Characterization of the Gene Encoding the Autotrophic ATP Sulfurylase from the Bacterial Endosymbiont of the Hydrothermal Vent Tubeworm *Riftia pachyptila*

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ATP sulfurylase is a key enzyme in the energy-generating sulfur oxidation pathways of many chemoautotrophic bacteria. The utilization of reduced sulfur compounds to fuel CO₂ fixation by the still-uncultured bacterial endosymbionts provides the basis of nutrition in invertebrates, such as the tubeworm *Riftia pachyptila*, found at deep-sea hydrothermal vents. The symbiont-containing trophosome tissue contains high levels of ATP sulfurylase activity, facilitating the recent purification of the enzyme. The gene encoding the ATP sulfurylase from the *Riftia* symbiont (*sopT*) has now been cloned and sequenced by using the partial amino acid sequence of the purified protein. Characterization of the *sopT* gene has unequivocally shown its bacterial origin. This is the first ATP sulfurylase gene to be cloned and sequenced from a sulfur-oxidizing bacterium. The deduced amino acid sequence was compared to those of ATP sulfurylases reported from organisms which assimilate sulfate, resulting in the discovery that there is substantial homology with the *Saccharomyces cerevisiae* MET3 gene product but none with the products of the *cysDN* genes from *Escherichia coli* nor with the *nodP* and *nodQ* genes from *Rhizobium meliloti*. This and emerging evidence from other sources suggests that *E. coli* may be atypical, even among prokaryotic sulfate assimilators, in the enzyme it employs for adenosine 5'-phosphosulfate formation. The *sopT* gene probe also was shown to specifically identify chemoautotrophic bacteria which utilize ATP sulfurylase to oxidize sulfur compounds.

The oxidation of reduced inorganic sulfur compounds by a variety of chemoautotrophic and photoautotrophic bacteria allows the conservation of energy by a chemiosmotic or substrate-level mechanism. In some of these bacteria, a portion of their ATP is derived from the oxidation of sulfite by the following reactions, termed the adenosine 5'-phosphosulfate (APS) pathway (14).

Together, the cytoplasmic enzymes APS reductase and ATP sulfurylase convert sulfite to sulfate. APS is produced as an intermediate by APS reductase (14). The final and ATP-generating step is catalyzed by ATP sulfurylase. Many phototrophic bacteria (2), some chemoautotrophic thiobacilli (14), and an autotrophic marine *Beggiatoa* strain (MS-81-1c) (12) found widely distributed in natural environments with high concentrations of sulfide or other reduced sulfur compounds use this pathway to generate ATP or supply electrons for CO₂ reduction. To date, no "autotrophic" ATP sulfurylase has been purified, nor has a gene encoding one been cloned, from any of these free-living autotrophic bacteria.

ATP sulfurylase activity has predominantly been studied in the assimilatory sulfate reduction pathways which incorporate sulfate for the biosynthesis of amino acids and other sulfur-containing metabolites. The best known of these are found in heterotrophic bacteria and eukaryotes such as fungi and plants. Sulfate is activated to the APS intermediate by ATP sulfurylase in an energy-requiring step. These organisms then use APS kinase to convert APS to 3'-phosphoadenosine 5'-phosphosul-

fate (PAPS) (16). Genes reported to encode the assimilatory ATP sulfurylase have only been identified by genetic complementation in the heterotrophic bacterium *Escherichia coli* (*cysDN*) (17, 18) and *Rhizobium meliloti* (*nodP* and *nodQ*) (28) and in the yeast *Saccharomyces cerevisiae* (*MET3*) (4). In none of these examples has complete biochemical characterization demonstrated that the catalytic properties of the encoded ATP sulfurylase resemble those of the well-characterized purified protein from fungi (30).

The third role ascribed to ATP sulfurylase is dissimilatory sulfate reduction (23). Some eubacteria and the thermophilic archaeobacterium *Archaeoglobus fulgidus* (5) use sulfate as a terminal electron acceptor for the anaerobic respiration of organic compounds or H₂. The two enzymes of the APS pathway are believed to operate in the reverse direction (23), with ATP sulfurylase catalyzing the activation of inorganic sulfate by ATP to give pyrophosphate and APS, which in turn is reduced to sulfite (31). Purification of the dissimilatory ATP sulfurylase was reported for several sulfate reducers (1, 5), but no amino acid or gene sequence data has yet been reported.

