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Genetic Analysis of the *modABCD* (Molybdate Transport) Operon of *Escherichia coli*†

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DNA sequence analysis of the *modABCD* operon of *Escherichia coli* revealed the presence of four open reading frames. The first gene, *modA*, codes for a 257-amino-acid periplasmic binding protein enunciated by the presence of a signal peptide-like sequence. The second gene (*modB*) encodes a 229-amino-acid protein with a potential membrane location, while the 352-amino-acid ModC protein (*modC* product) contains a nucleotide-binding motif. On the basis of sequence similarities with proteins from other transport systems and molybdate transport proteins from other organisms, these three proteins are proposed to constitute the molybdate transport system. The fourth open reading frame (*modD*) encodes a 231-amino-acid protein of unknown function. Plasmids containing different *mod* genes were used to map several molybdate-suppressible chlorate-resistant mutants; interestingly, none of the 40 mutants tested had a mutation in the *modD* gene. About 35% of these chlorate-resistant mutants were not complemented by *mod* operon DNA. These mutants, designated *mol*, contained mutations at unknown chromosomal location(s) and produced formate hydrogenlyase activity only when cultured in molybdate-supplemented glucose-minimal medium, not in L broth. This group of *mol* mutants constitutes a new class of molybdate utilization mutants distinct from other known mutants in molybdate metabolism. These results show that molybdate, after transport into cells by the ModABC proteins, is metabolized (activated?) by the products of the *mol* gene(s).

Although they are few in number, molybdoenzymes play an essential role in microbial metabolism. These enzymes (except dinitrogenase) contain a unique form of molybdopterin-nucleotide as the cofactor (33). In *Escherichia coli*, the main cofactor found in molybdoproteins (formate dehydrogenase, nitrate reductase, etc.) is molybdopterin guanine dinucleotide (33). The biosynthesis of molybdopterin guanine dinucleotide and thus active molybdoenzymes starts with the transport of molybdate into cells. Mutant strains which are defective in one molybdoenzyme, nitrate reductase activity, have been isolated from several microorganisms as chlorate-resistant strains (11, 19, 42). Pleiotropic molybdoenzyme-defective mutants whose phenotype can be suppressed by increasing the molybdate concentration in the growth medium were defined as transport-negative (*mod*; previously termed *chlD* [39]) mutants (11). By using this rationale, a fraction of chlorate-resistant mutants were identified as *mod* mutants (19, 42). Biochemical analysis of some of these mutants confirmed that the *mod* mutation decreased the rate of molybdate transport and thus its accumulation by cells (9, 16, 38). By complementing these mutants, the wild-type genes coding for various components of the molybdate transport system have been isolated from *E. coli* (16, 21, 35). Johann and Hinton (21) determined the DNA sequence of an internal segment of the *mod* operon (*modC* gene) from *E. coli*. However, the complete DNA sequence of the *E. coli mod* operon is not available, although a large number of presumptive *mod* mutants of *E. coli* have been described. Besides *E. coli*, *mod*$^+$ DNA was also isolated from *Azotobacter vinelandii* and *Rhodobacter capsulatus* and sequenced (27, 43). Analysis of the *mod* DNA sequences from these organisms suggests that the transport of molybdate into cells is achieved by a typical periplasmic binding protein and an ATP-dependent transport system similar to the ones reported for other solutes, like sulfate, histidine, maltose, etc. (2, 17, 40, 41).

In this communication, the complete DNA sequence of the *mod* operon from *E. coli* is presented. By using cloned *mod*$^+$ DNA, the mutations in a number of *mod* mutants were mapped within the *mod* operon. However, several chlorate-resistant mutants initially identified as Mod$^-$ had unique phenotypic characteristics, and on the basis of complementation analysis, the mutation in these mutants was found to be outside the *mod* operon.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are presented in Table 1 and are derivatives of *E. coli* K-12.

Media and growth conditions. L broth which served as the rich medium was supplemented with glucose (0.3%; LBG) (25), formate, or molybdate as needed at the concentrations indicated for each experiment. Glucose-minimal medium and low-sulfur medium (LSM) have been described previously (26). For molybdate-free glucose-minimal medium, sodium molybdate (normally present at a final concentration of 40 μM) was omitted. No attempt was made to remove contaminating molybdate from the ingredients of any of these media. The composition of glycerol-nitrate medium was the same as that of glucose-minimal medium, except that glucose was replaced by glycerol (1.5%) and sodium nitrate was added to a final concentration of 20 mM. The concentration of molybdate in glycerol-nitrate medium was 40 μM, and as indicated, this was increased to 0.5 mM. Cultures were grown at 37°C unless specified otherwise.

