Optimization of one-pot enzymatic UDP-galactose synthesis

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Uridine 5'-diphosphate-α-D-galactose (UDP-Gal) is one of the nucleotide sugars that are most relevant for glycoconjugate biosynthesis. And the regeneration of sugar nucleotides is a critical step in the biosynthetic pathway for the formation of oligosaccharides. To overcome the difficulties in the production of sugar nucleotides, we have developed a method to produce UDP-Galactose from relatively inexpensive starting materials i.e uridine 5'-monophosphate, galactose, glucose, acetylphosphate etc. For economical production of UDP-Gal, the process is composed of several steps as follows: i) conversion of UMP to UTP ii) production of UDP-Glc and glucose-1-phosphate from glucose and their regeneration iii) the main pathway to form UDP-Gal from galactose iv) ATP regeneration pathway catalysed by acetate kinase with acetylphosphate v) decomposition of pyrophosphate. The combined biosynthetic pathway involves eight enzymes, but we have used only four crude extracts from recombinant *Escherichia coli* that overexpress galactose-1-phosphate uridyltransferase (*galT*), UDP-Glc pyrophosphorylase (*galU*), galactokinase (*galK*), and uridine monophosphate (UMP) kinase (*umk*). The remaining enzymes involved in the synthetic pathway i.e acetate kinase (*ack*), glucokinase (*glk*), phosphoglucomutase (*pgm*), pyrophosphotase (*ppa*) are obtained from endogenous proteins which are available in *E.coli*'s metabolism. In previous study, the final yield of UDP-Gal was below 50%. However, we can find the rate-limiting step and increse the final yield through the optimization.

References

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