Supporting Information

Monomer-Targeting Affinity Peptide Inhibitors of Amyloid with No Self-Fibrillation and Low Cytotoxicity

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Experimental Section

Biopanning of Affinity Peptide Targeting Amylin Monomer

Ph.D.-12 Phage Display Library Kit was obtained from New England Biolabs, Inc. (NEB, Ipswich, MA, USA). The biopanning process was performed under the instructions Manual. Detailed steps can be referred to as the literature\(^1\). The concentrations of Tween-20 in the TBST washing buffer were 0.1%, 0.3%, and 0.5% for the first, second, and third rounds of biopanning, respectively. After three rounds of biopanning, individual phage clones were separated. The ssDNA of the phage clones was extracted using a Biomiga M13 Isolation Kit bought from Biomiga, Inc. San Diego, CA. and sequenced in Shanghai TSINGKE Biological Technology Co., Ltd. The affinity peptide sequences were obtained by reference to the codon table as indicated in the Instructions Manual.

The Pre-treated of Peptides

Lyophilized powder (Amylin and LA12, purity ≥95%, synthesized by Top Biotechnology Co., LTD., Shanghai, China) were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) to a concentration of 1 mg/ml followed by a 3-5 min sonication in bath ultrasonator at lower temperature, respectively. Then the solution was placed for 2 h at 4°C under static condition. The organic solvent (HFIP) was evaporated using a vacuum drying chamber to acquire a dispersed monomer. Treated peptides were stored at −20°C prior to use.

The Prediction of Binding Sites between Amylin Monomer and LA12

The original protein structure of amylin (5MGQ)\(^2\) was selected from the PDB database according to the similar culture conditions. The initial affinity peptide (LA12) structure was acquired from PEP-FOLD3\(^3\) through inputting the primary amino acid sequence. The binding sites between amylin and LA12 were calculated and analyzed according to the instructions of PEP-SiteFinder\(^4\), which identifies candidate regions for the protein-peptide interactions by performing series of blind docking experiments in which the protein and the peptide conformations are rigid.

The Preparation of Peptides Solution

The pre-treated peptides (amylin, Aβ42, PSMα3) were first dissolved in 10 mM by DMSO and then diluted to 25 μM using Tris-HCl buffer (10 mM, pH 7.3). LA12, GR12, IgG, and BSA were dissolved to the required concentration in the Tris-HCl buffer (10 mM, pH 7.3), respectively. Notably, GR12 was screened as Aβ42 monomer binding peptide in our previous work\(^1\). Prior to
incubation, the diluent was followed by a 3-5 min sonication in bath ultrasonator. Protein aggregation was initiated by incubating the diluent at 37°C without shaking. In the inhibitory experiment, LA12 was dissolved different final concentrations to reach the required molar ratio for testing inhibition efficiency, where amylin final concentration was set as 25 μM.

**Isothermal Titration Calorimetry (ITC) Characterization**

Affinity binding of different target-ligand systems was measured by MicroCal iTC200 (Malvern, USA). All solutions were centrifuged at 13,000 rpm/min for 10 min to remove the insoluble. Then the supernatant was taken to ensure that the volume of the target protein (amylin, Aβ42, BSA, IgG) solution was not less than 300 μL, and the volume of the ligand (LA12 and GR12) solution was not less than 60 μL. We comment that target protein concentration in the calorimetric cell was 25μM, while the ligand concentration in the syringe was set as 0.5 mg/mL (Figure S2a), 1 mg/mL (Figure S2b) and 2 mg/mL (Figure 2f, Figure S2c-e). To prove the specific interaction of target and ligand, either target or ligand was changed in the original amylin-LA12 systems. On the one hand, Aβ42, BSA, and IgG all are selected as control targets to detect their interaction with LA12. Among, Aβ42 has similar amyloidosis characteristics with amylin, and BSA was control proteins commonly used in affinity assay. Besides, IgG was the main component of serum immunoglobulin, and its interaction with LA12 may make a huge difference in inhibition efficiency in the blood. On another hand, GR12, Aβ42 monomer binding peptide screened in our previous work, was selected as control ligand to detect their interaction with amylin monomer. The isothermal titration calorimeter system temperature was set at 25°C, and the number of titrations was set as 20. The system compensation power was set 5 μcal/s, and the volume of the sample was set as 2 μL per titration. In addition, the stirring speed was set as 100 rpm/min. Amylin was titrated by repeated injections of 2 μL aliquots of LA12 solution. The least-squares method was used to determine the binding constant ($K_a$), the enthalpy change ($ΔH$) and entropy change ($ΔS$). The thermodynamic value is the average of at least three different experiments.