Hydrogen sulfide is abundant at deep-sea hydrothermal vents and provides an energy source for sulfur-oxidizing chemoautotrophic bacteria. Most of these bacteria are endosymbionts which provide the nourishment for a number of invertebrates which proliferate at these sites (3, 9, 10). There is considerable enzymological evidence that some of these symbionts utilize the APS pathway (9, 11). The first autotrophic ATP sulfurylase enzyme from any source was recently purified (24) from the symbiont-containing tissue of the giant vent tubeworm *Riftia pachyptila*. The gene encoding the autotrophic ATP sulfurylase was cloned in this study and has been given the designation *sopT* (sulfur-oxidation pathway, ATP-generating step). The ready supply in a single tubeworm of several hundred grams of symbiont-containing tissue having very high ATP sulfurylase activity, contrasted with the very low molar

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growth yields of free-living chemoautotrophs (15), made the initial enzyme purification and gene cloning from this novel source advantageous.

Two important questions arise which have been addressed in this study of the cloned *sopT* gene. (i) Do the ATP sulfurylase enzymes used in each of these diverse pathways have a common evolutionary origin? (ii) Can a single ATP sulfurylase gene be used as a probe to identify those sulfur-oxidizing bacteria which utilize the APS pathway?

MATERIALS AND METHODS

Bacterial strains and plasmids. *R. pachyptila* were collected at hydrothermal vent sites by using the Deep Submergence Vehicle *Alvin*. Bacterial symbionts were purified from homogenates of tubeworm trophosome tissue by Percoll gradient centrifugation (7). The purified pellets were kept at -80°C for storage on the support vessel and for shipping to the laboratory. *Riftia* symbiont samples Rs-8 and Rs-10 from individual tubeworms (dive no. 2340; 27°N , 111°W , 2,000-m depth) were collected at Guaymas Basin in 1991. Samples Rs-19 (dive no. 2519; $12^{\circ}48'\text{N}$, $103^{\circ}56'\text{W}$, 2,600-m depth) and Rs-30 (dive no. 2499; $9^{\circ}50'\text{N}$, $104^{\circ}17'\text{W}$, 2,500-m depth) were collected along the East Pacific Rise in 1992.

Strains screened by Southern analysis included *Chromatium vinosum* ATCC 17899, *Desulfovibrio desulfuricans* E1 Agheila Z NCIB no. 8380 (a gift from David Stahl), *S. cerevisiae* SEY6216; *Thiobacillus denitrificans* ATCC 25259, *Thiomicrospira crunogena* ATCC 35932, marine *Beggiatoa* strain MS-81-1c (22), and freshwater *Beggiatoa* strain OH-75-2a (21).

E. coli strains used for cloning were JM83 and XL-1 Blue (Stratagene) grown in LB medium and 2YT, respectively, supplemented with 100 mg of ampicillin per ml as needed. Plasmid pUC18 was used for constructing the genomic library. Plasmid pBluescriptKS⁺ (Stratagene) was also used for cloning and sequencing. The plasmid pTL3 contained the *cysDN* gene locus was obtained from George D. Markham (18). Plasmids pJSS38 and pJSS30 containing the *nodP* and *nodQ* genes, respectively, were obtained from Julie Schwedock (28).

DNA manipulations. Standard procedures were used for genomic extractions, transformation, restriction endonuclease digestions, ligations, and electrophoresis. Plasmid purification was performed by the alkaline lysis method described by Sambrook et al. (25), with further purification on p2523 columns (5 Prime-3 Prime). DNA fragments were extracted from low-melting-temperature agarose by heating them to 65°C in 0.25 volume of 1 M NaCl in TE (10 mM Tris-Cl [pH 7.4], 1 mM EDTA) followed by phenol extraction and precipitation in 0.1 volume of 3 M sodium acetate and 2.5 volume of 95% ethyl alcohol. PCR amplification was performed by standard procedures for one cycle of denaturation at 94°C for 5 min and 30 cycles at 94°C for 1 min, each followed by annealing at 50°C for 1 min and elongation at 70°C for 4 min.

