JOURNAL OF BACTERIOLOGY, July 1995, p. 4134–4136 Vol. 177, No. 14
0021-9193/95/$04.00+0

Sulfate and Thiosulfate Transport in *Escherichia coli* K-12: Evidence for a Functional Overlapping of Sulfate- and Thiosulfate-Binding Proteins

AGNIESZKA SIRKO, MALGORZATA ZATYKA, EWA SADOWY, AND DANUTA HULANICKA*

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-106 Warsaw, Poland

Received 5 December 1994/Accepted 4 May 1995

In *Escherichia coli*, sulfate and thiosulfate ions are transported by an ABC-type transporter consisting of both the membrane components (the products of *cysT*, *cysW*, and *cysA* genes) and the periplasmic binders (the products of *cysP* and *sbp* genes). The single *cysP* and *sbp* mutants are able to utilize both sulfate and thiosulfate as a sole sulfur source, while the inactivation of both genes leads to cysteine auxotrophy resulting from the block in the transport of both ions.

In members of the family *Enterobacteriaceae*, CysB protein and the sulfur source regulate the cysteine biosynthetic pathway, including its first step, sulfate and thiosulfate transport (13).

The fact that sulfate transport and sulfate-binding activity are repressed in bacteria grown on cysteine has long been known (4). The sulfate-binding protein (SBP) from *Salmonella typhimurium* was the first periplasmic binding protein identified (5). It has since been crystallized, and its tertiary structure has been studied in detail (17).

The sulfur source-dependent activity of sulfate binding in *S. typhimurium* suggested that the gene encoding SBP was also a part of the cysteine regulon (16); however, it has not yet been cloned. Its counterpart from *Escherichia coli*, identified accidentally by Hellinga and Evans at 89 min on the chromosome during their studies of phosphofructokinase, was denoted as *sbp* (6). We are unaware of further studies of this gene, and no data about the regulation of expression of the gene and the phenotypes of any *sbp* mutants are available. Recently, SBP isolated from *E. coli* has been shown to be identical in overall tertiary structure to its counterpart from *S. typhimurium* (11).

The sulfate-thiosulfate permease operon *cysPTWA*, located at 52 min on the *E. coli* chromosome, has recently been described (9, 19). This permease belongs to the class of permeases requiring specific, periplasmic, substrate-binding proteins (for review, see references 1 to 3). The first gene in the operon, *cysP*, encodes a previously unknown thiosulfate-binding protein (TSBP), and the others—*cysT*, *cysW*, and *cysA*—encode membrane-associated components of the sulfate-thiosulfate transport system. The fact that the *cysP* gene encoding TSBP belongs to the cysteine regulon has been well documented for both *E. coli* (8, 9) and *S. typhimurium* (7).

The cysteine prototrophy of the insertional *cysP* mutant (9) and the failure of systematic efforts to isolate transport-defective mutants specifically impaired in sulfate-binding activity (15) suggested that the activities of SBP and TSBP at least partly overlap.

In this paper, we report the construction and phenotypic analysis of a double *cysP sbp* mutant.

Cloning and sequencing of *sbp*. Since the chromosomal location of the *sbp* gene in *E. coli* was known, we used the respective clone (λ 540) from Kohara's library of the *E. coli* genome (12) to construct a plasmid, pBR15, containing the 1.3-kb *Bgl*II-*Eco*RI DNA fragment in pBR322. This fragment contains the entire *sbp* gene, including its putative promoter and transcriptional terminator. We have sequenced the cloned DNA fragment and have found several discrepancies with the published nucleotide sequence of the region inside the *sbp* gene (6). Most of these discrepancies entirely agree with the amendments of Jacobson et al. (11). The one discrepancy, resulting in the change of E-185 into V-185 in the SBP, was not