**Thioflavin T (ThT) Fluorescence Assay**

At designated time points, 100 μL cultures were removed and mixed with 100 μL 40 μM ThT in 10 mM Tris-HCl buffer (pH 7.3). ThT fluorescence emission spectrums of mixture were recorded using an F-4600 fluorescence spectrophotometer (Hitachi, Japan) under 438 nm excitation wavelength. The fluorescence intensity of amylin monomer without LA12 was set as control group (100%), while the
fluorescence intensity of experiment group including different molar ratio (1:1, 2:1, 3:1, 5:1, 10:1, 30:1, 50:1) of amylin monomer and LA12 were calculated as percentage of control group, where the final molar concentration of amylin monomer was 25 μM.

Circular Dichroism (CD) Spectrometry
The whole data of Circular dichroism (CD) Spectrometry were recorded utilizing a quartz cell with a 1 mm path length from 190 nm wavelength to 260 nm wavelength at a scan speed of 12 nm/min by Chirascan™ Circular Dichroism Spectrometry (Applied Photophysics, UK). The samples containing only Tris-HCl buffer were selected as the background which was subtracted in the following calculation. The averages of three consecutive scans per sample were selected as the final data. The secondary structures of peptides were predicted by CDNN software.

Atomic Force Microscope (AFM) Characterization
A 20 μL per culture was dropped on the unveiled smooth mica sheet, and after natural drying, it was rinsed gently with ultrapure water. Until the samples on the mica sheet surface were completely dried to be used for AFM characterization. AFM images were recorded using a Nanoscope VIIa microscope system (Bruker, USA) in Peak Force tapping mode. AFM height data of particles or fibers were analyzed by NanoScope Analysis.

Dynamic Light Scattering (DLS)
The size distribution of the clusters of LA12-amylin complex was measured at 25°C using a Nanosizer 2000 (Malvern, USA). LA12-amylin complexes were diluted to the appropriate concentrations using Tris-HCl buffer and then moved into a new disposable plastic cell. Each sample was measured three times.

Cytotoxicity Assay
The cell viability experiments in vitro were performed utilizing the INS-1 cell line, which has been widely used in the research of T2D. MTT method was used in this experiment, which was a way of measuring cell survival by chrominance ratio. INS-1 cells were incubated in RPMI medium including 0.11 g/L L-glutamine, 0.11 g/L sodium pyruvate, 5.6 mM glucose, 50 μM 2-β-mercaptoethanol, and 10% FBS. In MTT assay, INS-1 cells were spread out at the density of 5000 cells every well in 96-wells plate. 90 μL cell culture and 10 μL samples were added in each well and co-incubated 24 h. Therein, the samples utilized in the cell toxicity assay (Figure 5a) were just-
prepared LA12 monomers. And the samples used in the cytotoxicity assay (Figure 5b) were 7-day-cultures of amylin-LA12 complex in different concentration ratios, where the final concentration of amylin monomer was 2.5 μM in every sample and the final concentrations of LA12 were 0 μM (amylin fiber), 2.5 μM (1:1), 5 μM (2:1), 7.5 μM (3:1), 12.5 μM (5:1), 25 μM (10:1), 75 μM (30:1), and 125 μM (50:1) in terms of monomer concentration. To prove the specific interaction of amylin and LA12 in reasonable real-environment, BSA and IgG were added in the amylin-ligand system in the following cytotoxicity assay. Except for that, we utilized Aβ42 and PSMα3 to further verify the binding selectivity of LA12. The samples utilized in the cell toxicity assay (Figure S8a) were 7-day-cultures of complex (amylin-LA12, amylin-BSA, amylin-IgG, amylin-LA12-IgG, and amylin-LA12-BSA), where the final concentration of amylin monomer, BSA, and IgG were 2.5 μM in every sample and the final concentrations of LA12 were 25 μM (10:1), 75 μM (30:1) in terms of monomer concentration. And the samples utilized in the cell toxicity assay (Figure S8b) were 7-day-cultures of complex (amylin-LA12, Aβ42-LA12, and PSMα3-LA12), where the final concentration of amylin monomer, Aβ42 monomer, and PSMα3 monomer were 2.5 μM in every sample and the final concentrations of LA12 were 25 μM (10:1), 75 μM (30:1) in terms of monomer concentration. After that, 10 μL MTT solutions were added in the above system and the whole system was incubated for another 4 h. Then all solutions were removed and the leftover was suspended using DMSO and juggled for 10 min at 37°C. The cell viability was measured by a microplate reader at 490 nm wavelength. The wells containing 90 μL cell cultures and 10 μL Tris-HCl buffer were chosen as a control group with 100% cell viability and the data of other experimental groups were standardized as a percentage of data of control groups.

