A hybrid polymer to target blood group dependence of cholera toxin†

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Cholera is a potentially fatal bacterial infection caused by the cholera toxin (CT), an AB5 toxin secreted by Vibrio cholera. GM1 has long been known as the receptor of the cholera toxin in the intestine. However, increasing evidence is pointing towards the role of fucosylated conjugates as additional attachment options of the toxin. In the present paper we have synthesized a polymeric hybrid which can inhibit both modes of attachment.

Cholera is an acute diarrhoeal infection that is caused by the ingestion of water or food contaminated with the Vibrio cholera bacterium.1 Cholera is endemic in countries with poor sanitation and inadequate drinking water facilities, with 3 to 5 million reported cases every year.1 The current cholera epidemic in Yemen that began in 2016 has so far resulted in more than 3500 fatalities.1 It was caused by the cholera toxin (CT) which is a hybrid polymer to target blood group...
So far only one fucose-based polymer has been reported with an IC$_{50}$ of 1.5 μM derived from a cell-based assay.\textsuperscript{14} We set out to create a molecule that could block both GM1-based and fucose-based intoxication, by constructing a “hybrid” polymeric ligand. This was done in anticipation of multivalency enhancements as we have seen for other multivalent platforms.\textsuperscript{23–26} For this purpose, we used a dextran based polymer to which fucose and a galactoside were conjugated. Meta-nitrophenyl α-galactoside (MNPG) is an ideal candidate owing to its potency and we have demonstrated that when conjugated to polymers effective inhibition of cholera toxin is achieved in a GM1-based assay.\textsuperscript{27} In the present paper, we have synthesized a fucosylated and a hybrid polymer. The synthesized compounds were tested for their ability to inhibit the cholera toxin B-subunit by making use of the GM1-based ELISA assay along with the newly developed fucose-based version.

Results and discussion

Propargyl fucoside 1 and the MNPG derivative 2 were synthesized starting from L-fucose and galactose pentaacetate according to reported procedures (Fig. 1).\textsuperscript{27,28} Azido-functionalized dextran (M$_{w}$ = 155 kDa) with 6% azide functionalization was used as the polymeric scaffold.\textsuperscript{29} Copper-catalysed alkyne–azide cycloaddition was used for the conjugation of the dextran polymer to the fucoside 1 in order to obtain the fucosylated polymer i.e. 3. The hybrid polymer 4 was obtained by conjugating both MNPG propargyl and 1 in equimolar quantities to the dextran azide. Final polymers 3 and 4 were characterized by NMR and infrared spectroscopy, the latter of which was useful to see the disappearance of the azide signal at 2110 cm$^{-1}$ (Scheme 1) (see ESI$^\dagger$).

Cholera toxin inhibition

The polymers were evaluated for CTB inhibition in an ELISA-type assay by immobilising the GM1 ganglioside and using a cholera toxin–biotin conjugate. Galactose was used as the monovalent reference compound and showed weak inhibition as before\textsuperscript{30,31} (IC$_{50}$: 195 mM) whereas L-fucose was an extremely weak inhibitor with an IC$_{50}$ of 1.6 M (Table 1). Polymer 3 did not inhibit CTB in this assay up to 200 μM, while hybrid 4

![Scheme 1](image-url) Synthesis of fucose- and hybrid polymers. (i) Dextran azide, 1, CuSO$_4$, Na-CuAAC ascorbate, 100 °C, 75%, (ii) dextran azide, 1, 2, CuSO$_4$, Na-asorbate, 100 °C, 81%, (iii) dextran azide, 2, CuSO$_4$, Na-ascorbate, 100 °C, 51–58%.

![Table 1](image-url) Results of inhibition by multivalent carbohydrates in CTB-biotin ELISA assay$^a$

<table>
<thead>
<tr>
<th>Entry</th>
<th>Construct</th>
<th>Ligand</th>
<th>Valency (% functionalization of polymer)</th>
<th>IC$_{50}$ (μM)</th>
<th>Rel. pot.$^b$</th>
<th>Rel. pot. per sugar$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Galactose</td>
<td>α-Gal</td>
<td>1</td>
<td>195 000 ± 21000</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>L-Fucose</td>
<td>L-Fuc</td>
<td>1</td>
<td>158 000 ± 171000</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>L-Fuc</td>
<td>52 (5.6%)</td>
<td>No inhibition</td>
<td>7500</td>
<td>288</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>L-Fuc + MNPG (1:1)</td>
<td>52 (5.6%)</td>
<td>26 ± 10</td>
<td>61 000</td>
<td>1108</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>MNPG</td>
<td>55 (6%)</td>
<td>3.2 ± 0.9</td>
<td>61 000</td>
<td>1108</td>
</tr>
</tbody>
</table>

$^a$ Determined in an ELISA-like assay with CTB$_5$-biotin (40 ng mL$^{-1}$) and wells coated with GM1. $^b$ Relative to the potency of galactose. $^c$ Relative potency divided by the MNPG valency.
showed inhibition with an IC$_{50}$ of 26 µM. This represents a large potency enhancement in comparison with the millimolar inhibitory potencies of galactose and MNPG derivatives. The dextran azide polymer was also tested and did not show any inhibition in the assay.

Previously a fucosylated polymer has been synthesized and tested for aggregation based inhibition in an ELISA with T84 cells, Colo 205 cells and primary human jejunal epithelial cells. These cells, notably T84, are not easy to culture, so as an alternative assay unambiguously focused on fucose-CT interactions, we utilized immobilized polyacrylamide-conjugated l-fucose (PAA-fucose) and the same biotinylated toxin. PAA-fucose has been previously used to test fucosylated glycopolymers. We first compared the PAA-fucose assay with the T84 cell assay to evaluate the assay sensitivity and concomitant testing of the hybrid l-fucose (PAA-fucose) and the same biotinylated toxin. PAA-fucose was previously used to test fucosylated glycopolymers.

Notes and references


Table 2: Results of inhibition by multivalent carbohydrates in PAA-fucose ELISA assay

| Entry | Construct | Ligand | Valency | IC$_{50}$ (µM) | Rel. pot. | Rel. pot. | Valency
|-------|-----------|--------|---------|---------------|-----------|-----------|
| 1     | l-Fucose  | l-Fuc  | 1       | 11 730 ± 9000 | 1         | 1         | 1
| 2     | 3         | l-Fuc  | 52 (5.6%) | 0.63 ± 0.2    | 18 619    | 358
| 3     | 4         | l-Fuc + MNPG (1 : 1) | 52 (5.6%) | 1.1 ± 0.6    | 10 663    | 205
| 4     | 5         | MNPG   | 35 (6%)  | No inhibition | —         | —         |

Determinant in an ELISA-like assay with CTB$_4$-biotin (15.3 µg mL$^{-1}$ final concentration) and wells coated with PAA-fucose. Relative to the potency of l-fucose.


