Production of sialylated human milk oligosaccharides in optimized enzymatic one-pot reactions

Sabine Schelch\textsuperscript{a}, Barbara Petschacher\textsuperscript{a,b}, Stefanie Gross Belduma\textsuperscript{a,b}, Manuel Ebinger\textsuperscript{a}, Uma Reddy\textsuperscript{a}, Jürgen Kuballa\textsuperscript{a}, Bernd Nidetzky\textsuperscript{a,b}

\textsuperscript{a} Austrian Centre of Industrial Biotechnology, 8010 Graz, Austria.
\textsuperscript{b} Institute of Biotechnology and Biochemical Engineering, NAWI Graz, TU Graz, 8010 Graz, Austria
\textsuperscript{c} GALAB Laboratories GmbH, 21029 Hamburg, Germany

email: sabine.schelch@acib.at

Human milk oligosaccharides (HMOs) play a key role in the development of infants. Especially sialylated HMOs are an essential element for growth of the infant’s brain\textsuperscript{1} as CMP-N-acetyl neuraminic acid, which acts as a sialyl donor, is quite cost-intensive. Sialylated HMOs can be produced via enzymatic cascades in one-pot reactions from cheaper substrates, such as N-acetyl-O-mannosamine (ManNAc). The first reaction in the cascade from ManNAc to N-acetylneuraminic acid (NANA) can be catalyzed by two different enzymes, sialic acid aldolase and sialic acid synthase. So far only the aldolase has been studied in one-pot synthesis\textsuperscript{2}, here we compare both enzymes in a one-pot synthesis reaction.

Background

Enzymatic cascade

Figure 1: Reaction scheme of 3-step one-pot reaction using the enzymes sialic acid aldolase/synthase, CMP-sialic acid synthase and sialyltransferase for production of sialylated HMOs.

Preparation of enzymes

Enzymes for the production of sialylated HMOs from ManNAc were overexpressed in E.coli BL21, purified via His-tag and tested for activity.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Organism</th>
<th>Expression [mg/L cultivation]</th>
<th>Specific activity [U/mg]</th>
<th>$k_{cat} / K_{m}$ [s(^{-1})mM(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>sialic acid synthase</td>
<td>N. meningitidis</td>
<td>860</td>
<td>43</td>
<td>ManNAc: 7.07\textsuperscript{4} PEP: n.d.</td>
</tr>
<tr>
<td>sialic acid aldolase</td>
<td>L. plantarum</td>
<td>310</td>
<td>4.2</td>
<td>ManNAc: 0.03\textsuperscript{4} PEP: 0.11</td>
</tr>
<tr>
<td>CMP-sialic acid synthase</td>
<td>N. meningitidis</td>
<td>200</td>
<td>31</td>
<td>NeuAc 14\textsuperscript{4} CTP: 15\textsuperscript{10}</td>
</tr>
<tr>
<td>α,β-sialyltransferase</td>
<td>P. dactylus</td>
<td>150</td>
<td>5.6</td>
<td>Lactose: 16\textsuperscript{4} CMP: ManNAc: 14.5\textsuperscript{12}</td>
</tr>
</tbody>
</table>

Table 1: Properties of enzymes involved in production of sialylated HMOs

Results

Figure 2: Work flow of sequential 3’-sialylactose synthesis

Figure 3: TLC for qualitative analysis of sequential reaction
1. Reaction with aldolase after 7 h
2. Negative control without enzyme after 7 h
3. Reaction with aldolase after 7 h

Figure 4: Work flow of 3’-sialylactose synthesis in a one-pot

Future directions

- Identification of inhibiting factors for the synthase in one-pot reactions
- Quantitative analytics of one-pot reactions (monitoring of intermediates to detect potential inactivation and inhibition; closed balances)
- Recycling system for CTP
- Further optimization of one-pot systems to increase productivity

References:

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