Isolation of mutants. Mutants of strain BW545 defective in molybdate transport (*mod*) were isolated as chlorate-resistant mutants (3, 11). These Chl^r mutants were tested for the ability to respire in the presence of nitrate anaerobically by incubating the plates at room temperature in a vacuum desiccator under a dinitrogen atmosphere. After 4 days, colonies which had grown in this medium were selected. About 30% of the chlorate-resistant mutants were nitrate respi-

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TABLE 1. Bacterial strains used in this study

Strain	Genotype	Source and/or reference
BW545	Δ(*lacU*)*169 rpsL*	Laboratory collection
MC4100	*araD139* Δ(*argF-lacU*)*205 rpsL150 relA1 flbB5301 deoC1 ptsF25*	CGSC 6152
JBM239-7	MC4100 Φ(*chlD-lac*)*2002* [Φ(*mol'-'lacZ*$^+$)*2002*]	N. Amy (31)
JRG94	*leuB6 gal-31 mod-4 bioA2 rpsL129 thi-1*	CGSC 4458
RK4353	MC4100 *gyrA219 non-9*	V. Stewart
RK5202	RK4353 *chlD202*::Mu cts (*modC202*)	V. Stewart
VJS720	*chlD247*::Tn*10* (*modB247*)	V. Stewart
SE1325	*cysC43 srl-300*::Tn*10 thr-1 leu-6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 his-4 argE3 rpsL31 tsx-33 supE44 modB138*::Tn*5*	Laboratory collection
SE2010	MC4100 Φ(*modA'-'lacZ*$^+$)*101*	This study
SE2069	BW545 Φ(*modA'-'lacZ*$^+$)*102*	This study

ration-positive in glycerol-nitrate medium supplemented with 0.5 mM molybdate. A total of 34 such mutants were selected. These mutants also required molybdate (>0.1 mM) for the production of dihydrogen and were thus tentatively identified as molybdate transport (Mod$^-$) mutants.

mod-lac fusion mutants were isolated by using λp*lac*Mu53 and λpMu507 as described by Bremer et al. (5). The location of the *lac* fusion junction in strain SE2069 was determined after the cloning and sequencing of the *lac* fusion-containing DNA as described by Bremer et al. (6).

Enzyme assays. Cells were grown under anaerobic conditions and assayed for formate hydrogenlyase (FHL) or nitrate reductase activity with whole cells as described previously (25, 26, 29).

In vitro transcription-translation. These experiments were carried out with *E. coli* S30 extracts obtained from Promega (Madison, Wis.). Both linear DNA and plasmid DNA were used as templates, and the newly synthesized proteins were labelled with [^{35}S]methionine. To eliminate the synthesis of β-lactamase which interfered with the identification of other plasmid-coded proteins, the plasmid DNA was linearized by hydrolysis with restriction endonuclease *Sca*I, which has a unique site in the *bla* genes of plasmids pBR322 and pUC19. The labelled proteins were detected after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (24) and autoradiography.

DNA sequence. The plasmids, pSE1001, pSE1004, and pSE1009, used in the determinations of DNA sequences were described previously (Fig. 1) (26). The general procedures for plasmid isolation, manipulation, and transformation were described previously (26, 28, 29). Exonuclease III-mediated deletions (15) of plasmid pSE1009 were carried out as suggested by Promega. DNA sequences were determined by the Sanger dideoxy sequencing method (13, 37) with the three plasmids described above, plasmid deletion derivatives, and specific primers synthesized on the basis of DNA sequence. DNA primers were synthesized at the DNA synthesis core facility of the Interdisciplinary Center for Biotechnology Research of the University of Florida. DNA sequences were analyzed by using the computer software Genepro (Riverside Scientific, Seattle, Wash.) and the Genetics Computer Group program (1, 8, 10).

Materials. Biochemicals were from Sigma Chemical Co. (St. Louis, Mo.). Restriction endonucleases and DNA-modifying enzymes were from New England Biolabs (Beverly, Mass.), Promega, or U.S. Biochemicals (Cleveland, Ohio). All inorganic and organic chemicals were from Fisher Scientific and were analytical grade.

