Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

SUPPLEMENTARY INFORMATION

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

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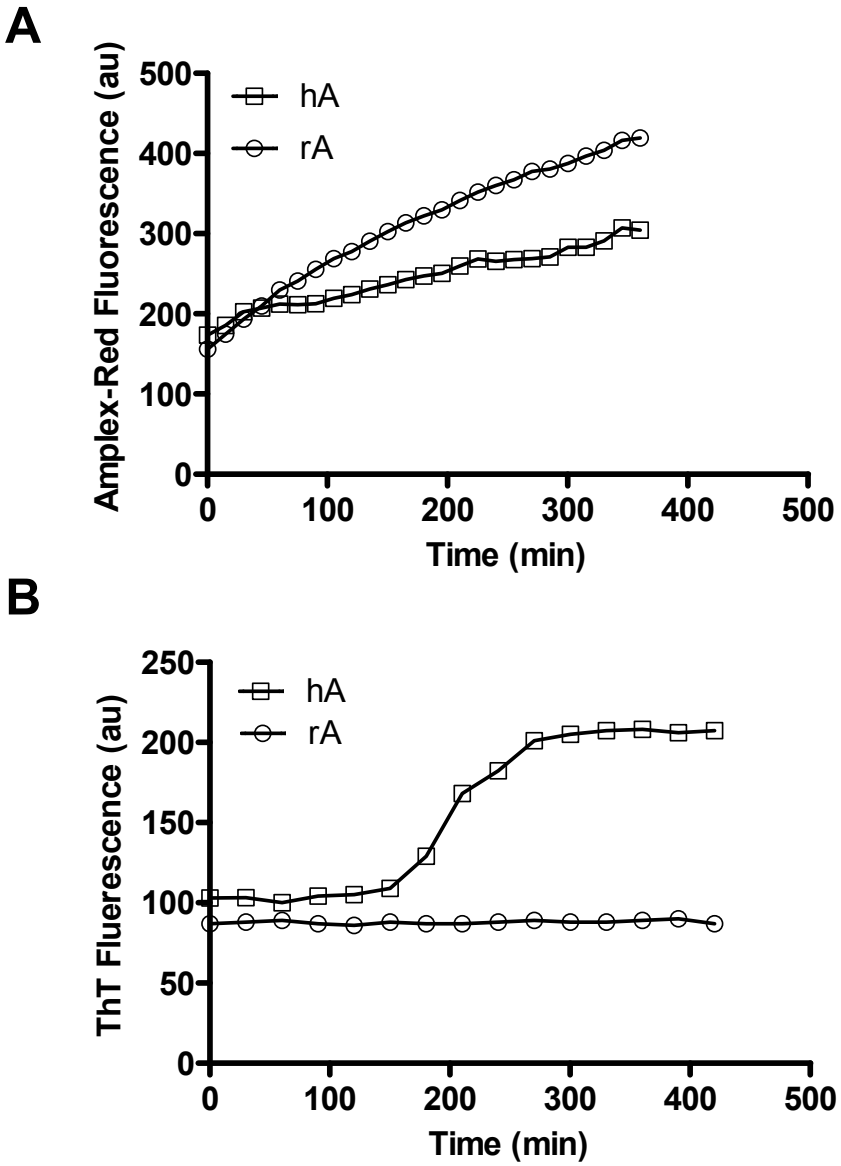

Figure S1. Amylin displays dissimilar aggregation and peroxide-forming kinetic rates. The human and rat amylin-induced peroxide formation (A) and aggregation rates (B) were investigated in real time by Amplex-Red and Th-T fluorescent assays, respectively. **(A)** Human and rat amylin (20 μM) generated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

