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Letter to the Editor: Backbone ^{1}H, ^{15}N and ^{13}C resonance assignments of the 28 kDa mature form of streptopain*

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Biological context

Streptococcus pyogenes (group A *Streptococcus* [GAS]) causes several human diseases, such as phyaryngitis, acute rheumatic fever, scarlet fever, post-streptococcal glomerulonephritis, and toxic-shock-like syndrome (Bisno 1991; Molinari et al., 1999). Virtually all strains of GAS isolated from patients with invasive disease express an extracellular cysteine protease known as streptopain (EC 3.4.22.10) (Gubba et al., 1998; Doran et al., 1999). Many reports also suggest that streptopain is an important virulence factor in streptococcal infections (Kuo et al., 1999; Tsai et al., 1998). Streptopain produced from GAS is released extracellularly to culture medium as a zymogen (proSCP) with a molecular weight of 40 kDa. The conversion of proSCP to 28 kDa active mature protease can be achieved by autoproteolysis and exogenous proteases (Doran et al., 1999; Liu et al., 1965; Nomizu et al., 2001). Since the 28 kDa active form of streptopain plays an important role in GAS pathogenesis, studies on the structure and function relationships of mature streptopain become a subject of interest (Doran et al., 1999; Liu et al., 1965; Nomizu et al., 2001).

The X-ray structure of the 40 kDa C192S mutant of streptopain has been determined, and the 3D structure is structurally homologous with proteins of the papain superfamily (Kagawa et al., 2000). In this study, we obtained the 28 kDa mature form of the streptopain C192S mutant by digesting the 40 kDa C192S mutant with wild-type streptopain. We determined the backbone ^{1}H, ^{13}C, and ^{15}N resonances for the 28 kDa C192S mutant of streptopain and deduced its secondary structures from multidimensional NMR spectroscopy because mature streptopain is one of the key participant in GAS pathogenesis (Kuo et al., 1999; Nomizu et al., 2001; Tsai et al., 1998).

Methods and results

The genomic DNA of GAS was extracted from strain A20. The structural gene of 371-residue proSCP was amplified by polymerase chain reaction (PCR) and then cloned into the pET-21a vector. The wild-type construct was used to produce C192S mutation using overlap extension PCR. The recombinant plasmid was transformed into the *E. coli* BL21(DE3)pLyS strain, and the system was inducibly expressed under control of a strong T7 promoter. The recombinant proteins were produced by growing cells at 28 or 37 °C for 12 hours in LB medium and purification by Ni^{2+}-chelating chromatography (Pharmacia Biotech). M9 minimal media was used and 1 g l^{-1} $^{15}NH_4Cl$ (99% ^{15}N), 2 g l^{-1} [^{13}C]-glucose (99% ^{13}C), and/or 98% D_2O were substituted for the unlabeled compounds in the growth media. Selective [α-^{15}N]-A-, -D-, -E-, -F-, -G-, -H-, -I-, -K-, -L-, -M-, -Q-, -V-, or, -Y-labeled proteins were prepared using the protocol described by McIntosh et al. (1990). The proteins were concentrated by amicon ultrafiltration using 10 kDa cutoff membrane and exchanged with PBS buffer. The 28 kDa C192S mutant of streptopain was obtained by digesting 20–40 mg ml^{-1} of the 42 kDa C192S mutant of streptopain with 50–100 μg ml^{-1} of wild-type streptopain. The reaction solution was then purified with Bio-Gel P-30 (Bio-Rad

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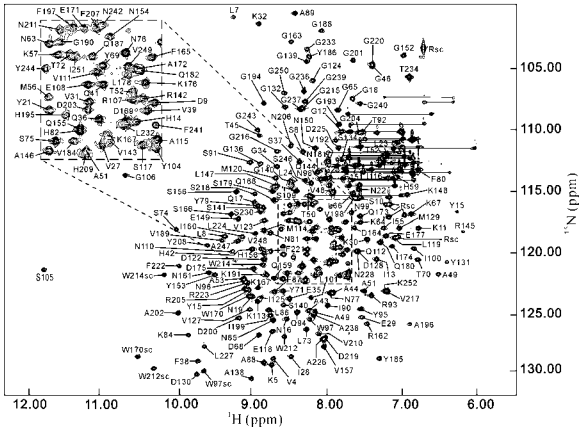

Figure 1. ^{1}H-^{15}N HSQC spectrum of the 28 kDa streptopain C192S mutant (253 residues) in 25 mM phosphate buffer, 100 mM NaCl, pH 6, which was acquired at 27 °C on a Bruker 600 MHz spectrometer. Resonances were labeled with the corresponding sequence positions and side-chain NH_2 resonances were connected with a line. In addition, the resonances of the side chains were labeled with an sc sign.

Lab) gel filtration chromatography. The final solution was also concentrated by amicon ultrafiltration using 10 kDa cutoff membrane.

NMR samples were made to 10% or 100% D_2O at pH 6.0 in 50 mM phosphate buffer. NMR experiments were performed on a Bruker Avance 600 spectrometer at 27 °C. Experiments of ^{15}N-edited TOCSY and NOESY, HCCH-TOCSY, HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HNCO, and HNHA were carried out for the purpose of proton, carbon, and nitrogen resonance assignments. Triple resonance experiments were recorded with 1.2–2.5 mM of ^{2}H (70%), ^{15}N/^{13}C (99%)-labeled proteins and deuterium decoupling was applied. Data was processed and analyzed using XWINNMR and Aurelia programs on an O2 Silicon Graphics workstation.

Extent of assignments and data deposition

The backbone ^{1}H, ^{15}N and ^{13}C resonance assignments for the 28 kDa mature form (253 residues) of the streptopain C192S mutant were obtained by analyzing triple resonances spectra. 237 out of 240 nonproline NH were observed. Thirteen [α-^{15}N]-selective amino acid-labeled proteins were used to confirm the assignments. The unobserved V3, S137, and Q245 residues are located at N-terminus or in the loop region, most likely absent due to rapid exchange with the solvent. 97% of Cα, 96% of Cβ, and 96% of Hα chemical shifts were assigned. Judging by the ^{1}Hα, ^{13}Cα, and ^{13}Cβ shifts, the 28 kDa C192S mutant of streptopain consists of five α-helices and five β-strands. They are essentially equivalent to that found in the X-ray structure (Kagawa et al., 2000). The backbone ^{1}H, ^{15}N and ^{13}C resonance assignments have been deposited in the BioMagResBank databank under accession number BMRB-5547.

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