Supporting Information

NMR Solution Structure of PisI, a Group B Immunity Protein that Provides Protection Against the Type IIa Bacteriocin Piscicolin 126, PisA[†,‡]

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Table SI: Bacterial strains and plasmids

Bacterial strain or plasmid	Relevant phenotype and properties	Reference or source
C. maltaromaticum UAL26	Producer of PisA and PisI	(*1*)
E. coli K12 TB1	F$^-$ *ara Δ(lac-proAB) [Φ80dlac Δ(lacZ)M15] rpsL(Str^R) thi hsdR*	New England Biolabs
E. coli BL21(DE3)	B F$^-$ *ompT hsdS*(r_B^- m_B^-) *dcm*$^+$ Tet^r gal λ(DE3) *endA* Hte	Stratagene
pMAL-c2x	6.6 kb expression vector, Amp^r, lacZ$^{'}$	New England Biolabs
pMAL-PisA	pMAL-c2x containing malE-pisA fusion	This study
pMAL-PisI	pMAL-c2x containing malE-pisI fusion	This study

Table SII: Primers used in this study for cloning and sequencing

Gene	Primer Name	Primer Sequence
PisA	pMALP126mF	5′-AAGTATTACGGAAATGGCGTTTCC-3′
PisA[a]	pMALP126mR	5′-AGAGAAGCTTTTATCCTTTGTTCCAACCA-3′
PisI	LGPIF1	5′-ATGGGTAAGTTAAAATGGTTT-3′
PisI[a]	LGPIR1	5′-AGAGAAGCTTCTAATATCCATATCTAAT-3′
MalE	LGMALE	5′-GGTCGTCAGACTGTCGATGAAGCC-3′
lacZα[a]	LGM13PUC	5′-CGCCAGGGTTTTCCCAGTCACGAC-3′

[a]Antisense primer

Expression of [^{15}N]PisI and [^{15}N]PisA in M9 Minimal Media. The initial clones expressing MalE-PisI and MalE-PisA utilized *E. coli* K12 TB1 as a host strain. However, this strain was unable to grow in minimal media. Thus, an alternate host strain, *E. coli* BL21(DE3) was used. Minimal media was prepared as follows: for 500 mL of media, 100 mL of 5x M9 salts (0.043 M NaCl, 0.11 M KH_2PO_4 and 0.25 M $Na_2HPO_4 \bullet 7H_2O$) was diluted with 400 mL of milli-Q water and sterilized. The following components were then added (all filter sterilized): 5 mL of 20% glucose, 1.25 mL of 20% $(^{15}NH_4)_2SO_4$, 1 mL of 1M $MgSO_4$, 0.5 mL of 0.1 M $CaCl_2$, 50 μL 10 mM $FeSO_4$ and 50 μL 10 mg/mL thiamine.

Purification of PisI by cation exchange chromatography. In order to separate PisI from MalE and Factor Xa following the cleavage reaction, cation exchange chromatography with SP sepharose resin was performed. The isoelectric point (pI) of PisI was estimated to be 7.9 (*2*) and the pI's of MalE and Factor Xa are both ~5.0 (according to manufacter's protocol). Following the cleavage reaction, the solution was first dialyzed against column buffer [50 mM sodium phosphate (pH 6.8) and 20 mM NaCl]. The solution was then applied to a column of SP-sepharose equilibrated with column buffer and monitored by UV absorbance at 280 nm. After the Factor Xa and MalE components and passed through the column, PisI was eluted by increasing the concentration of NaCl in the column buffer to 500 mM. The purity of the fractions containing PisI were confirmed by SDS-page electrophoresis, as well as MALDI-TOF spectrometry. When necessary, subsequent buffer exchange and concentration of PisI was accomplished with concentrating tubes (Amicon centrifugal filters, NMWL 5 kDa).

Purification of PisI by RP-HPLC. PisI was also purified by HPLC, using a C18 Protein & peptide preparative RP-HPLC column (Grace Vydac 218TP1022). The solvents used were milli-Q water, 0.1% TFA (Solvent A) and acetonitrile, 0.1 % TFA (Solvent B). The following method was employed: 40% solvent B for four minutes; increase solvent B to 70% over 13 minutes; maintain solvent B at 70% one minute; return to 40% solvent B over four minutes. A flow rate of 10 mL/min and a 1 mL injection were used. Separation and elution were monitored by UV absorption at 220 and 280 nm. PisI eluted at ~12 min.

