The Sulfur-Regulated Arylsulfatase Gene Cluster of 
_Pseudomonas aeruginosa_, a New Member of the cys Regulon

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A gene cluster upstream of the arylsulfatase gene (atsA) in _Pseudomonas aeruginosa_ was characterized and found to encode a putative ABC-type transporter, AtsRBC. Mutants with insertions in the atsR or atsB gene were unable to grow with hexyl-, octyl-, or nitrocatechol sulfate, although they grew normally with other sulfur sources, such as sulfate, methionine, and aliphatic sulfonates. AtsRBC therefore constitutes a general sulfate ester transport system, and desulfurization of aromatic and medium-chain-length aliphatic sulfate esters occurs in the cytoplasm. Expression of the atsR and atsBCA genes was repressed during growth with sulfate, cysteine, or thiocyanate. No expression of these genes was observed in the cysB mutant PAO-CB, and the ats genes therefore constitute an extension of the cys regulon in this species.

Sulfate esters make up a large proportion of the sulfur that is found in aerobic soils, and so it is not surprising that many soil microorganisms have evolved enzymes that catalyze the hydrolysis of these compounds, either to release the sulfate moiety as a sulfur source for growth, or as the first step in their mineralization. Bacterial sulfatases have been studied extensively in the past, with particular emphasis placed on those enzymes that lead to degradation of surfactants (5). Strains that are able to grow with alkylsulfates such as sodium dodecyl sulfate (SDS) as the source of carbon are widespread in the environment, even in samples isolated from uncontaminated sites (23). A variety of alkylsulfatases is responsible for the hydrolysis reaction, often even within one species. The best-studied such strain is _Pseudomonas_ sp. strain C12B (reviewed in reference 5), which displays a broad substrate tolerance even though the enzymes it contains are relatively substrate specific in terms of chain length and stereospecificity. Synthesis of these enzymes is controlled by a complex network of substrate and product induction (5).

Hydrolysis of aromatic sulfate esters, in contrast, is controlled in bacteria exclusively by the supply of sulfur to the cell, and is catalyzed by enzymes of the arylsulfatase family. These enzymes are common soil enzymes and, because they are easy to assay, are often used as a measure of soil quality (16). Synthesis of arylsulfatase is repressed during growth with inorganic sulfate or cysteine as the sulfur source and upregulated under sulfate-limiting conditions (e.g., during growth with sulfonates, sulfate esters, sulfamates, or methionine) (7). In _Pseudomonas aeruginosa_, the repressive effect in vivo was recently traced to two independent effectors—sulfite and either sulfate or cysteine (7)—whereas in _Klebsiella pneumoniae_, sulfate and cysteine repress arylsulfatase synthesis, also independently of each other (12).

The regulation of arylsulfatase synthesis is correlated with that of a group of so-called sulfate starvation-induced proteins, which were identified by differential two-dimensional electrophoresis (7, 13), and we have therefore used arylsulfatase as a model system for the sulfate starvation response. In this report, we show that, in _P. aeruginosa_, arylsulfatase is encoded together with a general transport system for both aliphatic and aromatic sulfur esters, and expression of this gene cluster requires the LysR-type transcriptional activator CysB.

**Cloning and sequence analysis of the ats gene cluster.** Previous studies of arylsulfatase in _P. aeruginosa_ led to the identification and characterization of the atsA gene (1), but complementation studies with a DNA fragment carrying only this gene were unsuccessful. We therefore cloned and sequenced a region upstream of the atsA gene, in order to identify the promoter region from which atsA is expressed. Screening of a cosmid bank of _P. aeruginosa_ yielded two cosmid clones carrying parts of the desired locus, and these were subcloned onto pBluescript (Stratagene) to give a 7-kb fragment on the plasmid pM4326. Sequencing of this fragment led to the identification of three further open reading frames, atsB, atsC, and atsR (Fig. 1). The genes atsB and atsC were carried as part of a putative operon with atsA, and overlapped each other by four nucleotides, whereas a fourth gene, atsR, was identified on the complementary strand, oriented divergently from atsBCA. The overall G+C content of the coding regions was 68.2%, although this dropped to 50% in the intergenic region between atsR and atsB.

