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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C NMR resonance assignments of staphostatin A, a specific *Staphylococcus aureus* cysteine proteinase inhibitor

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Abstract

The increasing antibiotic resistance of an important human pathogen *Staphylococcus aureus* calls for the development of new therapeutic strategies. Staphylococcal cysteine proteases have been suggested as targets for such therapies. The recent discovery of staphostatins, specific protein inhibitors of these enzymes, gives prospects for the design and production of synthetic, low molecular weight analogs which might become drugs. We have decided to structurally characterize staphostatin A, a representative inhibitor of staphylococcal cysteine proteases, and to assess its binding mode to the target protease with the view of clarifying the specificity determinants. Here we report the ^{1}H, ^{15}N and ^{13}C NMR resonance assignments of staphostatin A.

Biological context

Staphylococci, and in particular *Staphylococcus aureus*, are dangerous human pathogens with increasing antibiotic resistance infecting virtually every tissue of the host body. The mechanism of pathogenesis remains largely unknown since it is not obviously based on specific toxin production as in the case of other bacteria. A wide spectrum of secreted proteins have been proposed to facilitate the infection, among which cysteine proteases play an important part as shown by knock-out studies (Coulter et al., 1998; Rice et al., 2001). Based on these studies and accumulated complementary evidence these proteases have been proposed as suitable targets for the design of a novel anti-staphylococcal therapy. Recently, a group of endogenous, specific, staphylococcal cysteine proteinase inhibitors (staphostatins) has been described, giving prospects for the structure-based design and production of synthetic inhibitors. However, the structural features, the mode of action and mechanism of specificity of staphostatins remains unresolved (Massimi et al., 2002; Rzychon et al., 2003). Moreover, there is no visible sequence similarity between staphostatins and any other known proteins, including cystatins – the main group of cysteine proteinase inhibitors. In this context it seems interesting to assess whether staphostatins really constitute a novel class of cysteine protease inhibitors. To address these questions we have chosen to characterize by NMR the *Staphylococcus aureus* produced staphostatin A. Here we report the ^{1}H, ^{15}N and ^{13}C NMR resonance assignments of this inhibitor.

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Methods and experiments

Recombinant staphostatin A was expressed as the glutathione S-transferase (GST)-fusion protein using the pGEX-5T vector in a *E. coli* strain BL21 (DE3)pLysS. The bacteria were grown at 30 °C, in-

duced at OD_{600} = 1.2, harvested after 3 h, and lysed by sonication. After centrifuging out the debris, the lysate was applied to the glutathione-sepharose column to isolate the GST-staphostatin A. The fusion protein was digested with thrombin, yielding GST and staphostatin A. Cysteine residues of staphostatin A were oxidized in a buffer containing oxidized and reduced glutathiones as catalysts to produce a disulphide bridge. After digestion and oxidation, staphostatin A was recovered by gel filtration chromatography. Staphostatin A obtained by this protocol has a Gly-Ser insert at the N-terminus. Gel filtration, native electrophoresis and inhibition of protease in activity assays confirmed that the recombinant staphostatin A still bound and inhibited the target protease. The ^{15}N and $^{13}C/^{15}N$ uniformly labeled samples were obtained in minimal media containing $^{15}NH_4Cl$ and ^{13}C-glucose/$^{15}NH_4Cl$, respectively. The ^{15}N amino acid type selectively labeled proteins were produced in a completely defined medium including appropriate ^{15}N-labeled amino-acids. All NMR samples contained 0.7 ($\pm$ 0.2) mM staphostatin A in 140 mM NaCl; 2.7 mM KCl; 10 mM Na_2HPO_4; 1.8 mM KH_2PO_4; 0.05% NaN_3; pH 7.5 and 10% or 100% D_2O.

NMR experiments were performed at 27 °C on Bruker AMX500, DRX600 or DMX750 spectrometers. The following spectra were used for the ^1H, ^{15}N, $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ resonance assignments: ^1H-^{15}N HSQC (of uniformly and ^{15}N Tyr, Lys, Val, Ile, Gly/Ser, Leu and Phe selectively labeled samples), HNCA, CBCA(CO)NH, ^{15}N-edited 3D-NOESY, TOCSY and 2D NOESY. Additionally the HNHA experiment was acquired to obtain the $^3J_{HNH\alpha}$ coupling constants. The ^1H, ^{15}N and ^{13}C chemical shifts were referenced to DSS according to the IUPAC recommendation (Markley et al., 1998).

Extent of assignments and data deposition

The ^1H-^{15}N HSQC spectrum of staphostatin A shows well-separated signals with only few overlaps (Figure 1). The backbone resonance assignments were obtained for 95 residues of a total of 107 residues of the engineered staphostatin A (105 residues of a native protein). The resonances of the three N-terminal residues (of which the two most N-terminal were added in the cloning process), N17 to E19, and six residues distributed throughout the protein sequence

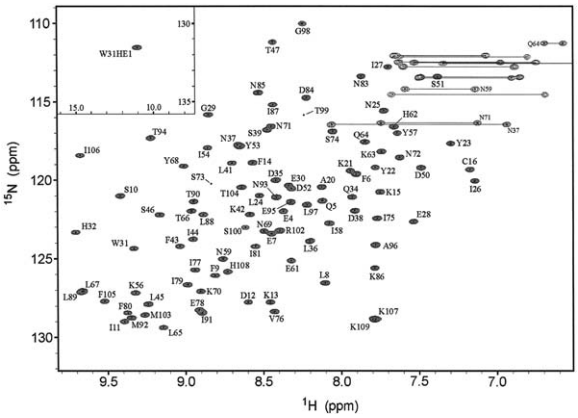

Figure 1. The 2D ^1H-^{15}N HSQC spectrum of staphostatin A. Assignments are shown alongside the corresponding cross-peaks. Signals connected by horizontal lines correspond to side chain amide groups of Asn and Gln.

did not appear in the NMR spectra. The data of the ^1H, ^{15}N and ^{13}C chemical shifts and $^3J_{HNH\alpha}$ coupling constants of staphostatin A have been deposited in the BioMagResBank under accession number 5810 (http://www.bmrb.wisc.edu).

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