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In Vitro Interactions of CysB Protein with the *cysJIH* Promoter of *Salmonella typhimurium*: Inhibitory Effects of Sulfide

JACEK OSTROWSKI AND NICHOLAS M. KREDICH*

Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

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The cysteine regulon of *Salmonella typhimurium* is positively regulated by the CysB protein and an inducer, which can be either *O*-acetyl-L-serine or N-acetyl-L-serine. In vivo experiments confirmed that sulfide and L-cysteine (supplied as L-cystine) interfere with induction by exogenously supplied *O*-acetyl-L-serine and also showed the same effects when *N*-acetyl-L-serine was used as an inducer. In a gel shift assay, purified CysB protein bound specifically to a 278-base-pair DNA fragment containing the *S. typhimurium cysJIH* promoter region. Binding occurred in the absence of inducer but did not stimulate in vitro transcription initiation, indicating that binding alone is insufficient to cause formation of a transcription initiation complex. Addition of *N*-acetyl-L-serine or *O*-acetyl-L-serine was required for transcription initiation and also stimulated binding three- to eightfold. Sulfide inhibited both transcription initiation and binding by interfering with the stimulatory effects of inducer in a competitive manner. These findings indicate that sulfide is an anti-inducer and may explain why full expression of the cysteine regulon requires sulfur limitation. L-Cysteine did not affect in vitro transcription initiation or binding of CysB protein to the *cysJIH* promoter region. The in vivo effects of L-cysteine may be secondary to its degradation to sulfide by the inducible enzyme cysteine desulfhydrase.

The cysteine regulon of *Salmonella typhimurium* consists of 14 or more genes necessary for the synthesis of *O*-acetyl-L-serine, the uptake and reduction of sulfate to sulfide, and the reaction of sulfide with *O*-acetyl-L-serine to form L-cysteine (19). Full expression of these genes requires a positive regulatory protein encoded by *cysB*, sulfur limitation, and a signal of sulfur limitation provided by either *O*-acetyl-L-serine or *N*-acetyl-L-serine, which function as internal inducers (16–18, 30). Highly purified CysB protein is a tetramer of identical 36-kilodalton subunits (25, 29) with a deduced amino acid sequence that predicts a helix-turn-helix structure similar to that found in a number of other DNA-binding, regulatory proteins (3, 31). CysB protein is a member of the LysR family of bacterial activator proteins, which have been so grouped on the basis of extensive amino acid sequence similarities (13). In vitro transcription initiation at the *cysJIH* promoter has been found to require CysB protein and either *O*-acetyl-L-serine or *N*-acetyl-L-serine (30), and it is hypothesized that these inducers stabilize or activate a complex formed between CysB protein and the *cysJIH* promoter. *N*-Acetyl-L-serine is derived from *O*-acetyl-L-serine through a nonenzymatic *O*- to *N*-acyl shift and is 10- to 30-fold more active than *O*-acetyl-L-serine as an in vitro inducer but less active in vivo (30).

Although the regulatory roles of CysB protein and acetyl-L-serine are well documented, it is still unclear why sulfur limitation is required for full expression of the cysteine regulon. L-Cysteine is a potent end-product inhibitor of serine transacetylase, the enzyme catalyzing synthesis of *O*-acetyl-L-serine (20, 22), and thereby plays a key role in modulating inducer levels. Therefore, growth on a readily available sulfur source, such as L-cystine or sulfide, which increases intracellular L-cysteine (40), would be expected to inhibit expression of the cysteine regulon by decreasing levels of *O*-acetyl-L-serine and its derivative *N*-acetyl-L-serine. Although this effect on the rate of inducer synthesis is probably very important, L-cysteine and sulfide must interact with the cysteine regulon in some other way as well, because either can prevent induction by exogenously supplied *O*-acetyl-L-serine (16, 18). Characterization of such an effect has been complicated by the fact that sulfide and L-cysteine are readily interconverted in the presence of *O*-acetyl-L-serine by the combined actions of *O*-acetylserine (thiol)-lyase, which catalyzes the reaction of sulfide with *O*-acetyl-L-serine to form L-cysteine (1, 22), and cysteine desulfhydrase, which degrades L-cysteine to pyruvate, ammonia, and sulfide (21). Theoretically, the ability of these sulfur compounds to prevent induction by exogenous *O*-acetyl-L-serine might be due to depletion of intracellular inducer through the activity of a futile cycle between L-cysteine and sulfide (19).