Hybridization procedures. Hybridizations were performed as described by Sambrook et al. (25). GeneScreen Plus membranes for Southern blots and Magma membranes (Micron Separations Inc.) for colony hybridizations were preincubated in hybridization solution which included formamide at 50, 35, or 25% for high-, medium-, or low-stringency conditions, respectively. The ^{32}P -labeled probe was added to the hybridization solution and incubated at 42°C , with rotating overnight. Following hybridization the membranes were washed under the following conditions: high stringency, (i) $2\times$ SSC ($1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0) (25)–0.1% sodium dodecyl sulfate (SDS); (ii) $0.5\times$ SSC–0.1% SDS; (iii) $0.1\times$ SSC–0.1% SDS for 15 min each at room temperature;

FIG. 1. Southern blot analysis of chromosomal DNA probed with *cysDN*. Lane 1 contains lambda/*Hind*III DNA molecular size markers. *Riftia* symbiont (lane 2) and *E. coli* (lane 3) DNA were digested with *Eco*RI and probed under low-stringency conditions.

medium or low stringency, (i) $2\times$ SSC–0.1% SDS; (ii) $2\times$ SSC–1% SDS for 30 min each at room temperature.

DNA sequence analysis. The DNA sequence was determined by the dideoxy-chain termination method of Sanger et al. (26) using the Sequenase version 2.0 kit (United States Biochemicals). Homology searches and sequence analyses were conducted by using DNA Strider and the Genetics Computer Group software packages (University of Wisconsin) (6). The FASTA search program was used to screen the SwissProt, GenBank, and GenEMBL data banks. The most closely matched sequences were evaluated with the GAP program.

Nucleotide sequence accession number. The sequence for the *sopT* gene encoding the ATP sulfurylase from the *Riftia pachyptila* symbiont is available from the GenBank database under accession number L26897.

RESULTS

Attempts to identify the ATP sulfurylase locus using a heterologous probe. The *cysDN* gene from *E. coli* hybridized to a 2.2-kb *Eco*RI band in *E. coli* (Fig. 1, lane 3) but showed no detectable homology with *Riftia* symbiont DNA (lane 2). Using the ATP sulfurylase genes *nodP* and *nodQ* from *R. meliloti* as probes also showed no detectable homology with symbiont DNA (data not shown). These represented all available prokaryotic heterologous probes, since no ATP sulfurylase gene from a sulfur-oxidizing autotrophic bacterium or sulfate reducer had previously been cloned.

PCR amplification of a 600-bp fragment. Two degenerate oligonucleotide 17-mer PCR primers were designed from peptide sequences derived from the purified ATP sulfurylase. One primer, ATGAT(T/C/A)AA(A/G)CCNGTNGG, corresponded to the first 6 of the 17 amino acids from the N terminus of the protein MIKPVGSDCLKPLFVYD (J. Schilling, University of California at Davis Protein Structure Laboratory). The second primer, GCNCA(T/C)GA(A/G)GA(A/

FIG. 2. Restriction map of the cloned *sopT* locus. The 600-bp fragment, including *Bam*HI and *Hind*III ends introduced by the primers, was derived by PCR amplification and cloned into pUC18, forming plasmid pBL5. The *Bam*HI-*Hind*III fragment used as the *sopT* probe in hybridization analysis is indicated by the arrow. A 4.5-kb *Eco*RI-*Hind*III fragment containing the entire *sopT* locus was cloned into pUC18, forming pBL20. The 1.2-kb *Eco*RI-*Sac*I fragment was subcloned into pBluescriptKS⁺, producing pBL23. The location of the 1.3-kb ORF detected by the computer is represented by the striped box. R, *Eco*RI; P, *Pst*I; A, *Acc*I; S, *Sac*I; N, *Nco*I; H, *Hinc*II; Av, *Ava*I; H3, *Hind*III; X, *Xho*I; Pv, *Pvu*II.

G)CTNTG, represented the first 6 amino acids of the 7-amino-acid internal peptide, AHEELCR, derived by tryptic digestion and identified by fluorescent labeling of the sulfhydryl group of the cysteine residue with monobromobimane (29a). These primers, used in the PCR amplification with purified *Riftia* symbiont DNA (Rs-10) as the template, yielded a single 600-bp fragment which was cloned into pUC18, forming plasmid pBL5 (Fig. 2). Sequencing from each end of the cloned fragment confirmed the presence of the primer sequences and additionally showed that the first 17 amino acids encoded at the amino terminus were identical to the 17-amino acid peptide sequence obtained from the purified protein.

