Binding of different ligands to sialyltransferase and the human blood group B galactosyltransferase (GTB) reveal common features of these glycosyltransferases

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Abstract: Glycostructures on eukaryotic cell surfaces play an important role in development and regeneration as well as in the pathogenesis of diseases. The terminal sugars found on these glycostructures, such as the sialic acids, are crucial in the control of many biological processes. The transfer of these sugars to glycoconjugates is controlled by specific glycosyltransferases.

Using saturation transfer difference nuclear magnetic resonance (STD-NMR) methods [1] it is possible to obtain information regarding protein-ligand interactions [2]. These experiments not only allow the detection of binding, but also, the determination of the binding epitope for each ligand at an atomic resolution. The binding epitope of CMP-Neu5Ac with different mammalian and bacterial sialyltransferases reveals that the nucleotide and sugar moiety are both in intimate contact with the protein surface. Further, a fragment based approach revealed that cytosine is the smallest fragment recognised by the enzymes.

An equivalent study conducted on the human blood group B galactosyltransferase (GTB) reveals that in this case uracil is the minimal recognition fragment and that the binding is essentially controlled by the base, with the sugar recognition being only involved in the determination of reaction specificity. This is illustrated by the fact that UDP, UDP-Glc and the natural substrate UDP-Gal all have the same binding affinity.

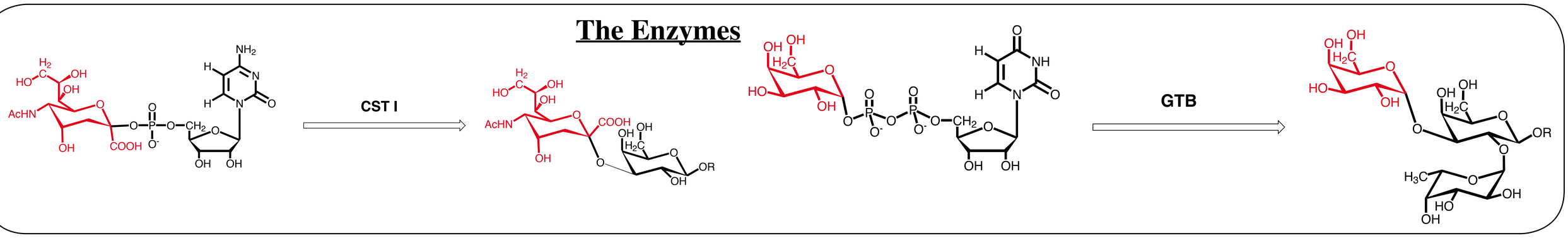
On the basis of these data, new insights into the structure and function of the active centers of sialyltransferases and GTB will be presented. In particular, the identification of common features and differences between these enzymes will be discussed.

The Method

What can STD-NMR tell us?

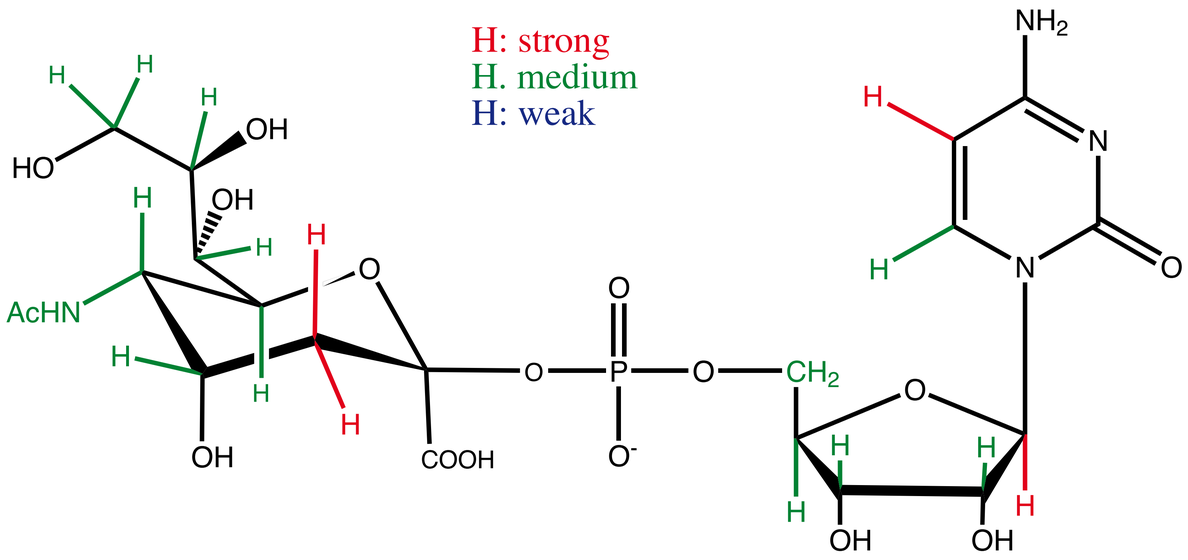
- Does a ligand bind?
- How does it bind (epitope)?
- Binding affinity