We report here that growth on either L-cysteine or sulfide also interferes with induction by exogenously supplied *N*-acetyl-L-serine, which is not a substrate for *O*-acetylserine (thiol)-lyase and cannot be converted to *O*-acetyl-L-serine under physiologic conditions. Therefore, the notion of a futile cycle that depletes *O*-acetyl-L-serine cannot totally account for the inhibitory effects of L-cysteine or sulfide. Instead, our results indicate that sulfide, but not L-cysteine, acts as an anti-inducer that inhibits formation of transcription initiation complexes in vitro.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. Wild-type *S. typhimurium* LT2 was used for studies of in vivo induction of the cysteine operon. *Escherichia coli* NM522 is *Δhsd-5 Δ(lac-pro)* (F′ *pro*$^+$ *lacI*^{q} *ZΔM15*) and was used as a host for plasmid pJOK201, which contains the *S. typhimurium cysJIH* promoter (30) and the first 100 base pairs (bp) of the *cysJ* coding region (27a) on a 509-bp *Kpn*I-*Eco*RI fragment inserted into the multicloning site of pUC19 (Fig. 1). The *Kpn*I site is 219 bp upstream of the transcription start site. This fragment was obtained from a large promoter-*cysJ* fragment in bacteriophage M13mp19 by the method of Dale et al. (5) and contained the sequence $(C)_{14}$GAATTC at the downstream *Eco*RI cloning site. Medium E of Vogel and

* Corresponding author.

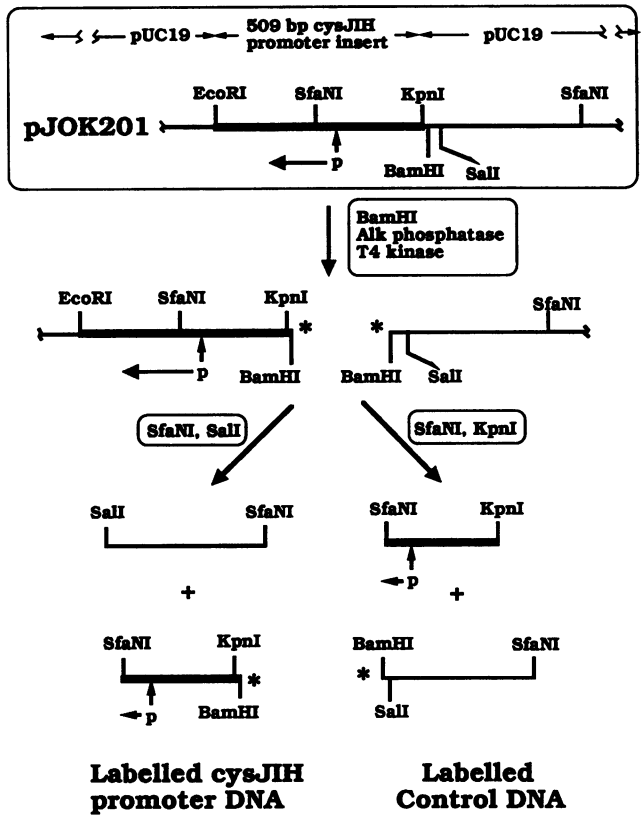

FIG. 1. Scheme for generating radiolabeled fragments from the *cysJIH* promoter insert of pJOK201. Plasmid DNA was linearized by digestion with *Bam*HI, treated with alkaline phosphatase, and 5′ end labeled by the use of T4 polynucleotide kinase. Symbols: *, ends radiolabeled with ^{32}P; ⟵ p, the *cysJIH* transcription start site and direction of transcription. Digestion with *Sfa*NI gave two radiolabeled products, one a 278-bp *Sfa*NI-*Bam*HI fragment containing *cysJIH* promoter sequences and the other a 477-bp *Bam*HI-*Sfa*NI fragment containing pUC19 sequences. Further treatment with *Sal*I removed radiolabel as a 12-bp segment from the pUC19 fragment and provided a source of radiolabeled *cysJIH* promoter DNA. A portion of the *Sfa*NI digest was treated with *Kpn*I to remove radiolabel from the *cysJIH* promoter fragment and was used as a source of radiolabeled control DNA.

Bonner (39) supplemented with 0.5% glucose was used as the minimal medium and was adjusted to pH 6.8 with HCl to minimize conversion of *O*-acetyl-L-serine to *N*-acetyl-L-serine (18).

Generation of radiolabeled fragments. *Bam*HI-digested pJOK201 was dephosphorylated with *E. coli* alkaline phosphatase, and after phenol extraction and addition of 0.5 volume of ammonium acetate, DNA was precipitated with ethanol. Linearized DNA was 5′ end labeled with [γ-^{32}P]ATP (3,000 Ci/mmol) and T4 polynucleotide kinase (24) and recovered by ammonium acetate-ethanol precipitation. Digestion with *Sfa*NI gave two radiolabeled fragments, one of 278 bp containing the *cysJIH* promoter and the other of 477 bp containing only pUC19 vector sequences (Fig. 1). One portion of this mixture was digested with *Sal*I to remove a 12-bp radiolabeled segment from the pUC19 fragment and was used as a source of labeled *cysJIH* promoter fragment; another portion was digested with *Kpn*I to remove a 12-bp radiolabeled segment from the *cysJIH* promoter fragment and was used as a source of labeled control DNA. After phenol extraction and precipitation with ammonium acetate-ethanol, both preparations were used without further purification.

