Large scale millisecond inter-subunit dynamics in the B subunit homopentamer of the toxin derived from \textit{E. coli} O157

Anna Yung, W. Bruce Turnbull, Arnout P. Kalverda, Gary S. Thompson, Steve W. Homans, Pavel Kitov and David R. Bundle

Introduction
The toxin derived from \textit{E. coli} O157 is a member of the AB\textsubscript{5} class of cytotoxins, comprising a catalytically active A subunit, and a torus-shaped homopentameric B subunit that binds to the cell-surface glycolipid globotriaosylceramide (Gb\textsubscript{3}) in a multivalent manner. The crystal structure of the B subunit shows similar inter-monomer $\beta$-sheet interactions between $\beta$2 of monomer n and $\beta$6 of monomer n+1, except between two monomers, resulting from a screw component of about 0.13 nm in the 5-fold rotation axis of the pentamer. In contrast, NMR measurements suggest a dominant conformer that is a symmetric homopentamer in solution, consistent with a single set of crosspeaks in $^1$H-$^{15}$N correlation spectra. However, $^1$H-$^{15}$N correlation spectra recorded on the protein in the presence of a five-fold molar excess of the bivalent inhibitor P$^k$-dimer, which is a bridged dimer analogue of the Gb\textsubscript{3} carbohydrate, are characterised by a reduction of resonance line-widths for a number of resonances. These data suggest an exchange contribution to the line-widths of the B subunit in the absence of P$^k$-dimer, which accordingly was probed by use of NMR relaxation dispersion measurements.

Slow conformational exchange between subunits
NMR relaxation dispersion experiments allow us to detect slow time-scale (millisecond) motions in proteins on a per-residue basis, and provide, inter alia, a quantitative measure of this time-scale. Our measurements showed significant motions for residues immediately adjacent to each monomer-monomer interface in the protein, each of which could be characterized by a single exchange rate of 1000/sec. The data can be interpreted structurally in terms of fast exchange between a symmetric conformation and a minor conformer that may be related to that observed in the crystal structure. On binding of P$^k$-dimer, which straddles binding-sites on adjacent monomers, these motions disappeared, indicating that this novel ligand effectively ‘locks’ the pentamer in a single conformation. Consequently, binding of P$^k$-dimer cannot be interpreted in terms of a simple two-state model, but is consistent with a sequential binding model involving co-operative effects. These effects involve a positive entropic component to co-operativity that has not been observed previously in multivalent systems to our knowledge.

Fig. 1: Side-view of \textit{E. coli} O157 B subunit in the asymmetric ‘excited’ (left) and symmetric ground states. These states exchange in solution on the millisecond time-scale.
Collaborators
David Bundle, Pavel Kitov, University of Alberta

Publications


Funding
This work has been funded by the Wellcome Trust and BBSRC