Supporting Information

Structural Conversion of Hunan Islet Amyloid Polypeptide Aggregates Under Electric Field

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Experimental

Samples preparation

2 mg hIAPP37 (amino acid sequence: KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY(1-37), disulfide bridge: 2-7, Science Biological Technology Co., Shanghai, China) powder was dissolved in 1 mL 1,1,1,3,3,3-hexafluoroisopropanol (HFIP, Tokyo Chemical Industry, Japan) and kept in a thermo-shaker at 350 rpm at 25℃ for 12 h before use, then aliquots of hIAPP were dried under vacuum. The dried hIAPP powder was dissolved into Milli-Q water to the final concentration of 20 μM and incubated for some time at 350 rpm at room temperature under the different intensity of electric field (0 mV/nm, 0.001 mV/nm, 0.002 mV/nm, 0.003 mV/nm).

Thioflavin T (ThT) Fluorescence assay

The Hitachi F-4500 fluorescence spectrometer is used to study on the dynamics of amyloid peptide aggregation by ThT assay. A total of sample solution (incubated peptide solution (25 μL), ThT fluorescence solution (25 μL) and solvents (150 μL) was added to a 0.1 cm quartz cells. ThT fluorescence spectra (excitation at 450 nm, emission at 485 nm) were measured from 0 h to 4 h. The measurement was repeated three times. The volume ratio of polypeptide solution and ThT (1 mM) was 1:1.

Circular Dichroism (CD) spectroscopy

CD spectra measurements were recorded on a spectropolarimeter (JASCO, Hachioji City, Japan) with a model No. PTC-348W1 (JASCO) using a quartz curette of 0.1 cm path length. All experiments were performed at room temperature and three scans at a scan speed of 50 nm min^{-1} with the spectral region from 190 nm to 250 nm was averaged. Besides, the band width was set at 1 nm. For each sample, the signal of Milli-Q water was subtracted as the baseline. The sample volume for each CD measurement was 300 μL and the concentration of hIAPP37 solution was 20 μM.

Atomic Force Microscopy

5 μL of incubated peptide solution with electric field (0 mV/nm, 0.001 mV/nm, 0.002 mV/nm, 0.003 mV/nm) were deposited onto the freshly cleaved mica surface and air-dried for 10 min, then the residues were removed. The sample was dried in ambient

condition before measurement. All AFM measurements were carried out by using a commercial AFM MultiMode VIII (Bruker, Santa Barbara, USA) in a Bruker Tapping mode with an ultra-sharp silicon probe with the spring constant of 26 Nm^{-1} under ambient condition. All AFM images were obtained at 512×512 pixels and analyzed with Scanning Probe Image Processor (SPIP) software.

MD Simulation details

In order to analyze the effect of electric field on secondary structure of amyloid peptide, we studied the effects of electric field on hIAPP37 pentamer model with β-sheet, which was kindly provided by Professor Tycko[1]. Three systems with different electric field intensities (0.003 mV/nm, 3 mV/nm, 300 mV/nm) was constructed, especially, the minimum system of 0.003 mV/nm was consistent with the electric field intensity (0.003 mV/nm) used in the experiment and the big system of 300 mV/nm was run for 3 times in the same condition. In addition, a blank control group without electric field was also set up for comparison. In these systems, hIAPP37 pentamer was placed at the center of the water box with the size of 9.04 x 9.04 x 9.04 nm^3. The electric field was implemented along the z direction perpendicular to hIAPP37 pentamer in all the systems. For each system, after the energy minimization, as well as the 100 ps NVT and 1 ns NPT MD simulations, 300 ns production simulation was performed under NPT ensemble. A time step was 2 fs. Pressure coupling was isotopically controlled to 1 bar by using a Berendsen barostat, and velocity rescaling coupling was utilized for adjusting the temperature to 310 K. Moreover, the AMBER99SB-ILDN force field[2] and TIP3P water model[3] were chosen for the protein and water, respectively. The LINCS algorithm was carried out for constraining all-bonds in the protein and water. Periodic boundaries conditions (PBC) were applied in all directions. The particle-mesh Ewald (PME) method and the Verlet cutoff scheme with a same cutoff of 1.0 nm were severally treated for the Long-range electrostatic interactions and Lennard-Jones interactions. All the molecular dynamics simulations were performed with GROMACS4 (GROningen MAchine for Chemical Simulations) package version 5.1.4.[4] The secondary structure identification of protein was determined by using the Define Secondary Structure of Proteins (DSSP) algorithm.[5]