Gel electrophoresis DNA-binding assay. Horizontal polyacrylamide gels (5% acrylamide, 0.061% bisacrylamide) were prepared in running buffer (10 mM Tris hydrochloride [pH 8.0], 1 mM disodium EDTA) and prerun at 10 V/cm for 30 min (10). The binding reaction was carried out at 37°C in a 20-μl mixture of binding buffer (40 mM Tris hydrochloride [pH 8.0], 0.1 M KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 100 μg of nuclease-free bovine serum albumin per ml) containing 0.1 pmol of radiolabeled DNA fragment (approximately 4×10^5 dpm of ^{32}P) and various amounts of purified CysB protein, *N*-acetyl-L-serine, *O*-acetyl-L-serine, sodium sulfide, or L-cysteine. A 0.5-mg/ml stock solution of CysB protein was diluted with binding buffer just before use. After 5 min of incubation, 3 μl of a bromophenol blue solution (0.05% in a 1:1 mixture of binding buffer and glycerol) was added with gentle mixing, and samples were loaded onto a gel with the current on. After 1 h at 10 V/cm, gels were dried, and radiolabeled bands were visualized by radioautography.

In vitro runoff transcription assay. Transcription runoff assays were performed as described previously (30). Template DNA containing the *cysJIH* promoter consisted of the 509-bp *Kpn*I-*Eco*RI fragment from pJOK201 (Fig. 1), which was purified by polyacrylamide gel electrophoresis and elution from DEAE-cellulose paper (7). A 458-bp DNA fragment containing the p_L promoter from phage λ was a gift from Deborah Steege and served as template DNA in control experiments, giving a 325-nucleotide transcription runoff product. The preincubation mixture consisted of binding buffer (see above) containing 0.1 mM ATP, 1 μg of purified template DNA per ml, 50 μg of nuclease-free *E. coli* RNA polymerase per ml, and various amounts of purified CysB protein, *N*-acetyl-L-serine, sulfide, or L-cysteine. Transcripts radiolabeled with [α-^{32}P]CTP were analyzed in sequencing gels.

Other techniques. Restriction enzymes and *E. coli* alkaline phosphatase were obtained with Bethesda Research Laboratories, Inc., and New England BioLabs, Inc., and were used according to the instructions of the suppliers. Nuclease-free bovine serum albumin, nuclease-free *E. coli* RNA polymerase, and T4 polynucleotide kinase were from Pharmacia LKB Biotechnology Inc. Sodium sulfide and L-cysteine hydrochloride (neutralized with NaOH) were prepared daily as 1 M stock solutions in 1 mM disodium EDTA and kept on ice in capped tubes until use. Our methods for the purification of *S. typhimurium* CysB protein (25), preparation of bacterial extracts (18), and assays for *O*-acetylserine (thiol)-lyase (1) and NADPH-cytochrome *c* reductase (37) have been described. *O*-Acetyl-L-serine (33) and *N*-acetyl-L-serine (26) were prepared as described elsewhere. Protein concentrations were determined by the dye-ligand method (2), with bovine serum albumin as a standard.

RESULTS

Repression of the cysteine regulon by sulfide and L-cystine. Wild-type *S. typhimurium* LT2 was grown on minimal medium containing either 1 mM reduced glutathione, 1 mM sodium sulfide, or 0.5 mM L-cystine as a sole sulfur source. At a density of 2×10^8/ml, either 1 mM *O*-acetyl-L-serine or 5 mM *N*-acetyl-L-serine was added, and bacteria were harvested after a two- to threefold increase in cell number. Cell extracts were assayed for NADPH-cytochrome *c* reductase as an indicator of *cysJ* expression from the *cysJIH* promoter (30) and for *O*-acetylserine (thiol)-lyase, which reflects

TABLE 1. Effects of *O*-acetyl-L-serine and *N*-acetyl-L-serine on induction of the cysteine regulon in wild-type *S. typhimurium* LT2[a]

Sulfur source	Addition	Activity[b]	
		Cytochrome *c* reductase (U/mg)	*O*-Acetylserine (thiol)-lyase (U/mg)
Glutathione	None	0.36	4.8
Sulfide	None	0.054	0.07
L-Cystine	None	0.035	0.06
Glutathione	1 mM *O*-acetyl-L-serine	0.44	7.2
Sulfide	1 mM *O*-acetyl-L-serine	0.115	0.14
L-Cystine	1 mM *O*-acetyl-L-serine	0.046	0.05
Glutathione	5 mM *N*-acetyl-L-serine	0.64	5.5
Sulfide	5 mM *N*-acetyl-L-serine	0.069	0.11
L-Cystine	5 mM *N*-acetyl-L-serine	0.054	0.08

[a] Cells were grown with vigorous shaking at 37°C in minimal medium adjusted to pH 6.8 containing 0.5% glucose and either 1 mM reduced glutathione, 1 mM sulfide, or 0.5 mM L-cystine as the sole sulfur source (15). Either *O*-acetyl-L-serine or *N*-acetyl-L-serine was added when cultures reached about 2×10^8 cells per ml, and bacteria were harvested by centrifugation at densities of 4×10^8 to 6×10^8 cells per ml. Enzyme assays were performed on crude extracts.

