Neutron-Enabled Probing of Virulence Factor Dynamics and Functional Insights for Infection Mechanisms

Understanding the molecular basis of streptococcal infection may lead to targeted therapies or vaccines against the bacteria's unique pathogenic mechanisms.

We often deal with the hassle of infections caused by Group A Streptococcus in our daily lives. This germ releases two powerful secret weapons, known as NAD+ glycohydrolase (NADase) and Streptolysin O (SLO). The challenge stems from the interplay between these two factors, creating a complication that brings complexity to our day-to-day existence.[1,2] To unveil its solution structure and its conformational flexibility, small-angle neutron scattering (SANS) experiments were conducted on the deuterated-NADase/SLO complex. This was imperative as its crystal structure lacked functionally crucial terminal regions.

Chun-Ming Wu from the NSRRC and Shuying Wang's research group at National Cheng Kung University have recently initiated a collaborative project focusing on SANS measurements using the **QUOKKA** instrument at Open-pool Australian lightwater reactor in Australian Nuclear Science and Technology Organisation (OPAL, ANSTO) with deuterated protein technology. The goal is to emphasize the dominance of NADase through contrast matching with SLO in a 42% D_2O solution. Deuterated-NADase/SLO aliquots were acquired through size-exclusion chromatography (SEC) utilizing a Superdex 200 Increase 10/300 column. However, efforts to examine the structural details of the SLO component faced challenges due to aggregation in the 100% D_2O sample, leading to difficulties in obtaining a clear interpretation. Despite this, valuable data were obtained, enabling the modeling of the complex's overall structure and dynamics.

