Design of metastable β-sheet oligomers from natively unstructured peptide

Supporting material

Preparation of Aβ40 oligomers and fibrils

Aβ40 peptide was purchased from the Keck Large Scale Peptide Synthesis facility, Yale University. 1 mg of peptide was resuspended in 1.5 mL of 50% acetonitrile/water mixture. The solution was divided into 3 Eppendorf tubes, each containing 500 µL (0.3 mg), and relyophilized. Soluble oligomers were prepared by dissolving 0.3 mg of the peptide in 200 µL hexafluoroisopropanol (HFIP) and incubating it for 10–20 min at room temperature, the samples were centrifuged for 15 min. at 14,000 x G and the solution was transferred to a new siliconized tube and subjected to a gentle stream of N_2 for 5-10 min to evaporate the HFIP. Then 700 µL dd H_2O were added and the resulting solution was stirred at 500 rpm using a Teflon-coated micro stir bar for 24-48 hr at 22 °C. Fibrils were prepared with dd H_2O (pH 3.8-4.2), containing 0.02% sodium azide. The final peptide concentration was 0.3mg/ml. The samples were stirred with a Teflon coated micro stir bar at 500 rpm at room temperature for 6 days; oligomers were A 11 positive and fibrils were OC positive (*1-5*).

Peptide Prp-G synthesis

The human prion protein segment (Prp 109-148) peptide with substitutions of M for G residues (Prp-G; GKHGAGAAAAGAVVGGLGGYGLGSAGSRPIIHFGSDYEDR-OH) was synthesized by using fluorenylmethoxycarbonyl (FMOC) automated solid-phase peptide synthesis using a continuous flow semiautomatic Applied Biosystems 433 peptide synthesizer. Peptide was purified by reverse phase high performance liquid

chromatography (RF-HPLC) and used only when it was 95% pure. The purity was also analyzed by matrix-assisted laser desorption ionization (MALDI) mass spectrometry.

Preparation of Prp mimic

The thioester Prp-G peptide (GKHGAGAAAAGAVVGGLGGYGLGSAGSRPIIHFGSDYEDR-OR) was synthesized as previously described [1, 5] . The first amino acid was manually coupled to the sulfamylbutyry-AM-PEGA resin (Novabiochem-Millipore, San Diego, CA), 1g (0.28mmole) of resin in 10 ml of dichloromethane (DCM), 5 eq of the first amino acid was added (Fmoc-Arg-OH), followed by the addition of 10 eq of DIEA, and stirred for 20 min at room temperature, then it was cooled using ice and salt to (-10 to -20 C), then 4.7 eq of ByBop was added and stirred for 8 to 9 h at (-10 to -20 C), the coupling efficiency was checked using the Kaiser test, the substitution level was found to be around 0.16 mmol /g, determined using Fmoc cleavage method. Acetylation was then performed using acetic anhydride. The sequence was elongated by standard FMOC automated solid-phase peptide synthesis. The fully protected peptide on the resin, was activated by treatment with iodoacetonitrile (100 mg, 0.16 mmol/g) in peptide synthesis vessel, and then washed 5 times with N-methyl-2-pyrrolidone (NMP). 5.0 ml of NMP, 185 µl of i-Pr2EtN (1.1 mmol), and 400µL of iodoacetonitrile, (previously filtered through an alumina basic plug in the dark) were added and the reaction mixture was shaken for 24h in the dark on a rotary plate. The resin was washed 5 times with NMP, 5 times with DMF, then 5 times with CH_2C_{l2} and dried. Trimethylsilyldiazomethane (100 mg resin 0.18 mmol/g) was washed 5 times with THF, then 2.7 ml of THF was added and then 2.7 ml of TMS-CH_2N_2 (50%, v/v in hexane) was added. After stirring for 2 h, the

resin was washed 5 times with 5 ml THF and 5 times with 5 ml of DMF and dried under vacuum, the displacement reaction was performed using ethyl-3-mercaptopropionate in CH_2C_{l2} (800μL) and ethyl-3-mercaptopropionate (120μL). The mixture was shaken on a rotary plate for 24 h. The resin was filtered, and then washed with 3x 3ml DMF. The filtrate and washes were collected and rotary evaporated at 34 °C. The yield was approximately 50%. The resulting, fully protected peptide was deprotected using standard methods, and purified by RF-HPLC. The purity was checked by analytical RP-HPLC and MALDI mass spectrometry and determined to be approximately 90%. Prp-G was coupled covalently to colloidal gold nanoparticles via a carboxy terminal thiol according to the protocol described to prepare Aβ40 oligomimics(*1, 5*). Briefly, gold colloids (mean diameter 5.3 nm) were obtained from Ted Pella, Inc. The colloid was collected at 40000 g and washed 2 times with distilled water to remove the preservatives. The washed colloidal gold particles were mixed with a freshly prepared solution of 0.2 mg/ml of Prp-G thioester, (pH 5.0-5.5), 25 ml of Prp-G solution (0.3 mg/ml) to 20 ml of washed gold colloids in water. After 3 hrs incubation at room temperature, the pH was adjusted to 7.4 with 100 mM Tris pH 8.0 (0.02% sodium azide). After incubation for 6 hr at room temperature, the antigen was collected by centrifugation at 30000 g at 4 °C for 30 min, washed three times with PBS pH 7.4 to remove any unincorporated Prp and then re-dispersed in distilled H2O. The resulting Prp mimics solution was stored at 4°C.

