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# Two enzymes, BtaA and BtaB, are sufficient for betaine lipid biosynthesis in bacteria

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**Abstract**

Betaine lipids are non-phosphorous glycerolipid analogs of phosphatidylcholine. The biosynthesis of the betaine lipid diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine has previously been studied in phosphate-starved cells of the purple bacterium *Rhodobacter sphaeroides*, and a genetic approach identified two proteins that are necessary for this process. Here, we show that all reactions of DGTS biosynthesis in *R. sphaeroides* are attributable to *Rs*BtaA and *Rs*BtaB, as co-expression of the respective genes leads to DGTS formation in *Escherichia coli*, which normally lacks this lipid. The recombinant *Rs*BtaA protein was membrane-associated and showed *S*-adenosylmethionine/diacylglycerol 3-amino-3-carboxypropyl transferase activity. *Rs*BtaA directed the transfer of label from 1-[$^{14}$C]*S*-adenosylmethionine or [$^{14}$C]diacylglycerol at equal rates into the betaine lipid precursor diacylglycerylhomoserine identifying both metabolites as the substrates of the reaction. Comparative analysis of *Rs*BtaA and its bacterial orthologs revealed a motif with similarity to the AdoMet binding pocket of methyltransferases, and allowed the prediction of residues involved in substrate binding.
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Many organisms, such as bacteria, plants, and fungi, rely on mineral nutrients taken directly from the soil or aquatic environment, and therefore tend to have exquisitely specialized mechanisms to cope with limitation of a given essential nutrient. For example, most organisms have well defined responses to phosphate limitation, including the replacement of cellular membrane phospholipids with non-phosphorous lipids. This last point has been well documented in the plants Arabidopsis [1,2] and oat [3], and most pronouncedly in the α-proteobacteria *Rhodobacter sphaeroides* and *Sinorhizobium meliloti*, which under phosphate stress become depleted of membrane phospholipids and induce the synthesis of non-phosphorous lipids, such as glycolipid species, the betaine lipid diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine (DGTS), and ornithine containing lipids. [4,5].

DGTS, which was first discovered in the unicellular alga *Ochromonas danica* [6] and thereafter in *Chlamydomonas reinhardtii* [7], and other lower plants and fungi [8–10], has been proposed to take the place of phosphatidylcholine (PtdCho) in membranes of these organisms. Studies of DGTS biosynthesis using radioisotope feeding experiments identified methionine as a source of both the $C_4$ homoserine moiety and methyl groups of DGTS in *Chlamydomonas* [11]. These results led to the conclusion that *S*-adenosylmethionine (AdoMet) is the active donor of the four carbon unit of methionine leading to the synthesis of the intermediate diacylglycerylhomoserine (DGHS), and that DGTS arises from lipid-linked methylation of DGHS by an AdoMet-dependent methyltransferase. A similar pathway was

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proposed for *R. sphaeroides* based on labeling experiments [12] (Fig. 1).

Recently, Klug and Benning [13] used a genetic approach in *R. sphaeroides* to gain access to genes that are required for the biosynthesis of DGTS. This work resulted in the identification of an operon containing two open-reading frames, designated *btaA* and *btaB*, which appeared to be necessary for DGTS accumulation during phosphate deprivation in this bacterium. The product of *btaA* was proposed to function as an AdoMet/diacylglycerol 3-amino-3-carboxypropyl transferase, producing the intermediate DGHS. *Rs*BtaB showed a high degree of similarity to methyltransferases, and was proposed to be a trifunctional AdoMet-dependent *N*-methyltransferase, adding three methyl units to the amino group of DGHS to form DGTS (Fig. 1). In fact, *Rs*BtaB shows a high degree of sequence similarity to *Rs*PmtA, which produces phosphatidylcholine by *N*-trimethylation of phosphatidylethanolamine [14], indicating that *Rs*BtaB and *Rs*PmtA may share analogous functions in separate pathways. However, a biochemical analysis of the respective proteins has not been previously done. To study DGTS biosynthesis at the level of the enzymes and understand bacterial DGTS biosynthesis in greater detail, we initiated a biochemical characterization of the BtaA and BtaB proteins from *R. sphaeroides*, focusing in particular on *Rs*BtaA due to the unusual nature of the proposed reaction.