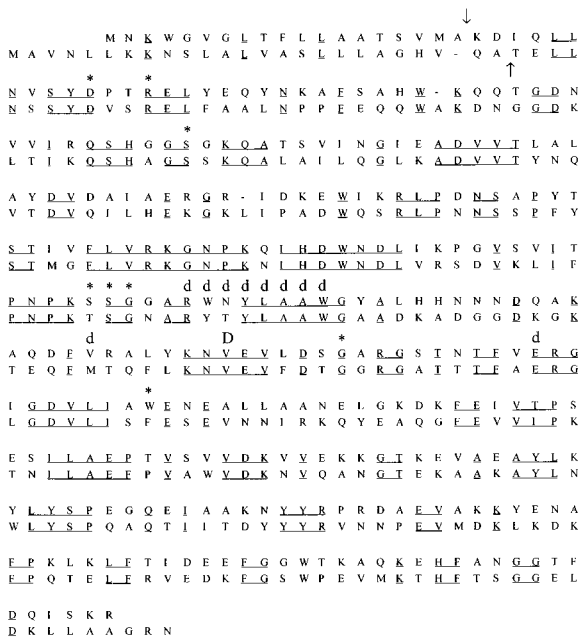

FIG. 1. Comparison of the amino acid sequences of SBP (upper sequence) and TSBP (lower sequence). Two gaps in SBP and one gap in TSBP sequences are included to maximize identity. The identical amino acids are underlined. The arrows indicate the ends of signal peptides. The letters above the aligned sequences show the differences between our data and the published SBP sequence (6). The differences observed by Jacobson et al. (11) are marked by a lowercase "d," and the differences not noted by Jacobson et al. (11) are marked by a capital "D." Asterisks mark the amino acids corresponding to the amino acids of SBP from *S. typhimurium* demonstrated to be critical for sulfate binding (17).

* Corresponding author. Mailing address: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, ul. Pawinskiego 5a, 02-106 Warszawa, Poland. Fax: (48) 39 12 16 23. Electronic mail address: asirko@ibbrain.ibb.waw.pl.

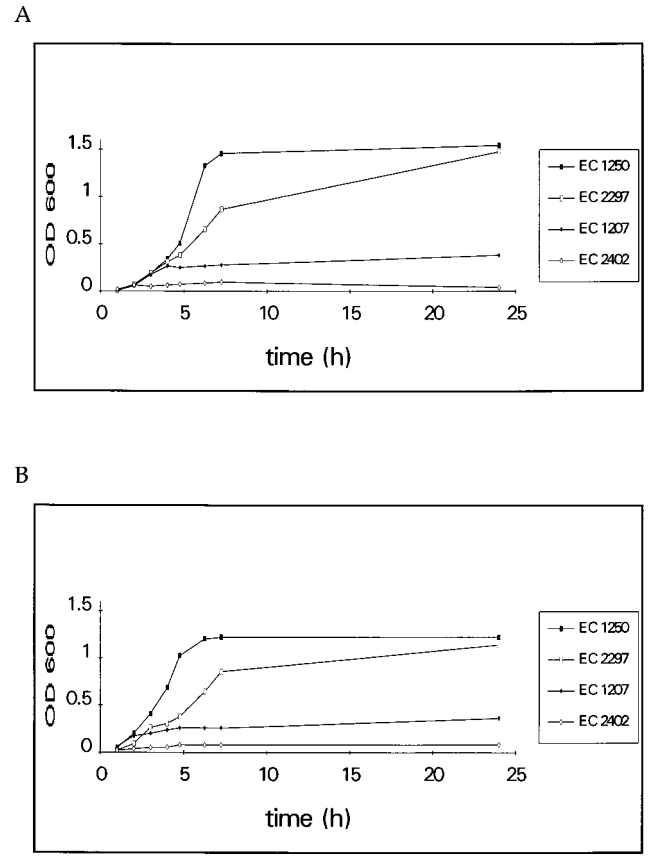

FIG. 2. Effects of the *cysP* and *sbp* mutations on the growth of bacterial cultures in minimal sulfur-free medium (10) supplemented with 0.1 mM sodium sulfate (A) or 0.05 mM sodium thiosulfate (B) as a sole sulfur source.

