Supporting Information

Conformational Dynamics of the Anthrax Lethal Factor catalytic center

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Materials and Methods

Sample preparation: The LF₆₇₂₋₇₇₆ construct was expressed in *E. coli*, labeled, isolated and purified as described elsewhere (1). In its metal-free form the recombinant polypeptide has a molecular mass of 12.1 kDa, pI 5.5; it consists of 106 residues corresponding to amino acids 672–776 at the C-terminus of the full length enzyme plus a N-terminal Gly as a cloning artifact (1). NMR measurements were carried out on 0.6–0.8 mM apoLF₆₇₂₋₇₇₆ samples in 300 μ l mixed solvent of 90% H₂O (50 mM KPi, 5 mM EDTA, 0.2M acetohydroxamic acid, pH 7.2) and 10% ²H₂O.

NMR experiments and structure calculations of apoLF₆₇₂₋₇₇₆: The complete set of heteronuclear NMR experiments used in this study is listed in Table S1. All resonance assignments have been deposited at the BioMagResBank (<http://www.bmrb.wisc.edu/>) under accession no. 16735. Using DYANA (2) the apoLF₆₇₂₋₇₇₆ solution structure (Figure S1) was determined from 1181 NOE constraints (541 constraints from ¹⁵N-edited [¹H-¹H]-NOESY and 640 constraints from ¹³C-edited [¹H-¹H]-NOESY; NOE cross-peak classification: 229 intra-residue, 952 inter-residue; 417 short-range, 326 medium-range and 209 long-range (Table S2 & Figures S2-S3), 56 backbone dihedral angle constraints and 28 H-bond constraints (2 upper distance limits plus 1 lower distance limit). The average target function for the final DYANA (2) ensemble of 30 models was $0.68 \pm 8.14 \times 10^{-2}$ Å². Each of the 30 lowest target function conformers was energy-minimized in vacuum with the program AMBER (3). For a superposition of the residue segments Arg678 to Asp701 and Phe714 to Ile770 (Figure S2), the RMSDs for the backbone and all heavy atoms of the energy minimized models were 0.81 ± 0.18 Å and 1.57 ± 0.19 Å, respectively. The statistical analysis of the energy-minimized family of 30 structures (Figure S1) and the average model of apoLF₆₇₂₋₇₇₆ are shown in Table S2.

The tautomeric states of the histidines in apoLF₆₇₂₋₇₇₆ were determined through a ¹H-¹⁵N correlation experiment optimized for the ²J_{HN} couplings in the imidazole ring (4). Three out of four histidine imidazole rings were observed in this experiment and appeared in the doubly protonated state (Figure S4). The ¹⁵N R1 and R2 relaxation rates (5) and {¹H^N}-¹⁵N NOEs values (6) were measured at 600 MHz (298 K) on a Bruker Avance spectrometer equipped with a TXI CryoProbe™ (Figure S5-S6). The LF₆₇₂₋₇₇₆ polypeptide contains only one proline (Pro760) with a predominant *trans* Xaa-Pro backbone conformation as manifested by the chemical shift difference of 4.1 ppm between ¹³C β and ¹³C γ . This value is close to the average value (4.51 ± 1.17 ppm for *trans*-Xaa-Pro) estimated from statistical analysis of ¹³C chemical shifts of proline residues in proteins (7). Molecular dynamics (MD) simulations were performed according to standard protocols described in detail elsewhere and results are presented in Figure S7 (8). For Zn(II)-LF MDs, a water molecule plays the role of the fourth ligand; thus the metal ion adopt tetrahedral geometry (8).

Table S2. Statistical analysis of the ensemble of 30 energy minimized models and the average model of apoLF₆₇₂₋₇₇₆.