Southern analysis of the *sopT* locus. The cloned PCR fragment containing the first 600 bp of the 5' terminus of the ATP sulfurylase gene was used as a probe to locate the complete gene in the *Riftia* symbiont genome. The insert was purified from plasmid pBL5, digested with *Bam*HI and *Hind*III, and ³²P labelled. Chromosomal DNA from *Riftia* symbiont Rs-8 was digested with restriction enzymes and probed with the *sopT* probe under high-stringency conditions. Southern analysis (Fig. 3) showed that there was a single band hybridizing in each lane, which was presumed to contain the complete gene. The probe hybridized to chromosomal DNA digested with *Eco*RI and *Hind*III (lane 1), *Eco*RI and *Bam*HI (lane 2), and *Eco*RI and *Sal*I (lane 3), showing bands of approximately 4.5, 6, and 4.5 kb, respectively. DNA from *Riftia* vestimentum which is non-symbiont-containing tissue (lane 4), showed no hybridization to the probe.

Cloning the *sopT* locus from the *Riftia* symbiont. The 4.5-kb *Eco*RI-*Hind*III fragment was cloned into pUC18, and the transformed colonies were screened by hybridization with the 600-bp *sopT* probe. The cloned 4.5-kb insert of plasmid pBL20 (Fig. 2) was mapped by restriction analysis and Southern analysis to confirm the identity between the insert and the probe and to localize the *sopT* gene within the insert. The 600-bp *sopT* probe hybridized to the region between the *Eco*RI and *Sac*I sites (Fig. 2). A 1.2-kb *Eco*RI-*Sac*I fragment which hybridized to the probe was subcloned into pBluescriptKS⁺, forming plasmid pBL23, to initiate sequencing.

Sequence analysis of the ATP sulfurylase gene. Sequencing the insert of pBL23 showed that the 5' terminus was located 600 bp upstream from the *Sac*I site. The nucleotide sequence corresponding to the internal primer overlapped the *Sac*I site (Fig. 4). The 602 bases upstream from the ATG translational start site of the gene, which presumably contained the regulatory components, were sequenced. An additional 800-base region, containing the rest of the open reading frame, was sequenced from the pBL20 plasmid by using a series of specifically synthesized oligonucleotide primers. The DNA sequence of the 2-kb region revealed the presence of an open reading frame of 1,317 nucleotides, starting with ATG and ending with the double termination codons TAATGA (Fig. 4).

FIG. 3. The 600-bp fragment of the *sopT* gene hybridized to *Riftia* symbiont DNA. Symbiont DNA was digested with *Eco*RI and *Hind*III (lane 1), with *Eco*RI and *Bam*HI (lane 2), and with *Eco*RI and *Sal*I (lane 3) and was probed under high-stringency conditions. The bands were approximately 4.5, 6, and 4.5 kb in size, respectively. Lane 4 contains *Riftia* vestimentum (host only) DNA, which did not hybridize to the probe.

Rs	MIKPVGSDELKPLFVYDPEEHHKLSHEAESLPSVVISQGP...VSSM	46
Sc	.MPAPHGGILQDLIARDALKKNELLSEASQ.SDILVWNLTPRQLCDIELI	48
Rs	MGAGYFSPAGFMNVADAMGAAEKMTLSGSSSCSVLCLLENTDAIGDAKR	96
Sc	LNGGFSPLTGFNLNENDYSVVDTSRLADGTLW.TIPITLDVDEAFANQIK	97
Rs	IALRDPNVEGNPVLAVMDIEAIEEVSDEQMAVMTDKVYRTTMDHIGVKT	146
Sc	PDTRIALFQDDEI..PIAILTVQDVYKPKNTIEAEKVFRGDPEHPAISYL	145
Rs	FNSQGRVAVSGPIQVLNFSYFQADFPTDFTAVEIRNEIKEHGWSKVVAF	196
Sc	FNVAGDYVVGGSLEAIQLFPQ.HYDYPGLRKTPAQLRLEFQSRQWDRVYAF	194
Rs	QTRNPMHRAHEELCRMPMESLDADGVVHMLLGLKKGDIAPVRDAAIR	246
Sc	QTRNPMHRAHEELTVRAAREANAK.VLIHPVVGLTGPGIDHHTR...VR	240
Rs	TMAEVY..FPPNVTMTGYGFDMLYAGPREAVLHAYFRQNMGATHFIIGR	294
Sc	VYQEIIKRYPNGIAFLSLPLAMRMSGDREAVWHAIIRKNYGASHFIVGR	290
Rs	E..PPAVVTTTVP..STPRFSSMTKCQAPWRSRSCSRPHGLLQEAQDC	340
Sc	DHAGPGKNSKGVDFYGPYDAQELVESYKHELDIEVVPFRMVYLPDEDRY	340
Rs	DDARRAGSHQGRRLTALRHQGREMLGGQIAPPPEFSRPEVAKILMD...	386
Sc	APIDQIDTTKTRTLNISGTLELRRLRVGGEIPWFYSYPEVVKILRESNPP	390
Rs	LLPVHQQLILIWFSKTRPGVGRW.....	410
Sc	RPKQGFISVLGNLSLTVSREQLSIALSTFLQFGGGYKIFEHNKTELL	440
Rs	..RVFLCAAGAL.....WPEAVAVAN...MEKRSSTG.....	437
Sc	SLIQDFIGSGSLIIPNQWEDDKDSVVGKQNVYLLDTSSADIQLESADE	490

