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## Nucleotide Sequence of S-Adenosyl-L-Methionine: Magnesium Protoporphyrin Methyltransferase from *Rhodobacter capsulatus*

David W. Bollivar and Carl E. Bauer

It is generally believed that Chl and bacteriochlorophyll biosynthesis are evolutionarily related. This supposition is supported by the observation that the biosynthetic pathway for Chl *a* involves intermediates that are common with bacteriochlorophyll *a* biosynthesis (reviewed in ref. 7) and by the observation that light-independent reduction of protochlorophyllide to Chlide involves an enzyme complex that is highly conserved between purple photosynthetic bacteria and plants (J. Suzuki and C. Bauer, unpublished data). Thus, photosynthetic bacteria offer a good model system for studying enzymes involved in Chl biosynthesis.

The bacterium *Rhodobacter capsulatus* has the capability to undergo rapid growth under nonphotosynthetic (heterotrophic) conditions and is, therefore, particularly amenable to a genetic analysis of pigment biosynthesis. Numerous mutants deficient in bacteriochlorophyll biosynthesis have been isolated that accumulate different intermediates in the Mg-tetrapyrrole biosynthetic pathway (9, 10). Of particular interest are mutations in the *bchH* gene which are reported to lack S-adenosyl-L-methionine: magnesium protoporphyrin methyltransferase activity which catalyzes the transfer of a methyl group from S-adenosyl-L-methionine to the sixth propyl group of Mg-protoporphyrin IX (6). The sequence of *bchH* (Table I, Fig. 1) encodes a large polypeptide of 1195 residues. The predicted protein has no apparent membrane-spanning region and its mean hydrophobicity (−0.116) is typical of soluble proteins (4). It has no readily identifiable homolog in the Genbank data base.

S-adenosyl-L-methionine: magnesium protoporphyrin methyltransferase is interesting from a biochemical point of

**Table I.** Characteristics of *bchH* Gene from *R. capsulatus*

Organism:	<i>R. capsulatus</i> , SB1003 (11).
Location on Chromosome:	Photosynthetic gene cluster; <i>BamD</i> and <i>BamF</i> fragments (9, 10).
Function:	Encodes S-Adenosyl-L-methionine: magnesium protoporphyrin methyltransferase (EC 2.1.1.11) (6).
Techniques:	M13 phage single stranded DNA sequencing, plasmid sequencing, restriction fragment subcloning, and dideoxy sequencing of both strands using Sequenase (U.S. Biochemical, Cleveland, OH).
Method of Identification:	Gene disruption published previously (10).
Structural Features:	Open reading frame 1195 amino acids; calculated <i>M<sub>r</sub></i> , 129,418.
Codon Usage:	82% XX G/C, (G+C) content 65%; exhibits typical codon preference for a translated <i>R. capsulatus</i> gene based on previously described codon usage table (10).
GenBank Accession No.:	M74001.

view because it has been shown to operate according to three different reaction mechanisms, depending on the organism from which it is isolated (7). The enzyme has not been purified to homogeneity; however, the biochemical reaction has been characterized in *Euglena gracilis*, *Hordeum vulgare*, *Triticum aestivum*, *Zea mays*, and *Rhodobacter sphaeroides* (1–3, 5, 8). It has been demonstrated to be noncompetitively inhibited by protochlorophyllide, Chlide, and its direct products, magnesium protoporphyrin monomethylester, and S-adenosyl homocysteine (1, 3).

