Biomolecular NMR Assignments
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ARTICLE

^{1}H, ^{15}N, and ^{13}C chemical shift backbone resonance NMR assignment of the accumulation-associated protein (Aap) lectin domain from *Staphylococcus epidermidis*

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Abstract
Staphylococcus epidermidis is the leading causative agent for hospital-acquired infections, especially device-related infections, due to its ability to form biofilms. The accumulation-associated protein (Aap) of *S. epidermidis* is primarily responsible for biofilm formation and consists of two domains, A and B. It was found that the A domain is responsible for the attachment to the abiotic/biotic surface, whereas the B domain is responsible for the accumulation of bacteria during biofilm formation. One of the parts of the A domain is the Aap lectin, which is a carbohydrate-binding domain having 222 amino acids in its structure. Here we report the near complete backbone chemical shift assignments for the lectin domain, as well as its predicted secondary structure. This data will provide a platform for future NMR studies to explore the role of lectin in biofilm formation.

Keywords Surface adhesion · Biofilm · Lectin · Solution NMR · Resonance assignment · Aap

Biological context

Staphylococcus epidermidis is an opportunistic pathogen and a leading cause of bacterial colonization and infection via biofilm on medical devices and implants (Yarawsky et al. 2020). *S. epidermidis* biofilms are difficult to eradicate owing to their resistance to physical, antibiotic, and host immune response factors. *S. epidermidis* biofilm is surrounded by a matrix composed of a polysaccharide intercellular adhesin (PIA), proteinaceous factors like Accumulation associated protein (Aap), Biofilm-associated protein (Bap), extracellular matrix-binding protein (Embp), and extracellular DNA (Mack et al. 1996; Williams et al. 2002; Rice et al. 2007; Rohde et al. 2007; Christner et al. 2010). The Aap protein plays an important role during the initial surface attachment and accumulation stage of biofilm development.

Aap is a cell wall anchored, multidomain protein consisting of an N-terminal A domain followed by a B domain, a P/G rich stalk, and a C-terminal LPXTG cell wall anchor motif (Fig. 1). Several studies suggest that Aap is a rod-like fibril extending from the cell, having a size of approximately 220 kDa; however, the size of Aap depends on the number of B-repeats present in the B-domain as well as proteolytic posttranslational processing (Sun et al. 2005; Banner et al. 2007; Gruszka et al. 2012; Conrady et al. 2013). Aap shares 54% of its identity with the SasG protein present in *Staphylococcus aureus* (Corrigan et al. 2007). Aap is involved in bacterial colonization on skin and adhesion to epithelial cells (Macintosh et al. 2009; Geoghegan et al. 2013). In most strains, the A domain includes a series of 16 amino acid A repeats followed by a 222 amino acid L-type lectin domain. This domain can be proteolytically cleaved by the SepA metalloprotease enzyme to expose the B domain (Rohde et al. 2005; Paharik et al. 2017). The B domain consists of 5–17 B repeats, and each of these units is composed of a 78 amino acid Zn^{2+} binding G5 domain and a 50 amino acid E

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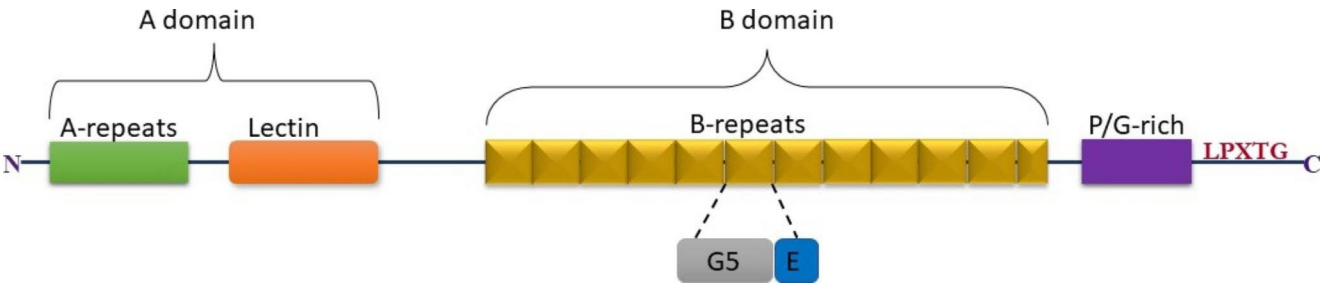

Fig. 1 Schematic representation of Accumulation associated protein (Aap).

domain (also known as a spacer domain). The final repeat in the B domain consists of a single G5 domain, which plays a role in the stabilization of the protein (Conrady et al. 2008), whereas the spacer domain prevents the misfolding of protein (Gruszka et al. 2012). Several crystallographic studies on a 1.5-B repeat construct have revealed that B repeats are rich in beta sheet and are monomeric in the absence of Zn^{2+}. However, in the presence of Zn^{2+}, these domains dimerize in an antiparallel fashion with no change in secondary structure (Conrady et al. 2008, 2013; Chaton and Herr 2017; Shelton et al. 2017). Following the B domain, Aap consists of a proline/glycine-rich region that is resistant to compaction and forms a highly extended stalk. At the C-terminus, the Aap is then anchored to the cell wall by the LPXTG recognition motif (Schneewind et al. 1993; Bowden et al. 2005).

