Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information for 'Characterizing the Assembly Behaviors of Human Amylin: A Perspective Derived from C-terminal Variants'

Mei-Sha Chen, De-Sheng Zhao, Ye-Ping Yu, Wei-Wei Li, Yong-Xiang Chen, Yu-Fen Zhao, Yan-Mei Li

Key Laboratory of Bioorganic Phosphorus Chemistry and Chemical Biology (Ministry of Education), Department of Chemistry, Tsinghua University, Beijing 100084, China

Contact information (corresponding author):
Yan-Mei Li
Email: liym@mail.tsinghua.edu.cn
Tel: 008610-62796197
Fax: 008610-62781695

SUPPLEMENTARY INFORMATION

Materials and Methods

Fresh Amylin^{37} Stock Solution Preparation and Peptide Aggregation

Amylin^{37}-$CONH_2$ (Genscript Corp., USA) and amylin^{37}-COOH (CASLO Laboratory Aps, Denmark) were dissolved in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP, Acros, USA) to 1 mg/ml, incubated overnight at room temperature, and then stored at -20°C. Before usage, HFIP was evaporated off under vacuum. For the cellular assay, peptide solutions were prepared by dissolved in the cell culture (RPMI-1640 with no fetal calf serum). For ThT fluorescence assay, PICUP and SDS-PAGE analysis, peptides were dissolved in hydrochloride acid (HCl, pH 2) and phosphate buffered saline buffer (PBS; 10mM; pH 7.4; Sigma, USA) to a final concentration (VHCl:VPBS=1:9). For TEM, the stock was prepared via dissolved in DMSO to 1mg/ml, and then added with PBS to final concentration. The samples were incubated at 37 °C for peptide aggregation.

MTT Assay

INS-1 cells were cultured in RPMI-1640 (GIBCO Invitrogen, USA) supplemented with 10% FBS (GIBCO Invitrogen, USA) in 96-well microplate at 37°C in a humidified 5% CO2 (5% CO2, 95% air) atmosphere for 24 h, and then the cells were exposed to different samples in RPMI-1640 without 10% FBS for 24 h. The medium was changed with RPMI-1640 containing MTT (1 mg/ml, AMRESCO, USA), and incubated for another 4 h. The absorbance of medium was measured at 560nm using a Synergy 4 Plate Reader (Bioteck Company, USA).

Thioflavin T (ThT) Fluorescence assay

Thioflavin T fluorescence assay was performed in 96-well black microplate (Corning Costar Corporation, USA) using a Synergy 4 Plate Reader. (λex=440 nm, slit width=5nm; λem=482 nm, slit width=10nm). Every well contained 10μl incubated solution with 190μl ThT solution (10μM) in 12mM phosphate buffer (pH 7.4). The final concentrations of peptides are 10μM.

Photo-Induced Cross-Linking of Unmodified Proteins (PICUP)

Amylin^{37}-$CONH_2$ (10μM) and amylin^{37}-COOH (10μM) solutions were prepared with different incubation time. For preparing crosslinking samples, peptide solution (10μl) was mixed with ammonium persulfate (20mM, 1μl) and tris (2,2'-bipyridyl) dichloro-ruthenium(II) hexahydrate (1mM, 1μl). Then the mixture was exposed to filament lamp for 10s. The reaction was stopped by dithiothreitol (1M, 1μl).

SDS-PAGE Analysis

The peptide samples after PICUP were mixed with SDS-sample buffer and heated to 90°C for 10 min. Then the samples were subjected to NuPAGE electrophoresis in 12% Bis-Tris gels with SDS-PAGE running buffer. Silver stain kit (Beyotime Company, China) was used to analyze the protein contents.

Transmission electron microscopy (TEM):

The TEM samples were prepared by incubating peptide (10μM) for 4 h. Then 8μl of each sample was placed on 300 mesh formvar-coated copper grids for 1.5 min before removing excess solution. After that the sample was stained with 1% fresh tungstophosphoric acid for another 1 min. The dried sample was examined with Hitachi-7650B electron microscope (Hitachi, Japan).

