Dissecting the cholera toxin-ganglioside GM1 interaction by isothermal titration calorimetry

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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 Gₛ-α. This modification prevents deactivation of Gₛ-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.

![Fig. 1.](image-url)  
*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 CTB.

Dissecting the “bivalent” interaction
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 Fig. 1). 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 have studied 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 either commercially available or could be accessed readily by chemical synthesis, larger fragments — including the full pentasaccharide — were most easily produced by stepwise enzymatic degradation of the natural ligand. Isothermal titration calorimetry (ITC) is a technique that exploits small stepwise changes in heat that is released during the course of a ligand-receptor titration to allow derivation of all thermodynamic parameters (free energy, enthalpy and entropy changes) in a single experiment. We have used displacement titrations to determine binding information for the low affinity fragments, whilst exploiting the high sensitivity of the high affinity GM1os interaction. Analysis of the intrinsic binding affinities for the ligands has shown that the terminal galactosyl and sialosyl residue each contribute just under 50% of the CTB-GM1os interaction, but paradoxically, sialic acid itself has no appreciable affinity for the receptor. Our results have thus also highlighted the origin of the high selectivity of CTB for GM1.

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