The Enzymes

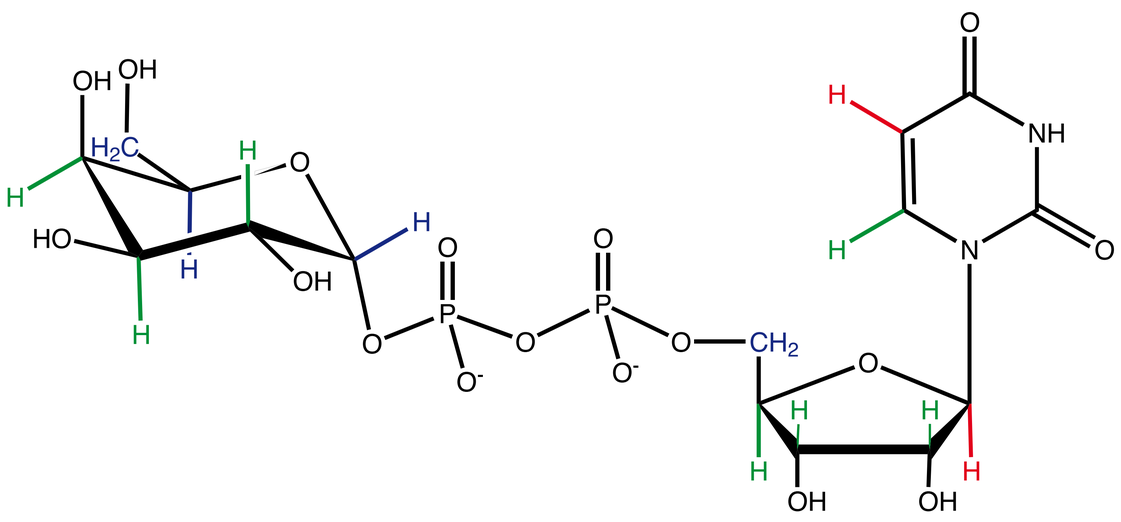


STD with natural substrates

Cst I



GTB



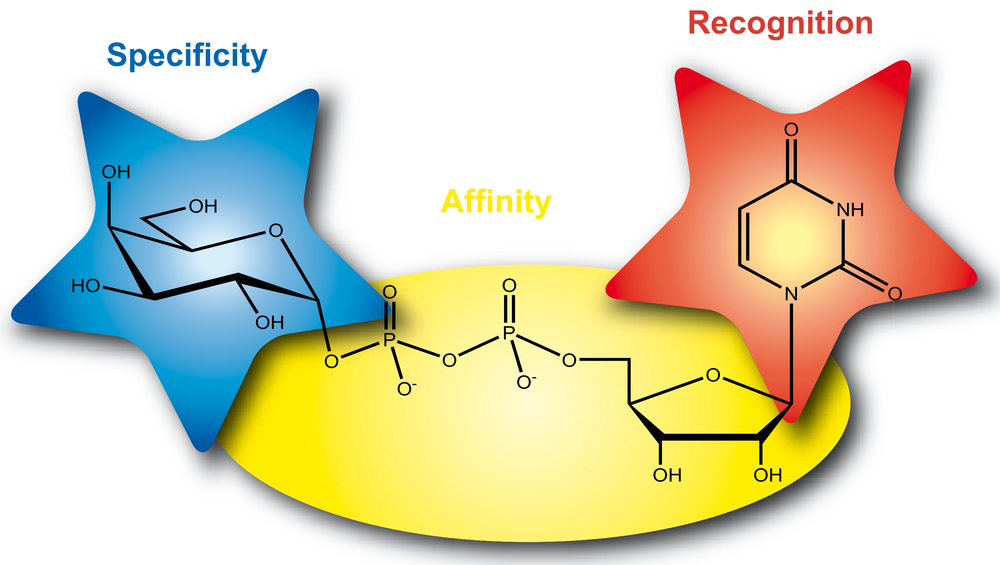
Conclusion:

- nucleotides have similar binding modes
- nucleotide + sialic acid are involved in binding to Cst I
- nucleotide dominates binding to GTB

STD & GTB

Relative binding affinity

UDP-Gal
UDP > **UMP**
Uridine > **Uracil**
Thymine



Conclusion:

- uracil smallest fragment recognised ⇒ base guides donor in binding pocket
- ribose + β-phosphate increase affinity
- several UDP-sugars bind to GTB, but only UDP-Gal is transfered ⇒ pyranose controls specifity

References:

[1] Mayer, M. and Meyer, B. (1999), *Angew. Chem. Int. Ed.* **38**, 1784-1787

[2] Meyer, B. and Peters, T. (2003), *Angew. Chem. Int. Ed.* **42**, 864-890