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Porphobilinogen Synthase in the Heme Biosynthesis Pathway of R. Capsulatus

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Poster Presentation P11

PORPHOBILINOGEN SYNTHASE IN THE HEME BIOSYNTHESIS PATHWAY OF R. CAPSULATUS

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Porphobilinogen synthase is an enzyme that is essential to the biosynthesis of tetrapyrroles, such as heme and chlorophyll, in organisms from bacteria to humans. The PBGS of purple non-sulfur bacteria of the genus *Rhodobacter* are thought to be unique in that they do not require zinc in the active site of the enzyme and that the enzyme is also not stimulated by the presence of magnesium, as it is in plants and the bacterium Escherichia coli. The hemB gene from Rhodobacter capsulatus was sequenced, and showed great sequence similarity to the related species Rhodobacter sphaeroides. The hemB gene was cloned into an expression plasmid to create an expression strain of E. coli to obtain large quantities of the PBGS protein. The protein was purified from the E. coli in milligram quantities and is in the process of being crystallized to determine the threedimensional structure. Activity assays have shown that zinc, while not required in the active site, does stimulate the activity. Potassium also stimulates the enzyme activity. Magnesium, which has been shown to stimulate in some species, does not seem to stimulate the R. capsulatus PBGS. More activity assays are planned and include βmercaptoethanol, and pH tests to find the optimal pH for the enzyme activity. Other work includes making a knockout mutation of the PBGS gene in R. capsulatus as there is evidence for viable mutants in Rhodobacter sphaeroides, which is significant since heme is required for life and this pathway is the only known mechanism for making heme