[b] Units of cytochrome *c* reductase activity is expressed as micromoles of NADPH oxidized per minute; units of *O*-acetylserine (thiol)-lyase activity are expressed as micromoles of cysteine formed per minute.

expression of *cysK* (4) and, to a lesser extent, expression of *cysM* (14). As expected, levels of these two enzymes were very low in cells grown on sulfide or L-cystine and 7- to 80-fold higher in cells grown on the limiting sulfur source, glutathione (Table 1). *O*-Acetyl-L-serine and *N*-acetyl-L-serine had little or no effect on cells grown on sulfide or L-cystine even though both compounds have previously been shown to be potent inducers of the cysteine regulon in sulfur-limited cultures of strains lacking serine transacetylase (17, 18, 30). *O*-Acetyl-L-serine did increase NADPH-cytochrome *c* reductase activity in sulfide-grown cells to 26% of fully derepressed levels but had no appreciable effect on *O*-acetylserine (thiol)-lyase (Table 1).

The relative inability of *O*-acetyl-L-serine to induce sulfur-replete cells has been reported previously (17, 18) and prompted speculation that sulfide, obtained either directly from the medium or indirectly from desulfhydration of L-cysteine (21), might deplete intracellular *O*-acetyl-L-serine in a reaction catalyzed by *O*-acetylserine (thiol)-lyase (19). However, since *N*-acetyl-L-serine is not a substrate for this enzyme, its inability to induce cells grown on sulfide and L-cystine indicates that these sulfur compounds act in some other way to inhibit expression of the cysteine regulon.

Binding of CysB protein to the *cysJIH* promoter region. The *cysJIH* operon belongs to the cysteine regulon and has the gene order promoter-*cysJ-cysI-cysH* (6, 15, 23, 27a, 28, 30, 30a), where *cysJ* and *cysI* code for the flavoprotein and hemoprotein components of NADPH-sulfite reductase (36, 38) and *cysH* encodes 3′-phosphoadenosine 5′-phosphosulfate sulfotransferase (8). A radiolabeled 278-bp *Sfa*NI-*Sal*I DNA fragment containing the *cysJIH* promoter region was used in a gel mobility shift assay (10, 11) to study in vitro interactions with purified CysB protein. A radiolabeled 477-bp *Sfa*NI-*Kpn*I DNA fragment lacking this promoter served as a control (Fig. 1). Mixtures of DNA and various amounts of CysB protein were incubated in the presence or absence of either 1 mM *N*-acetyl-L-serine or 1 mM *O*-acetyl-L-serine and analyzed for DNA-protein complexes by electrophoresis in polyacrylamide gels.

Addition of CysB protein to the *cysJIH* promoter fragment resulted in the formation of a DNA-protein complex with a mobility of 0.70 relative to that of free DNA (Fig. 2). Smaller amounts of complexes with mobilities of 0.53 and 0.44 were also observed at high concentrations of CysB protein. In the presence of 1.0 mM *N*-acetyl-L-serine, DNA-protein complexes were detected with as little as 0.1 μg of CysB protein per ml and comprised all of the DNA at 2 μg/ml (Fig. 2, lanes 2 to 7). DNA-protein complexes were also formed in the absence of *N*-acetyl-L-serine but accounted for less than half of the total DNA at 2 μg of CysB protein per ml (Fig. 2, lanes 8 to 13). A CysB protein concentration of 10 μg/ml was required to bind all of the DNA in the absence of *N*-