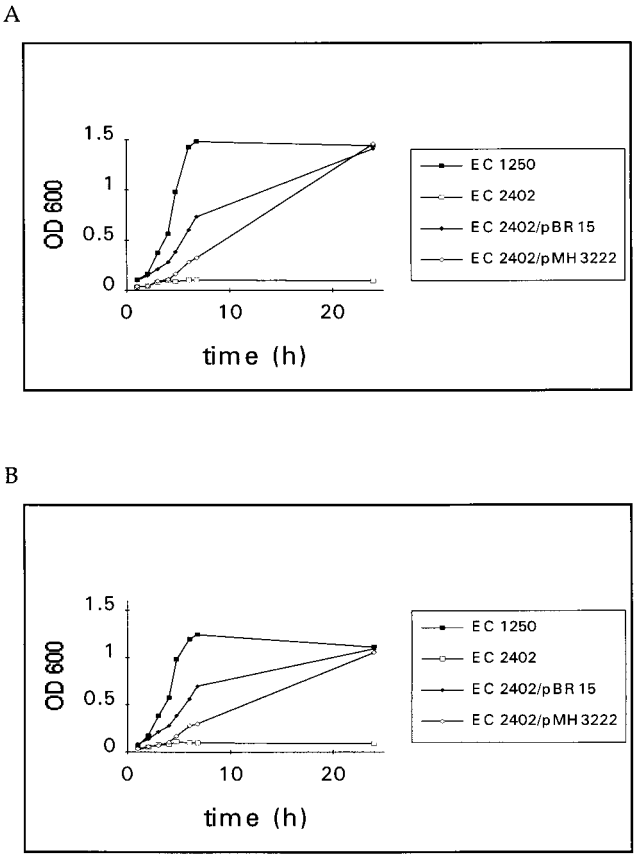

FIG. 3. Effects of plasmids with the intact *sbp* gene (pBR15) or the intact *cysP* gene (pMH3222) on the growth characteristics of the double *sbp cysP* mutant (EC 2402) in minimal medium supplemented with 0.1 mM sodium sulfate (A) or 0.05 mM sodium thiosulfate (B).

previously reported (11). On the nucleotide sequence level, with a numbering system consistent with that of Hellinga and Evans (6), A-1948 and A-1949 are, according to our data, changed into T and C, respectively. Comparison of the amino acid sequences of TSBP (9) and SBP (predicted from our corrected nucleotide sequence) is shown in Fig. 1.

Construction of mutants. The construction of the insertional *cysP-cat* mutant (EC 2297 strain) containing the chloramphenicol resistance gene (*cat*) inserted into the *cysP* gene has been described elsewhere (9). The *sbp-kan* insertional mutant (EC 1207 strain) has been constructed by the same method (22). This mutant contains the 1.6-kb *Bam*HI-*Bam*HI kanamycin-neomycin cassette isolated from pUC4-KIXX (Pharmacia Ltd.) inserted into the *Bcl*I site located in the *sbp* gene. The EC 2402 strain is the double *cysP-cat sbp-kan* mutant. All mutants were verified by genomic hybridization to the probes containing *cysP* and *sbp* coding regions (results not shown).

Growth characteristics of the mutants. We tested the rate and extent of growth of our mutants in minimal sulfur-free medium (10) supplemented with 0.1 mM sodium sulfate or 0.05 mM sodium thiosulfate (Fig. 2 and Fig. 3). Cells were incubated at 37°C with aeration, and at the times indicated, the optical density of the cultures at 600 nm (OD_{600}) was determined. The parental strain (EC 1250) served as a control.

The double *cysP sbp* mutant (EC 2402) is a cysteine auxotroph (Fig. 2). As expected, the cysteine prototrophy of the double *cysP sbp* mutant can be restored by the intact copies of either the *cysP* or *sbp* gene present on either the multicopy plasmid pMH3222 (9) or pBR15 (this paper), respectively (Fig. 3). Both single mutants are cysteine prototrophs able to utilize sulfate and thiosulfate as sole sulfur sources; both, however, have impaired growth compared with the wild-type strain (Fig. 2). This observation suggests that both SBP and TSBP are required for the normal transport of both ions (sulfate and thiosulfate).

