Process development for gram-scale synthesis of nucleotide sugars using in-vitro enzyme cascades

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Large-scale enzymatic synthesis of functional oligosaccharides and glycoconjugates such as human milk oligosaccharides and glycoproteins using Leloir glycosyltransferases is still limited by the price and availability of their building blocks, nucleotide sugars. We have successfully developed in-vitro multi-enzyme cascades consisting of up to six recombinant enzymes to produce the nucleotide sugars UDP-N-acetylglucosamine (UDP-GlcNAc), UDP-galactose (UDP-Gal) and UDP-N-acetylgalactosamine (UDP-GalNAc) from inexpensive precursors, i.e. uridine or UMP, polyphosphate and GlcNAc, Gal and GalNAc, respectively. For further cost reduction, the relatively expensive co-substrate ATP is in situ regenerated from polyphosphate. Utilizing a set of duet vectors, each cascade is expressed in one single E. coli strain.

Using cell lysate as biocatalyst formulation, UDP-GlcNAc was produced with a conversion yield approaching 100 % with respect to 100 mM UMP in a batch process at 100 mL scale within 24 h. The final product concentration was 61 g/L. Using the same set-up, UDP-Gal can be produced with a conversion yield of 90 % and a final product titer of 51 g/L. For the synthesis of UDP-GalNAc, a conversion yield of 86 % with respect to 50 mM uridine and a final product titer of 26 g/L were obtained within 24 h in a 50 mL batch process.

As an initial purification step, ultrafiltration with a cut-off of 10 kDa can be applied for protein removal. To further improve product purity, a scalable anion exchange chromatography protocol was established using HiTrap Q HP columns – a strong anion exchange resin. With a dynamic binding capacity of approximately 24 mgnucleotide/mLresin, milligram-scale purification per run utilizing mL-scale columns is possible.

Bibliographic references:

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