E345 Characterization of recombinant 6-pyruvoyltetrahydropterin synthase from *E. coli* and *Synechococcus* sp. PCC 7942

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6-pyruvoyltetrahydropterin synthase (PTPS) catalyzing the conversion of dihydroneopterin triphosphate to 6-pyruvoyltetrahydropterin is an essential enzyme involved in the biosynthesis of tetrahydrobiopterin (BH4). Recent progress in microbial genome sequencing revealed DNA sequences of ORFs highly homologous to PTPS in most of the species studied. The E. coli genome also contains a PTPS homolog even though no BH4 is synthesized in the organism. In order to investigate the genuine enzyme activity and its role in the prokaryote, the E. coli PTPS homolog was overexpressed as a His-tag recombinant protein. The purified recombinant enzyme was demonstrated to produce BH4 in the presence of its substrate dihydroneopterin triphosphate and sepiapterin reductase (SR) catalyzing the subsequent step of reduction to BH4, indicating that the enzyme is functionally equivalent to mammalian enzymes. The E. coli PTPS was kinetically compared with another recombinant enzyme of Synechococcus sp. PCC7942, in which PTPS has an obvious function of providing substrate for SR to produce BH4.

E346 Identification and Biosynthesis of BH4-glucoside in *Synechococcus* sp. PCC 7942

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Pteridine glycosides, which contains unconjugated pteridines and various kinds of sugars attached to the side chain of C-6 of pteridine rings, are abundant in cyanobacteria. However, their physiological functions have been unknown. Recently we isolated another pteridine glycoside which is presumed to have a structure of tetrahydrobiopterin (BH4)-glucoside in *Synechococcus* sp. PCC 7942. The structure was supported by the results of HPLC analysis and enzymatic assay of a sugar transferase producing BH4-glucoside in the presence of UDP-glucose and BH4. The biosynthesis of BH4 has been well established in higher animals and also in a photosynthetic bacterium, *Chlorobium limicola*. The genes encoding the biosynthetic enzymes for BH4 could be identified in the reported DNA sequences of the organism. The GTPCH gene was already defined. The PTPS and SR gene encoding the enzymes catalyzing the last two steps towards BH4 have been identified by homology search. Furthermore, PTPS gene was confirmed by heterologous expression in *E. coli*. Therefore, our results strongly support the biosynthesis of BH4-glucoside in Synechococcus sp. PCC 7942 and will provide a useful basis for our future research on the physiological role of the pteridine glycosides in cyanobacteria.