Fourier-Transformed Infrared (FT-IR) spectroscopy

To prepare samples for FTIR spectroscopy, proteins were centrifuged at 20000 g at 4ºC for 10 min and the pellet was washed with D_2O and placed between two calcium fluoride windows separated by a 50-μm Teflon spacer. The spectra were acquired at room

temperature on a Nicolet 6700 analytical FT-IR spectrometer. Overlapping infrared bands were partially resolved by Fourier self-deconvolution procedure.

Thioflavin T fluorescence

Thioflavin T (Sigma) binding was measured using a POLARstar OMEGA plate reader (BMG Labtechnologies, Melbourne, VIC, Australia) with 440-10 nm/520 nm excitation／emission filters set.1 μl of 0.3 mg/ml of protein sample and 250 μl of 5 μM ThT, 50 mM glycine buffer (pH 8.5). Fluorescence intensity values of samples were obtained by subtraction of blank.

Bis-ANS fluorescence

1,1'-bis(anilino)-4-,4'-bis(naphthalene)-8,8'-disulfonate (Sigma), bis-ANS binding was measured using a POLARstar OMEGA plate reader (BMG Labtechnologies, Melbourne, VIC, Australia) with 355 nm/520 nm excitation／emission filters set. 1 μl of 0.3 mg/ml of protein sample and 250 μl of 10 μM bis-ANS, 100 mM glycine buffer (pH 7.4). Fluorescence intensity values of samples were obtained by subtraction of blank.

Atomic force microscopy

10 μl of hPrp 109-148 oligomimics samples were spotted on mica and allowed to attach to the surface for 1 min. Mica was then dried with gentle air purge for 2 min. The mica surface was washed 10 times with 100 μl of distilled water to remove unbound protein and impurities. The samples were directly analyzed with Multimode 8 AFM (Veeco, CA) in a non-contact tapping method (ScanAsyst-air). Pictures were flattened and particle diameter was measured at half the maximal height with Nanoscope Analysis software version 1.2.

Size-exclusion chromatography

Prp mimic was analyzed using Shimadzu Ultra Fast liquid chromatography (UFLC) system fitted with a Superdex™ 10/300 GL Column (Tricorn). L × I.D. 10 × 300 mm, 13 μm particle size from GE Healthcare PBS, pH 7.4, was used as mobile phase, flow rate 0.8 mL/min. Gel filtration standard (Bio-Rad 51-1901) was used for calibrations. Excitation and emission wavelengths used for fluorescence detection were 280 nm and 350 nm, respectively.

Dynamic light scattering

Dynamic light scattering analysis was performed in a Zetasizer Nano ZS (Malvern, Worcestershire, UK) equipped with 4mW He–Ne 633nm laser with a detection angle of 173° backscatter at 25° C in a low volume (4μl) quartz suprasil cuvette (Hellma, Müllheim, Germany). Size distribution was obtained by intensity and volume measurements in multiple narrow mode. Measurements were performed at least in triplicate.

Circular dichroism measurements:

Circular dichroism (CD) spectra were recorded at room temperature using a J-720 spectropolarimeter (JASCO) equipped with a temperature controller. Spectra were measured at 0.20-nm intervals, spectral bandwidth of 1 nm, scan speed of 20 nm/min, response time of 8 s. Each spectrum represents the average of three scans in the range of 195 to 350 nm at 0.3 mg/mL protein in PBS. The quartz cells had path lengths of 0.1 mm and were washed with 30% HCl in EtOH, water, and MeOH before use.

Western blot

Peptide samples were electrophoresed on 4-12% Tris-HCl (Invitrogen) gels and were transferred onto nitrocellulose membranes, blocked with 10% nonfat dried milk for 1 h,

membrane was washed 3 times with Tris-buffered saline 1X-Tween 20 0.01% (TBS-T) and incubated with the primary antibody A11 (1:1000) in 5% nonfat dried milk in TBS-T for 1 hr and then washed 3 times with TBS-T. The membrane was incubated with anti-rabbit IgG conjugated with horseradish peroxidase (1:10,000, Jackson) at room temperature for 1 h, then washed 3 times with TBS-T. The blot was developed with super signal west pico chemiluminescence kit from Thermo Scientific.

Dot blot assay:

For dot blots, 0.36 µg of peptide (1.2 µL) was spotted on nitrocellulose membranes, after blocking with nonfat dried milk for 1 h, membranes were washed 3 times with TBS-T pH 7.4 and incubated with the primary antibody for 1 hr at room temperature. Antibody solutions were prepared in 5% nonfat dried milk in TBS-T, dilutions used 3F4 (1:10,000), Pri308 and [F89/160.1.5] (1:2,000), A-11 (1:1000) and OC (1:8000). The membranes were washed 3 times with TBS-T and incubated with anti-mouse IgG or anti-rabbit conjugated with horseradish peroxidase (1:10,000, Jackson) at room temperature for 1 h. The membranes were washed 3 times with TBS-T and the blots were developed with super signal west pico chemiluminescence kit from Thermo Scientific.

References

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