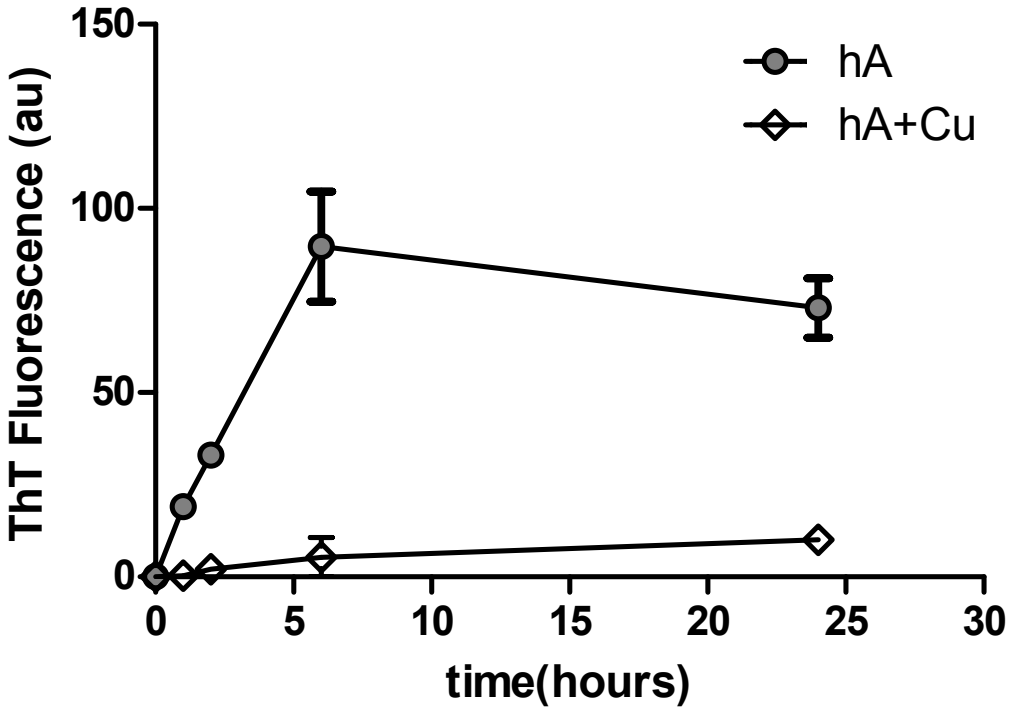

Figure S2. Cu^{2+} inhibits human amylin aggregation. 20 μM of human amylin was incubated in phosphate buffer, in the absence or presence of equimolar $CuCl_2$ and the extent of amylin-aggregation analyzed during 24h by end-point Th-T fluorescent assay.

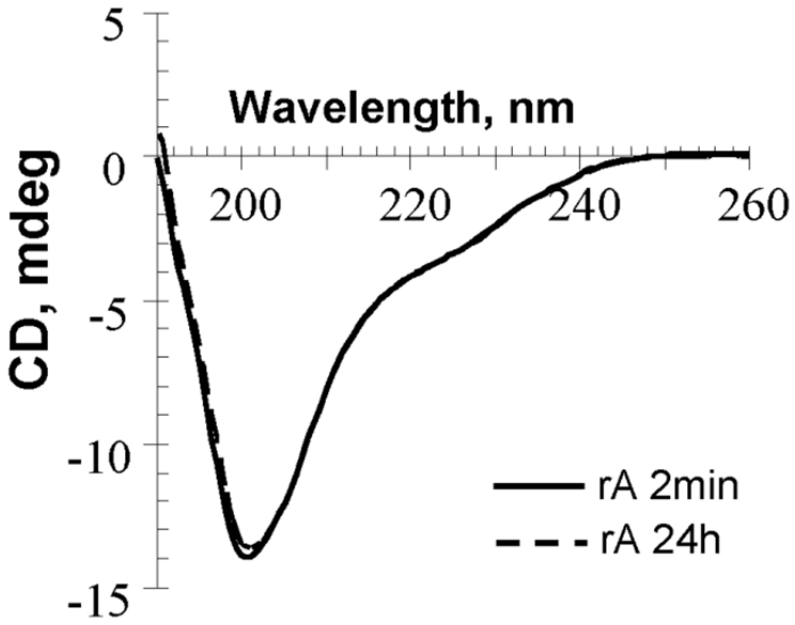

Figure S3. Dynamics of rat amylin secondary conformation in solution examined by CD spectroscopy. Following dissolution, conformation changes of rat amylin (20 μM) was monitored by CD during 24h period. Rat amylin retained random coil conformation throughout the experiment.