Purification of PisA by RP-HPLC. PisA was purified by HPLC using a C18 Protein & peptide preparative RP-HPLC column (Grace Vydac 218TP1022). The solvents used were milli-Q water, 0.1% TFA (Solvent A) and acetonitrile, 0.1 % TFA (Solvent B). The following method was employed: 20% solvent B for five minutes; increase solvent B to 50% over 15 minutes; increase solvent B to 70% over 3 minutes; return to 20% solvent B over one minute; maintain solvent B at 20% for four minutes. A flow rate of 10 mL/min and a 1 mL injection were used. Separation and elution were monitored by UV absorption at 220 and 280 nm. PisA eluted at ~17.5 min.

CD Experiments. All CD measurements were made on an OLIS DSM 17CD spectrophotometer (Bogart, GA) in a thermally controlled quartz cell with a 0.02 cm pathlength. The instrument calibration was checked against a 1 mg/mL solution of d-10-camphorsulfonic acid. The concentrations of PisA and PisI used were 0.5 mg/mL, corresponding to 0.11 mM and 0.045 mM respectively. In the first set of experiments, CD spectra of PisA and PisI in aqueous buffer (consisting of 20 mM sodium phosphate pH 6.0, 1 mM EDTA and 1 mM NaN_3) at 20^{o}C were

collected. In the second set of experiments, the CD spectra of PisA and PisI in 50% TFE and 50% aqueous buffer at 20^{o}C were recorded. Data was collected every 1 nm and were the average of ten scans. The bandwidth was set at 2.0 nm. For both set of experiments, baseline spectra of the appropriate solvent system were subtracted from the sample spectra prior to calculating molar ellipticities. Point-by-point integration was performed as a function of the high voltage readings on the photomultiplier detectors. Results were expressed in units of molar ellipticity (deg cm^{2} dmol^{-1}) and plotted against the wavelength. Analysis of the CD data and quantization of secondary structure was achieved using the CDPro software package (*3, 4*). For each separate experiment, the CD data was first converted from molar ellipticity into molar ellipticity per residue ($\Delta\varepsilon$/n) units using the program CRDATA. The SP43 basis set consisting of 43 soluble proteins was used as the reference data for structure analysis. Three programs (CONTINLL, SELCON3 and CDSSTR) were used to compare the experimental CD data against the reference data. All three programs employ a different algorithm to compute the secondary structure of the protein. The output was reported as a fraction of the following secondary structure classes: regular α-helix [H(r)], distorted α-helix [H(d)], regular β-strand [S(r)], distorted β-strand [S(d)], turns and unordered. The regular and distorted components of α-helix and β-strand, and turn and unstructured elements were summed for convenience. Since all three programs utilized the same basis set and values were similar, the fractional secondary structures calculated by each program were averaged.

