JOURNAL OF BACTERIOLOGY, July 1982, p. 495–499
0021-9193/82/070495-05$02.00/0

Vol. 151, No. 1

Identification of the *Salmonella typhimurium cysB* Gene Product by Two-Dimensional Protein Electrophoresis

EDWARD W. BAPTIST†, SCOTT G. HALLQUIST, AND NICHOLAS M. KREDICH*

Howard Hughes Medical Institute at Duke University and the Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

Received 28 December 1981/Accepted 10 March 1982

Examination of two-dimensional electropherograms of proteins from wild-type *Salmonella typhimurium* and 16 different *cysB* strains permitted the identification of a single 34,500-dalton polypeptide chain with a pI of 7.6 that was the product of *cysB*. Exclusion chromatography indicated that the native *cysB* protein is a multimer of at least two and probably four or more such subunits.

Control of L-cysteine biosynthesis in enteric bacteria is achieved both by feedback inhibition of serine transacetylase by L-cysteine (10, 11) and by a system of gene regulation in which a combination of sulfur starvation, *O*-acetyl-L-serine, and the *cysB* regulatory gene is required for derepression (8, 9). Recent investigations in *Salmonella typhimurium* and *Escherichia coli* indicate that *cysB* consists of a single cistron (2, 15), which codes for a protein (12, 14) that functions as an element of positive control (7). In this communication we provide additional direct chemical evidence for the protein nature and monocistronic origin of the *cysB* gene product in *S. typhimurium* by reporting the identification of wild-type and mutant *cysB* polypeptide chains on two-dimensional electropherograms of whole-cell extracts.

Our rationale was to analyze protein extracts of various *S. typhimurium* strains by the two-dimensional electrophoresis method of O'Farrell (13) and then to identify the wild-type *cysB* polypeptide spot by its absence or altered mobility in *cysB* mutants. Bacteria were grown in a sulfate-free minimal salts medium (16) containing 5 g of glucose per liter, appropriate supplements for auxotrophs, and a sulfur source that was either 0.5 mM glutathione for derepression of the cysteine regulon or 0.5 mM L-cystine for repression (9).

To radiolabel cell proteins, overnight cultures were diluted 20-fold into 10 ml of fresh medium containing 2 μCi of L-^{14}C-amino acids (New England Nuclear Corp.) per ml and then incubated at 37°C with vigorous shaking until density reached 0.8×10^9 to 1.0×10^9 cells per ml. After centrifugation and one wash with 10 mM Tris-hydrochloride (pH 7.6) containing 1 mM $MgCl_2$, cells were disrupted by sonication in a small volume of this same buffer. This crude extract

† Present address: Southern Biotech, Inc., Tampa, FL 33613.

TABLE 1. Gene product in *cysB* mutant strains[a]

Strain or mutation	Source[b]	*cysB* allele	Two-dimensional gel protein spot
Wild type		*cysB*$^+$	Present with normal pI[c]
cysB403	H/S	Δ*cysB403*	Absent
DW391	DU	*cysB*$^+$	Present with normal pI
DW392	DU	Δ*cysB403*	Absent
DW353	(2)	Δ*cysB1753*	Absent
DW367	(2)	Δ*cysB1767*	Absent
cysB517	H/S	*cysB517*	Absent
cysB661	H/S	*cysB661*	Absent
DW81	DU	*cysB267*	Absent
cysB70	H/S	*cysB70*	Present with basic pI shift
cysB45	H/S	*cysB45*	Present with basic pI shift
DW44	(9)	*cysB18*	Present with basic pI shift
DW46	(9)	*cysB484*	Present with basic pI shift
DW75	DU	*cysB27*	Present with basic pI shift
cysB25	H/S	*cysB25*	Present with acidic pI shift
cysB12	H/S	*cysB12*	Present with normal pI
DW43	(9)	*cysB16*	Present with normal pI
DW79	DU	*cysB88*	Present with normal pI
DW25	(9)	*cysB1352*	Present with normal pI

[a] Cells were grown on minimal medium with reduced glutathione as the sole sulfur source, and extracts were analyzed for a *cysB* spot by two-dimensional protein electrophoresis. All non-*cysB* mutants tested had a normal *cysB* spot and included *cysA3*, *cysA20*, *cysA197*, *cysCD519*, *cysE2*, *cysE6*, *cysE8*, *cysE11*, *cysE396*, *cysG439*, *cysHIJ383*, *cysK1751* (4), *cysK1772*, and *cysM1771* (3).

