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Multi-scale molecular dynamics study of cholera pentamer binding to a GM1-phospholipid membrane

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ABSTRACT

The AB_5 type toxin produced by the *Vibrio cholerae* bacterium is the causative agent of the cholera disease. The cholera toxin (CT) has been shown to bind specifically to GM1 glycolipids on the membrane surface. This binding of CT to the membrane is the initial step in its endocytosis and has been postulated to cause significant disruption to the membrane structure. In this work, we have carried out a combination of coarse-grain and atomistic simulations to study the binding of CT to a membrane modelled as an asymmetrical GM1-DPPC bilayer. Simulation results indicate that the toxin binds to the membrane through only three of its five B subunits, in effect resulting in a *tilted* bound configuration. Additionally, the binding of the CT can increase the area per lipid of GM1 leaflet, which in turn can cause the membrane regions interacting with the bound subunits to experience significant bilayer thinning and lipid tail disorder across both the leaflets.

1. Introduction

The disease cholera is often symptomized by excessive watery diarrhea and vomiting leading to dehydration, electrolyte imbalance and if untreated even death [1]. Despite advances in medicines, cholera continues to affect 3–5 million people worldwide, causing upto 130000 deaths yearly [2]. The main causative agent of the disease is the Cholera Toxin (CT) released in the intestinal lumen of humans [3,4] by the gram negative *Vibrio cholerae* bacterium [5,6].

The structure of the cholera toxin was first purified and studied by Finkelstein and LoSpalluto [7,8]. They identified the presence of two different parts of the toxin with different biological activity. The first part was identified to be cytotoxic while the other one was non-toxic and acted as a carrier. Subsequently, Zhang et al. [9] used X-ray crystallography to determine the crystal structure of the Cholera toxin. The toxin is classified as an AB_5-type toxin composed of a cytotoxic 'A' subunit and 5 non-toxic carrier 'B' subunits.

The 'A' subunit is composed of 240 amino acids and is separated into A1 (residues 1–192) and A2 (residues 193–240) [10]. A1 is the catalytic polypeptide responsible for the toxicity [11,12], while A2 is a kinked α-helix which tethers A1 to the B chain [12]. The 'B' subunits are composed of 103 amino acids each and together form a pentamer with a five-fold symmetry [12,13]. Several groups have isolated the 'B' subunit and have determined its crystal structure in complex with various ligands [14,15]. Fig. 1a shows the structure [9] of a cholera toxin with the A1 chain in blue, A2 in red and B subunits in green. Fig. 1b shows a bottom-up view of the pentameric distribution of the five B chains with the A1 and A2 subunits shown in grey.

For the cholera toxin to be effective, it needs to be internalized into the cell. Every human cell is enclosed within a thin membrane called the plasma membrane, which is about 5 nm wide and is the sole pathway for the transfer of substances in and out of the cell [16]. The plasma membrane is predominantly composed of a bimolecular layer of lipids, arranged such that the polar head groups face the surrounding aqueous environment, while the hydrophobic tails face each other [17]. It also contains proteins, gangliosides and other bioactive molecules that provide structure and function to the membrane. These components of the membrane are mobile and are capable of coming into proximity to engage in transient interactions [18].

The CT has been postulated to cross this lipid barrier through endocytosis [19,20], a process that first requires the toxin to bind to the extra-cellular membrane region. This initial binding is made possible by the specificity of the 'B' subunit for the extra-cellular membrane based monosialotetrahexosylganglioside (GM1) ganglioside [21,22]. The structure of GM1 can be described as an oligosaccharide head connected to two lipophilic tails. The lipophilic tails aid in anchoring the gan-

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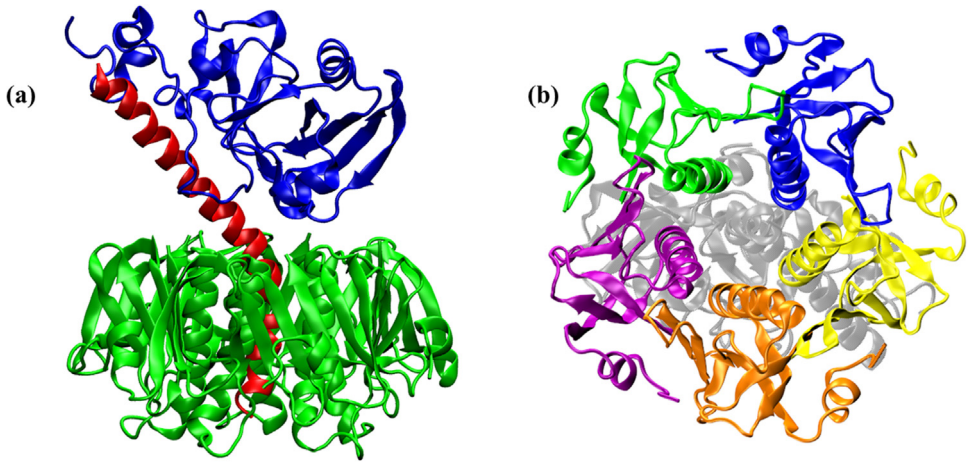

Fig 1. (a) A side-on view of the structure of the Cholera Toxin with the toxic A1 subunit in blue, linker A2 subunit in red and all five B subunits in green. (b) A bottom-up view of the structure of the Cholera toxin with the five B subunits shown in green, blue, yellow, orange and purple. The A1 and A2 subunits are shown in grey and have been made transparent and lighter for clarity.