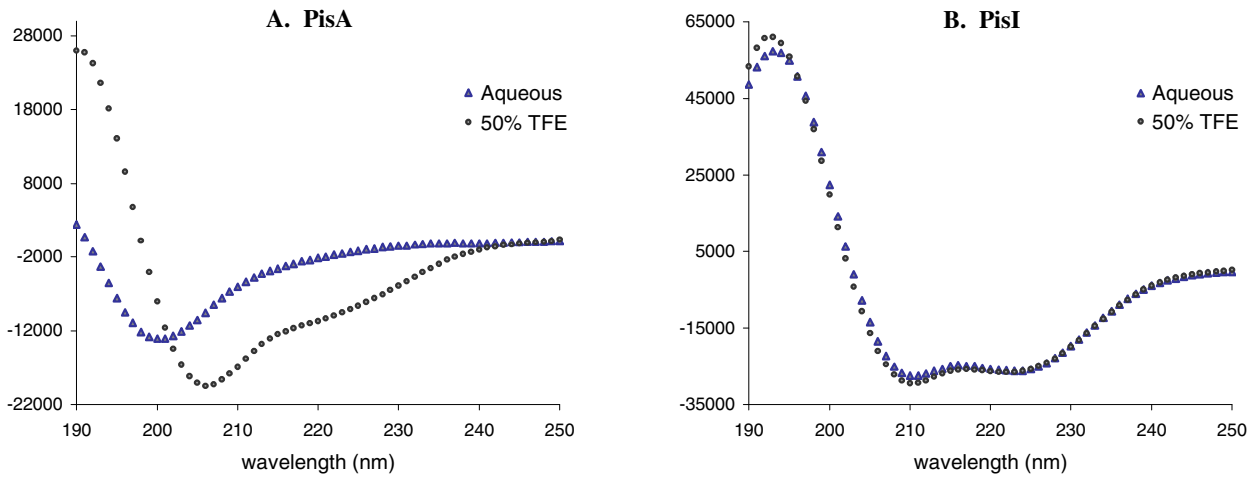

FIGURE S1: CD studies to determine the effect of TFE on the conformation of PisA (A) and PisI (B). PisA is random coil in aqueous conditions, but assumes a helical conformation in the presence of TFE. PisI has a high degree of helicity in aqueous conditions and is not significantly affected by the addition of 50% TFE.

Table SIII: CD Analysis of the Secondary Structure Fractions of PisA and PisI in aqueous conditions, or 50% TFE at 20°C (*3, 4*).

Protein	solvent	α-helix	β-strand	coil
PisA	aqueous	0.06	0.34	0.59
	50% TFE	0.41	0.15	0.44
PisI	aqueous	0.91	0.01	0.09
	50% TFE	0.91	0.03	0.11

Aqueous solvent consisted of 20 mM sodium phosphate (pH 6.0), 1 mM EDTA and 1 mM NaN_3. 50% TFE solvent consisted of a 1:1 mixture TFE and 40 mM sodium phosphate (pH 6.0), 2 mM EDTA and 2 mM NaN_3.

NMR Spectroscopy of [^{15}N]PisI and [^{13}C,^{15}N]PisI. NMR samples contained ~0.5 mM protein in 90% H_2O/10% D_2O or 100% D_20, 20 mM sodium phosphate (pH 5.9), 1 mM EDTA, 1 mM NaN_3 and 50 μM 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (DSS) (*5*). NMR spectra were acquired at 25°C on Varian Inova 500 MHz and 800 MHz spectrometers equipped with triple-resonance HCN cold probes and z-axis pulsed-field gradients (PFGs). The following experiments were used for backbone and side-chain ^{1}H, ^{13}C, and ^{15}N resonance assignments: ^{15}N HSQC, ^{15}N HSQC-TOCSY, ^{15}N HSQC-NOESY, HNHA, HNCACB, CBCA(CO)NH, C(CO)NH, ^{13}C HSQC, ^{13}C HCCH-TOCSY, ^{13}C HCCH-COSY, ^{13}C HSQC-NOESY (*6-17*). NMR spectra were processed using NMRPIPE and analyzed with NMRView (*18, 19*). Data were multiplied by a 90° shifted sine-bell squared function in all dimensions. Indirect dimensions were doubled by linear prediction and zero-filled to the nearest power of two prior to Fourier transformation.

Structure Calculations. NOE restraints were obtained from the ^{15}N- and ^{13}C- edited HSQC-NOESY experiments and ϕ angle restraints were derived from analysis of the diagonal-peak to

cross-peak intensity ratio in the HNHA experiment. The structure of PisI was calculated by using the CANDID module in the program CYANA 2.1 (*20*), using a combination of manually and automatically assigned NOEs (manual inspection of the peaks was necessary due to the presence of degraded material). Peaks were calibrated according to their intensities. A family of 100 random structures was generated and subjected to simulated annealing, with 10 000 torsion angle dynamic steps. After seven rounds of calculation, 2627 of the initial 2915 peaks were assigned. After accounting for symmetric peaks, a total of 1725 upper distance restraints and 76 ϕ dihedral angles were used in the final round of calculations. The twenty lowest energy conformations (no NOE violations of >0.3 Å with no residues in the disallowed region of the Ramachandran plot) were chosen as representative of the solution structure of PisI. The backbone r.s.m.d. for residues 13-92 was 0.56 Å. Structural statistics were calculated with CYANA and MOLMOL (*21*). Figures were generated with PyMOL (http://www.pymol.org), PDB2PQR (*22*), APBS (*23*) and MOLMOL. Coordinates have been deposited in the PDB data bank (http://www.rcsb.org/pdb), with the accession code 2K19. Chemical shift assignments have been deposited under BMRB accession number 15763 (http://www.bmrb.wisc.edu/).

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