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ARTICLE

Backbone and sidechain ^{1}H, ^{15}N and ^{13}C assignments of Tyrosine Phosphatase related to Biofilm formation A (TpbA) of *Pseudomonas aeruginosa*

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Abstract The backbone and side chain resonance assignments of the Tyrosine Phosphatase related to Biofilm formation A (TpbA) of *Pseudomonas aeruginosa* have been determined based on triple-resonance experiments using uniformly [^{13}C,^{15}N]-labeled protein. This assignment is the first step towards the determination of the 3-dimensional structure of TpbA.

Keywords TpbA · Biofilm · Phosphatase · NMR

Biological context

Bacterial biofilms are complex communities of bacteria that are highly resistant to antibiotics and are the cause of many recalcitrant infectious diseases diagnosed in the developed world (Donlan 2002). Biofilms are formed when communities of bacterial microorganisms adhere to a suitable surface and encapsulate themselves in an extracellular matrix composed of exopolysaccharides, proteins and DNA (Karatan and Watnick 2009). The bacteria within the colony communicate with each other through quorum sensing, and once the biofilm is formed they are virtually impossible to eliminate using traditional antibiotics (Davies 2002; Ueda and Wood 2009). As a result, biofilms pose a serious medical threat, especially when they infect an immunocompromised patient or colonize medical devices that must then be surgically replaced.

The pathogenicity of the free-living bacterium *Pseudomonas aeruginosa* is linked to its ability to form biofilms (Ryder et al. 2007). Infection from *P. aeruginosa* biofilms is a major problem for patients suffering from chronic diseases, especially cystic fibrosis (CF). In most CF patients, *P. aeruginosa* biofilm infections typically lead to respiratory failure and death, primarily due to the insensitivity of *P. aeruginosa* biofilms to antibiotics (Davies 2002). Tyrosine Phosphatase related to Biofilm formation A (TpbA) is a vital regulator of *P. aeruginosa* biofilm formation as it negatively regulates the concentration of the small molecule 3,5-cyclic diguanylate (c-di-GMP), a positive regulator of biofilm formation (Ross et al. 1990; Lee et al. 2007; Ueda and Wood 2009).

TpbA is a cysteine-based phosphatase that contains the protein tyrosine phosphatase (PTP) signature motif $C(X)_5R(S/T)$ and is capable of dephosphorylating serines, threonines and tyrosines (Ueda and Wood 2009; Pu and Wood 2010). In response to an external stimulus from the quorum sensing molecule oxo-C12-AHL, a signaling cascade within the cell triggers the expression and translocation of TpbA from the reductive cytosolic cellular environment to the more oxidative periplasmic space, where TpbA dephosphorylates and inactivates the membrane bound diguanylate cyclase TpbB. This reduces cellular c-di-GMP levels and, in turn, biofilm formation (Pu and Wood 2010). In this way, TpbA functions to link

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extracellular quorum sensing signals to biofilm formation. A structural study to elucidate the molecular basis of TpbA activity is therefore critical, as it will not only enable the mechanism by which the catalytic cysteine is protected from oxidation in the periplasm to be determined, but will also provide a basis for the development of novel chemical agents that modulate TpbA activity, and, in turn, biofilm formation (Messens and Collet 2006; Ueda and Wood 2009).

Methods and experiments

TpbA (residues 29–218) was subcloned into a modified pET28a-based vector (RP1B), which encodes a $Thio_6His_6$ expression/purification tag and a TEV (tobacco etch virus protease) cleavage site (MGSDKIHHHHHHENLYFQGH). The expression plasmid was transformed into BL21 (DE3) cells (Invitrogen) and cultures were grown to an optical density (OD_{600}) of 0.8–0.9 at 37°C with shaking at 250 rpm. Protein expression was induced by the addition of 1 mM isopropylthio-β-D-galactoside (IPTG), and the cultures were grown for an additional 18–20 h at 18°C with shaking at 250 rpm. Freshly transformed cells were grown in LB medium containing selective antibiotics. To prepare uniformly $^{13}C/^{15}N$-labeled and ^{15}N-labeled protein, bacterial cultures were grown in M9 minimal medium containing selective antibiotics and 4 g/l [^{13}C]-D-glucose and/or 1 g/l $^{15}NH_4Cl$ (Cambridge Isotope Laboratories, Cambridge, MA) as the sole carbon and nitrogen sources, respectively.

