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Backbone resonance assignments of innate immune evasion protein EapH2 from the *S. aureus*

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Abstract

Staphylococcus aureus is a ubiquitous and persistent pathogen of humans and livestock. The bacterium disrupts the host's innate immune system's ability to recognize and clear bacteria with optimal efficiency by expressing a wide variety of virulence proteins. Two single domain protein homologs (EapH1, EapH2) of the extracellular adherence protein (Eap) have been reported. Eap is a multidomain protein that participates in various protein-protein interactions that inhibit the innate immune response, including both the complement and Neutrophil Serine Proteases (NSPs). EapH1 and EapH2 are also inhibitors of NSPs (Stapels et al. 2014), but lack the ability to inhibit the classical, and lectin pathways of the complement activation system (Woehl et al. 2014).

We continue the characterization of Eap domains, here with the experiments on EapH2, we acquired a series of 2D and 3D NMR spectra of EapH2 in solution. We completed 99% of expected non-proline backbone ¹H, ¹⁵N, and ¹³C resonance assignments of EapH2 and predicted secondary structure via the TALOS-N server. The assignment data have been deposited in the BMRB data bank under accession number 27540.

Keywords

Backbone resonance assignment; *Staphylococcus aureus*; virulence protein; extracellular adherence protein homolog (EapH)

Biological context

Staphylococcus aureus is a ubiquitous and persistent pathogen of humans and livestock. The bacterium causes numerous infections of varying severity, including skin abscesses, endocarditis, and bacteremia, and receives particular attention due to increasing reports of

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antibiotic resistant strains. (Archer 1998; Lowy 1998; CDC 2011). Numerous studies have shown *S. aureus* secretes an array of virulence proteins whose activities block the central events required for bacterial opsonization by complement components and subsequent phagocytosis by neutrophils (Garcia et al. 2016; Kim et al. 2012; Lambris et al. 2008; Spaan et al. 2013). Furthermore, novel classes of *S. aureus* secreted proteins, such as the Extracellular Adherence Protein (Eap) family (Stapels et al. 2014) and Staphylococcal Peroxidase Inhibitor (SPIN) proteins (de Jong et al. 2017) have recently been identified as nanomolar-affinity inhibitors of NSPs and myeloperoxidase (MPO), respectively.

The multi-domain Eap protein has a mass of 50-70 kDa depending on the number of ~100 residue repeating domains found in its various isoforms (Geisbrecht et al. 2005). Eap contributes to the overall virulence of *S. aureus* by blocking both the classical and lectin complement pathways (Woehl et al. 2014) and Neutrophil Serine Proteases (NSPs) (Stapels et al. 2014). Two single domain protein homologs (EapH1, EapH2) of Eap have been also identified as inhibitors of NSPs (Stapels et al. 2014), but lack the ability to inhibit the classical, and lectin pathways of the complement activation system (Woehl et al. 2014). Recent work on Eap domains 3 and 4 reported their interaction with C4b and their ability to inhibit classical and lectin pathways (Woehl et al. 2014; Woehl et al. 2017). While the individual domains bound C4b with $K_D \sim 40 \mu\text{M}$, the construct containing both domains, Eap34, bound C4b with $K_D = 525 \text{ nM}$. The binding affinity is even higher for Eap, $K_D = 185 \text{ nM}$. Crystal structures of the second domain of Eap (Eap2), along with two homologs EapH1 and EapH2, revealed that the individual domains are characterized by a β -grasp type fold (Geisbrecht et al. 2005). Finding a structural basis for the difference in the inhibitory capabilities between Eap domains and EapH proteins becomes a relevant question.

Here we report the secondary structural features of EapH2 in the free form in solution. After assigning the backbone ^1H , ^{15}N , $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, and $^{13}\text{C}'$ resonances of EapH2, we predicted the secondary structure using the TALOS-N server along with the observed chemical shifts. These backbone assignments are the starting point for titrations with NSPs to identify the interaction site on EapH2 and other related Eap domains.

Methods and Experiments

Protein expression and purification

EapH2 was overexpressed following methods established at our lab and reported earlier (Geisbrecht et al. 2006; Herrera et al. 2018). A DNA fragment encoding the protein sequence was subcloned into the *SaI* and *NotI* sites of the prokaryotic expression vector pT7HMT. This vector encodes an N-terminal affinity His-tag that is used for Ni-affinity chromatography purification of the protein, but which can be removed by digestion with Tobacco Etch Virus (TEV) protease. The EapH2 protein resulting after cleavage retains an additional “Gly-Ser-Thr” sequence at the N-terminus. The plasmid containing this DNA fragment was further verified by sequencing and transformed into *Escherichia coli* BL21(DE3) cells.

Both uniformly ^{15}N and $^{13}\text{C}/^{15}\text{N}$ double-labeled EapH2 proteins were overexpressed in minimal medium (M9) enriched with $^{15}\text{NH}_4\text{Cl}$ (1 gram/litre) and ^{13}C -glucose (1g/litre) as

described in the protocol by Woehl et al. (2016). The purified protein yield from 1 L of *E. coli* culture was in the range of 5-10 mg for both ^{15}N and $^{13}\text{C}/^{15}\text{N}$ double-labeled EapH2.