All represent snapshots of hIAPP37 pentamer were displayed by using the Pymol6 program.[6]

References:

1 S. Luca, W. M. Yau, R. Leapman, R. Tycko, Biochemistry., 2007, 46, 13505-13522.

2 K. Lindorff-Larsen, S. Piana, K. Palmo, P. Maragakis, J. L. Klepeis, R. O. Dror, D. E. Shaw., Proteins, 2010, 78, 1950-1958.

3 W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey, M. L. Klein, J. Chem. Phys., 1983, 79, 926-935.

4 H. J. C. Berendsen, D. Vanderspoel, R. Vandrunen, Comput. Phys. Com., 1995, 91(1-3): 43-56.

5 W. Kabsch, C. Sander, Biopolymers, 1983, 22, 2577-2637.

6 W. L. DeLano, CCP4 Newsletter on protein crystallography, 2002, 40, 82-92.

Supporting Figures

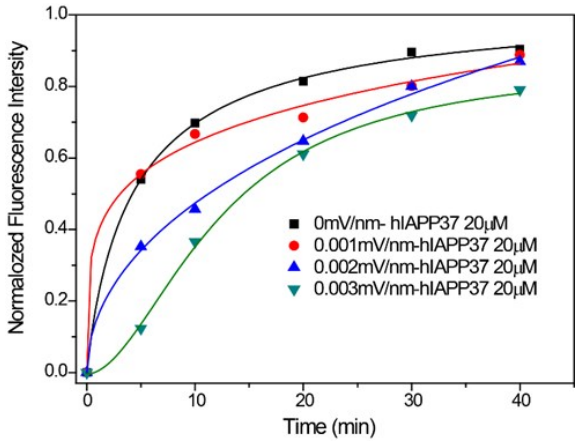

Fig S1 Aggregation kinetic of hIAPP37 peptide under EF (0 mV/nm, 0.001 mV/nm, 0.002 mV/nm, 0.003 mV/nm); The aggregation rate of hIAPP37 peptide was slightly inhibited in the presence of electric field.

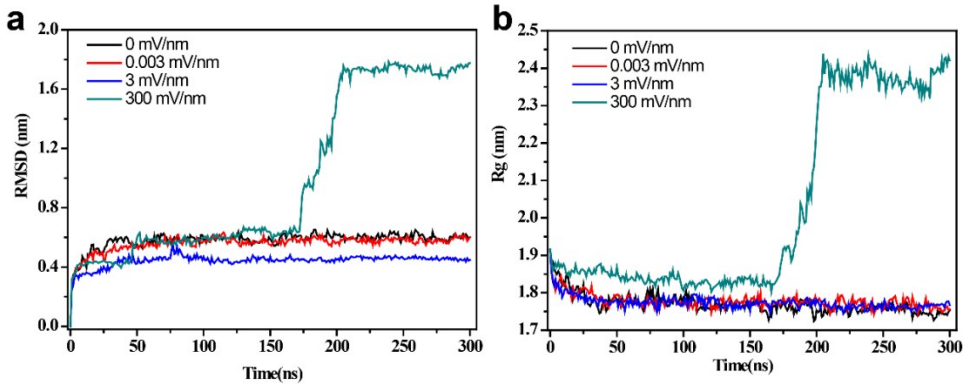

Fig S2 (a) the RMSDs and radius of gyration (Rg) (b) with time from 0 ns to 300 ns, obtained from AAMD simulations. The value fluctuation of RMSD and Rg of hIAPP37 pentamer under high electric field effect revealed the stable state of hIAPP37 pentamer with β-sheet was destroyed and then much more random coil structure was formed.

