The Bifunctional Enzyme, GenB4, Catalyzes the Last Step of Gentamicin 3',4'-Di-Deoxygenation via Reduction and Transamination Activities

CURRENT STATUS: ACCEPTED

Microbial Cell Factories BMC

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DOI:

10.21203/rs.2.18758/v1

SUBJECT AREAS

Applied & Industrial Microbiology

KEYWORDS

gentamicin, di-deoxygenation, GenB4, reduction activity, transamination activity

Abstract

Background

New semi-synthetic aminoglycoside antibiotics generally use chemical modifications to avoid inactivity from pathogens. One of the most used modifications is 3',4'-di-deoxygenation, which imitates the structure of gentamicin. However, the mechanism of di-deoxygenation has not been clearly elucidated.

Results

Here, we report that the bifunctional enzyme, GenB4, catalyzes the last step of gentamicin 3',4'-di-deoxygenation via reduction and transamination activities. Following disruption of *genB4* in wild-type *M. echinospora*, its products accumulated in 6'-deamino-6'-oxoverdamicin (**1**), verdamicin C2a (**2**), and its epimer, verdamicin C2 (**3**). Following disruption of *genB4* in *M. echinospora* Δ *genK*, its products accumulated in sisomicin (**4**) and 6'-N-methylsisomicin (**5**, G-52). Following *in vitro* catalytic reactions, GenB4 transformed sisomicin (**4**) to gentamicin C1a (**9**) and transformed verdamicin C2a (**2**) and its epimer, verdamicin C2 (**3**), to gentamicin C2a (**11**) and gentamicin C2 (**12**), respectively.

Conclusion

This finding indicated that in addition to its transamination activity, GenB4 exhibits specific 4',5' double-bond reducing activity and is responsible for the last step of gentamicin 3',4'-di-deoxygenation. Taken together, we propose three new intermediates that may refine and supplement the specific biosynthetic pathway of gentamicin C components and lay the foundation for the complete elucidation of di-deoxygenation mechanisms.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

Figure 1

Biosynthetic pathway of gentamicin.

Figure 2

HPLC-ELSD analysis of fermentation production by *M. echinospora* wild type and mutants.

Figure 3

Analysis of GenB4-catalyzed reactions. a) HPLC-ELSD analysis of GenB4 enzymatic assays. b) Proposed structure of the catalysate. c) Mass spectra analysis of compound (8). [M+H]⁺ m/z 449, [M+H₂O+H]⁺ m/z 467.

Figure 4

Mass-spectra analyses of compound (8) reduced with NaBH₄ or NaBD₄. a) Mass-spectra analysis of compound (8) reduced with NaBH₄. b) Mass-spectra analysis of compound (8) reduced with NaBD₄.

Figure 5

HPLC-ELSD analysis of GenB4-catalyzed verdamicin C2a (2) and verdamicin C2 (3) to gentamicin C2a (11) and gentamicin C2 (12).

Figure 6

HPLC-ELSD analysis of 6'-deamino-6'-oxoverdamycin (1) catalyzed by GenB4.

Figure 7

HPLC-ELSD analysis of the transformations of (1), (2), and (3).

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