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Backbone and side-chain resonance assignments of the membrane localization domain from *Pasteurella multocida* toxin

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Abstract

¹H, ¹³C, and ¹⁵N chemical shift assignments are presented for the isolated four-helical bundle membrane localization domain (MLD) from *Pasteurella multocida* toxin (PMT) in its solution state. We have assigned 99% of all backbone and side-chain carbon atoms, including 99% of all backbone residues excluding proline amide nitrogens. Secondary chemical shift analysis using TALOS+ demonstrates four helices, which align with those observed within the MLD in the crystal structure of the C-terminus of PMT (PDB 2EBF) and confirm the use of the available crystal structures as templates for the isolated MLDs.

Keywords

Membrane localization domain; *Pasteurella multocida* toxin; four helical bundle; C1 domain

Biological Context

Pasteurella multocida toxin (PMT) is an AB-type toxin that causes pleiotropic effects in intoxicated host cells (Wilson and Ho 2011). PMT protein has four functionally defined regions: a N-terminal region that contains the receptor-binding/translocation domains (residues 1-568) and a C-terminal region (Kitadokoro et al. 2007) that contains a C1 domain (residues 569-719) for membrane localization; a C2 domain (residues 720-1104) of unknown function; and a catalytic C3 domain (residues 1105-1284) with Gα-protein-deamidase activity (Wilson and Ho 2011).

Within the C1 domain of PMT, a four-helical bundle spanning residues 590-670 has been identified as a conserved membrane localization domain (MLD) (Kitadokoro et al. 2007; Kamitani et al. 2010) that is necessary to target the C3 deamidase domain to the plasma membrane when exogenously expressed in mammalian cells (Kitadokoro et al. 2007; Kamitani et al. 2010). A C-terminal fragment containing C1-C2-C3 and the MLD alone bind anionic phospholipids in lipid-binding assays (Kamitani et al. 2010; Brothers et al. 2011). These anionic phospholipids are found in high abundance in both the late endosome and in

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the inner leaflet of the plasma membrane, where the G-protein α -subunit target of PMT is located (Wilson and Ho 2011).

The MLD from PMT (MLD_{PMT}) is homologous to domains within at least 24 other bacterial toxins and effectors (Geissler et al. 2011). These domains are all thought to direct their associated catalytic domains to the host plasma membrane where their intracellular target is found. Importantly, only four proteins containing homologous MLDs have solved structures: PMT (PDB 2EBF), TcsL (PDB 2VL8), TcdA (PDB 3SS1), and TcdB (PDB 2BVL); all other structural models have been generated by structure prediction algorithms based on these four crystal structures (Geissler et al. 2010). Therefore, it is key to validate that the solution and membrane bound-states contain the same four helices that are present in the crystal structure to confirm the physiological relevance of these studies and the role of these bundles in the context of the larger toxins.

Here, we report the assignments of MLD_{PMT} in the solution state. Secondary chemical shift analysis using TALOS+ demonstrates that MLD_{PMT} has similar secondary structure elements to those found in the crystal structure of the C-terminus of PMT. Resonance assignments will facilitate further studies to examine the structure and dynamics of membrane binding by MLD_{PMT}.

Methods and Experiments

Recombinant protein expression and purification

We cloned residues 588-667 (residues 2-81 in this manuscript) flanked by an N-terminal methionine and a C-terminal GLE linker sequence attached to a hexahistidine tag into the plasmid pYCpet. Uniformly labeled ^{13}C , ^{15}N MLD_{PMT} was expressed in *E. coli* BL21(DE3) cells (Promega, Fitchburg, WI) from plasmid pYCpet. MLD_{PMT} was purified using a modification of the previously described method for other MLDs (Geissler et al. 2010; Geissler et al. 2011). Briefly, a 4 mL starter culture was grown at 37°C to OD₆₀₀ = 2 in LB. 1 mL of the bacteria were then harvested and resuspended in 1 mL of M9 salt buffer, and then inoculated into a flask containing 200 mL of the labeling medium containing ^{13}C , ^{15}N -BioExpress, U- ^{13}C -glucose, and ^{15}N -NH₄Cl (Cambridge Isotopes Laboratories, Inc., Andover, MA) (Studier 2005). Specifically, ^{13}C , ^{15}N -BioExpress (10X stock solution) was diluted to 0.1× in M9 minimal media at pH 7.5 containing 2 g/L U- ^{13}C -glucose and 2 g/L U- ^{15}N -NH₄Cl. The culture was grown at 37 °C until the OD₆₀₀ reached ~0.8. Expression of MLD_{PMT} was induced with 0.5 mM isopropyl β -D-1-thiogalactopyranoside for 4 h. The cells were then harvested and lysed in pH 6.0, 20 mM bis-tris, 500 mM NaCl buffer by sonication, and the cell debris pelleted. The supernatant was purified with Ni²⁺ affinity chromatography (HiPrep 16/160, GE Biosciences, Piscataway, NJ), concentrated using a Amicon Ultra-15 centrifugal filter unit with MWCO of 3K (Millipore, Billerica, MA), and then run through a Sephacryl-S100 size exclusion column to perform both buffer exchange as well as to further purify the protein using pH 6.0, 20 mM bis-tris buffer in 500 mM NaCl (GE Biosciences, Piscataway, NJ). The protein was then re-concentrated using an Amicon Ultra-2 centrifugal filter with MWCO of 3K (Millipore, Billerica, MA). This resulted in >99% purity, and a yield of 40–60 mg/L MLD_{PMT}.