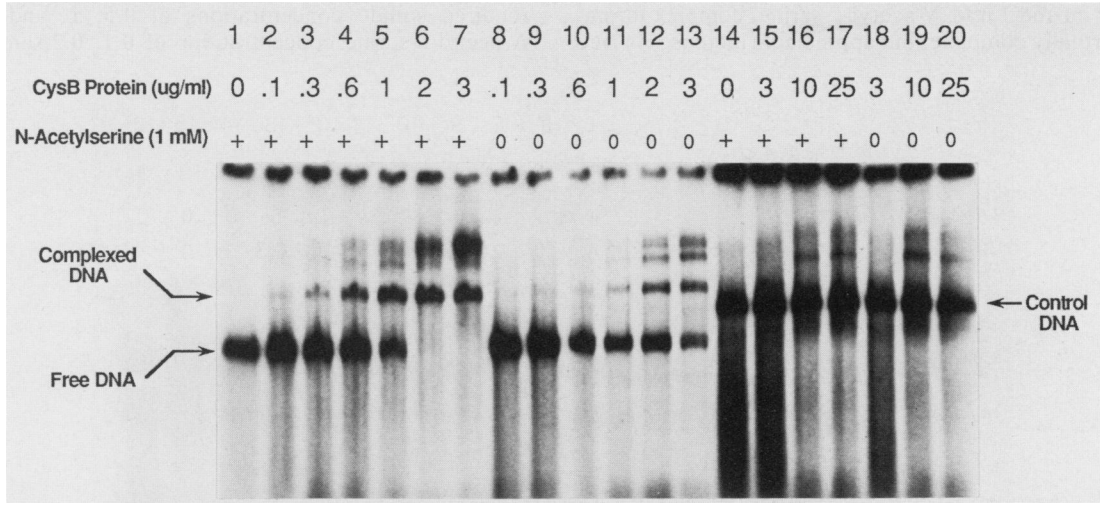

FIG. 2. Binding of CysB protein to the *cysJIH* promoter region in a gel mobility shift assay (10, 11). Radiolabeled DNA (0.5 μg/ml) was preincubated with various amounts of purified CysB protein for 5 min at 37°C in either the presence or absence of 1 mM *N*-acetyl-L-serine. Mixtures were then run in a 5% polyacrylamide gel as described in Materials and Methods. A 278-bp *Bam*HI-*Sfa*NI fragment containing the *cysJIH* promoter region was used in lanes 1 to 13; a 477-bp *Bam*HI-*Sfa*NI fragment that does not contain the *cysJIH* promoter region was used in lanes 14 to 20 as a control for nonspecific binding. CysB protein-DNA complexes were noted with the *cysJIH* promoter fragment but not with control DNA, except for a small contaminant of the 278-bp *Bam*HI-*Sfa*NI fragment that was more clearly noted in an underexposed film.

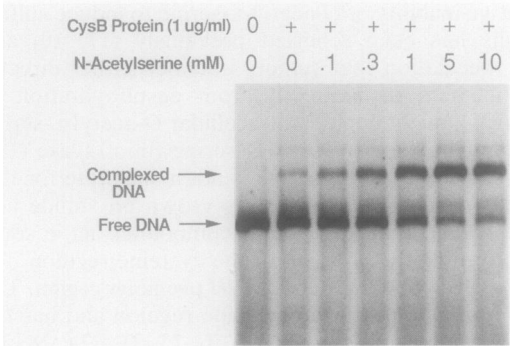

FIG. 3. Stimulation of CysB protein binding to a *cysJIH* promoter fragment by *N*-acetyl-L-serine. The gel mobility shift assay was performed as described in the legend to Fig. 2. Reaction mixtures contained 0.5 μg of a radiolabeled 278-bp *Bam*HI-*Sfa*NI fragment containing the *cysJIH* promoter region and 1 μg of CysB protein per ml except for lane 1. *N*-Acetyl-L-serine was varied from 0 to 10 mM as indicated.

acetyl-L-serine (not shown). At a fixed CysB protein concentration of 1 μg/ml, stimulation of binding was noted with as little as 0.1 mM *N*-acetyl-L-serine and reached a half-maximal value at 0.3 to 1 mM (Fig. 3). *N*-Acetyl-L-serine gave an estimated three- to eightfold increase in DNA-protein complex formation in different experiments. Similar results were obtained with *O*-acetyl-L-serine (data not shown). CysB protein at concentrations as high as 25 μg/ml did not form detectable complexes with the control DNA fragment but did bind a small contaminant of *cysJIH* promoter fragment that was present in this preparation (Fig. 2, lanes 14 to 20).

In vitro effects of sulfide and L-cysteine. The gel mobility shift assay was used to study in vitro effects of sulfide and L-cysteine on the binding of CysB protein to *cysJIH* promoter DNA. With 1.5 μg of CysB protein per ml and 1 mM *N*-acetyl-L-serine, sodium sulfide at 0.5 to 4 mM gave small but consistent decreases in the amount of DNA-protein complex formation (Fig. 4, lanes 2 to 6). With 3 μg of CysB protein per ml and 1 mM *N*-acetyl-L-serine, complex formation was virtually complete, but appreciable increases in free DNA were evident at 1 to 4 mM sodium sulfide. Sodium sulfide had no demonstrable effect on binding in the absence of *N*-acetyl-L-serine (Fig. 4, lanes 12 to 16) even at concentrations as high as 10 mM (not shown). The effects of *N*-acetyl-L-serine and sulfide on binding of CysB protein appeared to be competitive with each other, since inhibition by 1 to 4 mM sodium sulfide could be overcome by increasing *N*-acetyl-L-serine from 1 mM to 10 mM (data not shown). Although easily demonstrated, inhibition of CysB protein binding by sodium sulfide in the gel shift assay was only modest. The greatest effect noted was a 60% decrease in binding, which required 10 mM sodium sulfide at an *N*-acetyl-L-serine concentration of 0.7 mM (not shown). L-Cysteine at concentrations as high as 40 mM had no apparent effects on binding of CysB protein to the DNA fragment containing the *cysJIH* promoter region (data not shown).

