Identification of Molecular-Mimicry-Based Ligands for Cholera Diagnostics using Magnetic Relaxation

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ABSTRACT

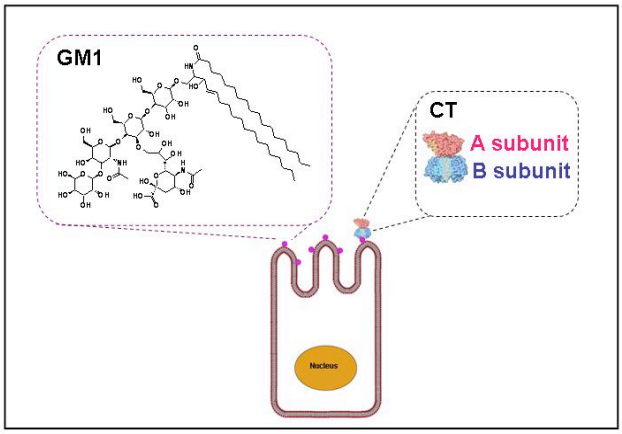
Magnetic nanosensors (MRnS) offer multiple benefits that can be used to design sensitive and robust diagnostic tools. High sensitivity, stability, and ease of surface modification make this approach a strong contender against the more commonly used heat-labile antibodies. Providing picomolar detection ranges, even at higher temperatures where antibodies are impractical, makes MRnS a good replacement for diagnostics in remote locations where specific storage requirements are not available. Amid the most widespread and effective virulent factors affecting humans are bacterial and fungal toxins. Hence, there is a need for sensitive toxin diagnostics that can be used in remote point-of-care locations and reduce the time required for validation of test results.

Keywords: iron oxide nanoparticles, small molecule probes, toxin diagnostics, Cholera

Using carbohydrate-conjugated MRnS[1], we were able to detect the cholera toxin (CT) with high sensitivity and specificity. Our method utilized molecular mimicry to screen the interaction of carbohydrates present in the known target (GM1 gangliosides) of the B subunit (CTB) of CT. Since, CTB binds GM1 ganglioside via interactions with its pentasaccharide moiety containing N-acetylgalactosamine (GalNAc), N-acetylneuraminic acid (Neu5Ac), glucose (Glc), and galactose (Gal) residues (**Scheme 1**) [2,3], we hypothesized that CTB would bind to magnetic nanoparticles coated with carbohydrate constituents of GM1. We anticipated that this interaction would generate changes in T2 that could be used for screening these interactions with the goal of identifying small molecule ligands useful for the development of sensors and potentially therapeutic agents for cholera.

Using this approach, we confirmed by both SPR and MRnS that galactose binds to CTB. Furthermore, we showed that an MRnS-galactose conjugate can detect CTB via magnetic relaxation with a detection limit of 40 pM. We further discovered via magnetic relaxation, and later confirmed by SPR, that dextran – a glucose polymer – can bind to CTB (**Figure 1**). For the magnetic relaxation assessment, a dextran-coated iron oxide nanoparticle was simply used as the MRnS probe, which was capable of achieving a CTB detection threshold of 16 nM. Further SPR analysis confirmed the interaction of CTB with dextran with a calculated dissociation constant (K_D) of 14 mM. The effect of ligand multivalency [4] was studied revealing that the valency of the MRnS affected the $\Delta T2$ pattern, but not the sensitivity of the system. Additionally, since binding between CTB and dextran has not been previously reported, we confirmed this interaction with a cell-based competition assay in which increasing concentrations of dextran led to the inhibition of CTB binding at the cell surface. This hints the potential use of dextran-based therapeutics in the treatment of cholera.

Taken together, our work suggests that magnetic relaxation can be used in the assessment and development of molecular-mimicking systems for potential MRnS-based diagnostics applications. Using molecular-mimicry-based MRnS and cholera toxin as a model system, we have discovered that dextran-coated nanoparticles can be used for the fast, cheap, homogeneous and single-step detection of cholera toxin.



Scheme 1. Structure of the GM1 ganglioside found on intestinal epithelial cells and binding of cholera toxin (CT) to the target cells. GM1 gangliosides, located on the surface of the targeted cells, are the known ligands that facilitate binding and internalization of CT. N-acetylgalactosamine (GalNAc), N-acetylneuraminic acid (Neu5Ac), glucose (Glc), and galactose (Gal) residues comprise the pentasaccharide moiety of the GM1 ganglioside facilitate interaction with the B subunit of cholera toxin (CTB).

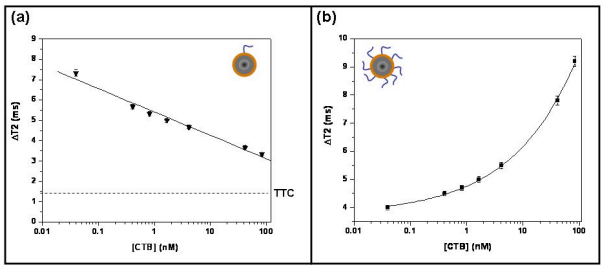


Figure 1. Specific detection of CTB with galactose-conjugated MRnS. Measurements involving the indicated concentrations of CTB were taken after a 5-min incubation with **(a)** Low Valency (LV) or **(b)** High Valency (HV) galactose-conjugated MRnS. The means ± SE of three independent experiments are shown. The background signal obtained from 38 nM of Tetanus toxin C-fragment (TTC) is presented in panel (a); no detectable signal was obtained from TTC with the HV MRnS.

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