FIG. 5. Amino acid sequence alignments of the ATP sulfurylase enzymes of the *Riftia* symbiont (from the *sopT* gene) and *S. cerevisiae* (from the *MET3* gene) (Rs and Sc, respectively). Lines between amino acids indicate identity, and dots indicate similarity. The boxed area indicates an extremely conserved region. Sequences that are overlined with dashed lines indicate sites of moderate homology.

binding site thought to make the sulfate reduction reaction more thermodynamically favorable, GSVDDGK, was found in both of these loci (17). This sequence was not found in either the *Riftia* symbiont or the *S. cerevisiae* ATP sulfurylase genes products.

Survey of other sulfur-metabolizing organisms by hybridization with the *sopT* probe. To test the effectiveness of the *Riftia* symbiont *sopT* gene as a general probe, several microorganisms were screened. Chromosomal DNA from each was digested with *EcoRI* and hybridized with the 600-bp *sopT* probe (the same results were obtained with the full-length 4.5-kb insert) under medium-stringency conditions. The results (Fig. 6) show hybridization of the *sopT* probe to DNA of the chemoautotrophs *T. denitrificans* (4.5-kb band, lane 3), *Beggiatoa* 81-1c (data not shown), and the photoautotroph *C. vinosum* (8-kb band, lane 4). Another sulfur-oxidizing bacterium, *T. crunogena* (lane 5), failed to hybridize with the *Riftia* symbiont probe. No hybridization was detected either with the dissimilatory sulfate-reducing bacterium *D. desulfuricans* (lane 6) or the sulfate-assimilating organisms *E. coli* (lane 7) and *S. cerevisiae* (lane 8) under these conditions.

DISCUSSION

The autotrophic ATP sulfurylase from the trophosome tissue of the tubeworm *R. pachyptila* was previously purified, and its properties were studied (24). It was logical, on the basis of kinetic parameters and the high enzyme activity, to believe that the protein was derived from the sulfide-oxidizing chemoautotrophic bacterial endosymbionts, but this was not proven

FIG. 6. Hybridization patterns of representative sulfur-metabolizing organisms probed with the *Riftia* symbiont *sopT* probe. Genomic DNA was digested with *EcoRI* and hybridized under medium-stringency conditions. Molecular size markers are shown in lane 1. Lane 2, *Riftia* symbiont Rs-30 DNA; lane 3, *T. denitrificans* DNA; lane 4, *C. vinosum* DNA; lane 5, *D. desulfuricans* DNA; lane 6, *T. crunogena* DNA; lane 7, *S. cerevisiae* DNA; lane 8, *E. coli* DNA.

directly because no purified symbionts were available. The *sopT* gene cloned and sequenced in this study clearly encodes the previously characterized ATP sulfurylase enzyme on the basis of the following evidence. First, the portion of the N-terminal (17-amino-acid) peptide sequence not used to generate the PCR primer matched that encoded by the cloned gene. Second, the *sopT* gene sequence predicts a 48-kDa protein, in agreement with previous data showing that the enzyme contains two identical 48-kDa subunits (24). Third, the cloned gene showed significant sequence homology only with another ATP sulfurylase gene (Fig. 5). Hybridization of the *sopT* probe to symbiont DNA, but not to DNA from symbiont-free vestimentum tissue (Fig. 3), indicated that the gene was derived from the bacterial symbiont rather than from the host. Furthermore, the mol% G+C for the *Riftia* symbiont, previously determined to be approximately 59% (8), was in excellent agreement with the value of 58% for the *sopT* coding region. DNA from host vestimentum tissue had a very different value, approximately 35% (8).

