

A complex reaction cascade for the biocatalysis of sialyllactose

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Motivation

- Sialyllactose (SL) is one of the human milk oligosaccharides (HMO's)
- HMO's support innate immunity by preventing attachment of potential pathogens to the intestinal lining and play an important role for the growth and development for infants especially in the first months of their life ^[1]
- More than 100 HMO's are identified and 10-30 % of them are sialylated, with SL as the most abundant sialylated oligosaccharide ^[1]
- SL has not been added to infant formula so far, while other HMO's like 2'-fucosyllactose or lacto-N-tetraose are already part of it ^[2]
- The development of a biocatalytic process reveals the opportunity for the application of SL in infant formula
- Lactose can be sialylated to 3'- and 6'-SL (see Fig. 1)
- The complex biocatalytic reaction cascade is shown in Fig. 2

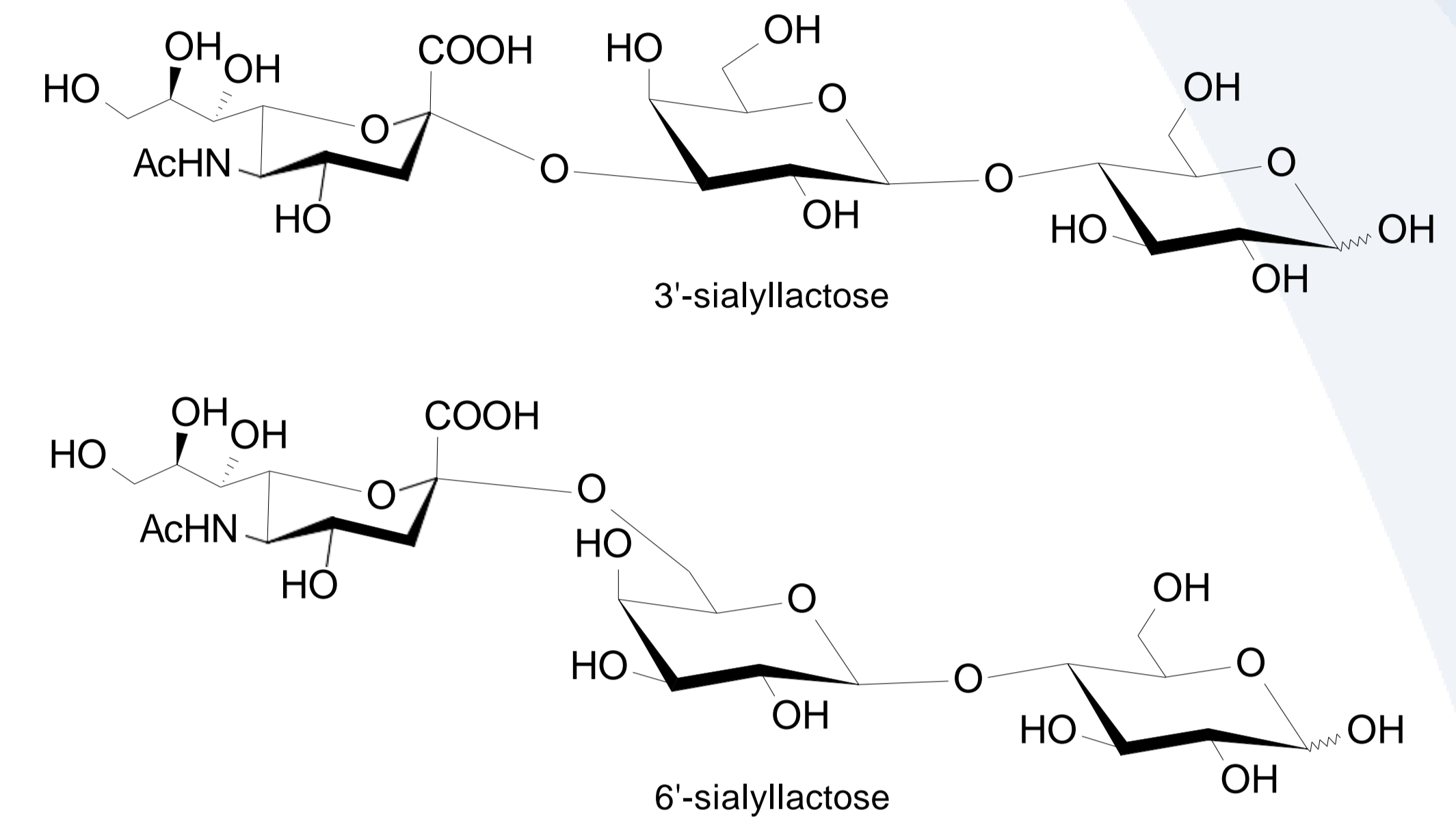


Fig. 1: Structure of 3'- and 6'-sialyllactose

Approach

- The cascade starts from N-acetylglucosamine (GlcNAc) as an inexpensive substrate

- Epimerization from GlcNAc to N-acetylmannosamine (ManNAc) catalyzed by GlcNAc-2-epimerase (EC 5.1.3.8) or by alkaline treatment ^{[3][4]}

- Biotransformation to the activated form of Neu5Ac: CMP-Neu5Ac

- Catalyzed by CMP-Neu5Ac-synthase (EC 2.7.7.43) with cytidinetriphosphate (CTP) ^[7]

- Sialyltransferases (EC 2.4.99.-) transfers Neu5Ac from CMP-Neu5Ac to lactose with sialyllactose as product ^[8]

