Full Paper

The Thioesterase Bhp is Involved in the Formation of β-Hydroxytyrosine during Balhimycin Biosynthesis in *Amycolatopsis balhimycina*

1. Sri Mulyani Dr.[1,2],
2. Ellen Egel[1],
3. Claudia Kittel Dr.[3],
4. Suada Turkanovic[4],
5. Wolfgang Wohlleben Prof.[3],
6. Roderich D. Süssmuth Prof.[4],
7. Karl-Heinz van Pée Prof.[1]

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Author Information

1 Biochemistry, TU Dresden, 01062 Dresden (Germany), Fax: (+49) 351-463-35506

[2] Prodi P. Kimia, FKIP, Universitas Sebelas Maret, Jl. Ir. Sutami 36 A, Surakarta 57126 (Indonesia)

[3] Lehrstuhl für Mikrobiologie/Biotechnologie, Eberhard-Karls-Universität Tübingen, Auf der Morgenstelle 28, 72076 Tübingen (Germany)

[4] Institut für Chemie/FG Organische Chemie, Technische Universität Berlin, TC Gebäude TC2, Strasse des 17. Juni 124, 10623 Berlin (Germany)

Email: Karl-Heinz van Pée Prof. (karl-heinz.vanpee@chemie.tu-dresden.de)

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Abstract

The putative hydrolase gene bhp *from the balhimycin biosynthetic gene cluster has been cloned and overexpressed in* Escherichia coli. *The corresponding enzyme Bhp was purified to homogeneity by nickel-chelating chromatography and characterized. Although Bhp has sequence similarities to hydrolases with "haloperoxidase"/perhydrolase activity, it did not show any enzymatic activity with standard "haloperoxidase"/perhydrolase substrates (e.g., monochlorodimedone and phenol red), nonspecific esterase substrates (such as* p*-nitrophenyl acetate,* p*-nitrophenyl phosphate and* S*-thiophenyl acetate) or the model lactonase substrate dihydrocoumarin. However, Bhp could be shown to catalyse the hydrolysis of* S*-β-hydroxytyrosyl-*N*-acetyl cysteamine thioester (β-OH-Tyr-SNAC) with 15 times the efficiency of* S*-*L*-tyrosyl-*N*-acetyl cysteamine thioester (*L*-Tyr-SNAC). This is in agreement with the suggestion that Bhp is involved in balhimycin biosynthesis, during which it was supposed to catalyse the hydrolysis of β-OH-Tyr-*S*-PCP (PCP=peptidyl carrier protein) to free β-hydroxytyrosine (β-OH-Tyr) and strongly suggests that Bhp is a thioesterase with high substrate specificity for PCP-bound β-OH-Tyr and not a "haloperoxidase"/perhydrolase or nonspecific esterase.*

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