Biochemical and molecular genetic data were used to compare the autotrophic ATP sulfurylase to other enzymes having ATP sulfurylase activity. The possibility that an active site of the autotrophic enzyme includes the highly conserved boxed region (Fig. 5) is consistent with the following observations: First, the internal peptide fragment (AHEELCR; Fig. 4) was initially identified by fluorescent labeling of the cysteine residue (Cys-210), indicating that this cysteine residue lies within an exposed area of the protein in its native conformation. Biochemical studies indicated that chemical modification of either an arginine by phenylglyoxal or a cysteine by DTNB [2',2'-dithiobis-(5-nitrophenyl)] eliminated the catalytic activity of the enzyme (24). Three arginine residues reside near the Cys-210 residue, which is thus both available and potentially essential for biological activity. Second, ligand binding of SO_4^{2-} , ATP, or APS protects against enzyme inactivation by DTNB (24), suggesting an active site location for the sulfhydryl group (24). Finally, this region has virtual identity with a sequence in the ATP sulfurylase (*MET3*) of *S. cerevisiae*, suggesting that it is a functionally conserved site.

Neither of the protein sequences contains a canonical P-loop motif of ATP-binding sites (GXXXXGK[TS]) (27). However, within a stretch of amino acids (Rs228-237; Fig. 5) which show a relatively high degree of homology, there is a GXKXG

which conforms to the following almost universal features of P-loops. First, virtually all contain the GXXXXG motif, and second, all have a conserved K. Thus, it is suggested that this site may be involved in ATP binding.

There are additional regions of the *Riftia* symbiont protein which show considerable homology with the *S. cerevisiae* protein (dotted lines in Fig. 5). However, none of these symbiont sequences includes a cysteine residue; thus, in the absence of further biochemical data, the role of these somewhat less highly conserved regions remains speculative. Although biochemical characterization of the *S. cerevisiae* ATP sulfurylase is incomplete, there is a very high degree of amino acid homology (67% identity and approximately 75% similarity in the first 400 bases) between it and the ATP sulfurylase from *Penicillium chrysogenum* (29a). Because the *P. chrysogenum* ATP sulfurylase has been characterized extensively (19), this strongly supports the view that the *MET3* gene encodes an enzyme having all the catalytic properties of an ATP sulfurylase (4). Considering the phylogenetic distance between the *Riftia* symbiont and *S. cerevisiae*, it is reasonable to expect that the 17-amino-acid conserved region will also be found in other ATP sulfurylase proteins as additional data become available.

There are significant differences in the biochemical properties of the autotrophic ATP sulfurylase compared to those of the fungal and plant enzymes (24), supporting the argument that these enzymes function in opposite directions. The highly conserved region of the products of *sopT* and *MET3* (Fig. 5) consists of 16 identical amino acids; 5 of these are basic and only 1 is acidic, suggesting that the region constitutes part of the catalytic site which interacts with a highly negatively charged substrate, e.g., APS, ATP, or sulfate. Perhaps significantly, the single amino acid difference is a basic residue (Arg-205) in *S. cerevisiae* interchanged with an acidic residue (Glu-207) in the *Riftia* symbiont. This slight change may be related to the fact that the enzymes function in opposite directions. A 31-amino-acid peptide, purified and sequenced from the *P. chrysogenum* ATP sulfurylase, contains a sulfhydryl group which seems to have a regulatory role (19). No region in the *Riftia* symbiont ATP sulfurylase sequence displayed significant homology to this peptide fragment. The *P. chrysogenum* protein is significantly larger (69 kDa) than that of the *Riftia* symbiont (48 kDa), thus it is likely that the regulatory region of the *P. chrysogenum* protein is located beyond the length of the symbiont ATP sulfurylase protein.

