

Dissecting the ligand binding energy for the *E. coli* heat-labile toxin

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Introduction

Cholera is a severe diarrhoeal disease that affects more than 130,000 people annually and is lethal in over 3% of cases. A further 6 million people per annum suffer from the less severe traveller's diarrhoea, largely on trips to southern Europe and developing countries. The causative agents of these two debilitating diseases are cholera toxin (CT) and heat-labile enterotoxin (LT) released by *Vibrio cholerae* and *Escherichia coli* bacteria, respectively. These two protein toxins have an AB₅-type multimeric structure, with essentially identical A-subunits and share 80% sequence identity in their B-subunits. The pentameric B-subunit (5 × 11.8 kDa) is a carbohydrate-binding protein that specifically recognises the oligosaccharide portion of a glycosphingolipid — ganglioside GM1 — which is present on the surface of cells forming the gut wall. On binding to five copies of this glycolipid, the A subunit (27 kDa) enters the cell through an as yet, unknown mechanism, where it catalyzes ADP-ribosylation of the signal transduction protein G_s-α. This modification prevents deactivation of G_s-α, and consequently leads to high intracellular levels of cAMP, which, in the small intestine, results in fluid loss and severe diarrhoea. As B-subunit adhesion to the surface of a target cell is a prerequisite for entry by the A-subunit, this protein-carbohydrate recognition event is a potential target for developing drugs against the toxic effects of these bacteria.

Dissecting “multivalent” interactions

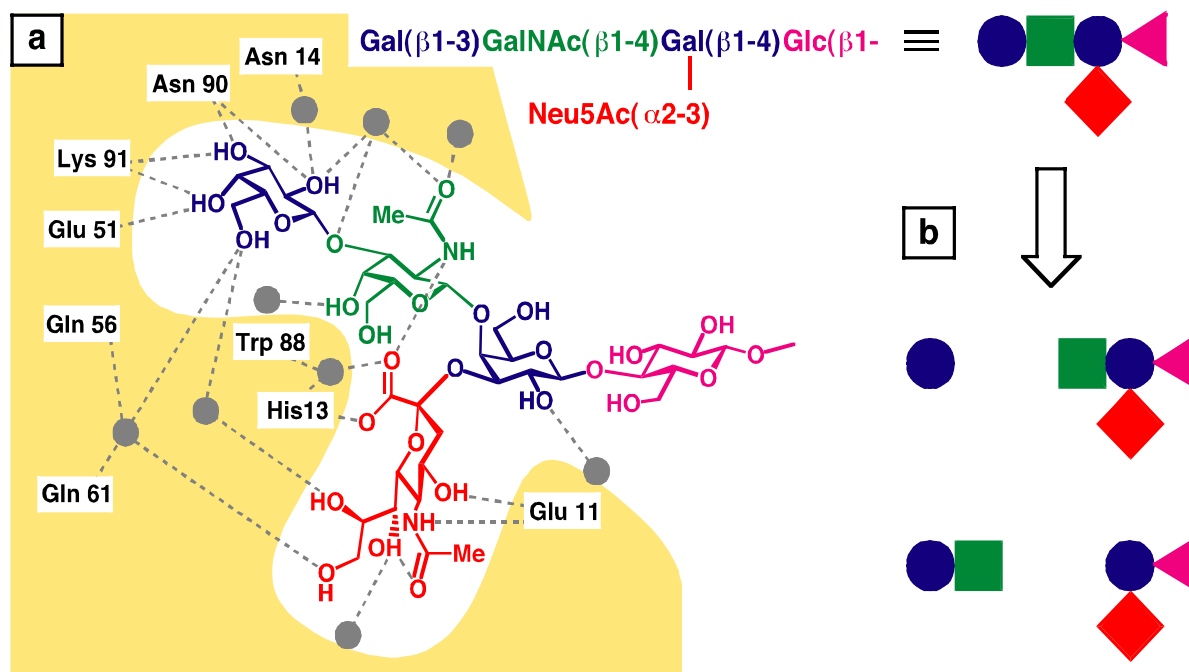


Figure. a) Complex of GM1 oligosaccharide with the cholera toxin B-subunit (CTB) with key hydrogen bonds between the ligand, protein and bound water molecules (grey circles) indicated as broken lines; b) Cartoon representation of the fragments of the oligosaccharide ligand that are being used in binding studies with LTB.

Although very important in cell surface biology, protein-carbohydrate interactions are notoriously weak, often having dissociation constants in the millimolar range. Nature circumvents this problem by displaying multiple copies of both the carbohydrate ligands and their protein receptors in such a way that many weak interactions reinforce one-another to

give a strong overall adhesion — not unlike molecular-scale velcro. In the case of CTB and LTB, this so-called multivalent effect manifests itself on two levels, most obviously in the pentavalent binding of the B₅ ring to five copies of GM1. However additionally, X-ray crystallography of the complex has previously revealed that on the smaller scale of an individual subunit, the branched oligosaccharide grabs hold of the protein in a “two fingered grip” (see Figure). These “bivalent” interactions rank among the highest intrinsic affinities in glycobiology and thus form a suitable model system for analysing the thermodynamics of interaction on a per-saccharide residue basis. Therefore, we are studying the binding abilities of fragments of the natural oligosaccharide ligand with the aim of dissecting the individual contributions from each monosaccharide to the overall interaction.

Synthesis and binding studies of GM1 fragments

Whereas smaller mono- and disaccharide fragments of ganglioside GM1 are commercially available or can be accessed readily by chemical synthesis, larger fragments — including the full pentasaccharide — are most easily produced by stepwise enzymatic degradation of the natural ligand. With these carbohydrates in hand, studies are underway to evaluate the ability of each fragment to bind to the B-subunit of LT. The methods being employed for binding studies include fluorescence spectroscopy, isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) spectroscopy. ITC, in particular, is useful for determining all thermodynamic parameters (free energy, enthalpy and entropy changes) in a single experiment. We aim to rationalise the binding affinities of individual carbohydrate fragments through NMR spectroscopic structural studies of their complexes.

Collaborators

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