Data Article

Structure of the GAT domain of the endosomal adapter protein Tom1

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ABSTRACT

Cellular homeostasis requires correct delivery of cell-surface receptor proteins (cargo) to their target subcellular compartments. The adapter proteins Tom1 and Tollip are involved in sorting of ubiquitinated cargo in endosomal compartments. Recruitment of Tom1 to the endosomal compartments is mediated by its GAT domain’s association to Tollip’s Tom1-binding domain (TBD). In this data article, we report the solution NMR-derived structure of the Tom1 GAT domain. The estimated protein structure exhibits a bundle of three helical elements. We compare the Tom1 GAT structure with those structures corresponding to the Tollip TBD- and ubiquitin-bound states.

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1. Specifications table

| Subject area | Biology |
|--------------|---------|
| More specific sub- | Structural biology |
| ject area | |
| Type of data | Table, text file, graph, figures |
| How data was acquired | Circular dichroism and NMR. NMR data was recorded using a Bruker 800 MHz |

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2. Value of the data

- The Tom1 GAT domain solution structure will provide additional tools for modulating its biological function.
- Tom1 GAT can adopt distinct conformations upon ligand binding.
- A conformational response of the Tom1 GAT domain upon Tollip TBD binding can serve as an example to explain mutually exclusive ligand binding events.

3. Data

Analysis of the far-UV circular dichroism (CD) spectrum of the Tom 1 GAT domain (Fig. 1) predicts 58.7% α-helix, 3% β-strand, 15.5% turn, and 22.8% disordered regions. The Tom1 GAT structural restraints yielded ten helical structures (Fig. 2A,B) with a root mean square deviation (RMSD) of 0.9 Å for backbone and 1.3 Å for all heavy atoms (Table 1) and estimated the presence of three helices spanning residues Q216-E240 (α-helix 1), P248-Q274 (α-helix 2), and E278-T306 (α-helix 3). Unlike ubiquitin binding, data suggest that conformational changes of the Tom1 GAT α-helices 1 and 2 occur upon Tollip TBD binding (Fig. 3A,B).

Fig. 1. Representative far-UV CD spectrum of the His-Tom1 GAT domain.
4. Experimental design, materials, and methods

4.1. Protein expression and purification

Human Tom1 GAT (residues 215–309) cDNA was cloned into both pGEX6P1 and pET28a vectors, and expressed as GST-tagged and His-tagged fusion proteins, respectively, using *Escherichia coli* [Rosetta (DE3) strain]. The $^{13}$C, $^{15}$N-labeled Tom1 GAT domain was expressed and purified as described previously [1].
4.2. Circular dichroism

Far-UV CD spectra of the His-Tom1 GAT domain were collected on a Jasco J-815 spectropolarimeter using a 1 mm path length quartz cell at room temperature. The protein (10 μM) was solubilized in 5 mM Tris–HCl (pH 7) and 100 mM KF. Spectra were obtained from five accumulated scans from 190 to 260 nm using a bandwidth of 1 nm and a response time of 1 s at a scan speed of 20 nm/min. Buffer backgrounds were employed to subtract the protein spectra. Data was processed using the Dichroweb server and the CONTIN algorithm (http://dichroweb.cryst.bbk.ac.uk/html/home.shtml).

4.3. NMR structure determination

NMR experiments were performed using 1 mM 13C, 15N-labeled Tom1 GAT domain in a buffer containing 20 mM d11-TrisHCl (pH 7), 50 mM KCl, 1 mM d18-DTT, and 1 mM NaN3. NMR spectra were recorded at 25 °C on a Bruker 800-MHz spectrometer (University of Virginia). The individual structure of Tom1 GAT was generated using CS-Rosetta (https://csrosetta.bmr.bire/wisc.edu/csrosetta). Chemical shift information

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![Fig. 3.](image_url)
(BMRB 26574) was used to obtain the structure calculation. The Rosetta calculations yielded 3000 structures of Tom1 GAT. From these, ten structures were selected based on their score and RMSDs, and converted to Protein Data Bank (PDB) format. NMR structural statistics for the ten lowest energy conformers of Tom1 GAT was generated using the Protein Structure Validation Suite. By using MolProbity, the Ramachandran analysis of the ten superimposed Tom1 GAT structures identified that 100% of the residues were in the most favored regions and there were no Ramachandran outliers in the allowed and disallowed regions. Protein structure images were obtained using PyMol (http://www.pymol.org). The structures of the ubiquitin- and Tollip TBD-bound states of the Tom1 GAT domain were obtained from data reported in Refs. [1] and [2].

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.02.042.

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