In vitro effects of sulfide and L-cysteine were also investigated in a transcription runoff assay using a 509-bp DNA template, which contains *cysJIH* promoter sequences extending from positions −219 to +290 relative to the major transcription initiation site (Fig. 1). As shown previously (30), synthesis of the 290-nucleotide *cysJIH* runoff transcript was found to be dependent on both CysB protein and *N*-acetyl-L-serine, the effects of which are presumed to occur at the level of transcription initiation. At 10 μg of CysB protein per ml and 1 mM *N*-acetyl-L-serine, sodium sulfide caused partial inhibition of transcript formation at 2 mM and almost complete inhibition at 4 mM (Fig. 5). L-Cysteine had no such effect. At 1 mM *N*-acetyl-L-serine and 5 μg of CysB protein per ml, the inhibitory effects of 4 mM sulfide could be partially overcome by increasing CysB protein to 10 μg/ml and were completely reversed at 40 μg/ml (Fig. 6). Inhibition by 2 mM sulfide at 10 μg of CysB protein per ml was reversed by increasing *N*-acetyl-L-serine from 0.3 mM to 0.7 mM (Fig. 7). Competition between *N*-acetyl-L-serine and sulfide is demonstrated in greater detail by the experiment shown in Fig. 8, where it can be seen that the amount of sulfide required to inhibit transcript formation was clearly a function of the *N*-acetyl-L-serine concentration. At 10 μg of CysB protein per ml, inhibition by approximately 90% required sulfide concentrations of 0.5, 1, and 2 mM at *N*-acetyl-L-serine concentrations of 0.1, 0.2, and 0.3 mM,

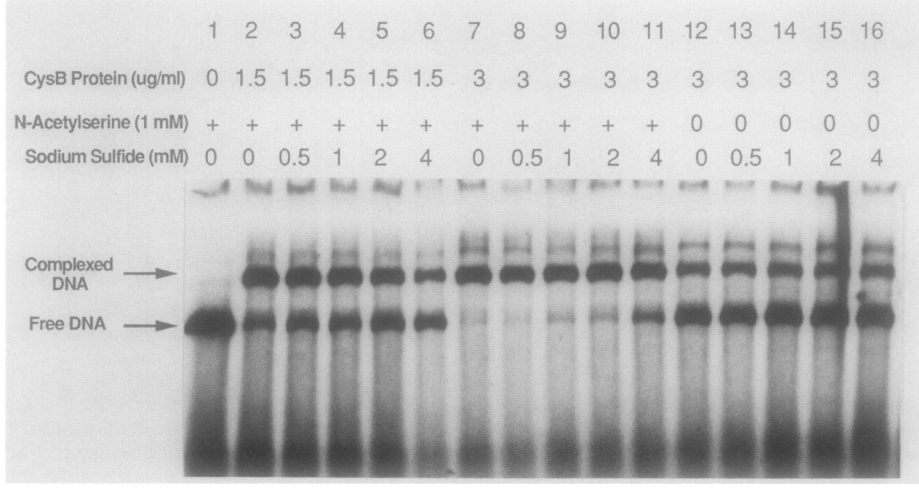

FIG. 4. Inhibition of CysB protein binding to a *cysJIH* promoter fragment by sulfide. The gel mobility shift assay was performed as described in the legend to Fig. 2. Reaction mixtures contained a 0.5-μg/ml concentration of rabiolabeled 278-bp *Bam*HI-*Sfa*NI *cysJIH* promoter fragment, CysB protein at 0 (lane 1), 1.5 (lanes 2 to 6), or 3 (lanes 7 to 16) μg/ml, and 1 mM acetyl-L-serine as indicated. Sodium sulfide was varied from 0 to 4 mM.

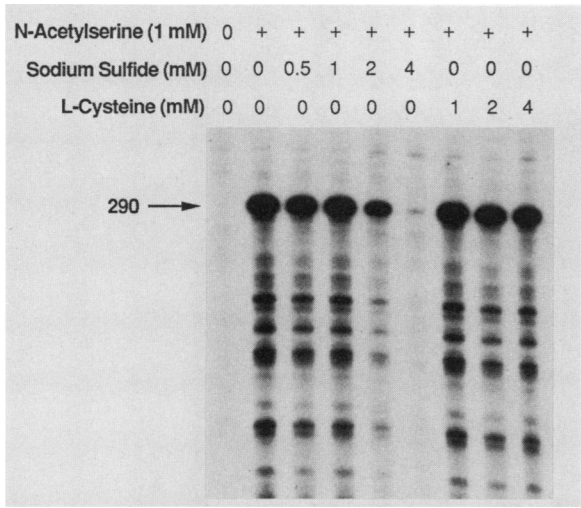

FIG. 5. Effects of sulfide and L-cysteine on transcription initiation in an in vitro runoff assay. All reactions contained RNA polymerase (75 μg/ml), CysB protein (10 μg/ml), and a 509-bp *Kpn*I-*Eco*RI fragment containing the *cysJIH* promoter and downstream sequences to +290 relative to the transcription start site (0.8 μg/ml). Mixtures were preincubated for 5 min at 37°C with various amounts of sodium sulfide or L-cysteine in the presence or absence of 1 mM *N*-acetyl-L-serine, and radiolabeled transcription runoff products were generated and analyzed on a sequencing gel as described in Materials and Methods. Transcript lengths were estimated by comparison with the G lane of a phage M13 DNA sequencing reaction (not shown) and corrected for the assumption that transcription products travel faster by one nucleotide than did the nonphosphorylated products of the standard. The arrow points to a 290-nucleotide transcript.