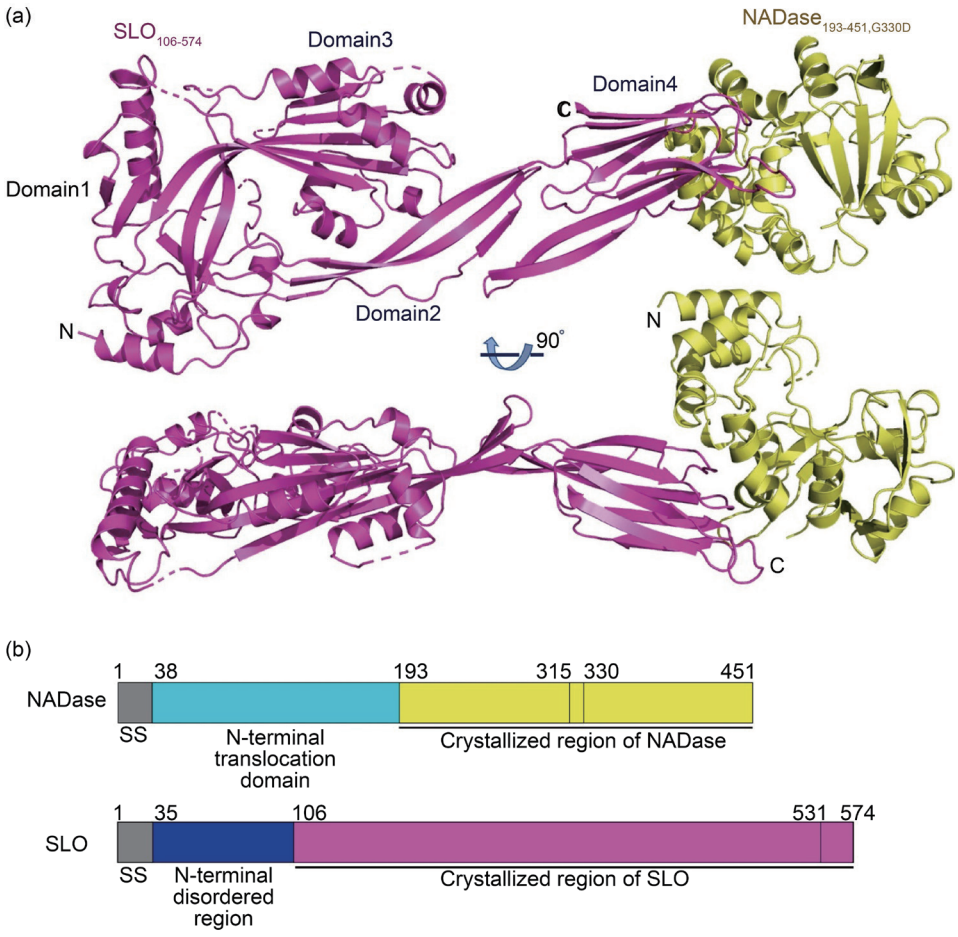

Fig. 1: Crystal structure of the $NADase_{193\text{-}451,G330D}/SLO_{106\text{-}574}$ complex. (a) Ribbon diagram of the crystal structure of the $NADase_{193\text{-}451,G330D}/SLO_{106\text{-}574}$ complex in two perspectives related by a rotation of 90° about the horizontal axis. where yellow is $NADase_{193\text{-}451,G330D}$ and magenta is $SLO_{106\text{-}574}$. (b) Schematic diagrams of NADase and SLO, where gray is the secretory signal (SS). [Reproduced from Ref. 3]

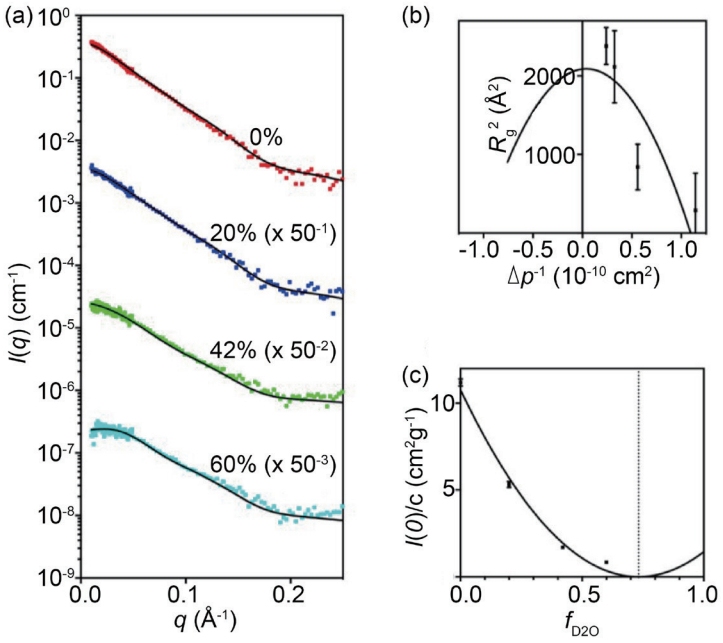

Fig. 2: SANS results: (a) SANS data (offset for clarity) collected from the ^{D}NADase/SLO complex with the multistate model scattering curves overlaid (solid black line); (b) Stuhrmann plot for the NADase/SLO complex A plot I(0) normalized by concentration as a function of D_2O content of the supporting solvent. (c) A plot I(0) normalized by concentration as a function of D_2O content of the supporting solvent. [Reproduced from Ref. 3]

Scattering data revealed a distinct upward shift in the dimensionless Kratky plot, signifying inherent flexibility. Rigid body modeling using the ensemble optimization method BILBOMD, which combines high-resolution structures with flexible linker regions, was employed (**Fig. 1**). Structural ensembles were selected based on combinations of conformers that best matched experimental data.

Results indicated that SANS data (**Fig. 2**) were consistent with a predominant compact state (~60%), where the terminal regions of both proteins interact, alongside a more extended form (~10%) with reduced contacts. The remaining ~30% represented free NADase, in agreement with previous binding studies. The compact state showcased contacts between SLO domain 4 and the NADase N-terminal translocation region, which is supported by mutagenesis data. Conversely, the extended form depicts separated terminal segments, likely corresponding to NADase dissociation from cell-bound SLO during delivery into host cells.

Validation of the conformational ensemble was sought through SANS measurements on the same samples in varying D_2O percentages. The resulting contrast variation series quantitatively aligned with the distribution of compact, extended, and free populations from initial dataset modeling.

In summary, neutron scattering experiments uncovered functionally relevant flexibility in the virulence factor complex (NADase/SLO), challenging assumptions derived from rigid crystal structures. These observations contribute to an integrated model that elucidates NADase delivery into host cells through dynamic association with SLO pores. Thus, SANS offers unique structural insights that are crucial for understanding the synergistic toxicity of these proteins in GAS infection. (Reported by Chun-Ming Wu)

This report features the work of Shuying Wang and her collaborators published in Commun. Biol. ***6****, 124 (2023).*

ANSTO QUOKKA – Small-angle Neutron Scattering

- SANS
- Protein Structure, Polymer, Biomaterial, Drug Delivery, Magnetism, Soft Matter

References

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