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Identification of the Key Enzyme of Roseoflavin Biosynthesis

Authors : V. Konjik, J. Schwartz, R. Sandhoff, M. Mack

Abstract : The rising number of multi-resistant pathogens demands the development of new antibiotics in order to reduce the lethal risk of infections. Here, we investigate roseoflavin, a vitamin B2 analogue which is produced by Streptomyces davawensis and Streptomyces cinnabarinus. We consider roseoflavin to be a 'Trojan horse' compound. Its chemical structure is very similar to riboflavin but in fact it is a toxin. Furthermore, it is a clever strategy with regard to the delivery of an antibiotic to its site of action but also with regard to the production of this chemical: The producer cell has only to convert a vitamin (which is already present in the cytoplasm) into a vitamin analog. Roseoflavin inhibits the activity of Flavin depending proteins, which makes up to 3.5 % of predicted proteins in organisms sequenced so far. We sequentially knocked out gene clusters and later on single genes in order to find the ones which are involved in the roseoflavin biosynthesis. Consequently, we identified the gene rosB, coding for the protein carrying out the first step of roseoflavin biosynthesis, starting form Flavin mononucleotide. Here we show, that the protein RosB has so far unknown features. It is per se an oxidoreductase, a decarboxylase and an aminotransferase, all rolled into one enzyme. A screen of cofactors revealed needs of oxygen, NAD+, thiamine and glutamic acid to carry out its function. Surprisingly, thiamine is not only needed for the decaboxylation step, but also for the oxidation of 8-demethyl-8-formyl Flavin mononucleotide. We had managed to isolate three different Flavin intermediates with different oxidation states, which gave us a mechanistic insight of RosB functionality. Our work points to a so far new function of thiamine in Streptomyces davawensis. Additionally, RosB could be extremely useful for chemical synthesis. Careful engineering of RosB may allow the site-specific replacement of methyl groups by amino groups in polyaromatic compounds of commercial interest. Finally, the complete clarification of the roseoflavin biosynthesis opens the possibility of engineering cost-effective roseoflavin producing strains.

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