Inhibiting bacterial toxin adhesion using protein aggregation

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Introduction

Cholera and travellers' diarrhoea are still life threatening diseases in many parts of the world. These two diarrhoeal diseases are caused by protein toxins that share over 80% sequence identity; cholera toxin and *E. coli* heat-labile toxin. Their AB_5 hetero-oligomeric structures comprise a single toxic A-subunit and a pentameric B-subunit that interacts with the cell surface glycolipid ganglioside GM1. Inhibitors of the B-subunit-GM1 interaction could provide a prophylactic treatment for these debilitating diseases. It has been shown that compounds bearing multiple copies of GM1 are potent inhibitors of B-subunit adhesion, and have been shown to give up to 47,500 times more efficient inhibition than monovalent GM1. Theoretical models describing multivalent interactions of cholera or heat-labile toxin usually assume that the protein does not aggregate on binding to multivalent ligands as the binding sites are all on the same face of the multimeric protein.

Isothermal titration calorimetry studies of GM1 dimers and tetramers interacting with the *E. coli* heat-labile toxin B-pentamer (LTB) have revealed little inherent increase in affinity compared to that of the monovalent GM1 ligand. Analytical ultracentrifugation experiments indicated that the ligands induce protein aggregation at sub-stoichiometric concentrations of ligand groups. Subsequent dynamic light scattering studies confirmed that space-filling networks of protein aggregates can form when as little as 10% of the binding sites are occupied. The mechanism and kinetics of the aggregation process are dependent on the valency of the ligands. Atomic force microscopy has been used to demonstrate that a divalent inhibitor induces head-to-head dimerisation of the protein toxin en route to higher aggregates. These studies suggest that designing ligands to have valencies that are mismatched with their protein receptors may provide a general strategy for receptor inhibition by aggregative mechanisms.

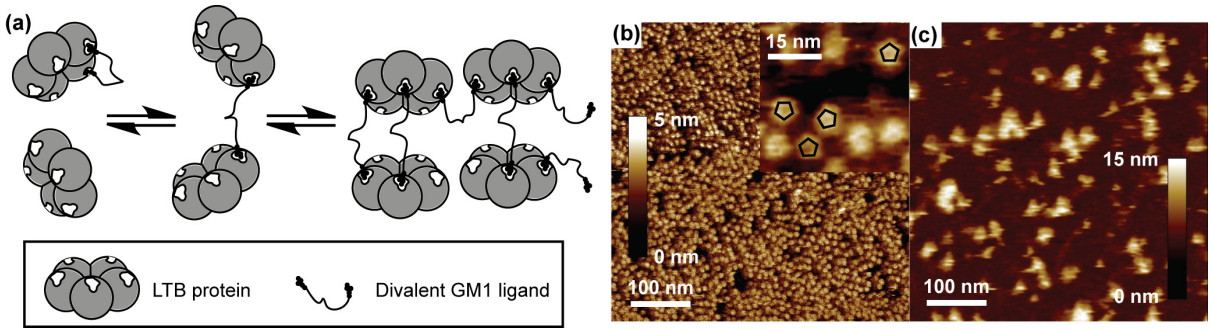

Fig. 1. (a) Cartoon representation of LTB aggregation by a divalent ligand. (b) AFM image of LTB pentamers on a mica surface. (c) LTB aggregates in the presence of one equivalent of the divalent ligand.

Collaborators

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Funding

This work is supported by the Royal Society and the European Union COST programme.