Chem Catalysis Preview

Producing alanine from methanol with a new cell-free synthetic pathway

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Summary:

Finding new alternative pathways to decouple the production of amino acids from the requirement of sugar feedstock would significantly increase the overall sustainability of such process. In this issue of *Chem Catalysis*, the Sieber group describes an elegant approach to the preparation of alanine starting from methanol.

Body:

Enzymatic cascades as a mean to efficient alternative synthetic approaches are rapidly growing in the literature and are not limited to academic achievements.[1] However, biocatalysis often appears to be more justified when it is applied to the synthesis of high value molecules such as the elegant production of the nucleoside analogue Islatravir reported recently by Merck,[2] because the cost of the development of multiple biocatalysts and the undeniable challenges of multi-step pathways should be offset by the value of the end product.

Of course, enzymes in nature are used also in many simpler metabolic and catabolic processes, and are able to assemble, *in-vivo*, a vast array of structurally complex molecules starting from extremely simple starting materials. With a more holistic view of sustainable chemistry and circular economy, the harnessing of biocatalysis to convert C1 building blocks into product of higher value brings new challenges onto the table. These processes must be efficient and broadly applicable. Excellent reviews have appeared in the literature on the use of formaldehyde, CO_2, methane, etc. as starting materials, [3–5] to name a few.

Methanol has also been explored as a feedstock mostly for microbial biotranformations,[6,7] it is highly abundant, can itself be produced from CO_2, and can be easily stored and transported. Cell-free systems which have been successful in the conversion of methanol to molecules of higher complexity are significantly more challenging and a much rarer occurrence in the literature.[8,9]

In this issue of *Chem Catalysis*, the Sieber group has assembled nine enzymes in a cascade which is capable of transforming methanol to alanine with high efficiency. The system is also cleverly recycling the cofactors (NAD and ATP) within the same multi-enzyme set-up (Fig. 1).[10]

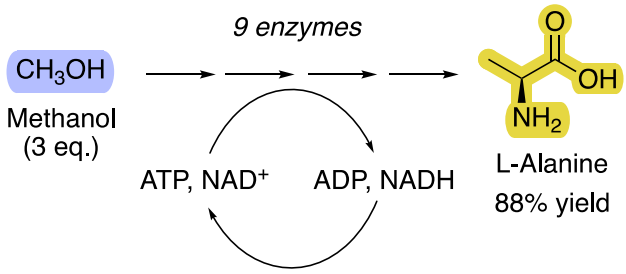

Fig. 1: Highly efficient synthesis of L-alanine from methanol

While the work describes here a nice route to one specific amino acid, as the penultimate product is pyruvate, this has major potential to develop into the synthesis of a variety of different products. The methanol to alanine pathway (MAP) expands a C1 to a C3 scaffold, requiring careful consideration of the potential inhibiting or denaturing effect of reagents such as ammonia and methanol itself, as well as the formaldehyde generated in the first step. In addition, the authors faced the problem of possible unfavourable thermodynamics of the overall cascade.
The selection of a number of extremophilic enzymes, known for their higher stability (five of the nine enzymes are from thermophilic organisms) was an excellent starting point to minimise inhibition from harsh reagents, and the initial simulation of the thermodynamic profile of the cascade showed in fact that the selected strategy was feasible.

In the designed synthetic MAP, methanol is first transformed into formaldehyde via alcohol oxidase (AOX), followed by a formolase (FLS)-mediated transformation into dihydroxyacetone. FLS low activity was compensated in the assembly by simply increasing the enzyme concentration. Effectively FLS represents the limiting step in the overall process, but this could be clearly overcome because the authors were not dependent on a much more complex whole-cell environment: the cell-free approach yielded full freedom of operation and individual adjustment of the different catalysts concentrations to deliver the final product. The optimisation of these first two steps enabled minimal accumulation of toxic acetaldehyde in the system.
In the second part of the cascade, dihydroxyacetone is phosphorylated by an ATP-dependent kinase (DhaK) and converted into D-glyceraldehyde 3-phosphate via isomerase (Tpi). DhaK is particularly sensitive to the presence of formaldehyde, the authors carefully checked the inhibitory concentration of this intermediate on all enzymes, and to further improve the system the authors envisage that a mutant variant with a modified active site to limit the binding of formaldehyde could be beneficial to improve the overall scalability of the system. D-glyceraldehyde 3-phosphate is oxidised to 3-phosphoglycerate by an NAD-dependent dehydrogenase (GapN) and transformed into 2-phosphoglycerate by a mutase (PgM). The last three steps of the lower cascade see the dehydration of 2-phosphoglycerate into phosphoenolpyruvate (by EnO catalysis) followed by dephosphorylation to pyruvate by pyruvate kinase (PyK) with concomitant regeneration of ATP. Finally, the reductive amination of pyruvate to alanine via alanine dehydrogenase (AlaDH) yields the final product with recycling of the NAD^+.

With such a complex system, the authors observed initially a 2 mM alanine formation from 20 mM methanol which, while being clearly suboptimal, offered plenty of room for

improvement. Many parameters were carefully optimised, such as the required manganese concentrations which was needed by PgM but impacted significantly both DhaK and PyK, or the effect of ATP/ADP concentration on the different kinases. The optimisation of the lower cascade drove the conversion of dihydroxyacetone to alanine up to 90%. Shifting back the attention to the first part of the cascade, and further adjustment of the enzyme concentration to better withstand the effect of methanol and formaldehyde, increased the alanine yield to 35% (7 mM from 60 mM methanol).
The authors fully revised the different enzyme concentrations to maximise the efficiency of the system. While this may appear straightforward, it was a very careful undertaking which had to consider the kinetics of the different catalysts as well as their stability. Interestingly, at higher concentrations of methanol (150 mM) the initial conversion of alanine had a lower rate (2.3 mM/h vs 2.7 mM/h at 60 and 90 mM of methanol), yet very similar final yields could be achieved. The swap of the initially selected FLS with a different, more thermostable variant, and further finetuning of reaction parameters such as rate of evaporation and oxygen supply, led to the production of 44 mM L-alanine from 150 mM methanol which is equivalent to a theoretical yield of 88%.

This interesting proof of concept clearly shows the potential of enzymatic cascades in the production of even simple molecules from renewable feedstock. Of course, this is not yet ready to be applied in larger than research lab scale, as the authors recognise that the quantity of enzymes required is too high to be feasible for production, but it is nonetheless an incredible step forward. Different enzyme variants with higher efficiency and stability would not be impossible to obtain, as it was shown for other lengthy cascades,[2] and an overview of alternative approaches for the synthesis of L-alanine from glucose for example, are not miles away.

Overall, the research presented by Sieber and co-workers is adding yet another piece of evidence that enzymes can be used for the production of not just high value molecules, but also could be exploited for the conversion of highly abundant and low cost reagents such as methanol to more complex, albeit still simple, building blogs in the context of a circular economy.

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