ATGCACGATGAGTCGATGAGCGGAACCATGCCGCTGCCGCCGACCGCTCCGGGGGGTATAACGTCGTGATCATCACGCTTGACCAGCATGCCGCCGCTCCGGTCCGCCGCGCTGCCG 120  
 M H D E S M S G T M P L P P H R P G G Y N V V I I T L D Q H A A G P A A R A L P  
 CGGCTGCAACACGACTTCCCGACATCCATGTGTCCTGCTCCATGCCGCCGCGGAATGGTCGGAACCCGCGCAAGCTGGCGGCTGCGAAAGCGCGCTCCTTGGCGCGAATATCGTTGTC 240  
 R L Q H D F P D I H V S V H A A A E W S E N P A K L A A A K A A V L G A N I V V  
 GCGAACCTTCTGTTTCATCGACGAGCATCTGACGGCGATCTGCCCGAGATGACCGCGTGCAGCAATCTGGATGCCCTTTGTCGGCATGGTTGCCGATCCGAGATCGTTCGTCTGACG 360  
 A N L L F I D E H L Q A I L P E M T A V R D N L D A F V G M V A D P Q I V R L T  
 AAGATGGCGATCTGGACATGACCAAGCCGCTTCGGGGCCGATGGCGCTTTTGAAGAGCTGCGCGCAAGTCCGAGCCGGCGCGGGATCGCGCGCAAGATCGATCGATCGCGC 480  
 K M G D L D M T K P A S G P M A L L K K L R G K S E P G A G S A E K Q M S M L R  
 ACGATCCCCAAGATGCTGAAATTCATCCCGGGCAAGGCGCAGGATCTGCGCGCTGGTTCCTGTCATGCAATATTGGCTGGCGGGTCCGAGGACAACATCGAATCGATGGTCCGCTAT 600  
 T I P K M L K F I P G K A Q D L R A W F L C M Q Y W L G G S E D N I E S M V R Y  
 CTGGTGGGCGCTATGCCGACAACCGGACTGGCGCGCATCAAGCGCGCGCGCGATCGACTACCCCGGGTCCGGCTTTATACCCCGACATGCCCGGCGGATCACCCGATCCCG 720  
 L V G R Y A D N R D W R G I K A A A P I D Y P E V G L Y H P D M P G R I T T D P  
 GCCAAGCTGCCGCAACCCGCAACCCGGTCCGCACGATCGGCATCTGATGCTGCGCAGCTATATCCTCGCAAGGACACCCGCGATTATGATCGCGTGATCCGCAATTGACGGCGCAT 840  
 A K L P Q P A N P V A T I G I L M L R S Y I L A K D T A H Y D A V I R E L Q A H  
 GCGCTCGCGCTTCTGCGCGCTTTGCCGGGGGGTGGATGGCGCGCGCGGATCGAGGAATCTCGACCGCAAGATCGACACGCTGCTGCTGCTCGCGCTTCTCGCTTGTGGGTGGC 960  
 G V A V L P A F A G G L D G R P A I E E F L H G K I D T L L S L S G F S L V G G  
 CCGGCTATAACGACGATGCGCGGTCGAAACGCGAGCTGGATGTGCTTACGTCACCGCGACCGCGTGGAAATTCAGACGCTTGGGCAATGGCGGGCTTGGCGCGG 1080  
 P A Y N D S D A A V E T L K R S W M C L T S P R S R W N S R R L G N G G P S G G  
 GGGCTCGGCGCGTGGAAACCACTGCTGATCGCGTGGCGGAAATCGACGGCGGACGAAACCCGCGCTTTTGGCGCGCGCATGATCCGCGGGCTGCCTGACCTGTGCGCGCGG 1200  
 G L G P V E T T M L I A L F E I D G A T N P T V F A G R H D P A G C R L D  
 TGCAACCCGATCCGGAAGCGAAAGCCACGCCATGGCCCGCTGCCCGAGCGGATCGAGACGCTGGTGGACAAGGTCGTCGCGATGGCGAAGTCCGCGCTCGAAGTCCGGAACCG 1320  
 C K P D P E A E S H A M A P C P E R I E T L V D K V V R M A N V R R S K V A E P  
 AAGTCCGCGATCGTCTTACGCGCTTCCCGCGAATGCGGGCGCTGCCGGGACCGCGCTTATCTCTCGTCTTGGAGCGCTTTTCAAGTGTGACCGCGATGAAGGCTCGGCTAC 1440  
 K V G I V L Y G F P P N A G A A G T A A Y L S V F E S L F N V M H A M K A S G Y  
 CAGATGGCGAGTGGCGAAAGCTGCGAGGCTGCGGATGCGGTGCTGTGCGGTCCGAACACGACGATGGCGAACCAGCGGAGATCGCGCGCGGATTCGCGCGCGCAATTCGTC 1560  
 Q M G E L P E S V Q E L R D A V L C G P N T T H G Q P A Q I A A R I P A R E F V  
 GCCCGCACGAATGGTCAAGACATCGAAGCGCTGGGCTCGACCCCGGCAAGCAGCAGACCGCGCGCGGAGCTGTTGTCCTTGGCGCTGAGTTCGGAATGCTTCTGCTCGG 1680  
 A R T K W L K D I E A A W G S T P G K H Q T D G R D V F V L G R Q F G N V F V G  
 CTCGAGCGGCTTTCGGCTACGAGGGGACCCGATGCGGCTCTTTTGGAGAAAGGCTTGGCGCGGACCCATGCTTTTGGCGCTTACCGCTGGCTGCGGAGGATTCGCGCGCGAC 1800  
 L Q P V F G Y E G D P M R L L F E K G F A P T H A F A F Y R W L R E D F A A D  
 ACGCTGTGCTTTCGGCATGACGGGGCGCTTGAATTCATCCCGGCAACAGCGGGCATGTCGCAAGCTGCTGGCGGATCGGCTGATCGGCAACTGCCGAAGCTCTATCTGTAT 1920  
