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BIOSYNTHETIC ENZYMES AND PHYSIOLOGICAL FUNCTIONS OF H_4-LIMIPTERIN FROM *CHLOROBIUM LIMICOLA*

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A new pteridine has been purified and identified from *Chlorobium limicola* F. *thiosulfatophilum* NCIB 8327 and is called limipterin named after *limicola*. The complete structure of limipterin is proposed as [1-O-(L-erythro-biopterin-2'-yl)-β-N-acetylglucosamine]. This study confirmed that tetrahydrolimipterin could be synthesized from GTP by GTP cyclohydrolase I, 6-pyruvoyltetrahydropterin (PTP) synthase, sepiapterin reductase and limipterin synthase. Biosynthetic intermediates such as H_2NTP, pyruvoyltetrahydropterin, and H_4-biopterin were identified *in vitro* using purified GTP CH I, PTP synthase, sepiapterin reductase and limipterin synthase by HPLC and TLC. The final biosynthetic enzyme, limipterin synthase had the activity that condenses H_4-biopterin with UDP-N-acetylglucosamine at the presence of 10 mM DTT and 10 mM $MnCl_2$. The molecular weight of limipterin synthase was calculated at 46,300 dalton from the calibrated Superdex 75 and subunit was estimated at 46,000 dalton. ^{3}H-limipterin added in medium was found in the cell wall-membrane portion after exponential growth. From these results, it may be reasonable to think that intracellular limipterin was released into the medium with adding sugar moiety to the cell wall during exponential cell growth. H_4-limipterin can also be used as a cofactor of aromatic amino acid hydroxylases instead of H_4-biopterin.

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PURIFICATION AND CHARACTERIZATION OF THE NUCLEAR RIBONUCLEASE P FROM ASPERGILLUS NIDULANS

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The Ribonuclease P, an endonuclease involved in 5'-end maturation of precursor-tRNA, was a ribonucleoprotein complex. *Aspergillus nidulans* had both nuclear and mitochondrial RNase P activities. These RNase P activities were separated with Q-Sepharose column and nuclear RNase P activity was distinguished from mitochondrial counterpart with precursor-$tRNA^{His}$ processing assay system. The nuclear RNase P was composed with seven polypeptides with masses of 125, 85, 45, 33, 30, 21, 19 kDa and a RNA (1039 nucleotides) component. Western blot analysis with anti-C5 antibody indicated that 125, 85, and 45 kDa polypeptides were bound with this antibody. On the basis of sequence analysis, nuclear RNase P RNA had two sequence regions that were very similar to consensus sequence regions in all RNase P RNA and the "cage-shape" model was constructed with these sequence regions like other RNase P RNA. The analysis of nuclear RNase P RNA indicated that nuclear RNase P RNA transcript was located from -79 to +960 nucleotide position in 18S rRNA of *A. nidulans*. It imply that a portion of 18S rRNA is involved in precursor-tRNA processing reaction as a RNA component of nuclear RNase P of *Aspergillus nidulans*.