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The synthesis of vitamin B₁₂ in mycobacteria

ABSTRACT

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. Every year, approximately 1.5 million people die due to this disease. Effective control of tuberculosis is recognized as one of the main goals of modern medicine. However, the growing drug resistance of bacteria difficult treating of this disease. There is the *rv2228c* gene in the *M. tuberculosis* genome which could be one of the potential molecular targets for new anti-mycobacterial drugs. The homolog of Rv2228c protein in the model strain of *Mycobacterium smegmatis* is MSMEG4305. The MSMEG4305 protein consists of an N-terminal domain homologous with eukaryotic and prokaryotic RNase H and a C-terminal domain with acid phosphatase activity with a potential role in the synthesis of vitamin B₁₂. A puzzling problem regarding *M. tuberculosis* is its metabolism of cobalamin. Vitamin B₁₂ also known as cobalamin is a water-soluble structurally complex molecule that is thought to affect *M. tuberculosis* metabolism through two mechanisms:

- i) acting as a cofactor for three enzymes: methionine synthase, methylmalonyl-CoA mutase, and ribonucleotide reductase and by
- ii) regulating gene expression by attaching to riboswitches in mRNA.

The aim of the study was to determine the role of vitamin B₁₂ for mycobacterial cells by:

- assessment of the possibility of vitamin B₁₂ synthesis by *M. tuberculosis*,
- determination of the contribution of CobC domain in the synthesis of vitamin B₁₂ in *M. smegmatis* cells,
- phenotypic analysis of *M. smegmatis* mutant with impaired the pathway of vitamin B₁₂ biosynthesis,
- identification of environmental factors affected on the biosynthesis of vitamin B₁₂.

The evaluation of the possibility of vitamin B₁₂ synthesis by *M. tuberculosis* was made by estimating the level of synonymous and non-synonymous substitution in genes involved in the metabolism of cobalamin in a population of 3798 clinical strains. We showed that genes associated with the biosynthesis and transport of vitamin B₁₂ and encoding enzymes requiring cobalamin for their activity in *M. tuberculosis* are under the influence of purifying selection. The results suggest that the presence of the B₁₂ synthesis pathway genes is adaptive to bacterial cell function. Furthermore, the presence of a purifying selection acting on the vitamin B₁₂ synthesis pathway in *M. tuberculosis* cells suggests that this pathway is functional.

In the next stage of work, we studied the contribution of MSMEG4305 protein in the synthesis of cobalamin in *M. smegmatis* strain. The growth of the *M. smegmatis* mutant with deletion of the *msmeg4305* gene was analyzed on medium with propionate, and control on medium with glucose as the only carbon sources. In addition, we inactivated the *prpR* gene in the Δ *msmeg4305* mutant strain. This gene encodes the protein that regulates the expression of enzymes involved in propionate degradation. The deletion of the *prpR* gene forces the cell to launch an alternative propionate metabolizing pathway - the methylmalonyl-CoA pathway, whose main enzyme - MCM which requires a cobalamin for its activity. We also prepared a Δ *cobIJ* mutant of *M. smegmatis* with a lack of the ability to synthesize enzymes with confirmed participation in the process of vitamin B₁₂ biosynthesis in mycobacteria. We observed total inhibition of the growth of the Δ *cobIJ*/ Δ *prpR* mutant on the medium with propionate as the only carbon source and the weakening of growth of the Δ *msmeg4305*/ Δ *prpR* strain. We also performed an analysis of vitamin B₁₂ concentration in cell lysates of Δ *msmeg4305* and Δ *cobIJ* *M. smegmatis* mutants in comparison to the wild type using an enzyme-linked immunoassay. We observed lower levels of vitamin B₁₂ in *M. smegmatis* Δ *msmeg4305* mutant cells and slowing growth of Δ *msmeg4305*/ Δ *prpR* on the propionate medium. It suggests that the *msmeg4305* gene product is involved in the synthesis of vitamin B₁₂ *in vivo*, but its function may be partly fulfilled by others, unknown enzymes.

We also analyzed the effect of the CobC domain of the MSMEG4305 protein on the RNase domain. Analysis of growth curves of mutants with complementation of the entire functional *msmeg4305* gene (Δ *rnhA*/ Δ *msmeg4305-attB::P_{ami}msmeg4305*) and the shortened gene lacking the C-terminal coding domain of CobC (Δ *rnhA*/ Δ *msmeg4305-attB::P_{ami}msmeg4305_{3'tr(199AA)}*) allowed to conclude that the CobC domain has little effect on the functioning of the RNase domain in the *M. smegmatis* mutant. This conclusion is based on the observed slight inhibition of mutant growth with a CobC domain deletion

($\Delta rn h A / \Delta m s m e g 4 3 0 5 - a t t B :: P_{a m i} m s m e g 4 3 0 5_{3' t r (199 A A)}$) relative to the mutant with the complemented functional *msmeg4305* gene. It was also shown that the level of DNA\RNA hybrids in cell isolates obtained from strains $\Delta rn h A / \Delta m s m e g 4 3 0 5 - a t t B :: P_{a m i} m s m e g 4 3 0 5$, $\Delta rn h A / \Delta m s m e g 4 3 0 5 - a t t B :: P_{a m i} m s m e g 4 3 0 5_{3' t r (199 A A)}$ *M. smegmatis* was identical to the wild type.

In the next stage of the work, we tested a phenotypic analysis of the *M. smegmatis* mutant with impaired vitamin B₁₂ synthesis pathway. Preliminary phenotypic studies of the *M. smegmatis* *msmeg4305* gene deletion mutant using the BIOLOG system identified a group of compounds that affect the mutant's metabolism, including a group of chemicals involved in folate metabolism in the cell. In the next experiment, the phenotype of $\Delta m s m e g 4 3 0 5$ mutant was analyzed using the "methylfolate trap" phenomenon. We observed the sensitization of the $\Delta m s m e g 4 3 0 5$ mutant in the presence of sulfamethazine in the medium. Supplementation with cobalamin in the presence of sulfamethazine caused phenotype regression suggesting that the *msmeg4305* gene product plays an important role in cobalamin metabolism and thus affects the proper functioning of the cell.

The final stage of the study was the analysis of environmental factors that may affect the biosynthesis and accumulation of cobalamin in the cell. QRT-PCR analysis showed constitutive or reduced expression of genes involved in cobalamin metabolism. The obtained results suggest that the increased level of vitamin B₁₂ in the cell is the result of its accumulation in mycobacterial cells, not the increased expression of genes involved in the cobalamin biosynthesis pathway in *M. smegmatis*.

In summary, the results obtained in this study indicate that although genes associated with vitamin B₁₂ are not necessary for the metabolism of mycobacteria, they are important for the proper functioning of the cell by regulating metabolic pathways - methylmalonyl cycle and folic acid metabolism. The results also suggest that the *msmeg4305* gene product is involved in the synthesis of vitamin B₁₂ *in vivo*.

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miejsowość i data

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podpis