 T L L H F G M H G A L E F M P G K Q A G M C E S C W P D R L I G N L P N V Y L Y  
 GCCGCCAACACCGTCGGAAGCGACGCTGGCGAAGCGCGCTGCAATGCGGTGATGCTGCGACCTGACCCCGCGCTCGCACAAATCGGGGCTGTACAAGGGCTTGGCGGAGATCAAG 2040  
 A A N N P S E A T L A K R R S N A V I V S H L T P P L A Q S G L Y K G L A E I K  
 GAAAGCCTTGGCGCTTGGCGCGCTTGGCGCGGCTGCGCGGAGCTGGAAGCTTGGAGCGGAGGCGGAGGCGGCGGCGGAGATGCTGGCGCTGATCGCGGAGATGCCGAC 2160  
 E S L G R L R L P P D S P E R E D L E A L V R E Q A K G V N M D A S D L S T L  
 TGGGAGAACTCTGGAACCGAGGGCGCTGATCACCAGAGCCTGATGCTGCTGGCGGCTCGATGACCGGAGAGGCGGCGGCGGAGATGCTGGCGCTGATCGCGGAGATGCCGAC 2280  
 W E K L L E T E G A L I T E G L H V V G R P M T G E A R A E M L A L M P E N A D  
 CGTGGCGGGCGGACAAGCTTCTGCAAGAGCATGAAATCGCGGGCTTTTGCACGCGCTCGATGGCGCTACGTGCGCGCGTGGCGGGGCGATCTGCTGCGCTCGCGGAGATC 2400  
 R A R D K L L Q E E H I A G L L H A L D G R Y V P P V P G G D L V R S P E I  
 CTGCCCACGGGCGCAACATCCACGCTTCGACCGCTTCCGATGCCGACCGCTTTGCGATCAAGGACGCGCGGCGGAGCGGCGGCTTGTGCGCACGACCCACCCCTGCCGCG 2520  
 L P T G R N I H A F D P F R M P T A F A I K D G A A Q A A G L L A T H P T L P R  
 TCGATCGCGCTGGTCTGTGGGGTTCGGAACAATCAAGTCGGACGCTGGTCCGATCGGTGAGGCGCTGGCGCTGATGGCGCGCGCGCGGCTTCAAGTACGGGCGCTGGCGCGG 2640  
 S I A L V L W G S D N I K S D G G P I G Q A L A L M G A R P R F D N Y G R L A G  
 GCGGAGTATCCCGCTTTCGGAATCGGGCGCTGCGCGCATCGACGTGGTGATGACGCTCTCGGGCATCTTCCGCGACCTGCTGCCCTTGCAGACGAAGCTTTCGGCGAGGCGCGCTAT 2760  
 A E L I P L S I D L G R P R I D V M T L S G I F R D L L P L Q T K L L A E A A Y  
 CTGTGCGGCGGCGGAAACGAGCCCTGGCGGAGAACTTCATCCGCGCAATGCTGCTGGCGACGATGACGAGACCGGAATGGAATTCGAGACCGCTCGCTGCGGGTGTCTCGAAT 2880  
 L C A S A E N E P L A Q N F I R A N V L A T M Q D T G M D F E T A S L R V F S N  
 GCCGAAGGGCTTACGGCTCGAACGCTCAACGCTGGTGGCTCGCGCGGCTTGGCGGACGAAGAGCTGGCGGATGCCTATGAGGCGCGCAAGTCTTGCCTATGGCGCGGACGCG 3000  
 A E G A Y S A N V N T L V G S A G F G D E D L A D A Y E A R K S F A Y G R D G  
 AAGTCGACGAACAGGTGAACCTGCTGCAAAACGCTGTCCAAGGTGATCTGGCGTATCAGAACCTTGAATCCGTCGAATGGCGGTGACACCGCTCGATCACTATTTTCGACACGCTG 3120  
 K S T K Q V N L L Q N V L S K V D L A Y Q N L E S V E L G V T T V D H Y F D T L  
 GCGGCGATCGCGCGGCGGCGAAACCGGCGCAGGGCGCAAGGAAACGCGGCTATATCGGGCAGCAGCGCGCGGCGCGGCGACGCTGCGCACGCTGCAAGACCGATCGCGCTGGAA 3240  
 G G I A R A T K R A Q G G K E T P V Y I G D Q T R G A G T V R T L Q D Q I A L E  
 ACCCGGGCGCTCGCTCAACCCGAAATTCACGAAGGTTGCTGAAACACGGGGCGAAGGCGTGGCGGAGTCAAGCGAGGTACGAACACCCCTTGGCTGGTCCGCGCACGAGG 3360  
 T R A N L R L K H G A G G V R Q I E A Q V T N T L G W S A T T G  
 CAGGTTGAGCTTGGGTCTATCAACGCTTTCGGAACCTTTTGCTTACGACGAGATGCGCGAGCGTCTGGCGAGCTGAACTCGGCTCGCTCAAGCGGATGGCGCAACGGCTTCTG 3480  
 Q V E P W V Y Q R L S E T F V L D D E M R E R L A S L N S A A S S R M A Q R L L  
 GAAGCTTCAAGCGCAACTACTGGCAGCGCTGGCGGCTTTCGAAGCGCGGCGGAGCTCGAAGACCGGATGGAAGCGCTTGGCGGCAATGAGATGGACGACTA 3600  
 E A S D R N Y W Q P D P A T L A A L Q A A A D E L E D R M E G V A A E \*

**Figure 1.** Nucleotide sequence and deduced amino acid sequence of *R. capsulatus bchH* gene. Underlined region corresponds to the sequence previously described by Yang and Bauer (10).

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