Several studies suggest that the A domain is responsible for the adhesion to abiotic surfaces and its proteolytic cleavage leads to the dimerization of B-repeats on the nearby bacteria, leading to intercellular adhesion and biofilm formation (Rohde et al. 2005; Conlon et al. 2014; Schaeffer et al. 2015; Paharik et al. 2017). Macintosh et al. identified Aap as a fibrillar adhesin and confirmed the role of the terminal A domain in corneocyte attachment (Macintosh et al. 2009). Later, Roy et al. concluded that the lectin subdomain of the A domain is responsible for corneocyte binding. These results are not surprising because lectins are the carbohydrate-binding proteins and the stratum corneum barrier is rich in glycans, proteoglycans, and glycoproteins (Rahmdel and Götz 2021; Roy et al. 2021). Here, we report the ^{1}H, ^{15}N and ^{13}C NMR backbone chemical shift assignments for the lectin domain of Aap. These assignments will be useful for future studies in discovering drug targets that bind to the lectin domain.

Methods and experiments

Protein expression and purification

A plasmid encoding the lectin domain was transformed into *E. coli* BL21 (DE3) for protein expression and purification. The plasmid encodes for a 6X-His tag, a maltose binding protein (MBP), and a tobacco etch virus (TEV) proteolytic cleavage site, followed by the Aap lectin domain, under the control of a IPTG-inducible promoter. These transformed cells were grown overnight in 25 mL M9 minimal media at 37 °C in an incubator with shaking at 200 RPM. This starter culture was then used to inoculate 1 L of M9 minimal media and incubated at 37 °C until the optical density at 600 nm (OD600) reached 0.8. M9 media contained 1 g L^{-1} ^{15}N ammonium chloride and 2.5 g L^{-1} D-glucose. Once the cells reached this state, they were placed in an ice bath to bring the temperature down to 10 °C. At this temperature, the cells were induced using 0.3 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) and 22 mL of 100% ethanol. Cells were grown for another 16–20 h at 20 °C. When the growth was finished, the cells were centrifuged at 8,000 *g* for 20 min, and the pellet was collected. This pellet was resuspended in 20 mL cold lysis buffer (20 mM HEPES buffer pH 7.4, 300 mM NaCl) and kept on ice with shaking after the addition of 0.5 mg/mL lysozyme. This mixture was then sonicated on ice in a Fisherbrand Model 505 sonicator at power level 6 for 6 min total processing time (30 s pulse, 30 s rest). After sonication, the sample was centrifuged at 100,000 *g* for 30 min, and the supernatant was collected. Lectin was purified using a 5 mL Ni-NTA HisTrap FF column (Cytiva Life Sciences, New Brunswick NJ) equilibrated with lysis buffer. The protein was eluted using a 60 mL linear gradient of elution buffer (lysis buffer with 300 mM imidazole). TEV protease and 2 mM tris(2-carboxyethyl)phosphine (TCEP) were added to the protein and dialyzed overnight in lysis buffer to cleave the MBP-Lectin and to remove excess imidazole. The sample was centrifuged at 8,000 *g* for 15 min, and the supernatant was re-applied to the Ni-NTA column. The flow through was collected, which contained the pure lectin domain.

NMR spectroscopy

NMR experiments were acquired at 298 K on a Bruker Avance III 600 MHz NMR spectrometer equipped with a 4-channel quadruple resonance cryoprobe (CP-QCI). All

NMR experiments were recorded on samples containing 0.4-0.6 mM lectin in 20 mM HEPES pH 6.8, 50 mM NaCl, 5 mM sodium azide, and 10% D_2O. Backbone assignments for the Aap-lectin domain were obtained from the analysis of the following heteronuclear two-dimensional (2D) and three-dimensional (3D): 2D ^{15}N-^{1}H HSQC, 3D HNCO, 3D HN(CA)CO, 3D HNCA, 3D HN(CO)CA, 3D HNCACB, and 3D HN(CO)CACB. All experiments were recorded using standard TROSY-resolved Bruker pulse sequences with TopSpin 3.6.4 (Salzmann et al. 1998), and the acquired NMR data were processed using NMRPipe (Delaglio et al. 1995). Analysis of the spectra and backbone resonance assignment were performed manually using CARA 1.8.4.2 (Keller 2004). Secondary structure estimation based on the chemical shifts and calculation of random coil index derived order parameters (RCI-S^2) were performed with TALOS+ (Shen et al. 2009).