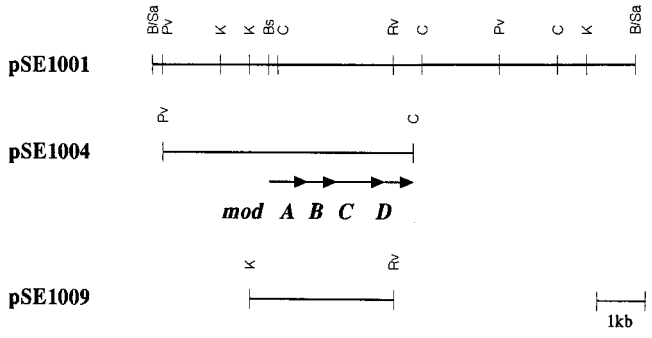

FIG. 1. Restriction map of *E. coli* chromosomal DNA in plasmids containing the *mod* operon. The direction of transcription is indicated by an arrow for each gene, as deduced from the orientation of the coding sequence. B/Sa, *Bam*HI/*Sau*3A junction used in cloning; Bs, *Bst*EII; C, *Cla*I; Rv, *Eco*RV; K, *Kpn*I; Pv, *Pvu*II.

RESULTS

Molybdate transport-defective mutants of *E. coli* have previously been described by several investigators (9, 11, 16, 19, 42). By using these mutants, *mod*$^+$ DNA was cloned and the DNA sequence of a part of the operon was determined (*modC*; 21). We previously constructed a recombinant clone (plasmid pSE1001; 26) which contained *E. coli* chromosomal DNA capable of suppressing the Mod$^-$ phenotype of a *molR-lac* fusion, strain SE1100. The Mod$^-$ phenotype of this strain is not understood and may depend on the fusion itself, which has been found (14) to map at 47 min rather than the 65 min position reported (26). The DNA sequence of the insert in plasmid pSE1001 (Fig. 1) was identical to that of the *modC* DNA cloned and sequenced by Johann and Hinton (21). In this section, the complete DNA sequence of the *mod* operon present in plasmid pSE1001, as well as the phenotypic properties of a set of molybdate-suppressible mutants (*mod* and *mol*), are presented.

modA gene. On the basis of the DNA sequence, the *mod* operon is composed of four genes, designated *modABCD* (Fig. 1 and 2). The first gene, *modA*, starts at position 294, extends for 774 bp, and codes for a protein of 257 amino acids. The apparent molecular weight of this protein as deduced from the DNA sequence is 27,363; this is in agreement with the 26,000-Da protein observed in the in vitro transcription-translation experiment (Table 2). The deduced N-terminal amino acid sequence of ModA is similar to the signal sequences found in periplasmic-solute binding proteins (2, 17, 20). It is likely that the ModA protein is targeted to the periplasm and that the preprotein is cleaved between the amino acids alanine and aspartate, leaving a 233-amino-acid mature protein. The ModA protein has sequence similarity to proteins from other organisms which also play roles in molybdate transport. The sequence identities are 26, 28, and 51% with the ModB protein of *A. vinelandii* (27), the ModA protein of *R. capsulatus* (43),

TABLE 2. Properties of *mod* genes and their products[a]

Gene	Size (bp)	M_r of protein		Predicted function
		Estimate	Observed	
modA	774	27,363	26,000	Periplasm binding
modB	690	24,938	24,000	Membrane channel
modC	1,059	39,045	37,500	Energizer
modD	696	26,336	ND[b]	Unknown

[a] See text for details.
[b] ND, not detected.