Sequence analysis of the deduced AtsRBC proteins suggested that they represented an ABC-type transporter of unknown specificity. AtsB encoded a 57.8-kDa polypeptide with 30 to 40% identity to known bacterial permeases. Hydrophobicity analysis with the program TMPred (6) predicted the presence of 12 membrane-spanning domains, and since the predicted AtsB protein is twice the size of related permeases (e.g., Taur of _Escherichia coli_, encoding the putative taurine permease [20], is 30 kDa in size), AtsB therefore corresponds as a monomer to the dimeric form of other permeases. The deduced AtsC protein was 31 kDa in size and was related (40 to 44% amino acid identity) to ATP-binding proteins of ABC-type transporters. The two Walker motifs which are characteristic of proteins of this family were present (GQSGGCGKST and LLLDEPF [consensus residues underlined]), as was the so-called ABC signature, LSGG (11). The third open reading frame identified, atsR, encoded a 34-kDa protein carrying a
putative N-terminal signal peptide. The AtsR protein was 25 to 42% identical to periplasmic substrate binding proteins involved in uptake of arylsulfatases or aliphatic sulfonates (21). These proteins are sufficiently similar that they have been proposed to form an independent family of binding proteins (21), adding to those previously defined by Tam and Saier (17).

AtsRBC proteins constitute a general sulfate ester transport system. To further characterize the AtsRBC transporter, mutations were introduced into the atsR and atsB genes. The promoterless xylE::Gm cassette from the plasmid pX1918GT (15) was ligated into the Eco47III site in atsR (nucleotide 8 of atsR) and between the BamHII and EcoRI sites in atsB, respectively (Fig. 1). The resulting constructs were subcloned onto the suicide plasmid pME3087 (22) and transferred onto the P. aeruginosa PAO1 chromosome by homologous recombination. This yielded strains JH1 (atsR::xylE/Gm), in which the xylE was present as a transcriptional fusion to the atsR gene, and JH3 (atsB::Gm), where unfortunately we were only able to generate Gm (atsB) and between the atsR and atsB respectively (11). The resulting constructs were subcloned onto the suicide plasmid pME3087 (22) and transferred onto the P. aeruginosa PAO1 chromosome by homologous recombination. These results demonstrate that the arylsulfatase is not involved in uptake of arylsulfatases or aliphatic sulfonates (21). Interestingly, JH1 and JH3 retained the ability to grow with SDS. This finding is consistent with previous studies of SDS degradation as a carbon source, which showed that the SDS sulfatase is periplasmically located (2, 5). In contrast to SDS sulfatase, however, the medium-chain-length sulfatase appears to be localized in the cytoplasm in P. aeruginosa, as is arylsulfatase (1).

Arylsulfatase and catechol oxygenase activities in strain JH1 were measured by standard methods (1, 8) and showed that expression of the atsR gene was repressed during growth with sulfate, and upregulated during growth with organosulfur sources such as pentanesulfonate or methionine (Fig. 2). Arylsulfatase synthesis was also regulated in the same way, although the arylsulfatase levels were not as high as in the wild-type strain (Fig. 2). As expected, no arylsulfatase activity was seen in strain JH3, due to the polar effect of the atsB::Gm insertion on transcription of the atsA gene.

