Second Generation Mimics of Ganglioside GM1 as Artificial Receptors for Cholera Toxin: Replacement of the Sialic Acid Moiety

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Abstract—In a program directed towards the design and synthesis of mimics of ganglioside GM1, the NeuAc recognition domain was replaced by simple hydroxoy acids, and the affinity of the new ligands to the cholera toxin was determined by fluorescence spectroscopy. The (R)-lactic acid derivative 4 was found to display the highest affinity of the series (K_D = 190 µM).

Carbohydrate–protein recognition events have emerged as a potential target for the cure of many human diseases, and the development of glycomimetic molecules, i.e., molecules that behave like structural and functional mimics of oligosaccharides, has attracted much attention. One of the best characterized protein–carbohydrate complexes is formed by ganglioside GM1 (Galβ1-3 GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ1-1 Cer) and two AB5 hexameric proteins, homologous by 80% and sharing a common mechanism of action: the cholera toxin from Vibrio cholerae (CT), or the heat-labile toxin of Escherichia coli (LT). Both toxins recognize the oligosaccharide headgroup of ganglioside GM1 (o-GM1) on the host cell epitelial surface using the B5 pentamer. Biochemical and structural data have shown that five binding sites are present on the toxin and that the carbohydrate binds to them using the two terminal sugars at its non-reducing end, a galactose (Gal) unit and a sialic acid (NeuAc) residue, the latter interacting mainly through its carboxy group. Finding new receptors for bacterial enterotoxins may lead to drugs that block their docking to the target cells, thus preventing the onset of the disease.

In the course of a program aimed to identify viable computational tools for the study of protein–carbohydrate interactions and to develop approaches to the design of glycomimetic molecules, the pseudo-oligosaccharide 2 was introduced as a functional and structural mimic of ganglioside GM1. For the design of 2, the Gal and NeuAc recognition determinants of GM1 were retained, and a new, conformationally restricted cyclohexanediol (DCCHD) was used as the scaffold element to replace the rest of the oligosaccharide. As a mimic of a 3,4-di-substituted galactose, DCCHD has many appealing features: it is easily available on a large scale and in enantiopure form; it contains two carboxy groups that represent convenient handles for conjugation to various supports, and, as a carbocycle, it should enhance the metabolic stability of the structure. Mimic 2 was found to bind CT as efficiently as o-GM1, as determined by competitive inhibition assays.

In order to simplify the molecular and synthetic complexity of 2, replacement of the NeuAc moiety by simple α-hydroxyacids was examined in the series of second generation mimics 3–5 (Scheme 1). Stereoselective sialylation is one of the unresolved problems in carbohydrate chemistry and, given the widespread occurrence of the sialic acid motif in protein-binding carbohydrates, the evaluation of functional substitutes for this residue in the context of the CT/LT model is worth pursuing. Indeed, sialylation of DCCHD represents the bottleneck of the entire reaction sequence leading to 2, and replacement of the NeuAc residue with more treatable...
chemical entities would considerably increase the accessibility of the artificial receptors. Conformational analysis of 3–5 showed that, although more flexible than 2, the (R)-lactic acid derivative 4, and to a minor extent, the glycolic acid derivative 3, should be able to simultaneously fit the galactose and the carboxy binding sites of CT using low-energy conformations. The three substrates were synthesized following the sequence reported in Scheme 1. Thus, from the enantiomerically pure diol 6, the monoethers 10–12 were synthesized using Bu₂SnO and the appropriate nucleophile (7–9). Glycosylation of the axial hydroxy group with the Galβl-3GalNAc donor 13 promoted with TMSOTf, to give the pseudotrisaccharides 14–16, from which standard removal of the protecting groups yielded 3–5.

The interaction of 1–5 with cholera toxin B5 pentamer (CTB) was studied using the intrinsic fluorescence of the Trp88 residue in the toxin binding site. Binding of 1–5 to CTB induces bathochromic shifts in the emission spectrum whose extent depends on the structure of the ligand. Thus, the resulting binding isotherms are more accurately compared by analyzing the intensity data, which requires a relatively high CTB concentration (0.5 μM, pentamer concentration). Wavelength titrations performed for 1 and 2 at lower toxin concentrations (0.1 μM) gave comparable results.

The normalized changes in fluorescence emission intensity in CTB upon titration with 1–5 are collected in

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**Scheme 1.** General sequence for the synthesis of 3–5. (a) Bu₂SnO, benzene, reflux, then Bu₂NI and 7, or 8, or 9 (50, 53, 76% yield). (b) 13 (0.5 equiv) and TMSOTf (0.3 equiv) in CH₂Cl₂, room temperature to reflux (48, 38, 38% yield). (c) H₂/Pd–C (90% yield); cat. MeONa in MeOH (80, 82, 85% yield).
calorimetric titrations, Freire and Schön have shown already occupied. In contrast, binding of a factor of 4 if an adjacent binding site on CTB is elicited by that the communications between CTB monomers does not display cooperative behavior. This suggests residue, which is present in In the series to CTB is known to occur cooperatively. Using appear to bind cooperatively to CTB, albeit with units of the toxin. Further studies are in progress to determine the mode of for CT.

Further studies are in progress to determine the mode of binding of 4, as well as to improve the affinity by varying the nature of the hydroxy acid side-chain. The hydroxy acid-containing mimics reported in this paper are easily accessible on a large scale and can be readily conjugated to aglycons. Thus they may be used to build multivalent ligands, which, ideally, should contain 5 pseudo-GM1 units capable of simultaneously interacting with the 5 B units of the toxin.

Figure 1. The effects of ligand binding on the fluorescence intensity of Trp88 in CTB. In A CTB (0.5 μM) was titrated with micromolar amounts of o-GM1 (open circles, dashed line) or of mimic 2 (closed circle, solid line), which are shown plotted against the normalized, absolute value of the relative fluorescence intensity (ΔI) measured at the emission maximum of Trp88. In B the same experiment was performed using 0.5 μM CTB and micromolar concentrations of 4 (open triangles, dashed line), 3 (closed circles, solid line) and 5 (open squares, dashed line) or 2 μM CTB and micromolar concentrations of Galβ1-3GalNAc-OAllyl 17 (closed triangles, dotted line).

Acknowledgements

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References and Notes

10. Calculations were performed using MacroModel (Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caulfield, C.; Chang, G.; Hendrickson, T.; Still, W. C. J. Comp. Chem. 1990, 11, 440) and following the protocols described for 1 and 2 in refs 5 and 6.

13. The cholera toxin B5 pentamer (CTB) was from List Biological Laboratories Inc. (Campbell, CA). CTB was resuspended to give the desired concentration in 0.1 M Tris, 0.4 M NaCl, 2 mM Na₂EDTA, 6 mM NaN₃, pH 7.5. Fluorescence measurements were made with a Perkin–Elmer LS-50 spectrofluorometer. The excitation wavelength was 280 nm, and the spectrum was recorded between 300 and 400 nm.

14. The observed emission maximum shifts (Δλ) under saturation conditions were as follows: 1 12.3 nm, 2 12 nm, 3 14.2 nm, 4 12 nm, 5 7.3 nm. The observed ΔΙ under saturation conditions at CTB 0.5 μM were: 1 +6.5, 2 −3.6, 3 −18.3, 1 −13.5, 5 −23.4.