^{Pk}–STARFISH: The Next Generation

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Background

Expression of Shiga like toxins (SLTs) by certain strains of pathogenic bacteria, i.e. E. coli 0157, is the major virulence factor, responsible for the onset of diarrheal diseases, and in some cases this leads to serious clinical complications such as Hemolytic Uremic Syndrome (HUS). Inhibition of binding of the pentameric B subunit of SLT to its natural receptor Gb_3 also called CD77, is envisioned as a means to alleviate or prevent the severe toxic effects and should offer a valuable therapy for this life−threatening disease.

Small oligosaccharides such as the Pk trisaccharide of Gb3 have intrinsically low affinity for SLT binding sites. Multivalent ligands often show enhanced activity that results both from an increased number of specific and non−specific interactions as well as from cross−linking of the receptor. These favourable factors are opposed by a generally unfavorable entropy of binding and merely increasing the number of ligands does not increase activity. As more ligands are added to a multimeric inhibitor, entropy losses and steric inaccessibility lead to increasingly smaller contributions to the activity of such inhibitors. Our design of a new class of structurally defined, receptor−tailored, multivalent inhibitors, STARFISH, presents us with the unique opportunity to explore the molecular basis of multivalent interactions in order to design tighter inhibitors.

Inhibitor design

We used the structural information[1] of the Shiga like toxin Type 1 (SLT−1) binding to the ^{Pk} trisaccharide epitope to design and then synthesize ^{Pk}–STARFISH, the first soluble and potent inhibitor of the toxin.[2] NMR, crystallographic data and comparison of data from site−directed mutants provides strong evidence that binding site 2 of SLT−1 is the most avid one followed by site 1 (c.a. 10% of site 2 activity) and the much less significant site 3. Although the original design had the objective of placing the two trisaccharides at the end of each arm of STARFISH in the two different binding sites 1 and 2, the actual mode of binding proved to be more uniform. As shown on Fig. 1 the decavalent ^{Pk}–STARFISH complexes with the 10 copies of binding site 2 on two toxin molecules and creates a 2:1 toxin:ligand complex. Thus, only 10 out of 30 putative ^{Pk} binding sites on the protein surface are engaged in binding, and the rest are hindered due to the sandwich or face−to−face arrangement. Although showing sub−nanomolar activity in solid−phase assays, ^{Pk}–STARFISH exhibited SLT−1 vero cell toxicity at only micro−molar concentrations. To enhance the specific binding of STARFISH to SLT we decided to utilize the subsidiary, less avid site 1. This iteration of the inhibitor design is based on the crystal structure of the complex between SLT−1B and decavalent STARFISH (Fig. 1). Additional Pk trisaccharide dimers were introduced to each arm of the STARFISH molecule that would allow the multivalent ligand to complex with the unoccupied binding site 1.

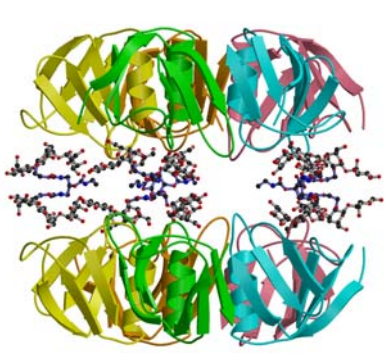
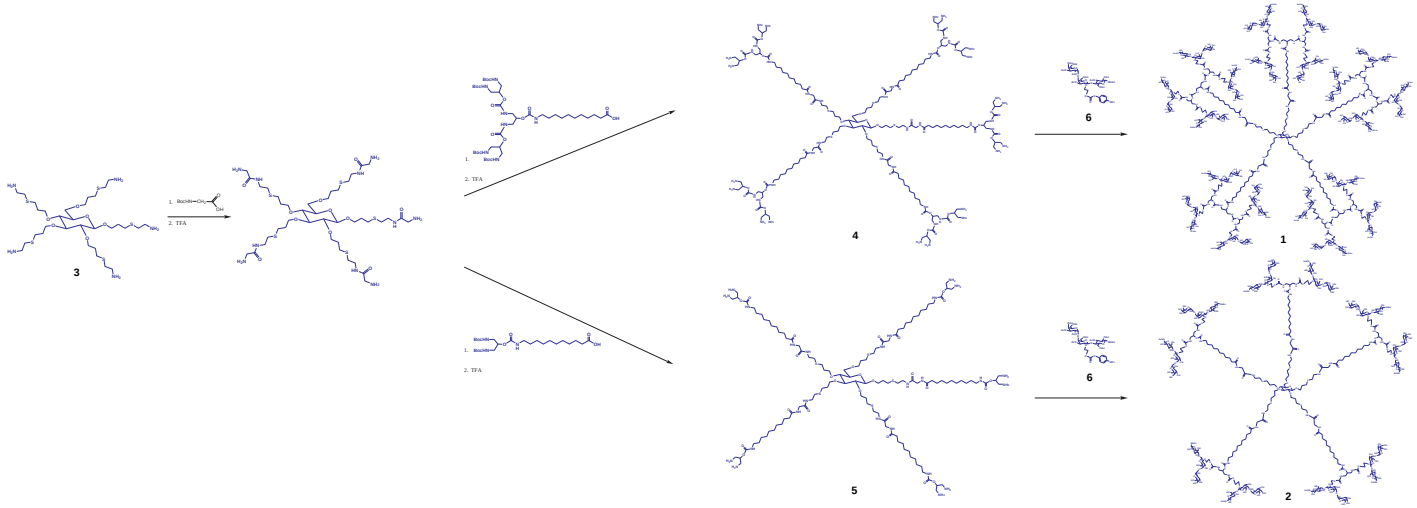