Extent of assignment and data deposition

The 2D ^{15}N-^{1}H TROSY NMR spectrum of Aap-lectin shows amide signals with good dispersion, indicating a properly folded tertiary structure of the protein domain in solution. The dispersed signals of Aap-lectin in the 2D ^{15}N-^{1}H TROSY NMR spectrum are shown in Fig. 2.

Analysis of the NMR spectra resulted in 225 (89%) out of 254 non-proline backbone amide resonance assignments and 88%, 86%, and 74% of all Cα, Cβ, and CO chemical shifts of the Aap-lectin, respectively. Backbone amide resonances of N380, Q391, T396, T397, N405, Q424, S425, N426, L452, R453, E454, Y470, N471, N472, D473, S497, T521, N522, W556, Q579, Y580, G581, N582, G583, N584, S585, G591, and H610 could not be identified. Nearly all peaks in the 2D TROSY spectrum are assigned, and this suggests that these residues may be broadened as a result of

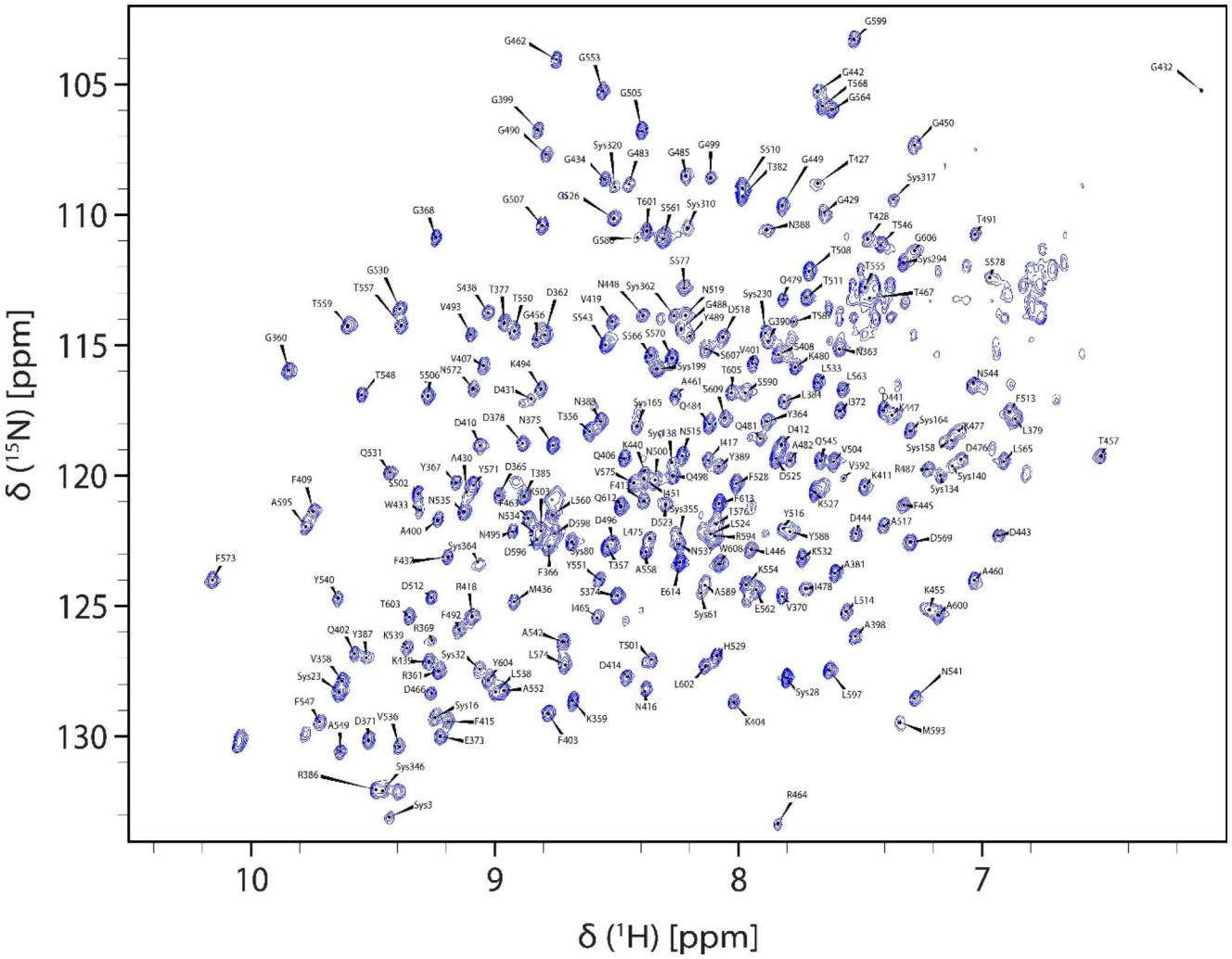

Fig. 2 Assigned 2D ^{15}N-^{1}H TROSY NMR spectrum of the Aap-Lectin domain.

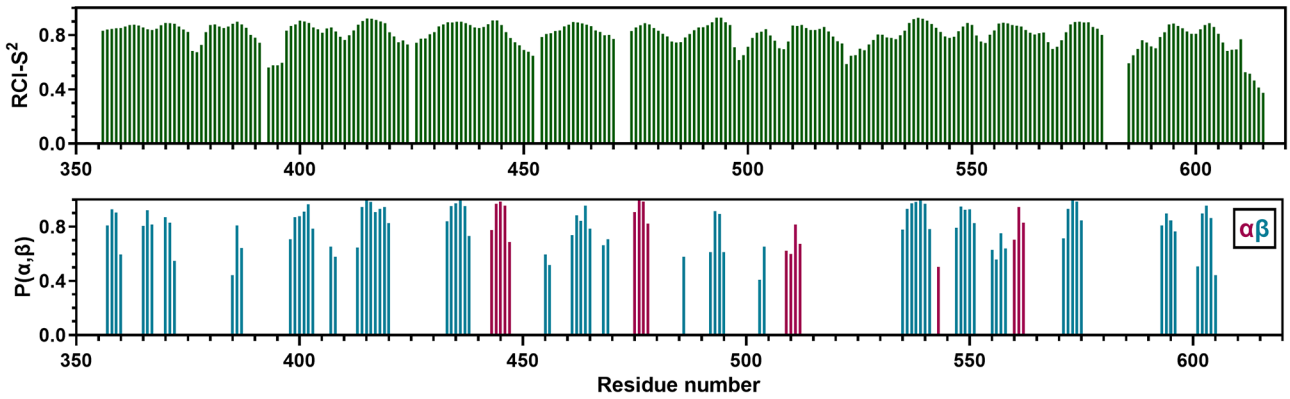

Fig. 3 Results from TALOS+. The top panel indicates predicted random coil index derived order parameters (RCI-S^2) and the bottom panel shows the possible secondary structure adopted by each residue.

chemical exchange or hydrogen exchange with the solvent. The chemical shift values for each of the assigned backbone H^N, N, Cα, Cβ, and CO atoms have been deposited in the Biological Magnetic Resonance Data Bank (https://www.bmrb.wisc.edu) under accession no. 51,293.

Secondary structure prediction of the Aap-lectin domain was performed using the chemical shift assignments of the atoms H^N, N, CO, Cα and Cβ, for each amino acid in the sequence using the TALOS+ server (Shen et al. 2009). TALOS+ prediction results (Fig. 3) indicate that secondary structure