The cells were harvested by centrifugation, resuspended in ice-cold lysis buffer (50 mM Tris pH 8.0, 500 mM NaCl, 5 mM imidazole, 0.1% Triton X-100, and EDTA-free protease inhibitor tablets (Roche)), and lysed by high-pressure cell homogenization (Avestin C3 Emulsiflex). The bacterial lysate was clarified by centrifugation at 16,000×g for 45 min at 4°C. After filtration, the supernatant was loaded onto a HisTrap HP column (GE Healthcare) equilibrated with 50 mM Tris pH 8.0, 5 mM imidazole, and 500 mM NaCl, and the His_6-tagged protein was eluted with a 5–500 mM imidazole gradient. Fractions containing

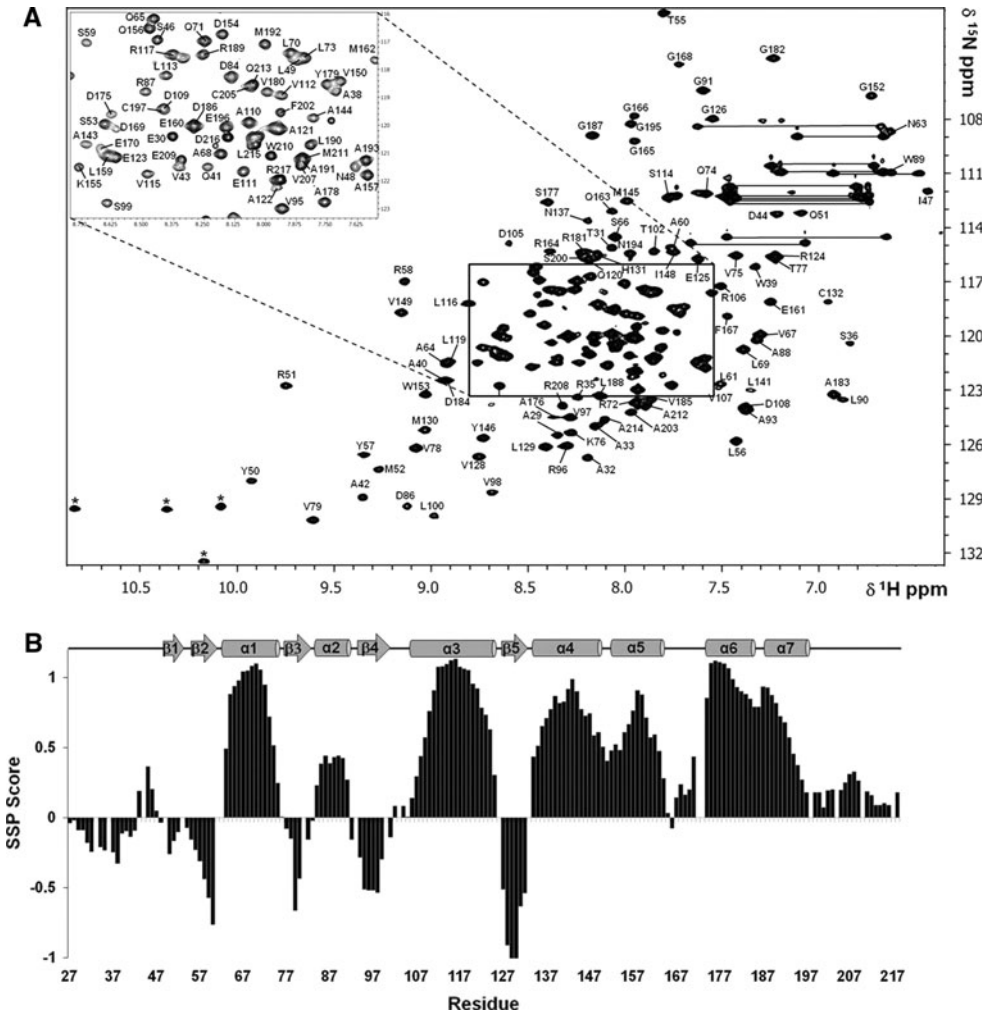

Fig. 1 **a** Fully annotated 2D [^1H, ^{15}N] HSQC spectrum of TpbA at 298 K (800 MHz ^1H Lamor frequency). Assigned residues are labeled with the residue name (single letter code) and number in the sequence. NH_2 groups of glutamine and asparagine side chains are indicated by a *solid line*—, and tryptophan side chain Nε1/Hε1 cross peaks are indicated by an (*). **b** Secondary structure propensity (SSP) scores for residues 29–218 of TpbA (Marsh et al. 2006). The RefDB random coil database was used for these calculations. α-helical regions are indicated by *gray cylinders*; β-strand regions are indicated by *gray arrows*

TpbA were pooled and digested with tobacco etch virus (TEV, in-house) protease overnight at 4°C while being dialyzed against 50 mM Tris pH 8.0, 500 mM NaCl. Cleaved protein was further purified using Ni^{2+}-NTA immobilized metal affinity chromatography followed by size exclusion chromatography (Superdex 75 26/60; GE Healthcare) equilibrated in 10 mM Tris pH 7.8, 100 mM NaCl, 0.5 mM TCEP to a purity of >98% and a final yield of ~25 mg protein/l culture. Purified protein was concentrated to 1.1 mM. For long-term storage, the protein was flash frozen in liquid nitrogen and stored at −80°C.