[b] H/S, From either P. Hartman or K. E. Sanderson; DU, constructed in this laboratory.

[c] In wild type the *cysB*$^+$ protein spot is "present with normal pI" by definition.

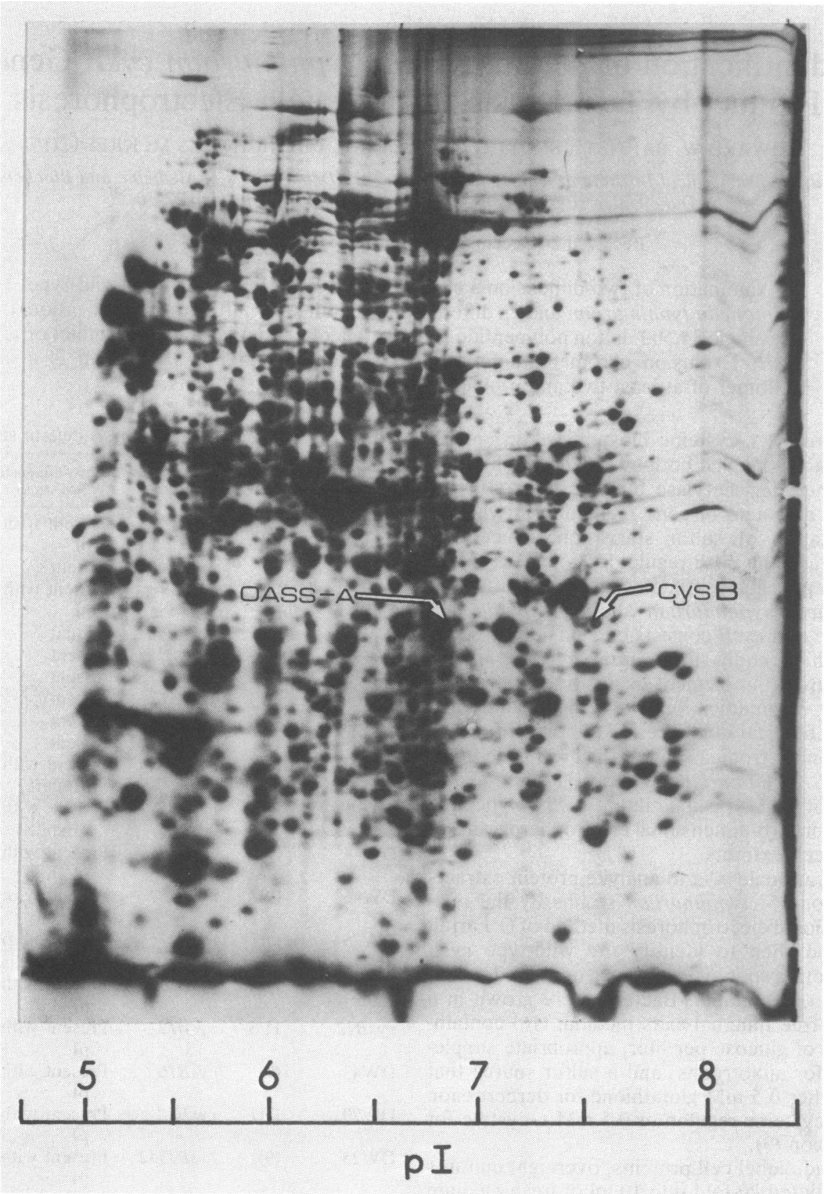

FIG. 1. Two-dimensional electropherogram of proteins from a crude extract of wild-type *S. typhimurium* grown on L-^{14}C-amino acids and on glutathione as sole sulfur source. The autoradiograph was developed after 7 days of exposure. The position of the spot corresponding to *O*-acetylserine sulfhydrylase A (OASS-A) was established from gels of purified enzyme. The *cysB* protein spot is estimated to have a molecular weight of 34,500 and a pI of 7.6.

was then analyzed on two-dimensional electrophoretic gels with pH 5 to 8 ampholines (LKB Instruments, Inc.) in the initial isoelectric focusing dimension. After fixation and staining with Coomassie brilliant blue G-250, the gels were exposed to Kodak No-Screen X-ray film for 3 to 14 days before development.

