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High-resolution crystal structure of Streptococcus pyogenes β-NAD+ glycohydrolase in complex with its endogenous inhibitor IFS reveals a highly water-rich interfaces

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One of the virulence factors produced by Streptococcus pyogenes is β-NAD+ glycohydrolase (SPN). S. pyogenes injects SPN into the cytosol of an infected host cell using the cytolysin-mediated translocation pathway. As SPN is toxic to bacterial cells themselves, S. pyogenes possesses the ifs gene that encodes an endogenous inhibitor for SPN (IFS). IFS is localized intracellularly and forms a complex with SPN. This intracellular complex must be dissociated during export through the cell envelope. To provide a structural basis for understanding the interactions between SPN and IFS, we overexpressed the complex between the mature SPN (residues 38–451) and the full-length IFS (residues 1–161), but it could not be crystallized. Therefore, we used limited proteolysis to isolate a crystallizable SPNct-IFS complex, which consists of the SPN C-terminal domain (SPNct; residues 193–451) and the full-length IFS. We have determined its crystal structure by single anomalous diffraction and refined the model at 1.70 Å resolution. Interestingly, our high-resolution structure of the complex reveals that the interface between SPNct and IFS is highly rich in water molecules and many of the interactions are water-mediated. The wet interface may facilitate the dissociation of the complex for translocation across the cell envelope.

1 Introduction

The gram-positive bacterium Streptococcus pyogenes causes a variety of human diseases such as superficial infections (pharyngitis and impetigo) and life threatening conditions (toxic shock syndrome and necrotizing fasciitis) [1,2]. The virulence of S. pyogenes is enhanced by its toxin β-NAD+ glycohydrolase (SPN; also known as Nga) [3]. The β-NAD+ glycohydrolase hydrolyzes β-NAD+, an important cofactor in numerous redox and energy-producing biological reactions, to produce nicotinamide and adenosine diphosphoribose (ADP-ribose) [4,5]. Strict β-NAD+ glycohydrolases are incapable of further catalysis of the products from the initial reaction [6]. SPN is comprised of two domains. SPN is also toxic to bacterial cells; therefore, *S. pyogenes* encodes the *ifs* gene, which encodes the *i*mmunity *f*actor for *S*PN (IFS) as an endogenous antitoxin [7,8]. We have solved the crystal structure of this SPN_{ct}-IFS complex by single anomalous diffraction and refined the model at 1.70 Å resolution. Much higher resolution of the data used in this study allowed us to identify a lot more water molecules bound to the protein complex, in particular, at the interface between SPN_{ct} and IFS.

2 Experiment

The contiguous spn (SpyM3_0128) gene covering the residues 38–451 and the full-length ifs (SpyM3_0129) gene of S. pyogenes M3 were PCR-amplified, and cloned into the pET-28b(+) vector (Novagen), using the NdeI/XhoI restriction enzymes. The selenomethionine (SeMet)-labeled complex protein was expressed and purified. The protein complex was concentrated to 50 mg ml^{-1} for crystallization. Best crystals of SPN_{ct}-IFS complex were obtained with the reservoir solution of 20% (w/v) tacsimate at pH 4.0 and 20% (w/v) polyethylene glycol (PEG) 3350. Single-wavelength anomalous diffraction (SAD) data were collected from a crystal of the SeMet-substituted SPN_{ct}-IFS complex at 100 K. Raw data were processed using the program suit *HKL2000*. The structure of SPN_{ct}-IFS complex was solved by Se SAD phasing. Phase calculation, density modification, and initial model building were carried out using *PHENIX AutoSol* and *AutoBuild*. And the model was refined with the program *REFMAC* and *PHENIX* . The coordinates and structure factors have been deposited in the Protein Data Bank under the accession code 4kt6.

3 Results and Discussion

We co-expressed the mature SPN (residues 38–451) and its endogenous inhibitor IFS (residues 1–161) from *S. pyogenes* but we could not crystallize the whole complex, because the SPN component was degraded slowly. Our crystals of the purified SPN_{ct}-IFS complex diffracted to high resolution and allowed us to solve the structure by the Se SAD method. The model of the SPN_{ct}-IFS complex was refined to yield R_{work} and R_{free} values of 19.7% and 23.5%, respectively, for 20.0–1.70 Å data. The model includes 830 residues in two copies of the complex (residues 193–446 of SPN and residues 1–161 of IFS) and 596 water molecules.

