Multivalent modulators of carbohydrate-processing enzymes

International context

Carbohydrate-processing enzymes (glycosidases = GH and glycosyltransferases = GT) are involved in a host of biological processes. During recent decades, tremendous efforts have been dedicated to designing inhibitors of carbohydrate-processing enzymes. Hundreds, if not thousands, of hit compounds with high affinities for their targets have been identified. However, the attention given to this unique class of compounds by both academic and industrial researchers has been poorly rewarded in terms of marketed drugs.

We explored an alternative strategy to the traditional “lock and key” concept for the design of glycosidase inhibitors. Iminosugars were grafted in a multiple fashion onto a common scaffold, potentially to provide cooperative effects, leading to a greater affinity enhancement with glycosidase targets than predicted from the sum of the constitutive interactions. This phenomenon, called the “multivalent” or “clustering” effect has been successfully exploited to design potent inhibitors of carbohydrate-binding proteins (lectins) but has rarely been investigated for carbohydrate-processing enzymes (GH and GT). In a first systematic study, we observed a small, but significant clustering effect on jack bean α-mannosidase with a gain in selectivity. Since then, several research groups have reported synthetic multivalent inhibitors for glycosidases and glycosyltransferases with strong activities. In the last five years, significant multivalent effects have been reported on biologically relevant enzymes, with a rapid growth of examples in the literature. A new part of the chemical space is currently being explored with this unique class of compounds which may provide new pharmaceutical opportunities to treat diseases involving carbohydrate-processing enzymes.

Specific developments by the group : multivalent inhibitors

After our first proof of concept for the multivalent inhibition of JBαMan, we wished to gain more insights into the binding mode operating with this enzyme. In a specific research study, we designed multivalent iminosugars with identical valencies but specific spatial presentation of the epitopes. Azido-armed iminosugars were grafted onto diverse scaffolds to form monovalent 1, tetravalent 2-6 and octavalent compounds 7-8 (Figure 8).
Very different inhibitory profiles were observed for compounds with identical valencies, indicating that the spatial distribution of the iminosugars is critical to fine-tune the enzymatic inhibitory activity. Compared to the monovalent reference 1, the best multivalent compound 6 showed a dramatic 800-fold improvement in the inhibitory potency for JBαMan, which is outstanding for just a tetravalent ligand. Atomic force microscopy performed with co-incubated solutions of the compounds with JBαMan shed light on the multivalent binding mode. The multivalent compounds were shown to promote the formation of JBαMan aggregates with different sizes and shapes (see examples with compounds 2 and 6 presented in Figure 9). The dimeric nature of JBαMan allows such intermolecular cross-linking mechanisms to occur. Multimeric glycosidases in their active pH range are therefore attractive targets for multivalent inhibition.

Specific developments by the group: polyvalent activators

We grafted iminosugars onto biocompatible dextrans to form linear and ramified polymers with unprecedentedly high valencies (from 20 to 900) to probe the evolution of the multivalent inhibition as a function of ligand valency. This study led to the discovery that polyvalent iminosugars can also significantly enhance, and not only inhibit, the enzymatic activity of specific glycoside-hydrolases, as observed on two galactosidases, a fucosidase, and a bacterial mannoside phosphorylase for which an impressive 70-fold activation was reached. The concept of glycosidase activation is largely unexplored, with only one recent example of small-molecule activators of a bacterial O-GlcNAc hydrolase (Angew. Chem. Int. Ed. 2014, 53, 13419). The possibility of using these polymers as “artificial enzyme effectors” may therefore open up new perspectives in therapeutics and biocatalysis.
Figure 9. Tetravalent iminosugars with different spatial presentations have significantly different inhibitory activities against the mannosidase JβMan and induce the formation of specific enzymatic aggregates (AFM images).

More recently, we designed magnetically recyclable glycosidase effectors boosting the activity of a α-galactosidase after 5 cycles (Figure 10).4

Figure 10. Recycling and boosting glycosidase activity. Step 1: The enzymatic substrate (paranitrophenyl-galactoside, pNP-Gal) is added to a mixture of glycosidase (AgaB) and SMan- or DNJ-coated magnetite 1 or 2. Step 2: The 2-AgaB complex enhances the kinetics of hydrolysis of the substrate. Step 3: The 2-AgaB can be recovered from the product by centrifugation and magnetic separation, allowing a re-engagement of the 2-AgaB complex in the next cycle and easy product recovery.
**Publications**


**Reviews**


**Highlight**

1- Comment augmenter l’activité enzymatique des glycosidases. CNRS website. 2015 http://www.cnrs.fr/inc/communication/direct_labs/gouin2.htm