Gels were run on extracts prepared from wild

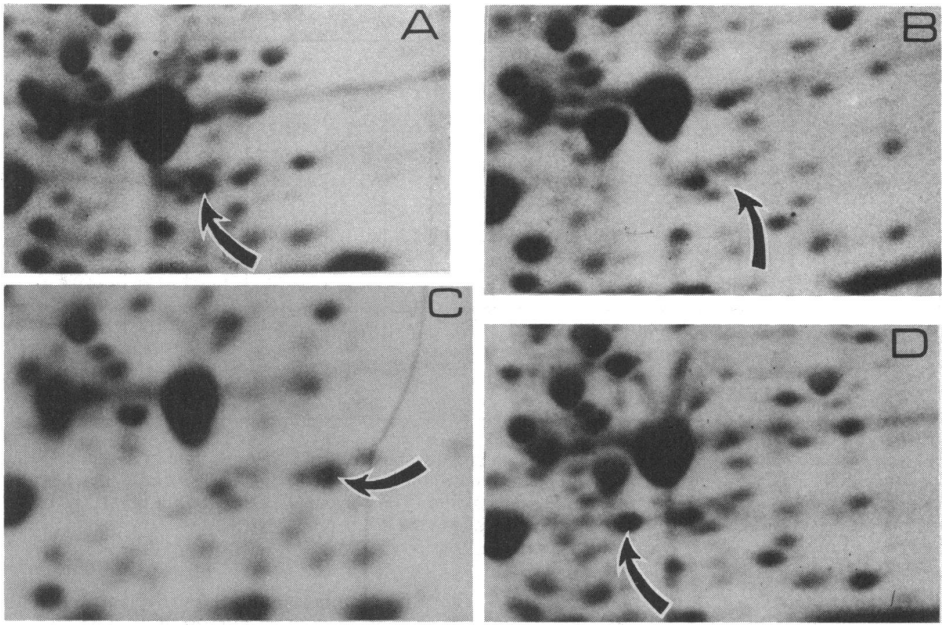

FIG. 2. Comparison of the region around the *cysB* spot in wild type and in three different *cysB* mutants. The autoradiographs show the *cysB* spot to be present in wild type (A); absent in *cysB403* (B); present with a more basic pI in *cysB70* (C); and present with a more acidic pI in *cysB25* (D). The *cysB70* gel was exposed a shorter time than the others, and ordinarily gives an altered *cysB* spot that is much more intense than that of wild type or other mutants.

type, from strains carrying 16 different *cysB* mutant alleles, and from strains carrying mutations in all known *cys* structural genes (Table 1). Extensive examination of the autoradiograms from these gels revealed only a single protein spot that could be identified as a product of the *cysB* gene by the following criteria: (i) present in wild type and all non-*cysB* mutant strains tested, whether cells were grown under conditions of repression or derepression for the cysteine regulon; (ii) absent in all of the three *cysB* deletion strains tested as well as in some strains carrying different *cysB* point mutations; (iii) appearance in several *cysB* point mutation strains of a new protein spot with the same molecular weight as the missing spot, but with a slightly altered pI. The position of this protein spot, shown for wild type in Fig. 1, corresponded to an apparent pI of 7.6 and a subunit molecular weight of approximately 34,500. The *cysB* gene product of *E. coli* is reported to have a pI of 7 and a subunit molecular weight of 39,000 (12).

