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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an enzyme complex that is highly conserved between purple photosynthetic bacteria and plants (J. Suzuki and C. Bauer, unpublished data).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</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).

ATGCAGATGAGTCGATGAGCGGAACCATGCCGCTGCCGCCGACCCGTCGGGGGGTATAACCTGCTGATCATCAGCTTGACCAGCATGCCGCCGCTCCGGTCCGCCGCCGCTGCCG 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
 CGGCTGCAACAGACTTCCCGACATCCATGTGTCGTCATGCCGCCGCGGAATGGTCGGAACCCGGCCAAAGCTGGCGGCTCGGAAAGCGGCCCTCTGGCGCGAATATCGTTGTC 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
 GCGAACCTTCTGTTATCGACGAGCATCTGACGGCGATCTGCCGAGATGACCCCGTGGCGACAATCTGGATGCCTTTGTCGGCATGGTTCCGATCCGAGATCGTTCGTGACG 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
 AAGATGGCGATCTGACATGACCAAGCCGCTTCCGGGGCGATGGCGCTTTGAAAAGCTGGCGCAAGTCCGAGCCGGCGGGGATCGCGCGAAAAGCAGATGTCGATGTCGCG 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
 ACGATCCCAAGATGCTGAAATTCATCCCGGGCAAGCGCAGGATCTGCCGCGCTGGTCTGTCATGCAATATTGGTGGCGGGTCCGAGGACAACATCGAATCGATGTCGCGTAT 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
 CTGGTGGCGGCTATGCCGACAACCGGACTGGCGCGCATCAAGCGCGCGCGCGATCGACTACCCCGGCTGGCGCTTTATCACCCGACATGCCCGGCGGATCACCCGATCCCC 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
 GCCAAGCTGCCGCAACCCGCAACCCGGTCCGCCAGTCCGATCTGATGCTGCGCAGCTATATCTCCGCAAGGACACCCGCGATTATGATCGGCTGATCCGCAATTCGACGGCGCAT 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
 GGGCTCCGCTTTCGCCCGCTTTGCCGGGGGGTGGATGGCCGCCCGCGATCGAGAAATCTCTGACGCAAGATCGACACGCTGCTGCTCGCTCCGGCTTCTCGCTTGTGGTGGC 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
 CCGGCTATAACGACAGCGATCGCGCGTGAACCGCTGAACCGCAGCTGGCTTACGTCACCGCGACCGCTGGAATTCAGACGCTTGGGCAATGGCGGGCTCCGGCGCGC 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
 GGGCTCCGCGGTTGAAACCACTATGTCGCGCTGCCGGAATCGACGGCGGACGAAACCCGCGCTTTGCCGGCGGCAATGATCCGGCGGGCTGCCTGACCTGTCCGGCGCGC 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 L T C A R G
 TGCAACCCGATCCGGAAGCCGAAAGCCACGCCATGGCCCGCTGCCCGAGCGGATCGAGACGCTGGTGGACAAGGTCGTCGCGATGGCGAAGTCCGCGCTCGAAGTCCGCGAACCG 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
 AAGTCCGATCGTCTTACGCGTCCCGCGAATCGCGCGCTGCCGGGACCGCGCTTATCTCTCGTCTTGGAGGCTTTTCAAGTGTGACCGCGATGAAGGCTCCGGCTAC 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
 CAGATGGCGAGTGGCGAAAGCTGCGAGGAGTGGCGATGGCGTGTGGTGGTCCGAAACAGCAGCATGGCCAAACCGCGCAGATCGCGCGCGGATCCGGCGCGCAATTCGTC 1560
 Q M G E L P E S V Q E L R D A V L C G P N T H G G Q P A Q I A A R I P A R E F V
 GCCCGCAAAATGGCTCAAAGCATCGAAGCCGCTGGGGCTCGACCCCGGCAAGCACCAGCAGCCGCGCGGCGGCGTGTGTCTGGCGCTGTCGGAATGTCCTTCGCGC 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