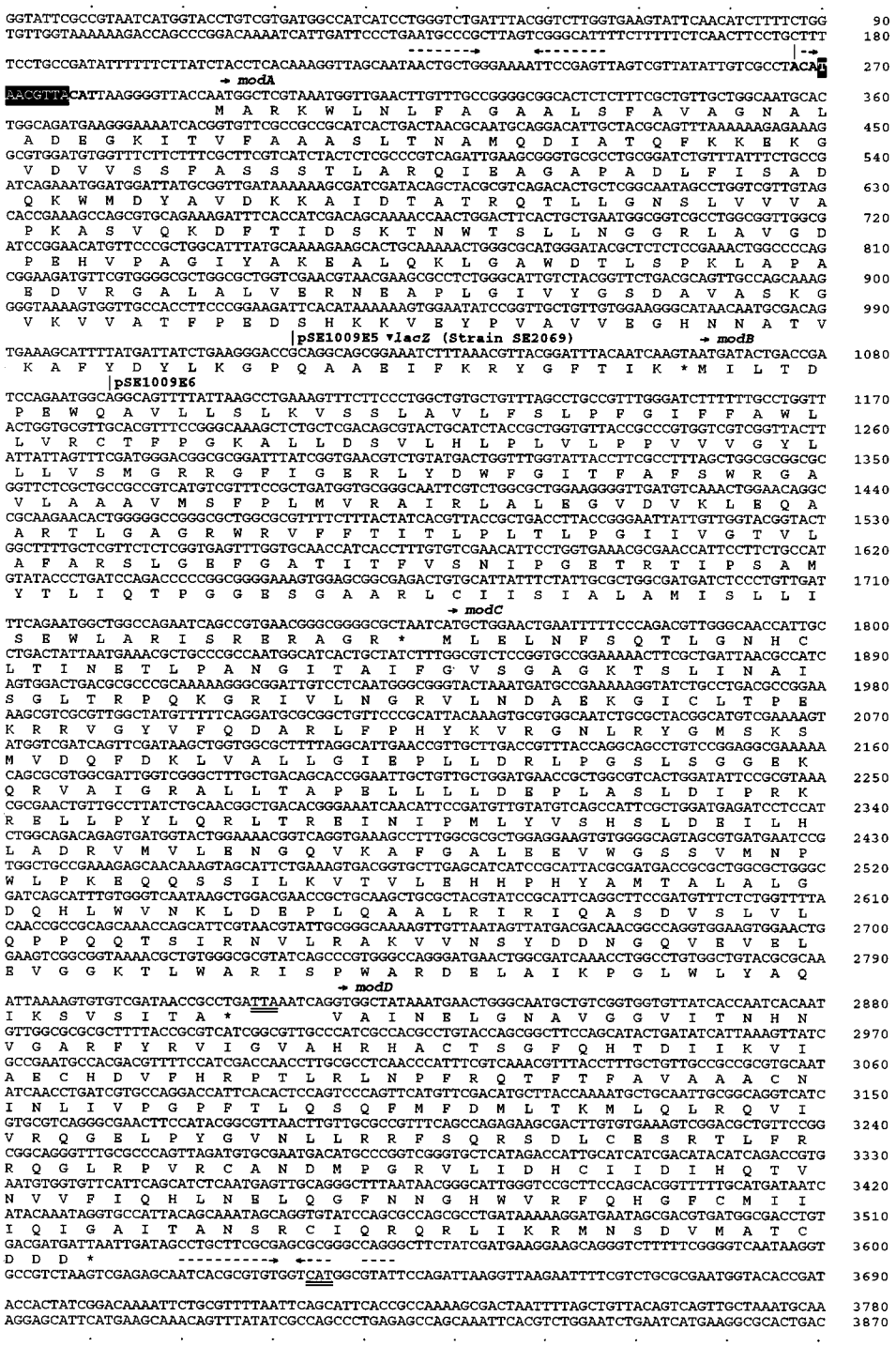

FIG. 2. DNA sequence and predicted translation products of the *modABCD* operon. The end positions of exonuclease III-mediated deletions of plasmid pSE1009 (pSE1009E5 and pSE1009E6) used in complementation analysis are indicated by vertical lines above the first bases in the DNA inserts of these two plasmids. The *lac* fusion junction in strain SE2069 is indicated by an arrowhead pointed down between the G and A bases (positions 1,032 and 1,033, respectively). In strain SE2069, phage Mu S end DNA (6) starts after the G at position 1,032 (*orfl-modA*). The transcription start site (position 267 [boldface A]) is indicated by a vertical line and a broken arrow above the sequence. Predicted stem-loop structures are indicated by broken arrows below lines. The CAT sequences are printed in bold. A 4-base inverted repeat between the CA sequences is printed in reverse print. The translation start and stop codons of an ORF in the opposite strand of the *modD* gene are indicated by double underline. Asterisks represent translation termination points. Dots above and below the sequence are located at intervals of 10 bases. The GenBank accession number for this sequence is U27192.

and the *orf8* gene product from the *lsg* region of *Haemophilus influenzae* (30), respectively.

***modB* gene.** The *modB* gene is 690-bp long and encodes a protein containing 229 amino acids. The ATG codon of the *modB* gene overlaps with the TAA codon of the *modA* gene (Fig. 2). The apparent molecular weight, based on the in vitro transcription-translation experiment, is 24,000, which is in agreement with the molecular weight of 24,938 predicted from the DNA sequence (Table 2). The ModB protein is highly hydrophobic and has five distinct hydrophobic regions which could serve as membrane-spanning areas. A sixth membrane-spanning segment that is not as extensive as the other five can be detected. In this regard, the ModB protein is similar to other membrane components of ATP-binding cassette transporters (18).

The ModB protein is 31 and 28% identical to the ModC protein of *A. vinelandii* (27) and the ModB protein of *R. capsulatus* (43), respectively. These similarities increase to 46 and 45% (*A. vinelandii* and *R. capsulatus*, respectively) if conservative substitutions in amino acid sequences are also included for these three proteins. Besides similarities to proteins in molybdate transport systems from other organisms, the ModB protein is also similar to the CysT and CysW proteins (33 and 31% identities, respectively) of *E. coli*, which constitute the membrane components of the sulfate-thiosulfate transport system (41). The ModB protein also shares amino acid sequence (32% identical) with the NifC protein of the dinitrogen fixation gene cluster from *C. pasteurianum* (44).

