University of Southampton

BipD – a Crucial Protein for Bacterial Invasion?

A Component of the Type Three Secretion System of *Burkholderia pseudomallei*

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Through the use of X-ray crystallography, it has been possible to determine the 3D atomic structure of the protein BipD. This has provided us with information about how it facilitates the insertion of other bacterial proteins into the plasma membrane of a target cell, enabling invasion by the bacterium.

Why Study BipD?

The protein BipD comes from the bacterium *Burkholderia pseudomallei*, which is responsible for the tropical disease **melioidosis**. It can kill within 48 hours and is on the US category B list of critical agents due to its potential as a **bioterrorist weapon**. It is therefore vital to not only understand how *B. pseudomallei* causes disease, but also how it infects cells.

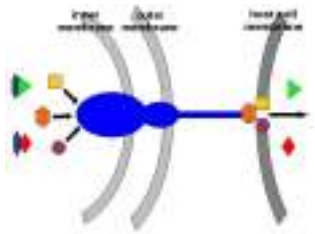
Melioidosis can cause blood disease, abscesses, lung disease, kidney disease, heart disease and more, hence *B. pseudomallei* has been referred to as **The Great Mimicker**

Understanding at a molecular level how *B. pseudomallei* invades a target cell will enable us to design drugs that specifically interfere with these processes, helping to prevent and treat the disease.

How Does BipD Work?

BipD is one of 3 **translocator** proteins secreted onto the surface of a target cell, by the bacterium, which then help to form a pore in the plasma membrane. This allows *B. pseudomallei* to insert its **effector** proteins into the cell, which act on the cellular machinery to subvert it for the bacterium's own replication and proliferation.

To transport the proteins across its membrane and secrete them, *B. pseudomallei* uses the equivalent of a ATP powered **molecular syringe** called a **Type Three Secretion System** (TTSS).



- BopB
- BopC
- BipC
- BipD
- BipB
- BopD

BipD assists the translocators, BipC and BipB, form a pore in the host cell membrane allowing the effectors, BopB and BopC, to invade the cell.

Methods

Expression of BipD

PCR was used to amplify the DNA out of its GSTag-vector and re-clone it into pET11a. The DNA was then inserted into BL21 *E.coli* for ease of expression and purification on the very large scale that is needed for crystallography. These were grown on **ampicillin** plates, to select for colonies that successfully took up the plasmid, then used to inoculate growth media.

Purification of BipD from Contaminants

Gel filtration: the beads within the column filter the molecules by size

The bacterial cells were broken open by **sonication**, then the soluble BipD protein purified from the cell debris. This was achieved primarily using **ion exchange** (*Q-sepharose anion exchange*) and **gel filtration** (*sephadex 200*) columns, which separate the molecules by charge and size respectively.

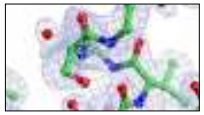
Growth of BipD Crystals

The purified BipD was **concentrated** to 6mg/ml. Crystal screens were set up using the **hanging drop method**. The crystals took 3-4 weeks to grow.

X-ray Crystallography

The crystals were **frozen** in liquid nitrogen and X-rays fired at them. The rays deflected by the electron clouds of repetitively packed protein molecules are recorded by a computer, producing a **diffraction pattern**.

Analysis of Data and Model Building



Computer programs are used to convert the pictorial diffraction patterns into numerical data, and build a 3D model of the protein structure.

the proposed atomic structure fits the electron density map

Results

The model was refined to 2.1Å. The longest helices form a 4-helix bundle and the remaining tertiary structure (3 helices and 2 sheets) is formed by the region linking the last two helices of the 4-helix bundle.

Conclusion

It was found that although BipD is monomeric in dilute solution, the two molecules in the crystallographic asymmetric unit associate by forming extensive contacts (involving the most highly conserved region of helix 8). This suggests the biologically active form may be a dimer.

There are other stretches of invariant sequence in helix 8 that are not involved in dimer formation and these may be involved in binding other TTSS proteins.

The electrostatic charges of BipD in its dimeric form
Blue = negative, Red = positive

Further Work…

We plan to make **mutants** and analyse the **conformational changes** in the BipD molecule, associated with its function, by X-ray crystallography and H-D exchange/mass-spectrometry. There is a homologue (LcrV) that has a very different structure, suggesting the molecule can change shape a lot depending on pH or whether it is in, on or out of the needle, bound to the membrane, in the translocon etc.

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