Figure 1. Crystal structure of 2:1 SLT−1/STARFISH adduct.

Synthesis

Synthesis of the key trisaccharide **6** will be described elsewhere. The poly−amino derivative **4** was obtained starting from a known penta−amine **3**. Conventional peptide chemistry was utilized to grow spacers of appropriate length, which are terminated by a tetra−amino dendrimer made from 1,3−diamino−2−hydroxy−propane. The resulting construct was coupled with activated ^{Pk}–trisaccharide derivative **6** to give after deprotection the target 20−mer STARFISH **1**. Decameric STARFISH **2** , which is a version of the original ^{Pk}–STARFISH, was obtained in an analogous fashion.



Biological Evaluation

Compounds **1** and **2** were evaluated in solid phase ELISA and vero toxicity assay as previously described[2] (Fig. 2). For SLT−1 the new ^{Pk}–STARFISH **1** inhibits the toxin at nano−molar concentrations. Presumably, the additional ligand−toxin contacts via site 1 account of the 1000 fold higher in vivo activity when compared to deca−mer **2**. Interestingly, while in case of SLT−1 the inhibition power of 20−meric STARFISH **1** is almost 3 orders of magnitude higher than that of 10−meric STARFISH **2**, for SLT−2, the activities of the two inhibitors are virtually identical. This suggests that SLT−2 may not possess the polymorphy of binding sites, which is characteristic of SLT−1/^{Pk}–trisaccharide interactions.

Crystallographic studies are in progress to confirm the ^{Pk}–trisaccharide occupancy of binding site 1 in a complex of **1** with SLT−1 and to reveal structural differences between SLT−1 and SLT−2 that account for the indiscriminate response of SLT−2 to multivalent inhibitors **1** and **2**.

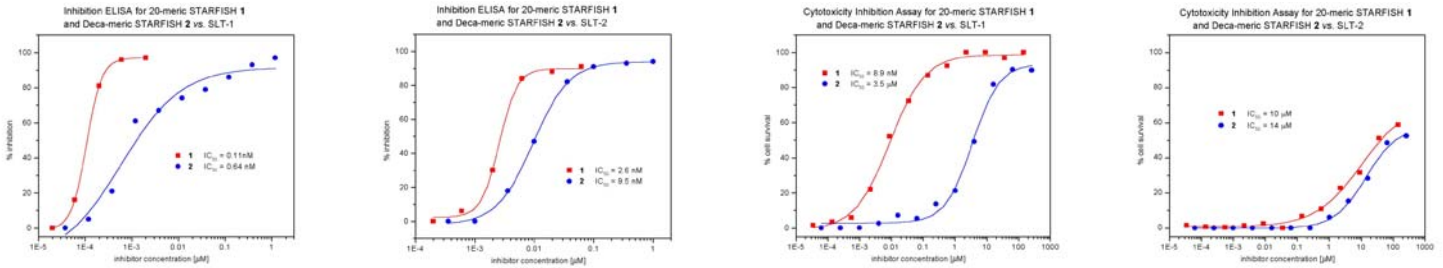


Figure 2. ELISA and Cytotoxicity Inhibition Assay.

Conclusions

- A modification of STARFISH has been synthesized, which shows SLT−1 in vivo inhibition at nano−molar concentrations.
- The activity of this 20−meric ligand with regard to Shiga−like toxin Type 2 is virtually identical to that of 10−meric STARFISH, and suggests a lack of diversity of binding modes for Pk−trisaccharide with SLT−2.

References

1. Ling, H.; Boodhoo, A.; Hazes, B.; Cummings, M. D.; Armstrong, G. D.; Brunton, J. L.; Read, R. J., *Biochemistry*., 1998, 37, 1777−1788.
2. Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; and Bundle, D. R., Nature, 2000, 403, 669−672.