***modC* gene.** The *modC* gene encodes a 352-amino-acid protein. In an in vitro transcription-translation system, a protein with an apparent molecular weight of 37,500 was produced; this size is comparable to the predicted anhydrous molecular weight of 39,045 (Table 2). The ModC protein has the typical ATP-binding domains observed in proteins which serve similar functions in other ATP-binding cassette transport systems (2, 17, 32, 40). This protein is 43 and 38% identical to the ModD protein of *A. vinelandii* (27) and the ModC protein of *R. capsulatus* (43), respectively. These similarities increase to 58 and 56%, respectively, with conservative substitutions. The *E. coli* ModC and CysA proteins are also 23% identical. The presence of nucleoside triphosphate-binding domains in the ModC protein suggests that this protein is the ATPase of the molybdate transport system and helps couple ATP hydrolysis to active molybdate transport.

***modD* gene.** A fourth open reading frame starting with the GTG codon can be seen immediately adjacent to the ModC gene. The *modD* gene starts at position 2,827 and ends at position 3,522. This 231-amino-acid protein has a predicted anhydrous molecular weight of 26,336. This protein is unique as no protein with a similar sequence was found in the sequence database. The possible physiological role of this protein is unknown. An open reading frame (ORF) immediately next to the *modC* gene (*modD*) was also reported for *R. capsulatus* (43). These *E. coli* and *R. capsulatus* proteins are not similar.

Other features from the DNA sequence. Besides the four ORFs coding for the *mod* operon, another ORF in the DNA sequence can also be detected. This ORF is contained within the opposite strand of DNA coding the *modD* gene (Fig. 2). The 30.2-kDa protein encoded by this ORF (YbhA) has sequence homology to another hypothetical *E. coli* protein (YidA) with a molecular mass of 29.7 kDa (7). These two proteins are 23% identical. In the N-terminal 45 amino acids, however, these two proteins are 44% identical (64% similar). This similarity also extends to the C-terminal 57 amino acids of the protein, for which the two proteins are 46% identical (56% similar). The cellular function of neither the YbhA protein nor the YidA protein is known.

Nitrate-respiration - defective mutants
Molybdate-suppressible
FHL$^+$ in LBG + Mo (1 mM) medium

Complementation by indicated plasmids

Plasmid	pSE1009	Plasmid	pSE1009E5	Plasmid or Plasmid	pSE1009E6 pSJE301
SE2010 (*modA101*)		SE1602 (*modB125*)		SE1594 (*modC117*)	
SE2069 (*modA102*)		SE1603 (*modB126*)		SE1595 (*modC118*)	
SE1592 (*modA115*)		SE1605 (*modB128*)		SE1597 (*modC120*)	
SE1593 (*modA116*)		SE1608 (*modB131*)		SE1599 (*modC122*)	
SE1596 (*modA119*)		SE1609 (*modB132*)		SE1600 (*modC123*)	
SE1598 (*modA121*)		SE1611 (*modB134*)		SE1604 (*modC127*)	
SE1601 (*modA124*)		SE1612 (*modB135*)		SE1606 (*modC129*)	
SE1607 (*modA130*)		SE1325 (*modB138*)		SE1613 (*modC136*)	
JRG94 (*modA4*)		VJS720 (*modB247*)		RK5202 (*modC202*)	

FIG. 3. Classification of molybdate transport-defective mutants of *E. coli*. Plasmid pSJE301 was described by Johann and Hinton (21) and carries a *modC*$^+$ gene. The parent of mutants not listed in Table 1 is strain BW545.

modABCD operon transcription starts at position 267, with A as the first base (34). Adjacent to the transcription start site, a CAT sequence can be seen; this sequence is repeated after 7 bases. An inverted repeat, TAAC.GTTA, is located between the two CA sequences. These unique sequences play a role in the regulation of *mod* operon transcription (34).

On the basis of the DNA sequence, the *modABCD* operon is physically located in the *E. coli* chromosome at 17 min (4) between 808.5 and 814 kbp according to the map of Kohara et al. (22). The availability of the *mod* DNA sequence and plasmids that contain different genes of the *mod* operon led to characterization of presumptive *mod* mutants. Such genetic analyses identified mutations in each of the first three *mod* genes (*modABC*) (Fig. 3) and also revealed another unique group of mutants, designated *mol* mutants.