Comparison of the area around the *cysB* protein spot in gels of 16 *cysB* mutants (Table 1) revealed that in all three deletion strains and in three point mutants the wild-type *cysB* spot was absent, and no new spot of similar molecular weight could be found (Fig. 2B). However, in five *cysB* point mutants the wild-type spot was also absent, but a new spot with the same molecular weight appeared with a basic pI shift of 0.15 pH unit (Fig. 2C). Only one mutant, *cysB25*, had a pI shift of 0.15 pH unit toward the acidic end of the gel (Fig. 2D). Four *cysB* point mutants could not be distinguished from wild type by two-dimensional gel electrophoresis. When the isogenic strains DW391 (*cysB*$^+$) and DW392 (*cysB403*) were compared by this technique, they were identical except for the absence in DW392 of the *cysB* protein spot and those proteins that were represssed by growth on L-cystine in the wild-type strain.

To estimate the molecular weight of the native *cysB* protein, a crude extract of the *cysB*$^+$ strain DW391 was fractionated with ammonium sulfate, and the material precipitating at 229 mg/ml was chromatographed on Ultrogel AcA34 (LKB Instruments, Inc.) together with a small amount of purified *O*-acetylserine sulfhydrylase A. Semiquantitative analysis of the effluent fractions by means of two-dimensional gel electrophoresis revealed that the *cysB* protein was eluted from the column at a K_v approximately one-half that of native *O*-acetylserine sulfhydrylase A, which is a dimer with a native molecular weight of 68,000 (1). This suggests that the

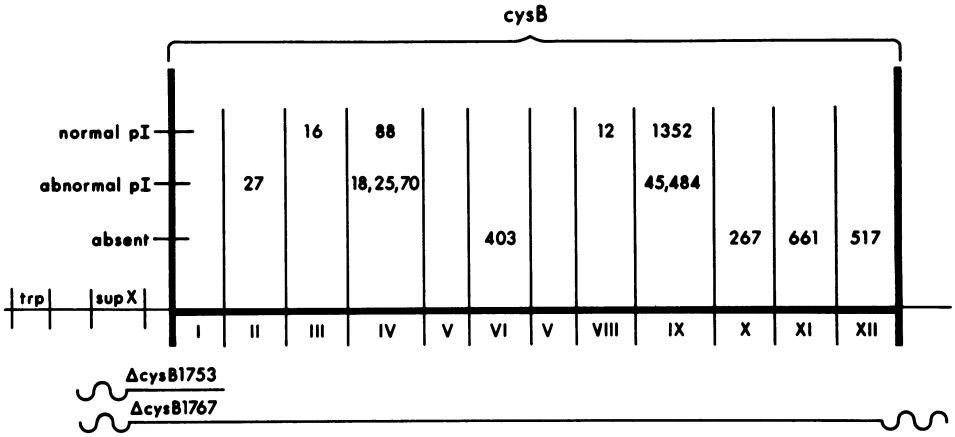

FIG. 3. Genetic map of the *cysB* region showing the relative positions of mutations used in this study. The Roman numerals indicate segments defined by various deletions (2). The other numbers indicate mutant *cysB* alleles, which are grouped according to whether they give a *cysB* polypeptide chain with a normal pI or an abnormal pI, or no spot at all. The deletions *cysB1753* and *cysB1767* give no *cysB* spot.

native *cysB* protein is a multimer of at least two, and most likely four or more, 34,500-dalton subunits, which agrees with data obtained from genetic experiments in *S. typhimurium* (5) and with studies on the *E. coli cysB* protein (12).

Comparison of the data summarized in Table 1 with a fine-structure map of the *S. typhimurium cysB* gene (2) shows that both a complete deletion and a partial deletion extending only a very short distance into the *trp*-proximal end of *cysB* result in loss of the *cysB* protein spot (Fig. 3). The same result was found in strains carrying either the internal deletion *cysB403* or any of the three *trp*-distal point mutations. Although the data are compatible with a monocistronic structure for this region, they might also be explained by polar effects on a multicistronic *cysB*. It is significant then that point mutations ranging in location from the *trp*-proximal *cysB27* to very near the opposite end of *cysB* (e.g., *cysB45* and *cysB484*) give *cysB* spots with altered pI values. Therefore, that portion of *cysB* extending from the *trp*-proximal end to *cysB484*, which includes about 80% of mapped point mutations (2), must be a single cistron, and it seems likely that all of *cysB* codes for a single polypeptide chain.