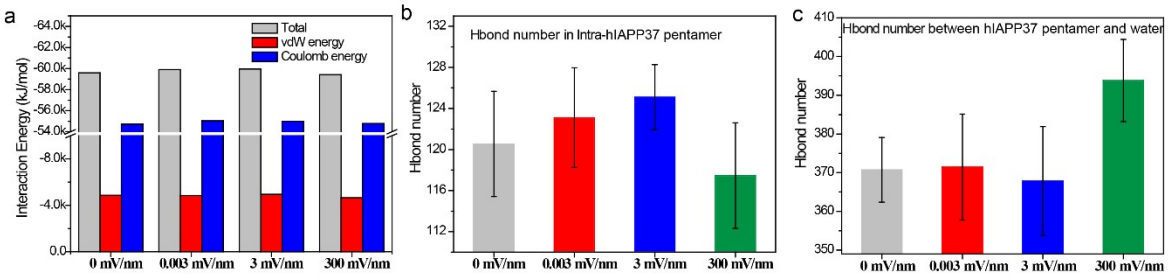

Fig S3 (a) The peptide-peptide interaction energy in hIAPP37 pentamer aggregates; (b) The number of hydrogen bonds of intra-hIAPP37 pentamer aggregates averaged after equilibration; (c) The number of hydrogen bonds between hIAPP37 pentamer aggregates water averaged after equilibration. There is no obvious variation in peptide-peptide interaction, but slight variation exists in the hydrogen bonds (HBs) among peptides, peptide and water molecular.

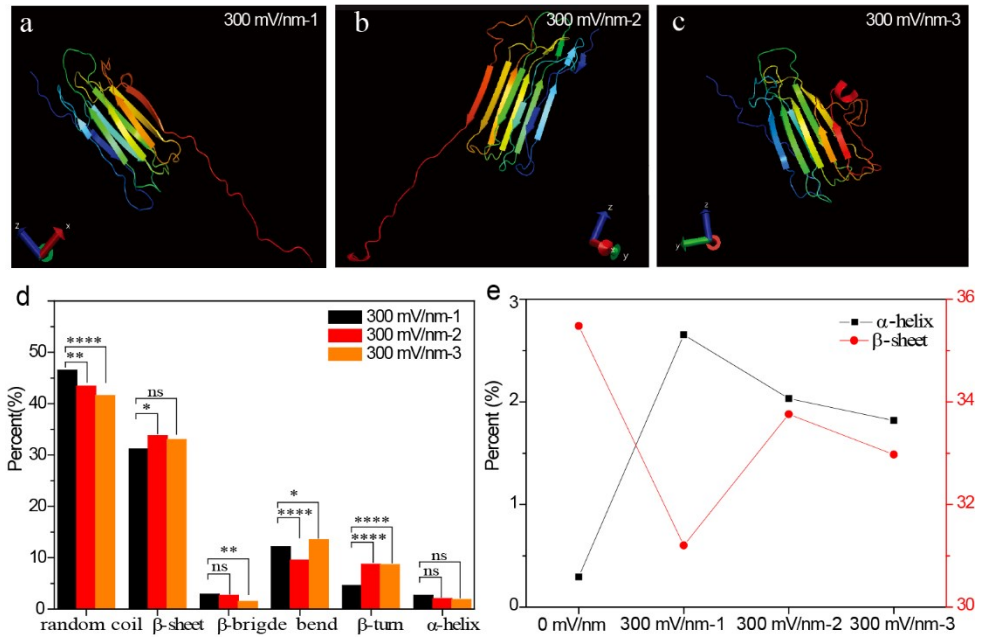

Fig S4 Conformation of hIAPP37 pentamer aggregates with the electric field intensity of 300 mV/nm(a-c), obtained from AAMD simulations at t=300 ns; (d) The corresponding secondary structural populations of hIAPP37 pentamer aggregates under electric fields (300 mV/nm); (e) The corresponding plot of the content of α-helix and β-sheet structure in Fig S4(d); One-way analysis of variance (ANOVA) with Tukey's correction, not significant (n.s.), *P < 0.05,**P < 0.01, ****P < 0.0001. The structure of hIAPP37 pentamer is unstable under EF intensity of 300 mV/nm.