Measurement of sulfate-binding activity. The cysteine auxotrophy of the double *cysP sbp* mutant (EC 2402) suggested that sulfate binding, and therefore its uptake, is impaired in this strain. We have performed assays of sulfate binding in the parental strain (EC 1250), the double mutant (EC 2402), and a single *sbp* mutant (EC 1207). Bacteria were grown to an OD_{600} of 0.3 to 0.4 in a sulfur-free minimal medium (10) supplemented with 0.5% glucose and 0.1 mM L-cystine or 1 mM djenkolic acid (conditions of cysteine starvation and, therefore, derepression of cysteine regulon) as sole sulfur sources. The sulfate-binding activities of osmotic shock fluids were determined as described previously (9). As shown in Table 1, sulfate binding is significantly reduced in both mutants tested. It is reduced about 3-fold in the *sbp* strain and almost 10-fold in the double mutant, compared with in the wild-type strain. The low level of sulfate-binding activity of the *sbp* mutant is sufficient, however, to maintain the cysteine prototrophy.

Conclusions. The hypothesis which was previously proposed in our laboratory (9) to explain the prototrophy of the *cysP*

TABLE 1. Activity of sulfate binding in mutants constructed for this study

Strain	Sulfur source	Sulfate binding (pmol/mg of protein)
EC 1250 (wild type)	Cystine	40
	Djenkolic acid	266
EC 1207 (*sbp*)	Cystine	41
	Djenkolic acid	77
EC 2402 (*sbp cysP*)	Djenkolic acid	29

mutant appears valid. Results reported here confirmed that the two binding proteins SBP and TSBP have partially overlapping activities and that a single mutation, inactivating only one of them, does not result in a lack of thiosulfate and sulfate binding and uptake. The double *cysP sbp* mutant can utilize neither sulfate nor thiosulfate as a sole sulfur source. Moreover, these results demonstrate that the sulfate-thiosulfate transporter consisting of the products of the *cysP*, *cysT*, *cysW*, *cysA*, and *sbp* genes is the only one responsible for the transport of both ions, in contrast to the multiple permeases for other substrates (e.g., for phosphate ions [18]).

Despite a striking similarity in function, size, and shape, the periplasmic binding proteins generally have only small amount of detectable primary sequence similarity to one another (21). SBP and TSBP are not the only examples of periplasmic binders having overlapping activities and interacting with the common membrane proteins (2, 14, 20). The availability of such pairs of proteins opens the way to experiments to determine protein domains responsible for interacting with membrane-associated portions of periplasmic permeases.

Finally, the puzzling growth characteristics of the single mutants might be explained by the very attractive hypothesis that at least one of these two binders, in addition to its role in the transport of sulfate and thiosulfate, can have an additional function. Recent evidence suggests that some of the periplasmic binding proteins of gram-negative bacteria can serve as receptors initiating sensory transduction pathways (21).

The initial experiments leading to the construction of the mutants were performed by A.S. in the laboratory of A. Böck, University of Munich, Germany. We thank A. Böck and G. Sawers for their hospitality and extensive help in the course of these experiments.