Expression of atsR and atsBCA is controlled by CysB in P. aeruginosa. Expression of the atsR and atsB genes was now examined in the wild-type strain PAO1, by using transcriptional lacZ fusions constructed by cloning the atsR::atsB intergenic region in both orientations into the promoter probe plasmid pQF120 (14), to yield the plasmids pME4334 (atsR::lacZ) and pME4337 (atsB::lacZ). β-Galactosidase activities in mid-exponential-phase cells during growth with various sulfur sources were measured with ONPG as a substrate and are shown in Fig. 3. Both atsR and atsB were upregulated during growth with organosulfur sources, and repressed during growth with inorganic sulfate, even when the latter was combined with an organosulfur compound. The degree of downregulation in the presence of sulfate was consistent with that previously observed with the chromosomal atsR::xylE fusion (Fig. 2), demonstrating that copy number did not have an effect on the regulation. This suggested that expression might not be regulated by a direct repressor (there was no evidence for titration of a repressor protein in the presence of a high-copy-number reporter plasmid), but might be mediated by a positive regulator, such as the CysB protein. CysB is a LysR-type transcriptional activator which has been well characterized in enteric bacteria (10), where it activates transcription of the cys biosynthetic genes in the presence of the coinducer N-acetylserine, and during sulfur limitation. It has recently also been reported in P. aeruginosa, where it plays a role in algD expression (3), and is required for growth with a variety of organosulfur compounds (8). We therefore tested expression of the atsB::lacZ and atsB::lacZ fusion constructs in the cysB::Gm mutant strain PAO-CB (8).

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<thead>
<tr>
<th>Sulfur source</th>
<th>Relative growth of strain:</th>
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<tbody>
<tr>
<td></td>
<td>PAO1S (wild type)*</td>
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<tr>
<td>Sulfate</td>
<td>++ +</td>
</tr>
<tr>
<td>Nitrocatechol sulfate</td>
<td>+ + +</td>
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<tr>
<td>Hexyl sulfate</td>
<td>+ + +</td>
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<tr>
<td>Octyl sulfate</td>
<td>+ + +</td>
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<tr>
<td>Dodecyl sulfate</td>
<td>+ + +</td>
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<tr>
<td>Pentanesulfonate</td>
<td>+ + +</td>
</tr>
<tr>
<td>Methionine</td>
<td>+ + +</td>
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* Strain PAO1S is a spontaneously streptomycin-resistant derivative of P. aeruginosa PAO1 (1).
Because this strain is auxotrophic for cysteine, we were unable to use sulfate as a repressing growth substrate, and we substituted it with thiocyanate, which also represses arylsulfatase expression in this species (7). No expression of the trans is observed under derepressing conditions in the absence of a functional CysB protein (Fig. 4). We therefore conclude that the atsR and atsBCA genes are new members of the cys regulon in *P. aeruginosa*, and the CysB protein clearly controls not just cysteine biosynthesis, but also the cleavage of organosulfur compounds to release inorganic sulfur for cysteine biosynthesis. However, when the atsRBCA cluster was introduced into *E. coli*, no synthesis of arylsulfatase was observed, and the cells were unable to grow with aromatic sulfates, despite the presence of an active *E. coli* cysB gene. It is not yet clear whether this effect is due to specificity of the *P. aeruginosa* CysB protein, but not the *E. coli* CysB protein, for binding sites in the atsR-atsB intergenic region, or whether additional species-specific factors are required for expression of the ats genes. Such factors are known for the sulfur-regulated sulfonatases systems in *Pseudomonas putida* (21) and *tats* in *E. coli* (18, 19), which in addition to CysB also require the LysR-type regulators AtsR and Cbl, respectively.

**FIG. 3.** Regulation of atsR::lacZ and atsB::lacZ expression in *P. aeruginosa* PA01S. Cells were grown in succinate-minimal medium with pentanesulfonate (Pn), methionine (Met), nitrocatechol sulfate (NCS), hexyl sulfate (Hx), or sulfate (SO₄) as the sulfur source and were harvested in the mid-exponential phase. β-Galactosidase specific activity (spec. act.) was measured with ONPG as the substrate. □, atsR::lacZ (pME4334); ▣, atsB::lacZ (pME4337); ■, vector control (pQF120).

**FIG. 4.** Effect of cysB on atsB and atsR expression in *P. aeruginosa*. Cells were grown in succinate-minimal medium with methionine (Met), thiocyanate (SCN), or both as the sulfur sources and were harvested in the mid-exponential phase. Expression of atsB::lacZ and atsR::lacZ was measured as β-galactosidase activity in the wild-type strain PA01S or in the cysB mutant PAO-CB. □, atsR::lacZ (pME4333); ▣, atsB::lacZ (pME4337); ■, vector control PA01S(pQF120).

**REFERENCES**