Analysis of Chl^r mutants. Twenty-two of the 34 Chl^r mutants isolated in this study produced FHL activities when grown in LBG-Mo medium. On the basis of complementation analysis with plasmids carrying different *mod* genes, the locations of mutations in these strains were identified (Fig. 3). None of the chlorate-resistant mutants had a mutation in the *modD* gene. Two *lac* fusion mutants, strains SE2010 and SE2069, had mutations in the *modA* gene. The *lac* fusion junction in strain SE2069 was cloned and sequenced. The *lac* fusion is between the G and A bases at positions 1,032 and 1,033, respectively, and is 11 amino acids from the carboxy-terminal end of the ModA protein (Fig. 2). After the mutations were mapped, two *mod* mutants from each subgroup were selected (SE1592 and SE1593 for *modA*, SE1602 and SE1603 for *modB*, and SE1595 and SE1597 for *modC*) for further analysis. The locations of the mutations in these mutants were confirmed by complementation with plasmids carrying the specific *mod* gene and by cotransduction by phage P1 with *gal* (70 to 80%).

Twelve of the 34 Chl^r mutants isolated and tentatively identified as Mod$^-$ did not produce dihydrogen even when grown in LBG-molybdate (1 mM) medium. When they were cultured in glucose-minimal medium or LSM with molybdate (50 μM), 11 of the 12 mutants produced dihydrogen. The 12th mutant, strain SE1590, was FHL$^-$ in all of the media tested and is probably a double mutant. The mutations in these 11 molybdate-suppressible *mol* mutants were not complemented by plasmids carrying *modABCD* DNA (data not shown).

DISCUSSION

The molybdate transport system of *E. coli* is composed of at least three proteins, a periplasmic molybdate-binding protein (ModA), an integral membrane protein (ModB), and an ATP-binding protein (ModC) encoded by the *modABC* genes (Fig. 2 and 3). In this regard, the molybdate transport systems in *E. coli*, *A. vinelandii*, and *R. capsulatus* are similarly constructed (27, 43). On the basis of similarity with the first 211 amino acids of the *E. coli* ModA protein, the *H. influenzae* ORF8 protein of the *lsg* operon (30) is possibly the periplasmic molybdate-binding protein in *H. influenzae*. However, the number of membrane components in *H. influenzae* is unknown since the available DNA sequence terminates within the "*modA*" gene. The fourth ORF in the *E. coli mod* operon (*modD*) codes for a unique protein with no defined function at this time. A mutation in or deletion of this gene (*modD*) produced no apparent phenotype (data not shown), suggesting that ModD is not needed for molybdate transport.

The molybdate transport system of *E. coli* has only one membrane protein; this is also true of the other two molybdate transport systems (*A. vinelandii* and *R. capsulatus*) analyzed so far (27, 43). In this regard, the molybdate transport system differs from many other ATP-binding cassette transporters, including the sulfate-thiosulfate transport system, which are known to have two very similar membrane proteins (17).

The *mod* operon is very poorly expressed in the wild-type mod^+ strain; only in a molybdate transport mutant or in the wild-type strain growing in a molybdate-deficient medium is *mod* operon expression elevated (34, 36). These results show that the *mod* operon is repressed in the presence of molybdate. A putative repressor-binding region was identified, and an alteration of this segment of DNA derepressed *mod* operon expression in the presence of molybdate (34).

The molybdate-suppressible *mol* mutants produced FHL and nitrate reductase activities only when cultured in glucose-minimal medium or LSM not in rich medium even with 1 mM molybdate. The results of transcription regulation experiments suggested that these mutants are capable of transporting molybdate through the native molybdate transport system when cultured in L broth (36). It is apparent from the phenotype that these *mol* mutants are defective in some step of molybdate metabolism after molybdate transport into cells. This defect is suppressed when cells are grown in LSM. *E. coli* is capable of using the sulfate transport system for transporting molybdate (26), and adenosine 5′-phosphosulfate sulfurylase from *E. coli* is also known to hydrolyze ATP in the presence of molybdate (45). The sulfate-to-cysteine pathway is derepressed only when cysteine is rate limiting for growth, as in a culture growing in LSM or glucose-minimal medium. The results obtained with *mol* mutants suggest that upon entrance into the cytoplasm of these mutants, molybdate is metabolized by appropriate proteins from the sulfate metabolic pathway (23) before insertion into molybdopterin to produce Mo-molybdopterin. In support of this possibility, double mutants carrying an additional mutation in the sulfate reduction (to sulfide) pathway failed to produce FHL activity in all of the media tested (12).