respectively. The reversal of sulfide inhibition by CysB protein or *N*-acetyl-L-serine indicates that such inhibition is specific for transcription initiated from the *cysJIH* promoter and is not due to nonspecific effects of sulfide on transcription in general. This conclusion is supported by our finding that transcript formation from a DNA template containing

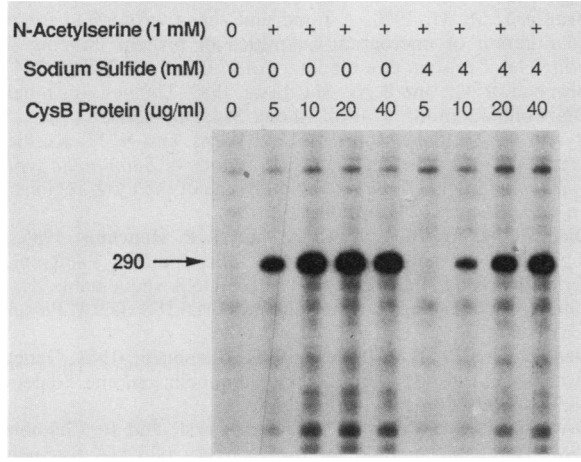

FIG. 6. Reversal of the inhibitory effects of sulfide on in vitro *cysJIH* transcription by CysB protein. Reaction mixtures contained 75 μg of RNA polymerase per ml, 0.8 μg of a 509-bp *cysJIH* promoter fragment per ml, 1 mM *N*-acetyl-L-serine, and various amounts of CysB protein with and without 4 mM sodium sulfide. Transcription runoff products were generated and evaluated as described in Materials and Methods and the legend to Fig. 5. The arrow points to a 290-nucleotide transcript.

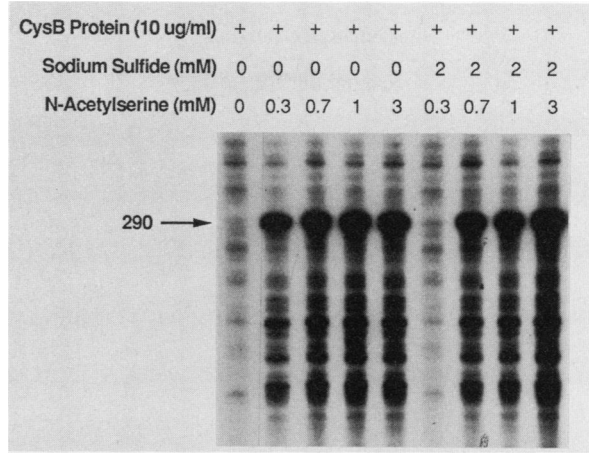

FIG. 7. Reversal of the inhibitory effects of sulfide on in vitro *cysJIH* transcription by *N*-acetyl-L-serine. Reaction mixtures contained 75 μg of RNA polymerase, 0.8 μg of a 509-bp *cysJIH* promoter fragment, and 10 μg of CysB protein per ml and various amounts of *N*-acetyl-L-serine with and without 2 mM sodium sulfide. Transcription runoff products were generated and evaluated as described in Materials and Methods and the legend to Fig. 5. The arrow points to a 290-nucleotide transcript.

the phage λ p_L promoter was not affected by sodium sulfide at concentrations as high as 4 mM (not shown).

DISCUSSION

The studies reported here demonstrate that CysB protein binds to the *cysJIH* promoter region in vitro and that such binding is stimulated by the inducers *N*-acetyl-L-serine and *O*-acetyl-L-serine. Although binding is complete at 10 μg of CysB protein per ml even in the absence of acetyl-L-serine, in vitro transcription initiation under such conditions requires the further addition of inducer (30). We conclude, therefore, that binding alone is insufficient to stimulate transcription and that the three- to eightfold increase in binding caused by inducer at lower CysB protein concentrations is a consequence of qualitative changes leading to formation of a transcription initiation complex. Binding of bacterial positive regulatory proteins to their DNA recognition sites in the absence of inducer has been observed for several other systems, including the AraC protein of *E. coli* (35), the NodD protein of *Rhizobium meliloti* (9), the MerR protein of transposon Tn*501* from *Pseudomonas aeruginosa* (12, 27), NtrC protein from *S. typhimurium* (32), and the NahR protein of *Pseudomonas putida* (34), and may represent a general property of positive regulation that allows a rapid response to regulatory signals provided by inducers.

