

Full Paper

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

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- Abstract
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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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