elements are composed of mainly β-strands with four α-helices. This is consistent with known folds of L-type lectin domains (Velloso et al. 2002). RCI-S^2 results indicated that the C-terminus is fairly flexible, with an RCI-S^2 value of less than 0.6. The region near 392–396 also has a comparatively low RCI-S^2 value, and this region corresponds to the 390 loop between strands four and five. Resonances were not observed for several loops in this protein, including residues 471–473 (in the Ca loop), residues 452–454 (in the basal loop), and residues 580–584 (in the binding pocket). These regions are all thought to have importance in substrate binding, and dynamic behavior of these residues may allow Aap-lectin to interact with multiple substrates (Yang et al. 2014). The broadening of these resonances therefore likely reflects functionally important motions, and work is ongoing to identify the functional importance of all of these regions.

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Authors' Contributions T.S., R.Y., and N.C.F wrote the main manuscript text. T.S. and R.Y prepared Figs. 1, 2 and 3. T.S., R.Y., and S.T. prepared protein samples. R.Y. and S.T. collected the NMR data. N.C.F. and A.B.H. designed the study. All authors reviewed the manuscript.

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Data Availabilty The assignments have been deposited to the Biological Magnetic Resonance Data Bank (BMRB) under the accession number: 51,293.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication The authors mutually consent for publication.

Competing Interests The authors declare that they have no competing interest.

Ethical Standards The experiments comply with the current laws of the USA.

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