Molecular dynamics simulation of amylin^{37}-COOH and amylin^{37}-$CONH_2$ monomers

The available NMR structure (PDB code: 2KB8)[1] was used as the initial structure of amylin^{37}-COOH, while the initial structure of amylin^{37}-$CONH_2$ was derived with amidation at the C-terminus. The molecular models were placed in the center of a 60×40×40 Å box, with enough sodium ions to ensure electrical neutrality. The box was filled with about 3000 SPC water molecules. The LINCS method was used to constrain bond lengths. Electrostatic interactions were calculated using the Particle-Mesh algorithm. The models were minimized by 700 steepest descent steps and heated to 300K during about 30ps and kept at 300K for 5ns. A constant pressure of 1bar was applied with a coupling constant of 1.0ps. Peptide, water, and sodium were coupled separately to a temperature bath at 300K with a coupling constant of 0.1ps. A time step of 3fs was used. Measurement of radius of gyration, distance, counts of hydrogen bonds and salt bridges were performed every 10ps by using facilities within the GROMACS package.[2, 3] The G43a1 force field was used in all of the calculations.

Molecular dynamics simulation of amylin^{37}-COOH and amylin^{37}-$CONH_2$ dimers

The simulations were based on the protofibril structure[4] sponsored by Dr. Robert Tycko from National Institutes of Health. The initial structure of amylin^{37}-$CONH_2$ parallel dimer was derived from modifying amylin pentamer, while the initial structure of amylin^{37}-COOH parallel dimer was derived from deamidation. Both dimer structures were placed in the center of a 60×40×40 Å box, with enough sodium ions to ensure the electrical neutrality. The box was filled with about 3000 SPC water molecules. LINCS method was implemented to constrain bond lengths. Electrostatic interactions were calculated via Particle-Mesh algorithm. The models were minimized by 700 steepest descent steps and heated to 300K in about 30ps and then kept at 300K for 50ns. A constant pressure of 1bar was applied with a coupling constant of 1.0ps. Peptide, water, and sodium were coupled separately to a temperature bath at 300K with a coupling constant of 0.1ps. A time step of 3fs was used. Measurement of radius of gyration, distance, counts of hydrogen bonds and salt bridges were performed every 10ps using the facilities from the GRPMACS package. The G43a1 force field was used for all the calculations.

Supplementary Figures

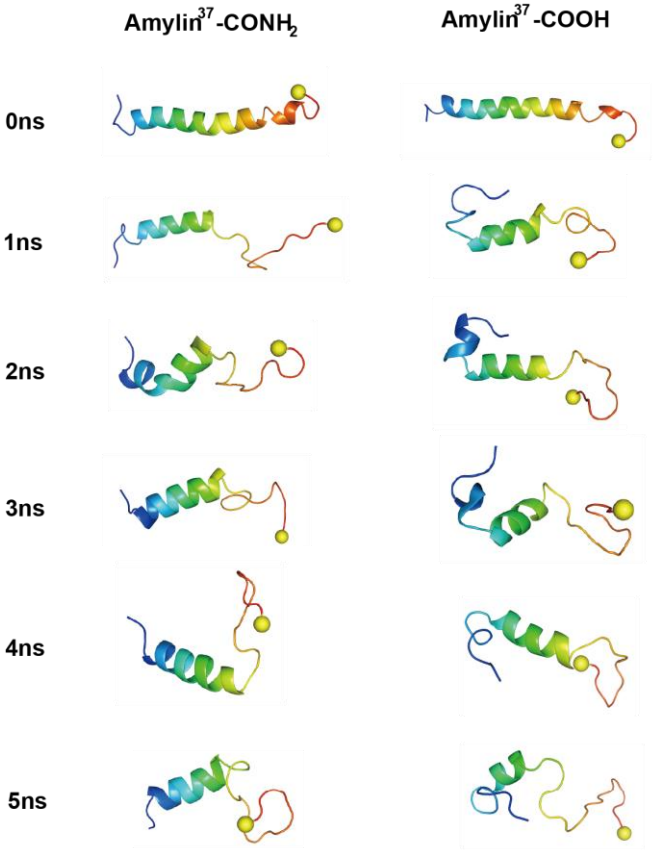


Figure S1. The MD simulation results of amylin^{37}-$CONH_2$ and amylin^{37}-COOH. Snapshots of amylin^{37}-COOH and amylin^{37}-$CONH_2$ were extracted from the MD simulation results of amylin^{37}-$CONH_2$ and amylin^{37}-COOH at 0, 1, 2, 3, 4, 5ns.