	ApoLF 672-776 ^a (30 Conformers)	ApoLF 672-776 ^b (average model)
Total number of meaningful NOE upper distance constraints	1181	
Intra-protein NOEs ^c		
Intra-residue	229	
Inter-residue	952	
Sequential (li-jl = 1)	417	
Medium-range (li-jl < 4)	326	
Long-range (li-j > 5)	209	
Total meaningful dihedral angle restraints ^c		
Phi	56	
Hydrogen bond derived restraints		
Intra-protein	28	
RMS violations per meaningful distance & angle constraints (Å)		
Intraresidue	0.0222 ± 0.0020	0.0252
Sequential	0.0199 ± 0.0022	0.0279
Medium range	0.0214 ± 0.0013	0.0240
Long range	0.0224 ± 0.0025	0.0196
Phi	0.4425 ± 0.4177	1.3370
Average number of violations per conformer		
Intraresidue	10.0667 ± 1.7114	13.000
Sequential	15.7667 ± 2.1861	22.000
Medium range	17.0000 ± 2.4900	23.000
Long range	7.8667 ± 1.6275	7.000
Phi	0.6000 ± 0.5538	1.000
NOE violations larger than 0.3 Å	0.0667 ± 0.2494	0.000
Average RMSD to the mean (Å)		
Residue range 678-701 & 714-770 (backbone atoms)	0.82 ± 0.18	0.57 ± 0.13
Residue range 678-701 & 714-770 (all heavy atoms)	1.59 ± 0.18	1.11 ± 0.11
Residue range 702-713 (backbone atoms)	2.28 ± 0.53	1.60 ± 0.33
Residue range 702-713 (all heavy atoms)	3.85 ± 0.68	2.71 ± 0.41
Secondary structure elements (backbone atoms) ^d	0.75 ± 0.18	0.52 ± 0.13
Secondary structure elements (all heavy atoms) ^d	1.48 ± 0.18	1.03 ± 0.10
residual DYANA Target Function (Å ²)	0.68 ± 8.13x10 ⁻²	

Structural analysis^e		
% of residues in most favorable regions	71,9	68.0
% of residues in allowed regions	21,7	21.6
% of residues in generously allowed regions	4.2	7.2
% of residues in disallowed regions	2.2	3.1 ^f
AMBER energy (kJ mol ⁻¹)	-5457.72 ± 271.16	-5787.21
^a Structure calculations were performed with the program DYANA (2). A total of 600 random conformers were subjected to 18000 steps of a simulated annealing process. Each member of the family was subjected to restrained energy minimization (REM) with the AMBER 5.0 package (3). Values of 32 kcal mol ⁻¹ Å ⁻² and 32 kcal mol ⁻¹ rad ⁻² were used as force constants for the NOE and torsion angle restraints, respectively. The data are calculated over the 30 conformers representing the NMR structure and on the energy minimized mean structure. The mean value and the standard deviation are given.		
^b Average model derived from the ensemble of 30 models and then subjected to restrained energy minimization as described above for the 30 conformers		
^c Number of meaningful constraints for each class. Three-bond coupling constants ³ J _{HNHA} were determined both by (i) the chemical shift index (CSI) and (ii) a 3D HNHA spectrum. They were translated into backbone dihedral angles restraints for the structure calculations using the corresponding Karplus curve.		
^d The peptide segments 680-698, 715-721, 729-731, 733-744, 749-758 & 760-770 were taken into account. These segments, according to the PROCHECK software, comprise the five helices of the polypeptide.		
^e As it results from the Ramachandran plot analysis performed with PROCHECK (11).		
^f The three residues that are found in disallowed region of the Ramachandran plot are Arg678, Lys712, & Asn775. These residues are found in regions that are characterized by the absence of NOE signals (N-terminal, L2 & C-terminal region, respectively).		

Table S3. RMSDs for backbone and heavy atoms between the apoLF₆₇₂₋₇₇₆ NMR model and all available X-ray structures of LF.

PDB id		1J7N ^a	1JKY ^b	1PWP ^c	1PWQ ^d	1PWU ^e	1PWV ^f	1PWW ^g	1YQY ^h	1ZXV ^j
2L0R	BB	1,26	1,37	1,32	1,41	1,32	1,27	1,29	1,39	1,26
2L0R	HA	2,39	2,53	2,44	2,51	2,44	2,50	2,43	2,51	2,44

^a Crystal structure of Zn-LF (wt, residues 27-776) (12).

