Racemic peptides from Amyloid beta and Amylin form rippled β-sheets rather than pleated β-sheets.

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SUPPORTING INFORMATION

Material and Methods

Peptide Synthesis and Crystallization

Peptides were purchased from Anaspec or synthesized on pre-loaded Wang resins by standard Fmoc based, solid-phase peptide chemistry. Syntheses were performed manually at 0.2 mM scale relative to resin loading. The peptides were cleaved and deprotected with a mixture consisting of trifluoroacetic acid (10 mL), tri-isopropylsilane (1 mL), and liquefied phenol (0.5 mL). The cleavage solution was added to the resins and agitated for 2 h. The solution was then evaporated to 2 mL under nitrogen gas, and the peptides precipitated with cold diethyl ether and centrifuged at 6000 rpm. The peptide pellet was washed with cold diethyl ether, dried, dissolved in 1:1 acetonitrile:water, flash frozen in liquid nitrogen, and lyophilized. No further purification was performed prior to crystallization.

MVGGVV:mvggvv HFIP

Stock solutions of the L-MVGGVV and D-mvggvv peptides in Nanopure water were prepared separately at concentrations of 2 mg/mL. Aliquots (100 ul) of each solution were combined and hexafluoroisopropanol (20 uL) was subsequently added. Colorless needles grew over the course of several days.

MVGGVV:mvggvv PFPA

Stock solutions of the L-MVGGVV and D-mvggvv peptides in Nanopure water were prepared separately at concentrations of 2 mg/mL. Aliquots (100 ul) of each solution were combined and pentafluoropropionic acid (20 uL) was subsequently added. Colorless needles grew over the course of several days.

KLVFFA:klvffa

Stock solutions of the L-KLVFFA and D-klvffa peptides in Nanopure water were prepared separately at concentrations of 2 mg/mL. Aliquots (100 ul) of each solution were combined and hexafluoroisopropanol (20 uL) was subsequently added. Colorless needles grew over the course of several weeks.

Ac-KLVFFAE-NH_2:Ac-klvffae-NH_2

Solutions of the L-Ac-KLVFFAE-NH_2 and D-Ac-klvffae-NH_2 peptides in Nanopure water were prepared separately at concentrations of 1 mg/mL. The solutions were combined and hexafluoroisopropanol (100 uL) was then added. A gel formed immediately. Over a period of several weeks, the gel separated and subsequently formed colorless needles.

AILSS:ailss

Stock solutions of the L-AILSS and d-ailss peptides in Nanopure water were prepared separately at concentrations of 40 mg/mL. Aliquots (200 uL) of each solution were combined and hexafluoroisopropanol (47 uL) was subsequently added. Colorless needles grew over the course of several hours.

Crystallographic Structure Determination.

Microfocus X-ray beam optics were required to measure crystal diffraction intensities from our crystals since they were needle-shaped, and less than 5 microns thick. We used microfocus beamline 24-ID-E of the Advanced Photon Source located at Argonne National Laboratory. Crystals were cooled to a

temperature of 100 K. Diffraction data were indexed, integrated, scaled, and merged using the programs XDS and XSCALE.[1] Data collection statistics are reported in Supplemental Table 1. Initial phases for KLVFFAE:klvffae were obtained by molecular replacement with the program Phaser[2] using PDB entry 3ow9 as the search model. Phases for the four remaining sets of diffraction intensities were obtained by direct methods using the program ShelxD[3] (for MVGGVV:mvggvv) and ShelxT[4] (for KLVFFA:klvffa and AILSS:ailss structures). Model building was performed using the graphics program Coot[5]. Atomic refinement was performed using the program Phenix.[6] Subsequent rounds of refinement were performed using the program Refmac[7] in all cases except MVGGVV:mvggvv with PFPA. Structure illustrations were created using PyMOL.[8]

Table S1. Data Collection and Refinements Statistics from Crystals of Amyloid Segments.

Data collection and refinement statistics from crystals of Amyloid segments

Peptide, racemic mixture Parent protein Co-solvent	**^{16}KLVFFA^{21}** Amyloid β TFA	**^{16}KLVFFAE^{22}** Amyloid β	**^{35}MVGGVV^{40}** Amyloid β HFIP	**^{35}MVGGVV^{40}** Amyloid β PFPA	**^{25}AILSS^{29}** IAPP
Data Collection					
Beamline	APS 24-ID-E	APS 24-ID-E	APS 24-ID-E	APS 24-ID-E	APS 24-ID-E
Space group	P-1	$P2_1/c$	P-1	$P2_1/c$	P-1
Resolution (Å)	1.50 (1.56-1.50)*	2.00 (2.15-2.00)	1.10 (1.16-1.10)	1.10 (1.16-1.10)	1.10 (1.16-1.10)
Unit cell dimensions: a,b,c (Å)	11.69,11.70, 18.51	24.92, 11.53, 21.54	9.42, 11.22, 21.12	9.56, 18.57, 20.83	9.50, 9.88, 16.29
Unit cell angles: α,β,γ (°)	89.96,91.78,110.51	90.0, 97.73, 90.0	96.57, 89.57, 94.95	90.0, 98.4, 90.0	90.45, 98.13, 94.38
Measured reflections	8550 (867)	1891 (234)	22565 (1253)	16074 (869)	9671 (428)
Unique reflections	1424 (139)	785 (113)	2980 (224)	2485 (155)	1898 (101)
Overall completeness (%)	97.0 (95.2)	87.3 (72.0)	85.8 (43.2)	82.5 (35.9)	80.3 (30.6)
Overall redundancy	6.0 (6.2)	2.4 (2.1)	7.6 (5.6)	6.5 (5.6)	5.1 (4.2)
Overall R_{merge}	0.157 (1.16)	0.140 (0.742)	0.174 (1.897)	0.099 (0.234)	0.142 (0.955)
$CC_{1/2}$	99.7 (76.7)	99.4 (76.7)	99.5 (62.7)	99.1 (97.7)	99.2 (65.1)
Overall I/δ	4.5 (1.4)	2.9 (1.1)	4.9 (0.8)	10.3 (5.3)	4.9 (1.4)
Refinement					
R_{work} / R_{free}	0.225 / 0.227	0.465 / 0.490	0.189 / 0.204	0.152 / 0.177	0.152/0.179
RMSD bond length (Å)	0.012	0.015	0.018	0.007	0.005
RMSD angle (°)	1.6	1.8	1.9	1.4	0.8
Number of peptide atoms**	52	64	43	38	34
Number of water atoms	3	0	2	1	5
Number of other solvent atoms	7	0	36	10	0
Average B-factor of peptide (Å^2)	22.8	37.3	20.5	5.8	11.8
Average B-factor of water (Å^2)	29.7	N/A	26.4	5.5	20.0
Average B-factor other solvent (Å^2)	41.3	N/A	26.4	9.8	N/A
PDB ID code	8T89	N/A	8T84	8T82	8T86

