

NMR studies on the dynamics and structure of Verotoxin-1 B subunits binding to its functional plasma membrane receptor, Globotriaosylceramide, Gb₃

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Introduction

Verotoxin (VT) was first reported by Konowalchuk *et al.* in 1977. Although VT-producing *E. coli* strains belong to several different serotypes, 0157:H7 is the dominant serotype isolated from patients suffering from food poisoning. The infection frequently develops into hemorrhagic colitis. Furthermore, about 10% of these cases develop into the haemolytic uremic syndrome, which is the leading cause of acute renal failure in children. Verotoxins belong to the AB₅ class of bacterial toxins. The enzymatic A-subunit inhibits protein synthesis in the cell, by selectively attacking the ribosome by removing the adenine residue at position 4324 of the 28S subunit. However, the A-subunit requires the presence of the homopentameric B-subunits to achieve toxicity. The role of the B-subunits is attachment to the target cells and the translocation of the A-subunit into the cytoplasm. The functional receptor of verotoxin is the glycolipid globotriaosylceramide [Gb₃, Gal α 1-4Gal β 1-4Glc-ceramide]. The binding affinity of VTB to Gb₃ is in the millimolar range ($K_d = 1 \times 10^{-3}$). Since binding to the cell surface has been shown to be a crucial step in the cytotoxicity of verotoxin, designing inhibitors to block toxin binding would be an effective approach to therapy development.

Crystal structures of the verotoxin B-pentamer (VTB) complex with the Gb₃ oligosaccharide have been reported. Three Gb₃-binding sites per B-monomer have been identified, giving a total of 15 binding sites in each VTB-pentamer. These sites are called sites 1 to 3. However, solution NMR data has demonstrated that site 2 is the major binding site.

NMR Relaxation Study

Presently, we are investigating the proteins dynamics using ¹⁵N NMR relaxation in order to examine differential dynamics upon binding. As the heteronucleus N, is bonded to a single proton, H, the dominant relaxation mechanism is the ¹⁵N-¹H dipolar interaction, with chemical shift anisotropy acting as a secondary, but significant relaxation mechanism. These nuclear spin relaxation rates are closely related to molecular motions.

Three backbone amide ¹⁵N relaxation parameters have been measured at two magnetic field strengths: (i) the longitudinal relaxation time constant T₁; (ii) the transverse relaxation time constant T₂ and (iii) the ¹H-¹⁵N nuclear Overhauser effect.

These relaxation parameters are being analysed using the model-free formalism of Lipari and Szabo. If an anisotropic model for the tumbling of the molecule is assumed, good model-free parameters can be extracted from the relaxation data: S, a generalised order parameter and an effective correlation time, τ_e . Once these numerical values have been calculated, they can be directly related to conformational entropies. We can use these values to analyse the conformational entropy of ligand binding on a per residue basis.

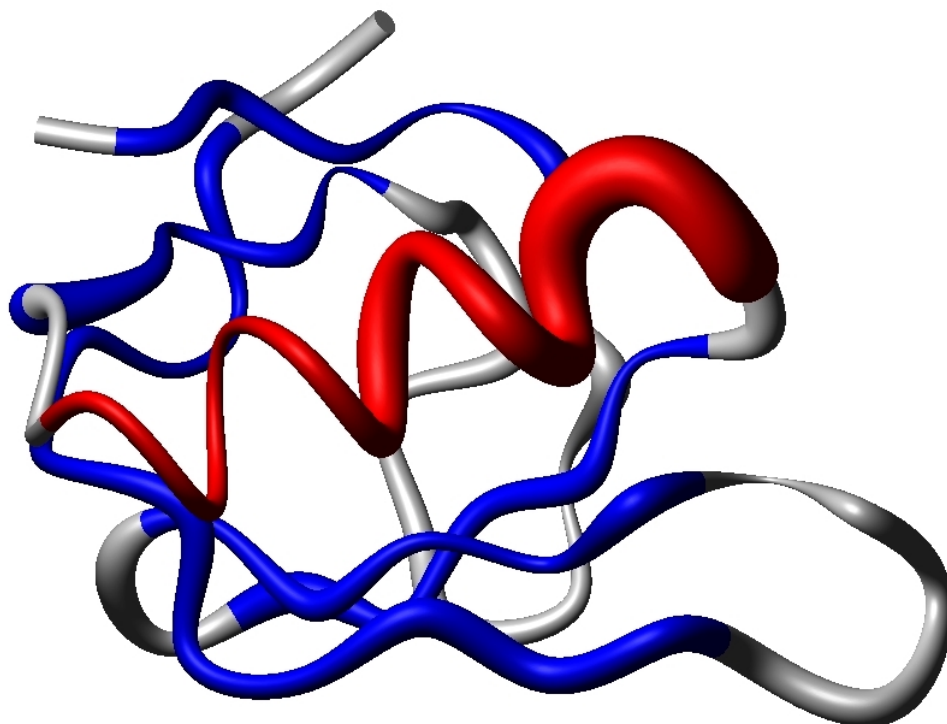


Figure: A backbone representation of the ^1H - ^{15}N heteronuclear nOe for a single subunit of VTB bound to the ligand S101; the binding site for S101 is in the loop at the bottom right corner of the picture.

References

Thompson, G. S., Shimizu, H., Homans, S. W. & Donohue-Rolfe, A. (2000) Localisation of the binding site for the oligosaccharide moiety of Gb3 on Verotoxin 1 using NMR residual dipolar coupling measurements. *Biochemistry*, **39** 13153-13156.

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