Abstract

Antitumor pyrrolobenzodiazepines (PBDs), lincosamide antibiotics, quorum sensing molecule hormaomycin, and antituberculotic griselimycin are structurally and functionally diverse groups of actinobacterial metabolites. The common feature of these compounds is the incorporation of L-tyrosine- or L-leucine-derived 4-alkyl-L-proline derivatives (APDs) in their structures. APD biosynthesis involves a set of up to six homologous proteins. According to their proposed order in the biosynthesis of 4-propyl-L-proline, a model APD of lincosamide lincomycin, the homologous proteins were named Apd1 – Apd6. Here, we report that the last reaction in the biosynthetic pathway of APDs, catalyzed by $F_{420}H_2$-dependent Apd6 reductases, contributes to the structural diversity of APD precursors.

Specifically, the heterologous overproduction and *in vitro* tests of six Apd6 enzymes demonstrated that Apd6 from the biosynthesis of PBDs and hormaomycin can reduce only an endocyclic imine double bond, whereas Apd6 LmbY and partially GriH from the biosyntheses of lincomycin and griselimycin, respectively, also reduce the more inert exocyclic double bond of the same 4-substituted Δ^1-pyrroline-2-carboxylic acid substrate, making LmbY and GriH unusual, if not unique, among reductases.

The two successive $F_{420}H_2$-dependent reduction steps proceeds through hydride transfer by a unique mechanism, which has not been described in the literature. In this work, we proposed and experimentally confirmed this mechanism. Furthermore, the work aimed to elucidate the nature of the different specificity of Apd6 homologs. The active site of the Apd6 proteins was proposed, then key amino acid residues were predicted and mutated. To obtain a direct evidence explaining the unusual distinction in the Apd6 protein reaction specificity, conditions for protein crystallization were developed.

Finally, a bioinformatic analysis of the Apd6 proteins revealed that bioactive metabolites bearing the APD motive in their structure are not as rare as previously presumed. It seems that Apd6 and their homologues play a role in the formation of new, yet undiscovered natural substances, and in the central metabolism of Bacteria and Archaea.

Apd6 reductases elucidated in this work establish the first $F_{420}H_2$-dependent enzymes from the luciferase-like hydride transferase protein superfamily from *Streptomyces* and in the biosynthesis of bioactive molecules at all. The universal contribution of this work is the extension of the still very limited understanding of the role of F_{420}-dependent enzymes in Actinobacteria, which contrasts with a significantly more solid knowledge in Archaea.

Key words

specialized metabolites, Actinobacteria, cofactor F_{420}, redox reactions, reaction specificity, lincomycin, pyrrolobenzodiazepines, hormaomycin, griselimycin