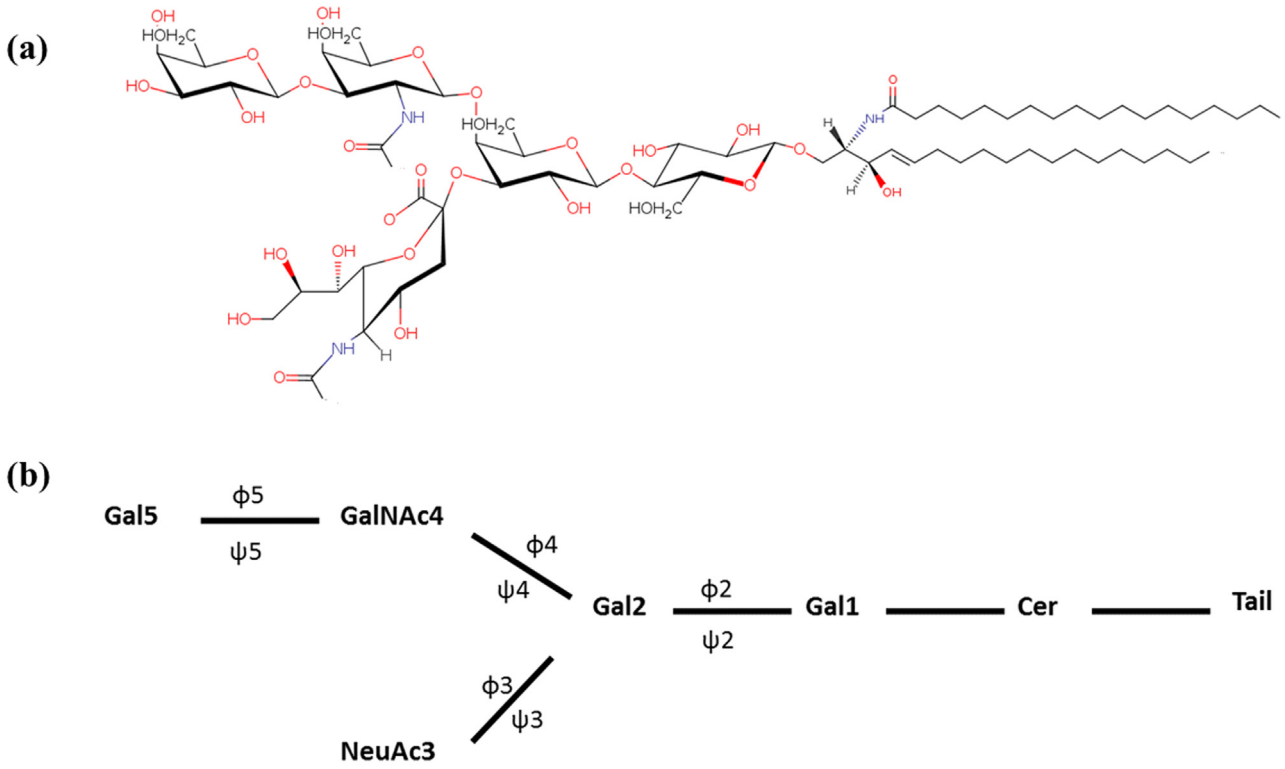

Fig 2. (a) Atomistic structure of the GM1 glycolipid. (b) The sequence of sugars in GM1 corresponding to the atomistic structure in (a) and their naming terminology followed in this paper. The inter-glycosidic linkage dihedral angles φ and ψ are also labelled.

glioside within the membrane bilayer. The oligosaccharide is a branched pentasaccharide with the D-sugars in the sequence Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glc [23]. Fig. 2 shows the structure of a GM1 molecule along with the saccharide naming terminology used in this paper. ϕ_i and ψ_i, the dihedral angles between the glycosides, are named according to the terminology used by Patel et al. [24] in their simulation study of GM1 in DPPC membranes .

The interaction between a glycolipid membrane and the CT has been studied extensively. Fishman et al. [25] showed back in 1978 that five GM1 molecules on the membrane are bound to the CT. Schoen et al. [26] proved that the binding of different GM1 molecules to the CT is cooperative with one binding aiding the next. More recently, Basu et al. [27] have postulated a tilted binding conformation and have also demonstrated that the lipids in contact with CT have a 'bent' head group orientation and greater tail disorder compared to bulk lipids.

The studies of glycolipid-pentamer interactions lead to the finding that galactose is crucial to GM1's affinity for the CT. The toxin does not bind as strongly to other glycolipids such as GM2 or GM3 which lack the galactose terminal sugar [28,29]. Bernardi et al. [30] also observed the importance of galactose when trying to design sugar based artificial receptors of CT.

The importance of galactose has led many groups to develop galactose based molecules to inhibit CT's endocytosis. These inhibitors aim to occupy the GM1 binding site of the 'B' pentamer