Protein modification reagents and binding studies on bacterial toxins

Thomas Branson, Martin Fascione, Edward Hayes, Pintu Mandal, James Ross, Daniel Williamson, Michael Webb and Bruce Turnbull

Introduction

Sortase A (SrtA) catalyses the reversible attachment of virulence factors to the cell walls of Gram positive bacteria via C-terminal modification of proteins at an LPXTG recognition sequence. The enzyme has been widely exploited for introducing labels at the C-terminus of recombinant proteins, but the reversibility of the reaction necessitates the use of large excesses of the labelling reagents and the method has rarely been used for N-terminal labelling of proteins.

Results

We have developed new reagents for efficiently labelling any protein that has at least one unhindered glycine residue at its N-terminus (Figure 1). By using depsipeptide substrates (i.e., containing an ester bond) the trans-peptidase reaction becomes effectively irreversible and allows quantitative labelling of proteins with only a small excess of the labelling reagent.

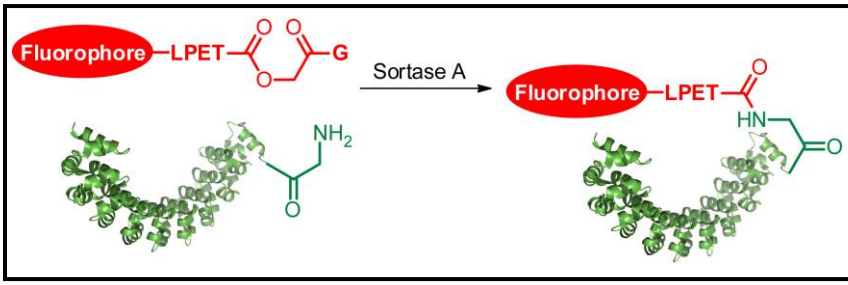

Figure 1: schematic depiction of labelling a protein using a fluorescent depsipeptide substrate for the Sortase A enzyme.

In other studies we have continued to investigate the carbohydrate-binding specificity of the cholera toxin. It has long been known that patients with blood group O are more severely affected by El Tor cholera than those in blood groups A or B. Krengel and co-workers discovered a secondary carbohydrate binding site for blood group oligosaccharides in the closely related *E. coli* heat-labile toxin (Figure 2) which led to the hypothesis that blood group A and B oligosaccharides that are shed from the surface of cells could help prevent the toxin from reaching its primary ligand in the cell membrane.

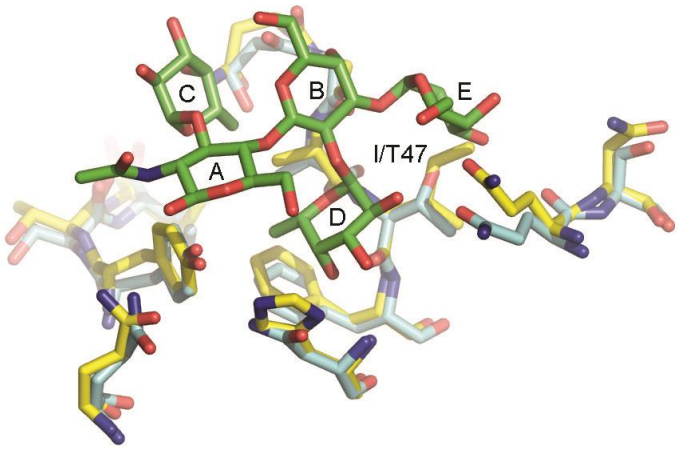

Figure 2: overlay of structure of *E. coli* heat-labile toxin (light blue; 2O2L.pdb) and a model of El Tor CTB (yellow) constructed using the structure of classical biotype CTB (3CHB.pdb). The blood group B Lewis-y oligosaccharide is shown in green. Galactose E, which is absent in the equivalent blood group O oligosaccharide, sits in close proximity to residue 47 in the protein which is either an isoleucine or threonine residue.

We have chemically synthesised blood group O and blood group B oligosaccharides of the Lewis-y series and used isothermal titration calorimetry and saturation-transfer difference NMR spectroscopy to show that both carbohydrates are ligands for the *E. coli* version of the

toxin, but only the blood group O oligosaccharide can bind to the toxin responsible for El Tor cholera. The difference in specificity appears to arise from a single mutation at residue 47 in the binding site (Figure 2) in which a threonine residue allows blood group B binding, whereas an isoleucine residue does not. This result suggests that surface mutations on the protein could be used to engineer cell/tissue selectivity into the protein, e.g., for targeting tumours that overexpress certain oligosaccharides on their surface.

Publications

Mandal, P., Branson, T., Hayes, E., Ross, J., Gavin, J., Daranas, A. & Turnbull, W. (2012) Towards a structural basis for the relationship between blood group and the severity of el tor cholera. *Angew. Chem. Int. Ed. Engl.* **51:** 5143-5146.

Turnbull, W. & Stalford, S. (2012) Methylthioxylose - a jewel in the mycobacterial crown? *Org. Biomol. Chem.* **10:** 5698-5706.

Turnbull, W. (2012) Biophysical studies on the interactions of bacterial toxins, in "Glyco-bioinformatics", Eds. M. G. Hicks and C. Kettner, Beilstein Institute, Frankfurt, pp 55-68.

Williamson, D., Fascione, M., Webb, M. & Turnbull, W. (2012) Efficient N-terminal labeling of proteins by use of sortase. *Angew. Chem. Int. Ed. Engl.* **51:** 9377-9380.

Funding

This work is funded by the Royal Society, EPSRC, Newton International Fellowships, AstraZeneca and the University of Leeds.

Collaborators

External: A. Daranas and J. Gavín (University of La Laguna, Tenerife).