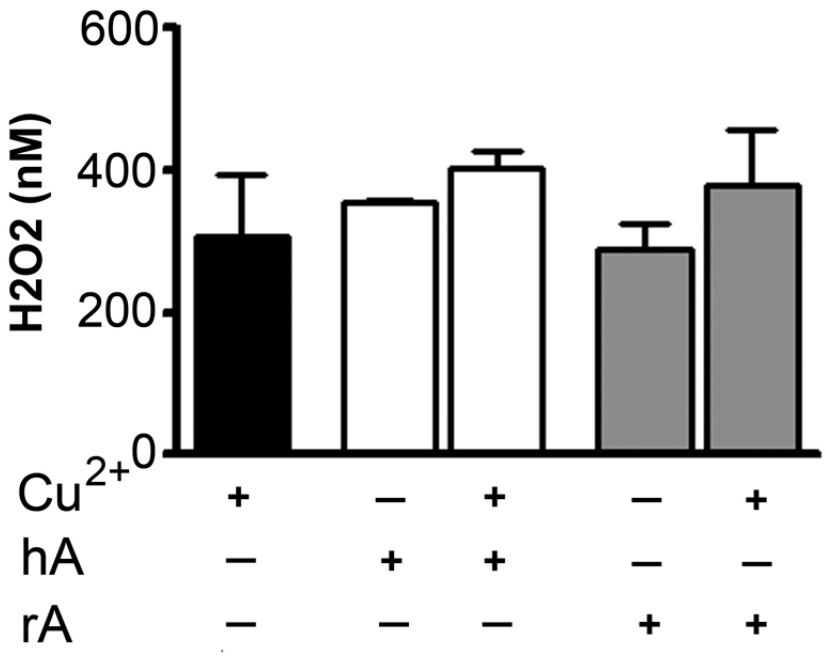

Figure S4. Effect of Cu^{2+} on amylin-evoked peroxide production in vitro. 20 μM of human amylin was added to phosphate buffer that was or was not supplemented with 20 μM $CuCl_2$ and the extent of H_2O_2 production analyzed by Amplex-Red fluorescent assay. Supplementation of Cu^{2+} had no significant effect on amylin-evoked H_2O_2 production and accumulation in solution.

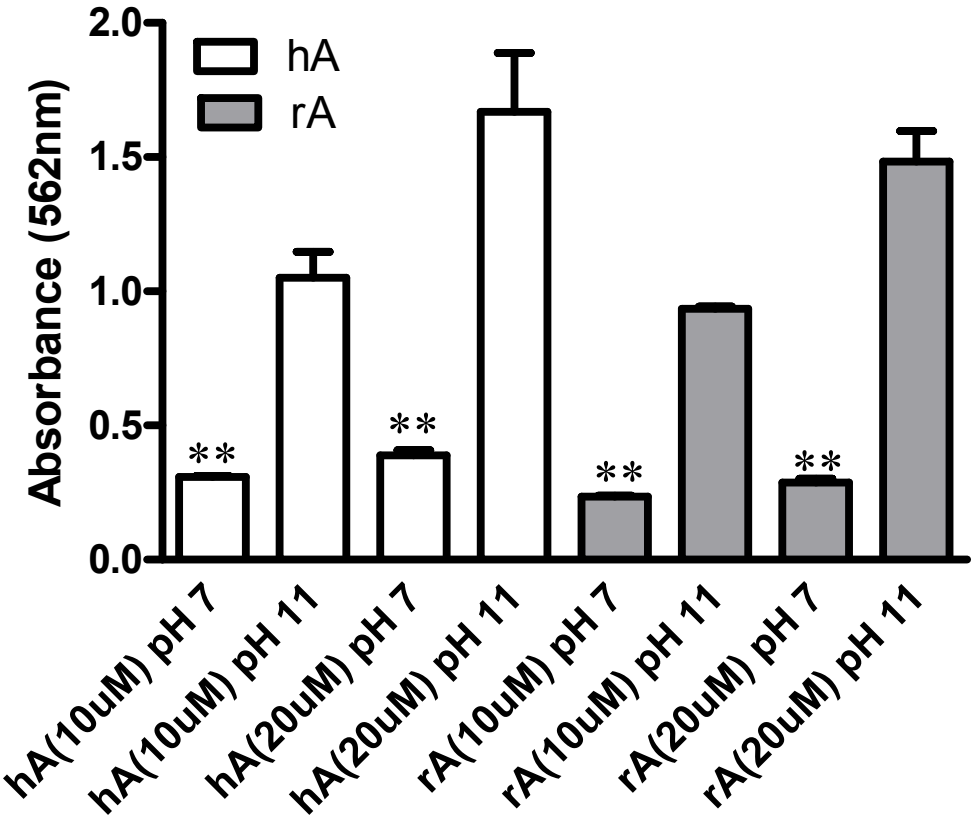

Figure S5. Reduction of Cu^{2+} by human and rat amylin is pH-dependent. Bicinchoninic (BCA) reagent was used to detect the reduction of Cu^{2+} to Cu^{1+} by human and rat amylin under alkaline and neutral conditions. Human and rat amylin (10 and 20 μM) were mixed with $CuSO_4$ and BCA reagent solution (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.