**Supplement section**

![Figure S1. Amino acid sequence and molecular structures of the affinity peptides LA12. Within the LA12, histidine residues (His, H) may dominate the interactions with aromatic amino acid side groups in the](image-url)
amylin monomer via π-π stacking. The proline residue (Pro, P) increases the stability and rigidity of LA12.

Electrostatic interaction may also play a role in mediating intermolecular interactions with an amylin monomer due to the presence of abundant positively charged residues in LA12.

Figure S2. CD curves of amylin monomer and LA12. (a) amylin monomer (25 μM). (b) LA12 (50 μM).

Figure S3. The molecular structure of amylin monomer 5MGQ from PDB (left). The reported core region of amylin monomer (right).

Figure S4. Typical ITC titration curves (top panel) and binding isotherms (bottom panel) for affinity peptide.
(a) 0.5 mg/mL and (b) 1 mg/mL LA12 at 25°C interactions with 25 μM amylin in 10 mM Tris-HCl at pH 7.3.

Figure S5. Typical ITC titration curves (top panel) and binding isotherms (bottom panel) for affinity peptide (a) 2 mg/mL LA12 interactions with 25 μM Aβ42, (b) 2 mg/mL LA12 interactions with 25 μM BSA, (c) 2 mg/mL GR12 interactions with 25 μM Amylin, (d) 2 mg/mL LA12 interactions with 25 μM IgG in 10 mM Tris-HCl at pH 7.3.

Figure S6. (a) CD curves of amylin after 7 days incubation without adding LA12. (b) The percentages of secondary structure of LA12 alone after 0 and 7 days incubation predicted by CDNN.
Figure S7. AFM characterization of LA12 aggregates after 7 days incubation at different molar concentrations. (a) 250 μM. (b) 750 μM. (c) 1250 μM. The scale bars for the images in the left column are 1.0 μm. The molecular binding patterns in the right columns were speculated by comparing the actual size of the molecules with the height of the aggregates in the AFM. (d) Particle size statistics of aggregates in AFM characterization different molar concentrations (250 μM, 750 μM, 1250 μM) of LA12 alone after 7-day-incubation.

Figure S8. The specific inhibitory effect of LA12 on amylin-induced cytotoxicity. (a) The addition of BSA and IgG nearly have no disturbance on the INS-1 cell protection activity of LA12 using MTT assay. (b) LA12 nearly has no inhibitory effect on Aβ42-induced cytotoxicity and PSMα3-induced cytotoxicity utilizing MTT assay. Amylin concentration was 25 μM in the above assays and cell viability for treatment with Tris-HCl buffer alone was set to 100% as a control in MTT assay.
Table S1. The binding constant ($K_a$) of different target-ligand systems.

<table>
<thead>
<tr>
<th>Target-ligand Systems</th>
<th>$K_a$</th>
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<tbody>
<tr>
<td>Aβ42 and LA12</td>
<td>$1.04 \times 10^5$ M$^{-1}$</td>
</tr>
<tr>
<td>BSA and LA12</td>
<td>$7.06 \times 10^4$ M$^{-1}$</td>
</tr>
<tr>
<td>IgG and LA12</td>
<td>-</td>
</tr>
<tr>
<td>Amylin and GR12</td>
<td>$4.45 \times 10^3$ M$^{-1}$</td>
</tr>
<tr>
<td>Amylin and LA12</td>
<td>$2.28 \times 10^5$ M$^{-1}$</td>
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Table S2. DLS analysis of the clusters of amylin-LA12 complex

<table>
<thead>
<tr>
<th>Sample</th>
<th>Size (nm)</th>
<th>PDI</th>
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<tbody>
<tr>
<td>Amylin alone (25 μM)</td>
<td>785±170</td>
<td>1.08</td>
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<tr>
<td>Amylin:LA12=1:1</td>
<td>678±147</td>
<td>0.94</td>
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<tr>
<td>Amylin:LA12=1:2</td>
<td>585.5±126.5</td>
<td>0.90</td>
</tr>
<tr>
<td>Amylin:LA12=1:3</td>
<td>585.5±126.5</td>
<td>0.89</td>
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<tr>
<td>Amylin:LA12=1:5</td>
<td>325.5±126.5</td>
<td>0.84</td>
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<tr>
<td>Amylin:LA12=1:10</td>
<td>135±29</td>
<td>0.63</td>
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<tr>
<td>Amylin:LA12=1:30</td>
<td>67.6±23.75</td>
<td>0.6</td>
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<tr>
<td>Amylin:LA12=1:50</td>
<td>505.5±109.5</td>
<td>0.79</td>
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References