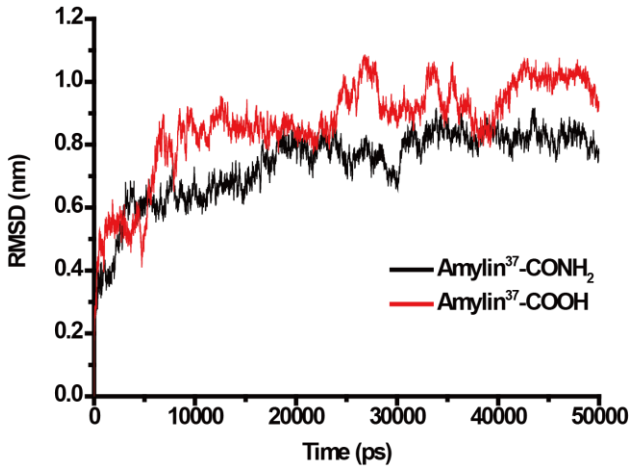


Figure S2. The evolution of RMSDs of amylin^{37}-$CONH_2$ and amylin^{37}-COOH dimers during 50000ps MD simulation at 298 K.

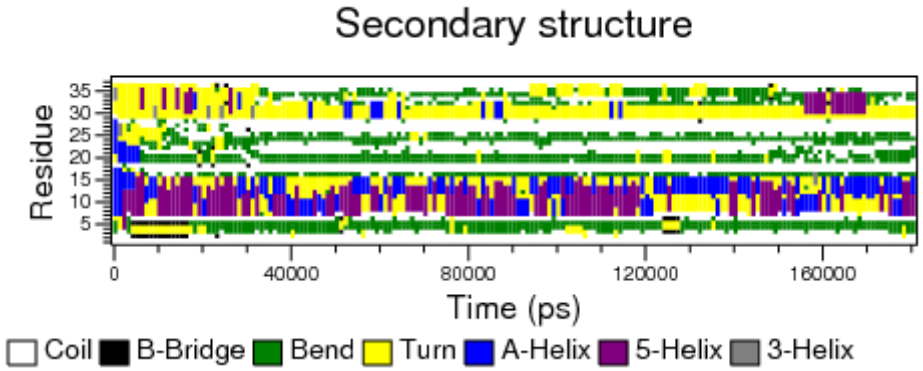


Figure S3. Secondary structures as function of time for Amylin^{37}-NH_2 in the new MD trajectory as calculated by DSSP.

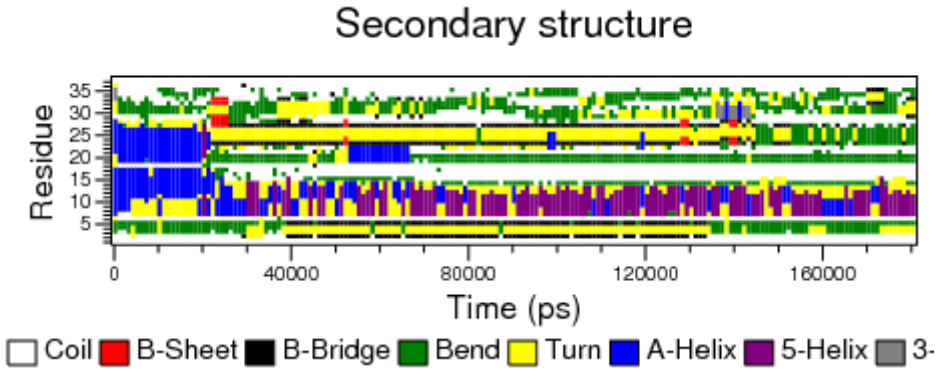
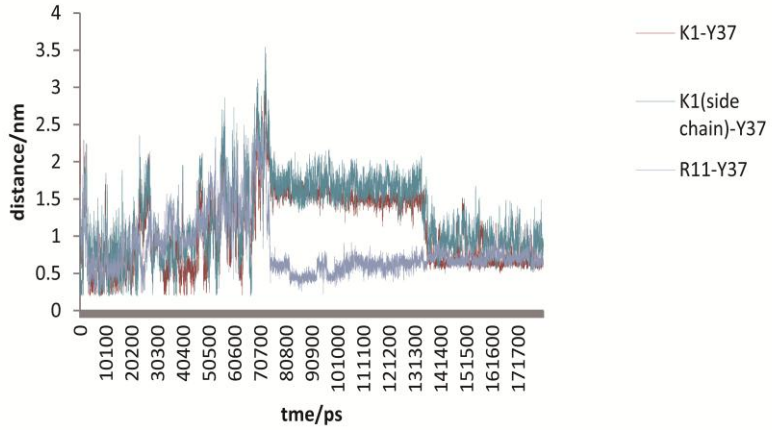


Figure S4. Secondary structures as function of time for Amylin^{37}-COOH in the new MD trajectory as calculated by DSSP



Figrue S5. Intra-molecule salt bridge as function of time for Amylin^{37}-COOH in the new MD trajectory.

References

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