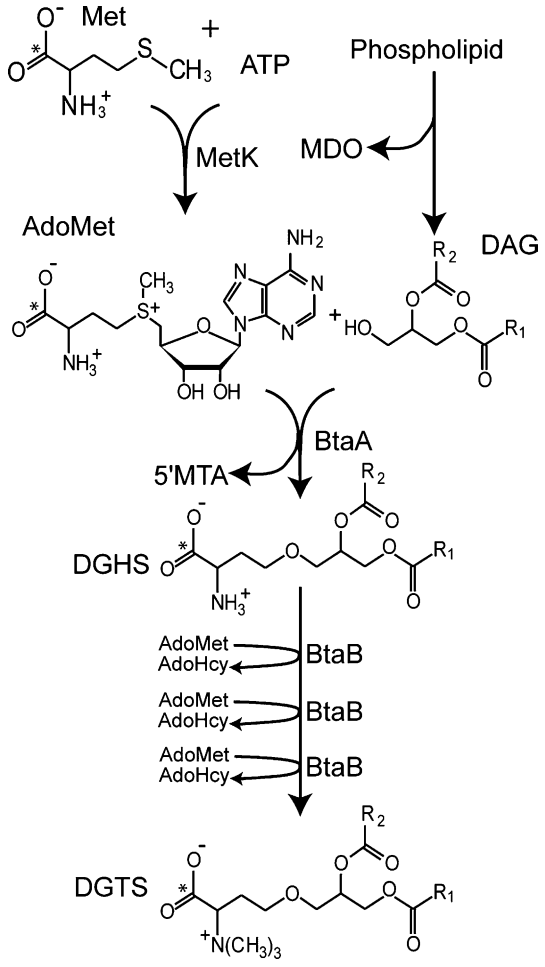


Fig. 1. DGTS biosynthetic pathway and involvement of *Rs*BtaAB in bacteria. BtaA is proposed to catalyze the transfer of the 3-amino-3-carboxypropyl group of AdoMet to the 3-hydroxyl of DAG to form the intermediate DGHS. DGHS is then sequentially *N*-methylated by the BtaB methyltransferase to form DGTS. AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; DAG, diacylglycerol; DGHS, diacylglycerylhomoserine; DGTS, diacylglyceryl-(*N*,*N*,*N*)-trimethylhomoserine; MDO, membrane-derived oligosaccharides; MetK, AdoMet synthetase; 5′MTA, 5′-methylthioadenosine.

## Materials and methods

### *Materials*

Phospholipase C from *Bacillus cereus* was from Sigma (St. Louis, MO), mouse monoclonal anti-His tag antibody was from Qiagen (Valencia, CA, USA). Restriction enzymes, T4 DNA ligase, calf-intestinal phosphatase, and Klenow fragment were from New England Biolabs (Beverly, MA, USA), and *Taq* DNA polymerase was from Roche (Indianapolis, IN, USA). Labeled precursors were purchased from American Radiolabeled Chemicals, St. Louis, MO, USA. All other chemicals and solvents were of reagent grade and were from Sigma, EM Science (Gibbstown, NJ, USA) or J.T. Baker (Phillipsburg, NJ, USA).

### *Expression of RsbtaA and RsbtaB*

A summary of all strains and plasmids used in this study is presented in Table 1. The coding regions of *RsbtaA* and *RsbtaB* were PCR-amplified from plasmid pRKL323 [13] for expression in pQE-31 (Qiagen) or pACYC-31 [15], respectively, with the following primers (restriction sites underlined): *btaA* forward (*Sph*I), 5′-ACATGCATGCAGTGACGCAGTTCGCCCTC-3′; *btaA* reverse (*Kpn*I), 5′-CGGGGTACCAGGACGAT CCGCTCGAACCG-3′; *btaB* forward (*Bam*HI), 5′-GC GGATCCGATGACCGACGCCACCCAT-3′; *btaB* reverse (*Hin*dIII), 5′-GCAAGCTTCTCTCACCGCGT GAGCGTG-3′. PCR was carried out with *Taq* DNA polymerase (Roche) according to the manufacturer's specifications, except that 10% DMSO (v/v) was added to each reaction to overcome difficulties in PCR due to the high G+C content of *R. sphaeroides* DNA. PCR products were first cloned into PCR2.1-TOPO (Invitrogen, Carlsbad, CA, USA) and sequenced at the MSU Genomics Technology Support Facility, followed by subcloning into the expression vectors using restriction sites as outlined in the PCR primers (see Table 1). The resulting constructs were thus designated pBtaA (*btaA* sequence in pQE-31), and pBtaB (*btaB* in pACYC-31).

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