*Numbers in parentheses report statistics in highest resolution shell.

**Count excludes hydrogen atoms.

Table S2. Torsional angles of the various rippled and pleated MVGGVV X-ray structures

Rippled MVGGVV:mvggvv HFIP | ***8T84.pdb***

residue	Phi	psi	mirror image phi	mirror image psi
M35	-	135.49	-	-140.35
V36	-116.45	115.49	116.45	-115.49
G37	-69.04	151.79	69.04	-151.79
G38	-74.76	168.62	74.76	-169.62
V39	-129.56	136.71	129.56	-136.71
V40	-112.93	-	112.93	-

Rippled | MVGGVV:mvggvv | PFPA | ***8T82.pdb***

residue	Phi	psi	mirror image phi	mirror image psi
M35	-	146.95		
V36	-110.01	111.67	110.01	110.01
G37	-68.15	153.07	68.15	68.15
G38	-68.76	168.97	68.17	68.17
V39	-133.94	124.52	133.94	133.94
V40	-107.35	-	107.35	-

Pleated | L-MVGGVV | ***2ona.pdb***

residue	phi sheet 1	psi sheet 1	phi sheet 2	psi sheet 2
M35	-	132.62	-	140.82
V36	-137.37	140.44	-136.85	134.61
G37	-165.44	155.39	-148.83	151.62
G38	-146.02	145.39	-158.83	159.17
V39	-134.25	123.76	-141.54	137.17

V40	-109.36	-	-144.16	-

Pleated | L-MVGGVV | ***2okz.pdb***

residue	phi sheet 1	psi sheet 1	phi sheet 2	psi sheet 2
M35	-	156.79	-	138.86
V36	-145.95	133.30	-128.52	119.38
G37	-133.02	147.55	-138.60	146.96
G38	-140.74	139.48	-134.19	126.70
V39	-131.19	131.12	-113.89	116.80
V40	-128.20	-	-135.39	-

Table S3. Sheet-Sheet Interface Properties.

Table of Sheet-Sheet interface Properties

Chirality	Peptide Crystal	Area Buried (Å^2)	Shape Comple-mentarity	Sheet structure	Strand orientation	Strand registration	Sheet-to-sheet orientation	Dry or solvated?	PDB ID
	Crystal structures containing MVGGVV sequence								
L+D	MVGGVV in HFPA	63	0.75	Rippled	Antiparallel	In-register	Face-to-face	Solvated	8t84
L+D	MVGGVV in PFPA	78	0.75	Rippled	Antiparallel	In-register	Face-to-edge	Solvated	8t82
L	MVGGVV-form 1	92	0.76	Pleated	Antiparallel	In-register	Face-to-face	Dry	2ona
L	MVGGVV-form 2	103	0.82	Pleated	Antiparallel	In-register	Face-to-face	Dry	2okz
	Crystal structures containing KLVFFA sequence								
L+D	KLVFFAE	137	0.49	Rippled	Antiparallel	Out-of-register	Face-to-face	Dry	N/A
L+D	KLVFFA	96	0.85	Rippled	Antiparallel	Out-of-register	Face-to-face	Dry	8t89
L	KLVFFA-form1	113	0.58	Pleated	Antiparallel	In-register	Face-to-face	Dry	2y2a
L	KLVFFA-form2	131	0.54	Pleated	Antiparallel	In-register	Face-to-face	Dry	3ow9
L	KLVFFA-form3	114	0.55	Pleated	Antiparallel	In-register	Face-to-face	Dry	2y29
	Crystal structures containing AILSS sequence								
L+D	AILSS	104	0.85	Rippled	Antiparallel	In-register	Face-to-face	Dry	8t86
L	AILSST	112	0.57	Pleated	Antiparallel	In-register	Face-to-face	Dry	3fod
	Classical steric zipper structures, select examples								
L	GNNQQNY from Sup35	143	0.86	Pleated	Parallel	In-register	Face-to-face	Dry	1yjo
L	LVEALYL from Insulin	205	0.81	Pleated	Parallel	In-register	Face-to-face	Dry	3hyd
L	VVTGTVA from α-Synuclein	162	0.94	Pleated	Parallel	In-register	Face-to-face	Dry	4r0w
L	VQIVYK from Tau	114	0.72	Pleated	Parallel	In-register	Face-to-face	Dry	2on9
L	VQIINK from Tau	146	0.85	Pleated	Parallel	In-register	Face-to-face	Dry	6odg
L	NFGAILS from IAPP	180	0.81	Pleated	Antiparallel	Out-of-register	Face-to-face	Dry	5e5v

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