The results of our in vivo experiments indicate that the ability of sulfide and L-cystine to prevent induction of the cysteine regulon by exogenous acetyl-L-serine cannot be due entirely to the depletion of intracellular inducer in the *O*-acetylserine (thiol)-lyase reaction, because *N*-acetyl-L-serine is not a substrate for this enzyme. Our in vitro experiments provide an alternative explanation for the role of sulfur limitation in the regulation of the cysteine regulon by showing that sulfide inhibits transcription initiation from the *cysJIH* promoter. Inhibition is competitive with the stimulatory effects of acetyl-L-serine, suggesting that sulfide interacts directly with CysB protein, perhaps at the same binding site as that for inducer. Thus, sulfide may be thought of as an anti-inducer. Although intracellular sulfide concen-

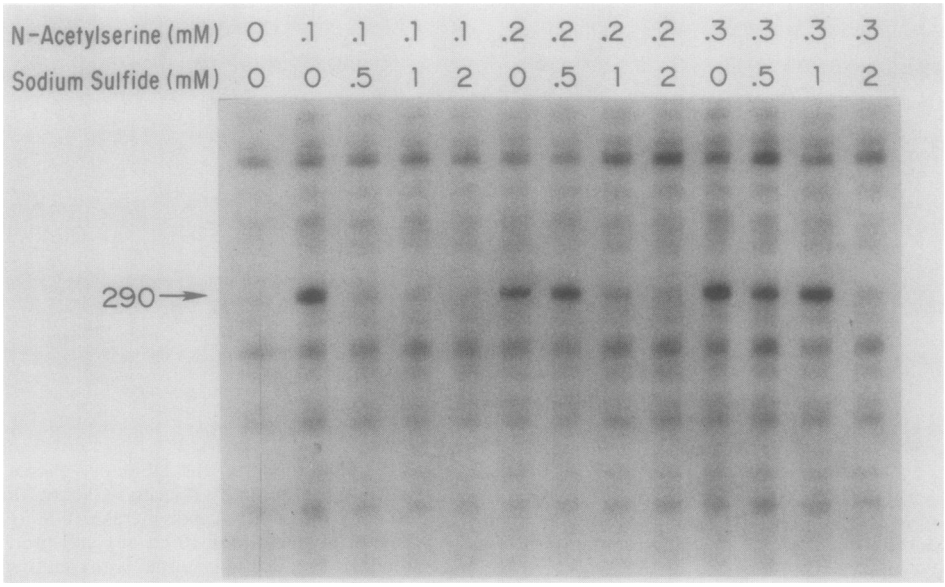

FIG. 8. Inhibition of *cysJIH* transcription by sulfide at different concentrations of *N*-acetyl-L-serine. Conditions were identical to those described in the legend to Fig. 7 except for the concentrations of *N*-acetyl-L-serine and sodium sulfide used. CysB protein was present at 10 μg/ml. The arrow points to a 290-nucleotide transcript.

trations have not been measured in cells grown on this sulfur source, the 1 mM concentration used in our growth medium is twice that required to completely inhibit the stimulation of in vitro transcription initiation by 0.1 mM *N*-acetyl-L-serine. Since L-cysteine does not inhibit in vitro transcription initiation, we assume that the effects of growth on L-cystine are due to generation of sulfide by the inducible enzyme cysteine desulfhydrase (21).

The ability of sulfide to inhibit binding of CysB protein to the *cysJIH* promoter parallels its effects on transcription initiation and is competitive with *N*-acetyl-L-serine. Sulfide reverses binding that has been stimulated by *N*-acetyl-L-serine but does not affect binding that occurs in the absence of inducer. These results are consistent with a model in which the effects of inducer and anti-inducer on binding are secondary to qualitative changes that determine whether the complex is active in transcription initiation. Inhibition of binding by sulfide is less dramatic than inhibition of transcription initiation, but quantitative comparisons are technically difficult because the gel shift assay allows loss of sulfide by evaporation and ionic migration. Loss of sulfide occurring before entry of the sample into the gel might allow reassociation of DNA-CysB protein complexes and lead to underestimates of the ability of sulfide to inhibit CysB protein binding. Furthermore, RNA polymerase, which itself may influence CysB protein binding, is present in transcription runoff experiments but not in gel shift binding assays.

The competitive interactions of acetyl-L-serine and sulfide with CysB protein are of unknown physiologic significance, since accumulation of the inducer *O*-acetyl-L-serine should be prevented through its reaction with sulfide in the *O*-acetylserine (thiol)-lyase reaction to form L-cysteine and by feedback inhibition of serine transacetylase by L-cysteine (20, 22). *N*-Acetyl-L-serine formation would be similarly affected because it is derived from *O*-acetyl-L-serine. Once *N*-acetyl-L-serine is formed, however, its removal would not be stimulated by sulfide, since it is not a substrate for *O*-acetylserine (thiol)-lyase. Therefore, the ability of sulfide to interact directly with CysB protein could be important in rapidly down-regulating expression of the cysteine regulon when shifting from conditions of sulfur limitation and *N*-acetyl-L-serine accumulation to a state of sulfur sufficiency.

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