In our high-resolution structure of the SPN_{ct}-IFS complex, IFS interacts with SPN_{ct} through numerous hydrogen bonds and electrostatic interactions, many of which are water-mediated. The complex buries a large surface area at the interface between SPN_{ct} and IFS (3,210 $Å^2$ and 3,280 $Å^2$ for A:B and C:D interfaces,

respectively). Our higher resolution (1.70 Å) structure reveals that the interface is very rich in water molecules; 67 and 71 water molecules are identified at the A:B and C:D interfaces, respectively. Many of these water molecules are conserved and common to both interfaces.

Our high-resolution crystal structure of the SPN_{ct}-IFS complex reveals that many interactions between SPN_{ct} and IFS are water-mediated. A prominent example is the α2–α3 loop of IFS, which points toward the NAD binding cavity of SPN. Compared to the unbound IFS structure (PDB entry 3qb2) [9], the α2–α3 loop of IFS bound to SPN_{ct} is considerably moved toward the active site cavity of SPN in our SPN_{ct}-IFS complex, with an r.m.s. deviation of 0.52–0.94 Å for 20 C^{α} atoms. The side chain of Arg40 on the IFS α2–α3 loop protrudes into the NAD binding cavity of SPN_{ct} (Fig. 1), blocking the binding of the substrate β-NAD^{+}. Arg40 of IFS interacts with SPN_{ct} through extensive water-mediated interactions (Fig. 1). It makes an extensive water-mediated hydrogen-bond networks with the residues located on α2, α8, α8–β3 loop, and α9–β4 loop of SPN_{ct} (Gln216 on α2; Ile328 and Lys329 on α8; Gly330 and Asp332 on α8–β3 loop; Gly368, Asn370, Asn373, Ile374, Gln378, Thr379, Trp380, Glu389, and Glu391 on α9–β4 loop).

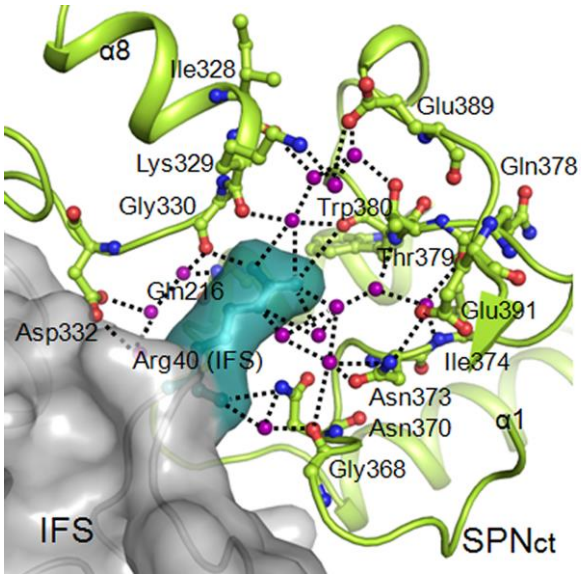

Fig. 1: Water-mediated interactions between Arg40 of IFS (gray surface) and the active site of SPN (in green ribbon).

SPN_{ct} was shown to be an atypical member of the ADP-ribosyltransferase superfamily with the characteristic Arg/His (R/H) motif (His273) and the *A*DP-*r*ibosylating *t*urn-*t*urn (ARTT) motif. The Ser-Thr-Ser (STS) motif of the ADP-ribosyltransferase superfamily is missing in SPN. It has an α-helical linker subdomain, which is absent in other ADP-ribosyltransferase superfamily enzymes [9].

The ARTT motif is important for the substrate specificity and recognition of the ADP-ribosyltransferase superfamily [10]. The Q/E-X-E sequence of the ARTT motif provides the key catalytic glutamic acid to stabilize an oxocarbenium ion intermediate [10]. The second Gln or Glu (Q/E), located two positions upstream from the catalytic Glu in the ARTT loop, is essential for the ribosyltransferase activity of ADP-ribosylating toxins. It may be important for recognizing the target residue of substrate proteins [10]. It was suggested that the different conformation of the ARTT loop in SPN as well as SPN's unique α-helical linker subdomain does not allow accommodation of protein substrates in the canonical mode of other ADP-ribosyltransfereases [9].

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