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

Xiao tang Chen
Shenyang Pharmaceutical University

✉ 376416460@qq.com *Corresponding Author*
ORCID: <https://orcid.org/0000-0003-0467-1488>

Hui Zhang
Shenyang Pharmaceutical University

Shaotong Zhou
Shenyang Pharmaceutical University

Mingjun Bi
Shenyang Pharmaceutical University

Shizhou Qi
Shenyang Pharmaceutical University

Huiyuan Gao
Shenyang Pharmaceutical University

Xianpu Ni
Shenyang Pharmaceutical University

Huanzhang Xia
Shenyang Pharmaceutical University

DOI:

10.21203/rs.2.18758/v2

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 gen B4 in wild-type *M. echinospora*, its products accumulated in 6'-deamino-6'-oxoverdamycin (1), verdamicin C2a (2), and its epimer, verdamicin C2 (3). Following disruption of gen B4 in *M. echinospora* Δ gen K, 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'-oxoverdamicin (1) catalyzed by GenB4.

Figure 7

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

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

fig S13.pdf
 fig S5.pdf
 fig S12.pdf
 fig S11.pdf
 fig S9.pdf
 fig S8.pdf
 fig S10.pdf
 fig S4.pdf
 table S1.pdf
 fig S7.pdf
 fig S6.pdf
 fig S2.pdf
 fig S1.pdf
 fig S3.pdf