NMR spectroscopy

Solution NMR spectra were acquired at the School of Chemical Sciences NMR Facility (University of Illinois at Urbana-Champaign) on a Varian INOVA 600 spectrometer equipped with a 5 mm, triple resonance (^1H - ^{13}C - ^{15}N) triaxial gradient probe, using VNMRJ version 2.3 with the BioPack suite of pulse programs. Spectra were acquired at 30°C on U- ^{13}C , ^{15}N samples at 1 mM in 20 mM bis-tris buffer, 100 mM NaCl at pH 6.0 with 10%

D₂O (v/v) and 0.01% DSS. 2D ¹H-¹⁵N HSQC spectra were interleaved with the measurements for assignments, which consisted of the standard suite of gradient-based heteronuclear triple resonance 3D spectra (HNCO, HN(CA)CO, HNCACB, CBCA(CO)NH) (Clubb et al. 1992; Kay et al. 1990; Grzesiek et al. 1993; Grzesiek and Bax 1992a). Further, ¹³C-HCCH-TOCSY, CC(CO)NH, and ¹³C-NOESY-HSQC were utilized to obtain side-chain resonances (Bax et al. 1990; Grzesiek et al. 1993; Marion et al. 1989). Spectra were processed with NMRPipe (Delaglio et al. 1995) and analyzed in Sparky (Goddard and Kneller 2006).

Extent of assignments and data deposition

The data sets enabled assignment of 99% of all backbone and side-chain carbon atom assignments including 99% of the H^N, N, C α , C β , C' backbone assignments (excluding proline amides) for MLD_{PMT}. The assignments are deposited in the BMRB under accession number 18565. The ¹H-¹⁵N gradient HSQC spectrum (Fig. 1) demonstrates good dispersion, consistent with a folded structure. All but two expected (non-proline) resonances (77) corresponding to residues 588-667 are observed in the HSQC.

TALOS+ (Shen et al. 2009) analysis demonstrates four helices, consistent with the homology model of MLD_{PMT} based on the crystal structure of PMT (Fig. 2). The helices consist of residues 6-12 (H1), 22-36 (H2), 40-61 (H3) and 67-81 (H4), connected by loops 13-21 (L1), 37-39 (L2), and 62-66 (L3). The majority of unassigned residues belong to the N-terminal leader sequence or C-terminal His-tag. The exception is Glu65, for which the amide H^N and N signals are missing. TALOS+ analysis indicates that Glu65 is in loop L3; this loop is proposed to be important for membrane binding through hydrophobic and electrostatic interactions (Geissler et al. 2010; Geissler et al. 2011). Resonance assignments will facilitate further studies to understand the dynamics and conformations of these residues in the free and membrane-bound states.

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Abbreviations

MLD	Membrane localization domain
MLD_{PMT}	Membrane localization domain from <i>Pasteurella multocida</i> toxin
PMT	<i>Pasteurella multocida</i> toxin

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Figure 1. ^1H - ^{15}N 2D HSQC spectrum of MLD_{PMT} acquired at 600 MHz (^1H frequency) at pH 6.0. 79 amide resonances are observed and assigned.

Figure 2.

Secondary structure elements of the MLD_{PMT} identified by TALOS+ compared to the secondary structure from the crystal structure of the MLD from the C1 domain of PMT (Kitadokoro et al. 2007, Shen et al. 2009). Plotted are the Phi (Φ) (black circles) and Psi (Ψ) (gray squares) backbone torsion angles as predicted by TALOS+ (Shen et al. 2009). Along the *x*-axis is a cartoon representation of the secondary structure based on the crystal structure [2EBF (grey) (Kitadokoro et al. 2007)] and the secondary structure predicted by TALOS+ (black). The residue numbers from the construct and those from the crystal structure of PMT (in parenthesis) are both listed along the *x*-axis.