Jagura-Burdzy and Hulanicka (6) have recently reported that in *E. coli cysB* is autoregulated by a process that is independent of whether cells are grown under conditions of repression or derepression for the cysteine regulon. We too noted no appreciable differences in the intensity of the wild-type *cysB* spot between repressed and derepressed cells. We did find, however, that the *cysB70* spot was consistently several-fold more intense than any other *cysB* spot. If there is a property of the *cysB* protein that is responsible for autoregulation it may be altered in such a way in *cysB70* to allow overproduction of the mutant *cysB* gene product.

This work was supported by grant AM12828 from the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases.

LITERATURE CITED

1. **Becker, M. A., N. M. Kredich, and G. M. Tomkins.** 1969. The purification and characterization of *O*-acetylserine sulfhydrylase A from *Salmonella typhimurium*. J. Biol. Chem. **244**:2418–2427.
2. **Cheney, R. W., Jr., and N. M. Kredich.** 1975. Fine-structure genetic map of the *cysB* locus in *Salmonella typhimurium*. J. Bacteriol. **124**:1273–1281.
3. **Hulanicka, M. D., S. G. Hallquist, N. M. Kredich, and T. Mojica-A.** 1979. Regulation of *O*-acetylserine sulfhydrylase B by L-cysteine in *Salmonella typhimurium*. J. Bacteriol. **140**:141–146.
4. **Hulanicka, M. D., N. M. Kredich, and D. M. Treiman.** 1974. The structural gene for *O*-acetylserine sulfhydrylase A in *Salmonella typhimurium*: identity with the *trzA* locus. J. Biol. Chem. **249**:867–872.
5. **Jagura, G., D. Hulanicka, and N. M. Kredich.** 1978. Analysis of merodiploids of the *cysB* region in *Salmonella typhimurium*. Mol. Gen. Genet. **165**:31–38.
6. **Jagura-Burdzy, G., and D. Hulanicka.** 1981. Use of gene fusions to study expression of *cysB*, the regulatory gene of the cysteine regulon. J. Bacteriol. **147**:744–751.
7. **Jones-Mortimer, M. C.** 1968. Positive control of sulphate reduction in *Escherichia coli*: the nature of the pleiotropic cysteineless mutants of *E. coli* K12. Biochem. J. **110**:597–602.
8. **Jones-Mortimer, M. C., J. F. Wheldrake, and C. A. Pasternak.** 1968. The control of sulphate reduction in *Escherichia coli* by *O*-acetyl-L-serine. Biochem. J. **107**: 51–53.
9. **Kredich, N. M.** 1971. Regulation of L-cysteine biosynthesis in *Salmonella typhimurium*: effects of growth on varying sulfur sources and *O*-acetyl-L-serine on gene expression. J. Biol. Chem. **246**:3474–3484.

10. **Kredich, N. M., M. A. Becker, and G. M. Tomkins.** 1969. Purification and characterization of cysteine synthetase, a bifunctional protein complex, from *Salmonella typhimurium*. J. Biol. Chem. **244**:2428–2439.
11. **Kredich, N. M., and G. M. Tomkins.** 1966. The enzymatic synthesis of L-cysteine in *Escherichia coli* and *Salmonella typhimurium*. J. Biol. Chem. **241**:4955–4965.
12. **Mascarenhas, D. M., and M. D. Yudkin.** 1980. Identification of a positive regulatory protein in *Escherichia coli*: the product of the *cysB* gene. Mol. Gen. Genet. **177**:535–539.
13. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007–4021.
14. **Tully, M., and M. D. Yudkin.** 1975. The nature of the product of the *cysB* gene of *Escherichia coli*. Mol. Gen. Genet. **136**:181–183.
15. **Tully, M., and M. D. Yudkin.** 1977. Fine-structure mapping and complementation analysis of the *Escherichia coli cysB* gene. J. Bacteriol. **131**:49–56.
16. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.