The samples for NMR experiments contained 0.5 – 0.8 mM uniformly ^{15}N or $^{13}\text{C}/^{15}\text{N}$ double-labeled EapH2 protein in 50 mM sodium phosphate buffer (pH 6.5) containing 5 % (v/v) D_2O (used as a lock solvent). The purity and mass of the labelled protein was verified using mass spectrometry (Ultra Flex III TOF, Bruker Daltonics) prior to NMR data acquisition.

NMR spectroscopy

NMR spectra were acquired at 25°C on a Bruker Avance III NMR spectrometer equipped with a 5mm cryogenically cooled TCI probe operating at 800 MHz for ^1H frequency. Backbone resonance assignments were achieved following standard procedure (Whitehead et al. 1997) using 2D ^1H - ^{15}N HSQC and 3D HNCQ, HN(CA)CO, HN(CO)CA, HNCA, CBCA(CO)NH and HNCACB spectra. Only 12% points of Nyquist grid in the indirect dimension were sampled non-uniformly using Poisson-Gap sampling scheme. These non-uniformly sampled (NUS) spectra were reconstructed using hmsIST (Hyberts et al. 2012), processed using NMRPipe (Delaglio et al. 1995), and analyzed with CARRA software (Keller 2004). The ^1H chemical shift assignments were referenced by using 2,2-dimethyl-2-silapentane-5-sulphonic acid (DSS) at 25°C as a standard. The ^{13}C and ^{15}N chemical shift were referenced indirectly to DSS, using the absolute frequency ratios. The acquisition parameters for all the NMR experiments are mentioned in Table 1. All the triple resonance NMR experimental data were acquired using non-uniform sampling.

Results

Extent of Assignments and Data Deposition

Signals well dispersed and with adequate intensity were detected in the acquired 2D ^1H - ^{15}N HSQC spectrum of EapH2 (Fig. 1). Amino acid numbering is based upon the EapH2 sequence, with an extra “Gly-Ser-Thr” sequence at the N-terminus. A total of 99% of backbone ^1H and ^{15}N resonances of 113 non-proline residues, and 95% of $^{13}\text{C}\alpha$, 97% of CO, and 71% of the expected $^{13}\text{C}\beta$ resonances have been unambiguously assigned based on a standard set of triple resonance spectra described above. The backbone amide residues that could not be assigned are G1 and K16. G1 lies in a loop region of the artificial N-terminus as a result of the subcloning procedure.

The secondary structure elements of EapH2 were predicted by the TALOS-N program (Shen et al. 2013) using the resonance assignments of $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, and $^{13}\text{C}'$ (Fig. 2). This prediction is in agreement with the secondary structures observed in EapH1, EapH2, and Eap2, as determined by X-ray crystallography (Geisbrecht et al. 2005), and the structural characteristics described by NMR for Eap3 and Eap4 (Herrera et al. 2018, Woehl et al. 2016). The chemical shift assignments have been deposited in BioMagResBank (<http://www.bmrb.wisc.edu>) under the accession number 27540.

Acknowledgements

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Fig. 1. 2D ^1H - ^{15}N HSQC spectrum of 0.7 mM $^{13}\text{C}/^{15}\text{N}$ -labeled EapH2 recorded at 298 K, pH 6.5 on a Bruker 800 MHz Avance III spectrometer equipped with a TCI cryoprobe. Sequence specific assignments of backbone amide groups are indicated by single letter residue name and sequence number. An expanded section of the central, overlapped region is shown. The unlabeled cross peaks correspond to a fragment product of the expression system and side chains ^1H - ^{15}N .

Fig. 2.

Secondary structure prediction for the EapH2 domain based on the TALOS-N program using obtained chemical shift values. β -strand probabilities are given by positive values, α -helices are given by negative values, and loop regions are given by values approximately from -0.3 to 0.3 . At the top the prediction is further illustrated with α -helices shown as cylinders and β -sheets shown as arrows.

Table 1.

Acquisition parameters for NMR experiments for backbone resonance assignments.

Experiment Parameter	2D ^{15}N , ^1H HSQC		3D HNCB 3D HN(CA)CO			3D HNCA 3D HN(CO)CA			3D HNCACB 3D HN(CO)CACB		
	^1H	^{15}N	^1H	^{15}N	^{13}C	^1H	^{15}N	^{13}C	^1H	^{15}N	^{13}C
No. of Complex Points (Real + Imaginary)	2048	256	2048	184	128	2048	184	128	2048	184	128
Offset (ppm)	4.7	118	4.7	117	176.7	4.7	117	57.7	4.7	117	44.7
Spectral Width (ppm)	16	35	14	34	12	14	34	30	14	34	62
Inter-scan delay	1 sec										
No. of scans	8		HNCO = 8 HN(CA)CO = 16			8			16		