On the basis of phenotypic properties and complementation analysis, strain JBM239-7, a *lac* fusion mutant described by Amy and her coworkers (31), is similar to the *mol* mutants described above. This strain differed from other *mol* mutants in its ability to produce high levels of β-galactosidase activity in L broth, a property observed only with *mod* mutants (36). It is possible that strain JBM239-7 carries two mutations, a *lac* fusion mutation in the *mod* operon and a second mutation in an unidentified (*mol*) gene essential for molybdate metabolism.

In summary, the transport of molybdate is effected by three proteins which are analogous to other known periplasmic binding protein and ATP-dependent transport systems. Upon entrance into the cytoplasm, molybdate is further metabolized (reduced?) to produce activated Mo either by the native molybdate system or by enzymes in the sulfate reduction pathway before insertion into molybdopterin. Current experiments are directed toward identifying the biochemical nature of the activation system.

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REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tools. J. Mol. Biol. **215:**403–410.
2. **Ames, G. F.-L.** 1986. Bacterial periplasmic transport systems: structure, mechanism and evolution. Annu. Rev. Biochem. **55:**397–425.
3. **Andersen, K., and K. T. Shanmugam.** 1977. Energetics of biological nitrogen fixation: determination of the ratio of H_2 to NH_4^+ catalyzed by nitrogenase of *Klebsiella pneumoniae* in vivo. J. Gen. Microbiol. **103:**107–122.
4. **Bachmann, B. J.** 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54:**130–197.
5. **Bremer, E., T. J. Silhavy, and G. M. Weinstock.** 1985. Transposable λ p*lac*Mu bacteriophages for creating *lacZ* fusions and kanamycin resistance insertions in *Escherichia coli*. J. Bacteriol. **162:**1092–1099.
6. **Bremer, E., T. J. Silhavy, J. M. Weisemann, and G. M. Weinstock.** 1984. λ p*lac*Mu: a transposable derivative of bacteriophage lambda for creating *lacZ* protein fusions in a single step. J. Bacteriol. **158:**1084–1093.
7. **Burland, V. D., G. Plunkett III, D. L. Daniels, and F. R. Blattner.** 1993. DNA sequence and analysis of 136 kilobases of the *Escherichia coli* genome: organizational symmetry around the origin of replication. Genomics **16:**551–561.
8. **Chou, P. Y., and G. D. Fasman.** 1978. Prediction of the secondary structure of proteins from their amino acid sequence. Adv. Enzymol. **47:**45–147.
9. **Corcuera, G. L., M. Bastidas, and M. Dubourdieu.** 1993. Molybdenum uptake in *Escherichia coli* K12. J. Gen. Microbiol. **139:**1869–1875.
10. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
11. **Glaser, J. H., and J. A. DeMoss.** 1971. Phenotypic restoration by molybdate of nitrate reductase activity in *chlD* mutants of *Escherichia coli*. J. Bacteriol. **108:**854–860.
12. **Hasona, A., and K. T. Shanmugam.** Unpublished data.
13. **Hattori, M., and Y. Sakaki.** 1986. Dideoxy sequencing method using denatured plasmid templates. Anal. Biochem. **152:**232–238.
14. **Healy, F., R. Ray, and K. T. Shanmugam.** Unpublished data.
15. **Heinkoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28:**351–359.
16. **Hemschemeier, S., M. Grund, B. Keuntje, and R. Eichenlaub.** 1991. Isolation of *Escherichia coli* mutants defective in uptake of molybdate. J. Bacteriol. **173:**6499–6506.
17. **Higgins, C. F.** 1992. ABC transporters: from microorganisms to man. Annu. Rev. Cell Biol. **8:**67–113.
18. **Higgins, C. F., M. P. Gallagher, S. C. Hyde, M. L. Mimmack, and S. R. Pearce.** 1990. Periplasmic binding protein-dependent transport systems: the membrane associated components. Philos. Trans. R. Soc. Lond. B **326:**353–365.
19. **Hinton, S. M., and D. Dean.** 1990. Biogenesis of molybdenum cofactors. Crit. Rev. Microbiol. **17:**169–188.
20. **Izard, J. W., and D. A. Kendall.** 1994. Signal peptides: exquisitely designed transport promoters. Mol. Microbiol. **13:**765–773.
21. **Johann, S., and S. M. Hinton.** 1987. Cloning and nucleotide sequence of the *chlD* locus. J. Bacteriol. **169:**1911–1916.
22. **Kohara, Y., K. Akiyama, 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.
23. **Kredich, N. M.** 1987. Biosynthesis of cysteine, p. 419–428. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
24. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of