It is puzzling that the autotrophic ATP sulfurylase from the *Riftia* symbiont and the assimilatory ATP sulfurylase from the eukaryote *S. cerevisiae* share regions of virtual identity and significant overall homology with each other but not with the gene products previously reported to encode assimilatory ATP sulfurylases in other prokaryotes. On the basis of sequence comparisons both by computer and by Southern hybridization analyses (Fig. 1 and 6 and data not shown), the *sopT* gene does not share detectable homology with *cysDN* in *E. coli* or with *nodP* and *nodQ* in *R. meliloti*. The encoded ATP sulfurylases of *E. coli* and *R. meliloti* are composed of two very differently sized subunits (18, 28), while other well-characterized ATP sulfurylases, involved in either sulfur-based energy generation or sulfate assimilation in eukaryotes, contain two or more identical subunits (24). Further evidence of a fundamental difference comes from the observation that the *cysDN* product, purified to apparent homogeneity, showed an ATP sulfurylase specific activity of 0.23 U/mg in ATP synthesis (18). This is less than 1% of the activity of comparably assayed ATP sulfurylases purified from all other sources (1, 5, 20, 24). These discrepancies might previously have been attributed to the evolutionary distance between the prokaryote *E. coli* and the eukaryotic

TABLE 1. Hybridization of the ATP sulfurylase gene from the *Riftia* symbiont (*sopT*) and *E. coli* (*cysDN*) to DNA from other organisms

Organism	Mode of nutrition	Hybridization ^a to:	
		<i>sopT</i>	<i>cysDN</i>
Sulfur-oxidizing bacteria			
<i>Riftia</i> symbiont	Chemoautotrophic	+	—
<i>C. vinosum</i>	Photoautotrophic	+	—
<i>T. denitrificans</i>	Chemoautotrophic	+	—
<i>T. crunogena</i>	Chemoautotrophic	—	—
<i>Beggiatoa</i> 81-1c	Chemoautotrophic	+	ND ^b
Sulfate-reducing bacteria			
<i>D. desulfuricans</i>	Heterotrophic	—	—
Sulfate-assimilatory organisms			
<i>E. coli</i>	Heterotrophic	—	+
<i>Beggiatoa</i> 75-2a	Heterotrophic	—	ND
<i>S. cerevisiae</i>	Heterotrophic	—	—

^a Assayed under low stringency conditions.

^b ND, not done.

sulfate assimilators. However, the discovery of the conserved region in the prokaryotic *sopT* and the eukaryotic *MET3* ATP sulfurylase proteins, plus the extremely low specific activity of the *E. coli* ATP sulfurylase even compared to other prokaryotic assimilatory enzymes (20), suggests that *E. coli* may be atypical in the enzyme it employs for the first step in sulfate assimilation. This perhaps reflects the low-redox environment which it inhabits, where demand for sulfate assimilation might be expected to be minimal. A locus with homology to *sopT* and *MET3* may still exist in *R. meliloti*, since sulfate assimilation independent of *nodP* and *nodQ* was recently discovered to be encoded by the as yet unsequenced *saa* locus (29).

The results of this study indicate that there is not sufficient homology between *sopT* and a gene encoding ATP sulfurylase from *D. desulfuricans* to give a hybridization signal under low-stringency conditions; nor is there positive hybridization between *cysDN* and any DNA sequence from *D. desulfuricans*. Thus, until gene or protein sequence data are available, conclusions about its evolutionary relationship with autotrophic or assimilatory ATP sulfurylases is not possible.

Table 1 summarizes the hybridization studies in which organisms previously assayed for ATP sulfurylase activity were screened with the *Riftia* symbiont *sopT* and *E. coli* *cysDN* probes. These results show a correlation between the *sopT* probe hybridization and the use of ATP sulfurylase in autotrophic nutrition. The *sopT* probe clearly hybridized to DNA from diverse sulfur bacteria (Fig. 6) which are deduced from enzyme studies to use the APS pathway: *T. denitrificans* (29a), *Beggiatoa* MS-81-1c (12), and *C. vinosum* (23). *sopT* does not share detectable homology with DNA from the chemoautotroph *T. crunogena* nor with that from the heterotroph *Beggiatoa* OH-75-2a, both sulfur bacteria which seemingly do not use the APS pathway (11a, 12). Thus, a significant finding of this study is the indication that the *sopT* gene will be useful in the identification of sulfur-oxidizing chemoautotrophic bacteria which use the APS pathway, including sulfur-oxidizing bacterial endosymbionts of other marine invertebrates (unpublished data), with no apparent danger of false positives from sulfate reducers or sulfate assimilators.

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