NMR spectroscopy

All NMR measurements were performed at 298 K. The sequence-specific backbone assignment was obtained from the following experiments performed on a Bruker Avance 500 MHz spectrometer: 2D [^{1}H,^{15}N] HSQC, 2D [^{1}H,^{13}C] HSQC, 3D HNCA, 3D HNCACB, 3D CBCA(CO)NH, 3D (H)CC(CO)NH (τ_m = 12 ms), 3D HBHA(CO)NH, and a 3D ^{15}N-resolved [^{1}H,^{1}H] NOESY spectrum (τ_m = 65 ms). A HNCACB spectrum was also recorded on a Bruker Avance 800 MHz spectrometer. The side chain assignment of all aliphatic residues was obtained from a 3D HC(C)H-TOCSY (τ_m = 12 ms) spectrum recorded on a Bruker Avance 800 MHz spectrometer, with assistance from 3D (H)CCH-TOCSY (τ_m = 12 ms) and 3D HC(C)H-COSY spectra recorded on a Bruker Avance 500 MHz spectrometer. The aromatic side chains were assigned using 2D [^{1}H,^{1}H] NOESY (τ_m = 65 ms) and 2D [^{1}H,^{1}H] TOCSY (τ_m = 50 ms) (800 MHz ^{1}H Lamor frequency) acquired with a TpbA sample dissolved in 100% D_2O solution after complete H/D exchange of all labile protons. The NMR spectra were processed with Topspin 1.3 (Bruker, Billerica, MA) and analyzed using the CARA software package (www.nmr.ch).

Assignment and data deposition

Assignments were obtained for 92% of the backbone nuclei (^{13}Cα, Hα, ^{15}N, and H_N) and 93% of side chain $^{13}CH_n$ moieties. Of the 183 expected backbone amide N–H pairs (10 prolines), 164 were identified. Accurate side chain assignment was uncertain for 9 residues, due to spectral overlap, low signal to noise and undetectable peaks. These residues were P62, F81, C132, K133, H134, D172, M173, S198 and W210. Furthermore, due to chemical shift similarities between Qγ resonances and other NOE cross peaks, the ^{13}Cε methyl groups of M28, M145, M162, M192 and M211, and the side chain ^{15}NH groups of Q65, Q71, Q92, N136, N137, Q163, and Q213 could not accurately be assigned. Side chain ^{15}NH moieties of arginines, lysines, and histidines were not assigned. Figure 1 shows the secondary structure propensity (ssp) scores (Marsh et al. 2006) based on $\Delta C\alpha$–$\Delta C\beta$ chemical shift values, showing that TpbA has a mixed α-helical and β-sheet structure, which is typical for phosphatases. All chemical shifts were deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 18228.

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References

Davies JC (2002) Pseudomonas aeruginosa in cystic fibrosis: pathogenesis and persistence. Pediatr Respir Rev 3(2):128–134

Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8(9):881–890

Karatan E, Watnick P (2009) Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol Mol Biol Rev 73:310–347

Lee VT, Matewish JM, Kessler JL, Hyodo M, Hayakawa Y, Lory S (2007) A cyclic-di-GMP receptor required for bacterial exopolysaccharide production. Mol Microbiol 65:1474–1484

Marsh JA, Singh VK, Jia Z, Forman-Kay JD (2006) Sensitivity of secondary structure propensities to sequence differences between alpha- and gamma-synuclein: implications for fibrillation. Protein Sci 15(12):2795–2804

Messens J, Collet JF (2006) Pathways of disulfide bond formation in Escherichia coli. Int J Biochem Cell Biol 38:1050–1062

Pu A, Wood TK (2010) Tyrosine phosphatase TpbA controls rugose colony formation in Pseudomonas aeruginosa by dephosphorylating diguanylate cyclase TpbB. Biochem Biophys Res Commun 402(2):351–355

Ross P, Mayer R, Weinhouse H, Amikam D, Huggirat Y, Benziman M, de Vroom E, Fidder A, de Paus P, Sliedregt LA et al (1990) The cyclic diguanylic acid regulatory system of cellulose synthesis in Acetobacter xylinum. Chemical synthesis and biological activity of cyclic nucleotide dimer, trimer, and phosphothioate derivatives. J Biol Chem 265:18933–18943

Ryder C, Byrd M, Wozniak DJ (2007) Role of polysaccharides in Pseudomonas aeruginosa biofilm development. Curr Opin Microbiol 10:644–648

Ueda A, Wood TK (2009) Connecting quorum sensing, c-di-GMP, pel polysaccharide, and biofilm formation in *Pseudomonas aeruginosa* through tyrosine phosphatase TpbA (PA3885). PLoS Pathog 5:21000483