- An α -2,3-sialyltransferase is used to produce 3'-sialyllactose

- An α -2,6-sialyltransferase is used to produce 6'-sialyllactose

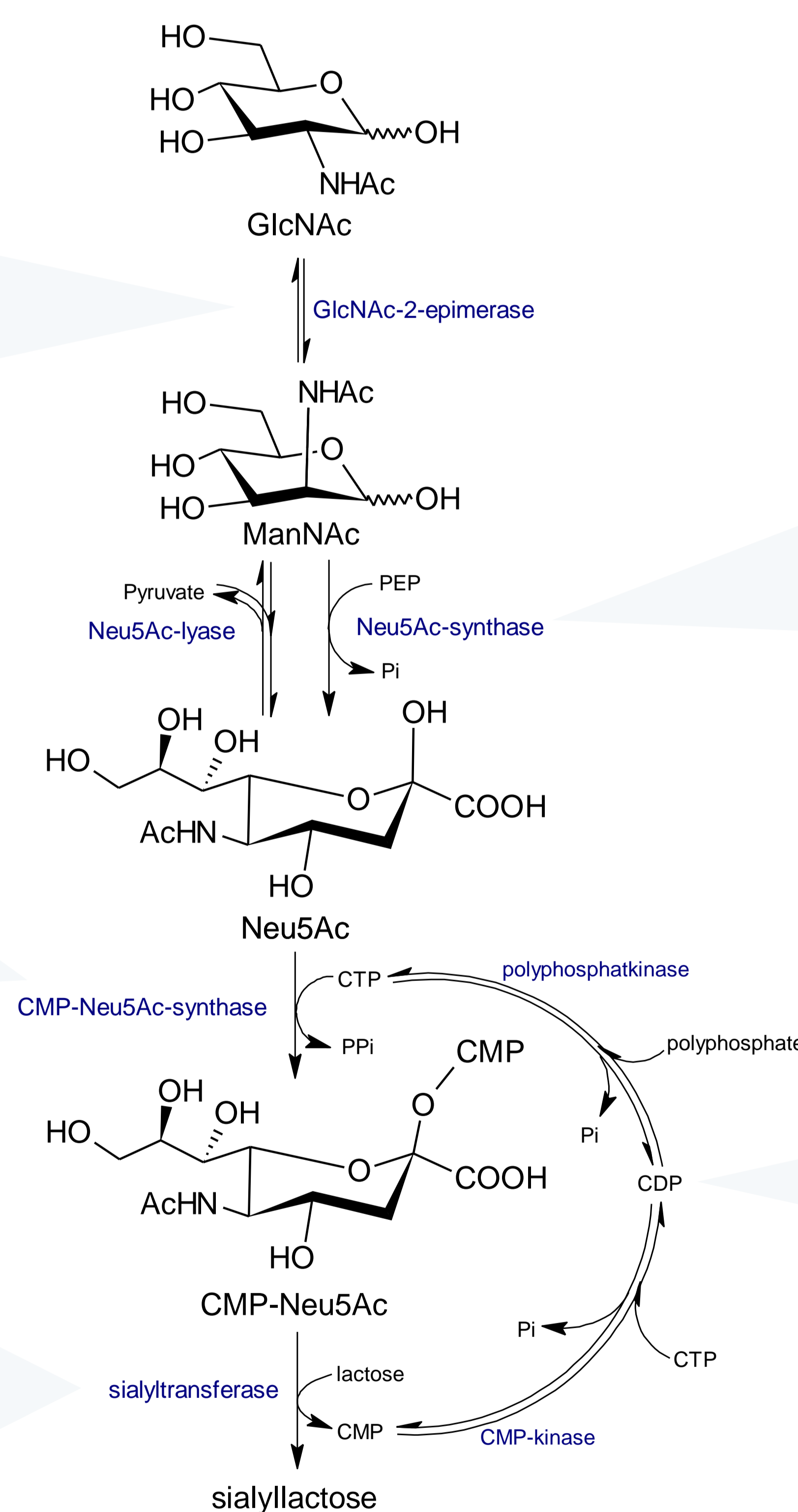


Fig. 2: Biocatalytic synthesis of sialyllactose

- N-acetylneuraminic acid (Neu5Ac) is synthesized as a precursor of SL
- Two possible ways:
 - catalyzed by Neu5Ac-lyase (E.C. 4.1.3.3) with pyruvate ^[5]
 - catalyzed by Neu5Ac-synthase (EC 2.5.1.56) with phosphoenolpyruvate (PEP) ^[6]

- A CTP regeneration system is necessary to reduce the costs of the expensive substrate CTP

- CTP can be regenerated in a two step enzyme cascade with CMP-kinase (EC 2.7.4.25) and polyphosphatkinase (EC 2.7.4.1) ^{[9][10]}

Summary & Outlook

The biocatalytic synthesis of sialyllactose is a complex reaction cascade with different enzymes starting from GlcNAc as an inexpensive substrate. Several possible ways for biocatalytic synthesis of Neu5Ac as a precursor for SL have been examined in the last 30 years, while the step from Neu5Ac to SL is not well investigated so far. Many different sialyltransferases have been characterised, but rarely implemented for SL production.

Inhibitions and enzyme stabilities might be a bottleneck for the process, where individual solutions have to be found. This reaction cascade can be implemented in different ways. An application of free enzymes, immobilized enzymes or whole-cells can be considered for the process. The establishment of a cost-efficient process opens many possibilities for the future.

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