the head of bacteriophage T4. Nature (London) **227:**680–685.
25. **Lee, J. H., P. Patel, P. Sankar, and K. T. Shanmugam.** 1985. Isolation and characterization of mutant strains of *Escherichia coli* altered in H_2 metabolism. J. Bacteriol. **162:**344–352.
26. **Lee, J. H., J. C. Wendt, and K. T. Shanmugam.** 1990. Identification of a new gene, *molR*, essential for utilization of molybdate by *Escherichia coli*. J. Bacteriol. **172:**2079–2087.
27. **Luque, F., L. A. Mitchenall, M. Chapman, R. Christine, and R. N. Pau.** 1993. Characterization of genes involved in molybdenum transport in *Azotobacter vinelandii*. Mol. Microbiol. **7:**447–459.
28. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Maupin, J. A., and K. T. Shanmugam.** 1990. Genetic regulation of formate hydrogenlyase of *Escherichia coli*: role of the *fhlA* gene product as a transcriptional activator for a new regulatory gene, *fhlB*. J. Bacteriol. **172:**4798–4806.
30. **McLaughlin, R., Y. A. Kwaik, R. Young, S. Spinola, and M. Apicella.** 1992. Characterization and sequence of the *lsg* locus from *Haemophilus influenzae*. GenBank accession number M94855.
31. **Miller, J. B., D. J. Scott, and N. K. Amy.** 1987. Molybdenum-sensitive transcriptional regulation of the *chlD* locus of *Escherichia coli*. J. Bacteriol. **169:**1853–1860.
32. **Mimura, C. S., S. R. Holbrook, and G. F.-L. Ames.** 1991. Structural model of the nucleotide-binding conserved component of periplasmic permeases. Proc. Natl. Acad. Sci. USA **88:**84–88.
33. **Rajagopalan, K. V., and J. L. Johnson.** 1992. The pterin molybdenum cofactors. J. Biol. Chem. **267:**10199–10202.
34. **Rech, S., U. Deppenmeier, and R. P. Gunsalus.** 1995. Regulation of the molybdate transport operon, *modABCD*, of *Escherichia coli* in response to molybdate availability. J. Bacteriol. **177:**1023–1029.
35. **Reiss, J., A. Kleinhofs, and W. Klingmuller.** 1987. Cloning of seven differently complementing DNA fragments with *chl* functions from *Escherichia coli* K12. Mol. Gen. Genet. **206:**352–355.
36. **Rosentel, J. K., F. Healy, J. A. Maupin-Furlow, J. H. Lee, and K. T. Shanmugam.** 1995. Molybdate and regulation of *mod* (molybdate transport), *fdhF*, and *hyc* (formate hydrogenlyase) operons in *Escherichia coli*. J. Bacteriol. **177:**4857–4864.
37. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
38. **Scott, D., and N. K. Amy.** 1989. Molybdenum accumulation in *chlD* mutants of *Escherichia coli*. J. Bacteriol. **171:**1284–1287.
39. **Shanmugam, K. T., V. Stewart, R. P. Gunsalus, D. H. Boxer, J. A. Cole, M. Chippaux, J. A. DeMoss, G. Giordano, E. C. C. Lin, and K. V. Rajagopalan.** 1992. Proposed nomenclature for the genes involved in molybdenum metabolism in *Escherichia coli* and *Salmonella typhimurium*. Mol. Microbiol. **6:**3452–3454.
40. **Shuman, H. A.** 1987. The genetics of active transport in bacteria. Annu. Rev. Genet. **21:**155–177.
41. **Sirko, A., M. Hryniewicz, D. Hulanicka, and A. Bock.** 1990. Sulfate and thiosulfate transport in *Escherichia coli* K-12: nucleotide sequence and expression of the *cysTWAM* gene cluster. J. Bacteriol. **172:**3351–3357.
42. **Stewart, V.** 1988. Nitrate respiration in relation to facultative metabolism in enteric bacteria. Microbiol. Rev. **52:**190–232.
43. **Wang, G., S. Angermüller, and W. Klipp.** 1993. Characterization of *Rhodobacter capsulatus* genes encoding a molybdenum transport system and putative molybdenum-pterin-binding proteins. J. Bacteriol. **175:**3031–3042.
44. **Wang, S. Z., J. S. Chen, and J. L. Johnson.** 1990. A nitrogen-fixation gene (*nifC*) in *Clostridium pasteurianum* with sequence similarity to *chlJ* of *Escherichia coli*. Biochem. Biophys. Res. Commun. **169:**1122–1128.
45. **Wilson, L. G., and R. B. Bandurski.** 1958. Enzymatic reactions involving sulfate, sulfite, selenate and molybdate. J. Biol. Chem. **233:**975–981.