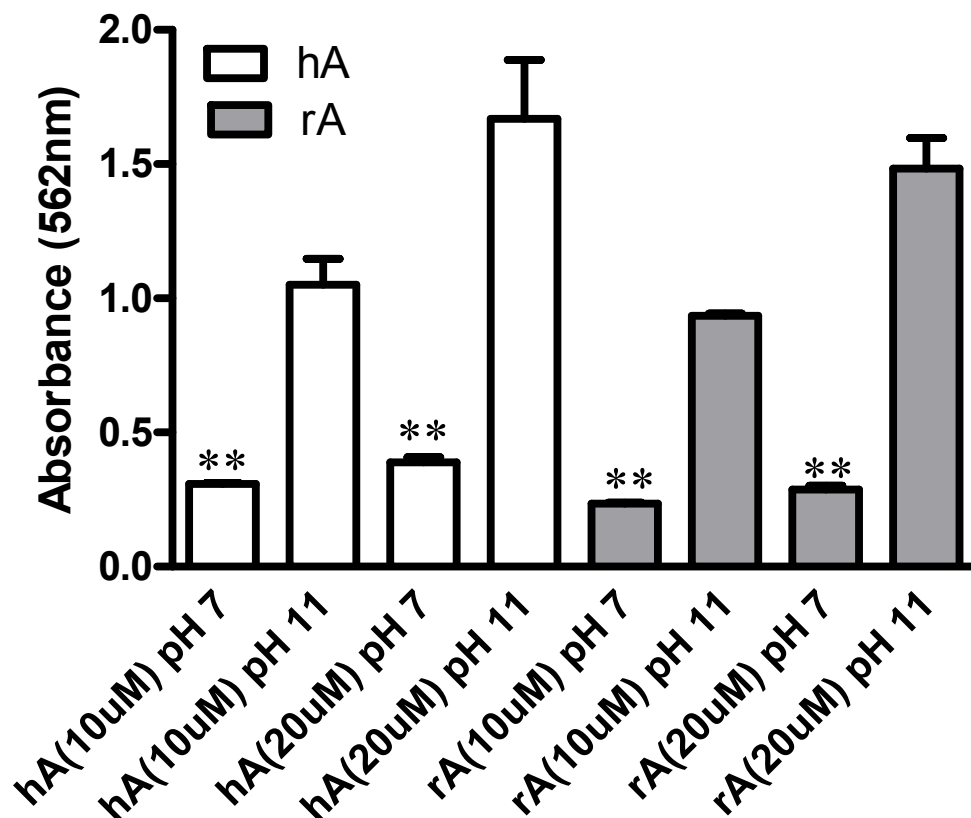


Figure S5. Reduction of Cu^{2+} by human and rat amylin is pH-dependent. Bicinchoninic (BCA) reagent was used to detect the reduction of Cu^{2+} to Cu^{1+} by human and rat amylin under alkaline and neutral conditions. Human and rat amylin (10 and 20 μM) were mixed with CuSO_4 and BCA reagent solution (Pierce) at pH=11 and pH=7 for 60 min. Following the treatments, changes in BCA absorbance were determined. At pH=11, both amylin isoforms reduced Cu^{2+} to Cu^{1+} indicated by an increase in BCA absorbance at 562 nm (absorption maximum for Cu^{1+} -BCA complex). In contrast, amylin was much less effective in reducing Cu^{2+} at a biologically-relevant pH value (pH=7). Significance established at ** $P < 0.01$, hA, pH=11 vs. hA, pH=7, Student's t-test, n=6.