^b Crystal structure of apo-LF (wt, residues 29-776) in complex with N-terminal sequence of MAPKK2 (12).

^c Crystal structure of Zn-LF in complex with small molecule inhibitor, NSC 12155 (13).

^d Crystal structure of Zn-LF (wt, residues 27-776) in complex with Thioacetyl-Tyr-Pro-Met-amide (14).

^e Crystal structure of Zn-LF (residues 30-776; in complex with (3-(N-hydroxycarboxamido)-2-isobutylpropanol-Trp-methylamide), a known MMP inhibitor (14). *Please note that in this PDB entry it is mentioned that this is the E720C LF mutant, while the PDB atomic coordinates file reveals a E687C mutation.*

^f Crystal structure of apo-LF (wt, residues 27-776) in complex with an optimized peptide substrate (14).

^g Crystal structure of Zn-LF in (mutant E687C, residues 28-776) complex with an optimized substrate (14).

^h Crystal structure of Zn-LF (domains II-IV, residues 265-776) in complex with a hydroxamate inhibitor (15).

^j Crystal structure of Zn-LF (wt, residues 27-776) in complex with a small molecule inhibitor, BI-MFM3 (16).

Figure S1. The family of 30 solution NMR structures of LF₆₇₂₋₇₇₆ (PDB 2L0R). Helix numbering is according to the X-ray structure of Zn(II)-LF (PDB 1J7N). Figure generated with MOLMOL (17).

Figure S2. (Upper) NOEs per LF residue. White bars indicate intraresidue connectivities, grey and dark-grey sequential and medium range connectivities, respectively, while black bars indicate long range connectivities (asterisk indicates the N-terminal Gly residue which is a cloning artifact). (Lower) RMSD for backbone (●) and heavy atoms (○) per LF residue. Secondary structure elements and residue accessibility according to PROCHECK are shown at the top of each plot (light blue: residues with higher solvent accessibility; dark blue: buried residues).

Figure S3. (A) Sequential and medium range NOE connectivities (asterisk indicates the N-terminal Gly residue which is a cloning artifact). **B**) $^3J_{\text{HNHA}}$ coupling constants (red bars indicate residues in helical conformation in the solution NMR models).

Figure S4. 600 MHz ^1H - ^{15}N HSQC NMR spectrum of ^{15}N -labeled apoLF₆₇₂₋₇₇₆ pH 7.0 at 25°C. Acquisition parameters: (see Table S1; $^2J(^{15}\text{N}$ - $^1\text{H}) = 22$ Hz, 1s relaxation delay). Assigned cross peaks for His686, His690, and His745 are labeled. The peak pattern is indicative of the doubly protonated histidine tautomer (inset) (4). The analogous pattern for His749 (located at the transition between L4 and helix 4 α 8; see Figure S1) was too weak to be detected possibly due to exchange broadening.

Figure S5. (A) R_1 (B) R_2 (C) heteronuclear $\{^1\text{H}^{\text{N}}\}-^{15}\text{N}$ NOE. Secondary structure and solvent accessibility properties per residue according to PROCHECK (light blue: residues with higher solvent accessibility; dark blue: buried residues) are shown at the top.

Figure S6. (A) Order Parameters S^2 and (B) and (C) atomic rms fluctuation recorded during a 10 ns MD of the energy minimized average NMR model and the NMR model in complex with Zn(II) (and a H₂O as a 4th metal ligand; see Materials & Methods), respectively. Secondary structure and solvent accessibility properties per residue according to PROCHECK (light blue: residues with higher solvent accessibility; dark blue: buried residues) are shown at the top.

Figure S7. Snapshots taken during the 10ns MD of apoLF₆₇₂₋₇₇₆ at (A) 0 ns, (B) 4.3 ns, (C) 5.5 ns, (D) 7.0 ns, (E) 8.5 ns and (F) 10 ns. Helix annotation is shown in panel (A). His686, His690, Asp693, Thr709, Thr731 and Glu735 bonds are shown in gold, oxygen atoms in red and nitrogen atoms in blue. Figure generated with MOLMOL (17).

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