 CTCAGCCGCTTCCGGTACGAGGGGACCCGATGGCGCTCTTTTGGAAAGGCTTCCGCGCGACCCATGCCTTTCCGCGCTTACCGCTGGCTGCGGAGGATTCGCGCGCGC 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 A F Y R W L R E D F A A D
 ACGTGTGTCATTCGGATGACGGGGCGCTTGGTTCATGCCCGCAACAGCGGGCATGTGCGAAAGTCTGCGCGGATCGGCTGATCGGCAACTGCCGAAGCTTATCTGTAT 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
 GCGGCAACAACCGTCGGAAGCAGCTGGCGAACCGCGCTGAAATCGGATCGTCTGACCCCTGACCCCGCGCTCGCACAAATCGGGGCTGTACAAGGGCTCGCGGAGATCAAG 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
 GAAAGCTTGGCGCTTGGCGCGCTGCCCGGACTGCCCGGAGTGAAGACTGGAAGCCTTGTGCGGAGCAGGCCAAGGGCGTGAACATGGACCGCAGCGACCTTCCACGCTG 2160
 E S L G R L R A 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
 TGGAGAACTCTGGAACCGAGGGCGCTGATCCCGAAGCGCTGATGCTGCGCGCGTCCGATGACCGGAGGAGCGCGGGCGGAGATGCTGGCCCTGATCCCGGAGAATGCCGAC 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
 CGTGGCGGGCGCAAGCTTCTGACGAAAGCATGAAATCGCGGGCTTTTGCACCGCTCGATGGCGCTACGTGCGCGCTGCGCGGGCGGATGCTGCTGCGCTCCCGGAGATC 2400
 R A R A D K L L Q E E H E 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
 CTGCCACGGGGCAACATCCACGCTTCCGACCGCTTCCGATGCCGACCGCTTTGCGATCAAGGAGCGCGCGCGCAGCGCGCGGTTGCTCGCCACGCACCCACCTTCCCGCGC 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
 TCGATCGCGCTGTTCTGTGGGGTCCGACAACATCAAGTCGGACGCTGGTCCGATCGGTCAGGCGCTGGCGCTGATGGCGCGCGCGCGGTTCCGACAATACGGGCGCTGGCGGG 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
 GCGGAGTATCCCGCTTCCGAACTGGCGCGTCCCGCATCGACGCTGGTGTGACGCTTCCGGCATCTTCCCGACCTGCTGCCCTTCCAGACGAAGCTTCCGCGGAGGCGCGCTAT 2760
 A E L I P L S E 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
 CTGTGGCGCAGCGGAAAACGAGCCCTGGCGGAGAATTCATCCCGCAATGCTGCGGACGATGACGAGACCCGGAATGGACTTCGAGACCGCTCGTGGCGGTGTTCTCGAAT 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
 GCCGAAGGGCTTACGGCTCGAAGCTCAACCGCTGGTCCGCTCCGCGGGTTGGCGCAGAAGCAGTGGCCGATGCCTATGAGCCCGCAAGTCTTCCCTATGGCCCGACGGC 3000
 A E G A Y G S N V N T L V G S A G F G D E D E L A D A Y E A R K S F A Y G R D G
 AAGTCGACAAACAGTGAACCTGCTGCAAAACGCTGTCCAAGTTCATGCGCTATCAGAACCTTGAATCCGTCGAACTGGCGGTGACCCGCTCGATCACTATTTCGACACGCTG 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
 GCGGCTCCGCGGGCGCGAAAACCGGGCGAGGGCGCAAGGAAACCGCGTATATCGGGCACGACCCGCGCGCGCGGCGCGGCGCGGCGCGGCGCGGCGCGGCGCGGCGGCGG 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
 ACCCGGCGCGCTCGTCAACCCGAAATTCACGAAGGTTGCTGAAACACCGGGCCGAGGCTGCGCGAGATCGAAGCGGAGTACGAAACCCCTTGGCTGGTCCGCGCACGAGG 3360
 T R A R S L N P K F Y E G L L K H G A E G V R Q I E A Q V T N T L G W S A T T G
 CAGTTGAGCTTGGTCTATCAACCGCTTTCGGAACCTTTGCTTACGACGAGATGCGCGAGCGTTCGGCCAGCTGAACTCGGCTGGTCAAGCCGGATGGCGCAACGGCTTCTG 3480
 Q V E P W 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
 GAAGCTTACAGCCGAACTACTGGCAGCGGATCCCGGCGAGCTGGCGGCTTGGCAAGCAGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG 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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