REFERENCES

1. **Adams, M. D., and D. L. Oxender.** 1989. Bacterial periplasmic binding protein tertiary structures. J. Biol. Chem. **264:**15739–15742.
2. **Ames, G. F.-L.** 1986. Bacterial periplasmic transport systems: structure, mechanism and evolution. Annu. Rev. Biochem. **55:**397–425.
3. **Ames, G. F.-L., C. S. Mimura, S. R. Holbrook, and V. Shyamala.** 1992. Traffic ATPases: a superfamily of transport proteins operating from *Escherichia coli* to humans. Adv. Enzymol. Relat. Areas Mol. Biol. **65:**1–47.
4. **Dreyfuss, J.** 1964. Characterization of a sulfate- and thiosulfate-transporting system in *Salmonella typhimurium*. J. Biol. Chem. **239:**2292–2297.
5. **Dreyfuss, J., and A. B. Pardee.** 1965. Evidence for a sulfate-binding site external to the cell membrane of *Salmonella typhimurium*. Biochim. Biophys. Acta **104:**308–310.
6. **Hellinga, H. W., and P. R. Evans.** 1985. Nucleotide sequence and high-level expression of the major *Escherichia coli* phosphofructokinase. J. Biochem. **149:**363–373.
7. **Hryniewicz, M., and N. M. Kredich.** 1991. The *cysP* promoter of *Salmonella typhimurium*: characterization of two binding sites for CysB protein, studies of in vivo transcription initiation, and demonstration of the anti-inducer effects of thiosulfate. J. Bacteriol. **173:**5876–5886.
8. **Hryniewicz, M., A. Sirko, and D. Hulanicka.** 1989. Identification and mapping of the sulphate permease promoter region in *Escherichia coli*. Acta Biochim. Polon. **36:**353–363.
9. **Hryniewicz, M., A. Sirko, A. Pałucha, A. Böck, and D. Hulanicka.** 1990. Sulfate and thiosulfate transport in *Escherichia coli* K-12: identification of a gene encoding a novel protein involved in thiosulfate binding. J. Bacteriol. **172:**3358–3366.
10. **Hulanicka, M. D., T. Klopotowski, and D. A. Smith.** 1972. The effect of triazole on cysteine biosynthesis in *Salmonella typhimurium*. J. Gen. Microbiol. **72:**291–301.
11. **Jacobson, B. L., J. J. He, P. S. Vermesch, D. D. Lemon, and F. A Quiocho.** 1991. Engineered interdomain disulfide in the periplasmic receptor for sulfate transport reduces flexibility. Site-directed mutagenesis and ligand-binding studies. J. Biol. Chem. **266:**5220–5225.
12. **Kohara, Y., K. Akijama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50:**495–508.
13. **Kredich, N. M.** 1992. The molecular basis for positive regulation of cys promoters in Salmonella typhimurium and Escherichia coli. Mol. Microbiol. **6:**2747–2753.
14. **Nikaido, K., and G. F.-L. Ames.** 1992. Purification and characterization of the periplasmic lysine-, arginine-, ornithine-binding protein (LAO) from *Salmonella typhimurium*. J. Biol. Chem. **267:**20706–20712.
15. **Ohta, N., P. R. Galsworthy, and A. B. Pardee.** 1971. Genetics of sulfate transport by *Salmonella typhimurium*. J. Bacteriol. **105:**1053–1062.
16. **Pardee, A. B.** 1966. Purification and properties of a sulfate binding protein from *Salmonella typhimurium*. J. Biol. Chem. **241:**3962–3969.
17. **Pflugrath, J. W., and F. A. Quiocho.** 1988. The 2A resolution structure of the sulfate-binding protein involved in active transport in *Salmonella typhimurium*. J. Mol. Biol. **200:**163–180.
18. **Rao, N. N., and A. Torriani.** 1990. Molecular aspects of phosphate transport in *Escherichia coli*. Mol. Microbiol. **4:**1083–1090.
19. **Sirko, A., M. Hryniewicz, D. Hulanicka, and A. Böck.** 1990. Sulfate and thiosulfate transport in *Escherichia coli* K-12: nucleotide sequence and expression of the *cysTWAM* gene cluster. J. Bacteriol. **172:**3351–3357.
20. **Speiser, D. M., and G. F.-L. Ames.** 1991. *Salmonella typhimurium* histidine periplasmic permease mutations that allow transport in the absence of histidine-binding proteins. J. Bacteriol. **173:**1444–1451.
21. **Tam, R., and M. H. Saier, Jr.** 1993. Structural, functional, and evolutionary relationships among extracellular solute-binding receptors of bacteria. Microbiol. Rev. **57:**320–346.
22. **Winans, S. C., S. J. Elledge, J. H. Krueger, and G. C. Walker.** 1985. Site-directed insertion and deletion mutagenesis with cloned fragments in *Escherichia coli*. J. Bacteriol. **161:**1219–1221.