Precisely Patterned Nanofibers for
High Performance Bioseparations

BIOSYNTHESIS OF L-AZIDOHOMOALANINE

Contents

Biosynthesis of L-azidohomoalanine

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Abbreviations

Abbreviation / Acronym	
Aha	L-azidohomoalanine
HSAT	L-homoserine acetyl transferase
ncAA	non-canonical amino acid
OAHS	*O*-acetyl-L-homoserine sulfhydrylase

1. Introduction

The non-canonical amino acid (ncAA) L-azidohomoalanine (Aha) is a methionine analog that can be introduced into proteins in *E. coli* instead of methionine by the supplementation of a methionine-auxotrophic strain [1]. The azido group in the side chain of Aha is reactive and selectively reacts with ligands carrying terminal alkynes in the presence of copper (I) (copper-catalyzed azide-alkyne cycloaddition) or strained alkynes (copper-free, strain-promoted azide-alkyne cycloaddition) [2]. Such bioorthogonal conjugation reaction allows the directed and controlled modification of proteins [3].

Deliverable 2.1 includes the biosynthesis of at least 500 mg of Aha. To achieve this deliverable, we employed the published biosynthesis pathway for Aha [4] and produced Aha in fed-batch *E. coli* bioreactor cultures.

The biosynthesis pathway transforms the cellular metabolite L-homoserine to Aha in two enzymatic steps: First, the cellular metabolite L-homoserine is *O*-acetylated with acetyl-CoA to *O*-acetyl-L-homoserine by the enzyme L-homoserine acetyl transferase (HSAT). In the second step, *O*-acetyl-L-homoserine is transformed into Aha in the presence of sodium azide (NaN_3) by the enzyme *O*-acetyl-L-homoserine sulfhydrylase (OAHS).

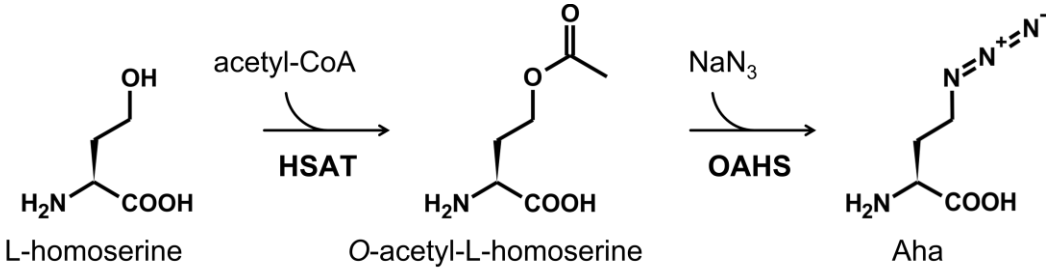

Figure 1 Biosynthesis of L-azidohomoalanine from L-homoserine, acetyl-CoA and sodium azide, NaN_3.

2. Production of Aha in fed-batch bioreactor cultures

We performed fed-batch cultivations of an *E. coli* strain producing HSAT and OAHS at the 1.2 L scale and supplemented the cultures with sodium azide. A negative control culture remained without NaN_3 supplementation.

The accumulation of Aha was analyzed in the medium supernatant using thin layer chromatography run under published conditions [5].

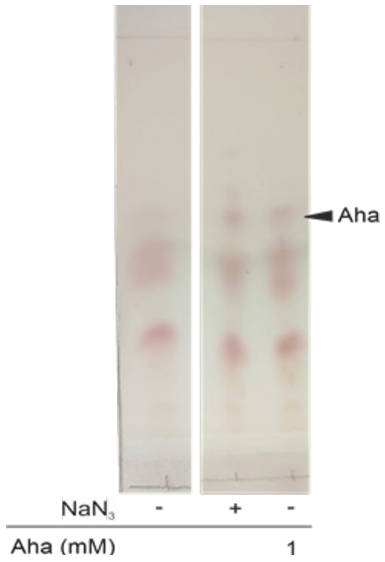

Figure 2 Biosynthesized L-azidohomoalanine in culture supernatants of fed-batch E. coli cultures equipped with the corresponding biosynthesis pathway. NaN_3 was added to the culture (+) or not (-). For the concentration determination, the culture supernatant of the culture without NaN_3 treatment was spiked with 1 mM commercial Aha.

Formation of Aha was detected in HSAT/OAHS producing strains only when the medium was supplemented with sodium azide. In total, we were able to biosynthesize at least 500 mg Aha.

3. Summary

At least 500 mg of Aha were biosynthesized from sodium azide in fed-batch cultures of *E. coli* equipped with the corresponding biosynthesis pathway.

References

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