JOURNAL OF BACTERIOLOGY, Feb. 1993, p. 892–897
0021-9193/93/030892-06$02.00/0

Vol. 175, No. 3

Altered Regulation of the *glnRA* Operon in a *Bacillus subtilis* Mutant That Produces Methionine Sulfoximine-Tolerant Glutamine Synthetase†

HAROLD J. SCHREIER,[1,2]* CHRISTINE A. ROSTKOWSKI,[1] AND ELLEN M. KELLNER[1]‡

Center of Marine Biotechnology, The University of Maryland, 600 East Lombard Street, Baltimore, Maryland 21202,[1] and Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, Maryland 21228[2]*

Received 21 September 1992/Accepted 16 November 1992

A *Bacillus subtilis* mutant that produced glutamine synthetase (GS) with altered sensitivity to DL-methionine sulfoximine was isolated. The mutation, designated *glnA33*, was due to a T · A-to-C · G transition, changing valine to alanine at codon 190 within the active-site C domain. Altered regulation was observed for GS activity and antigen and mRNA levels in a *B. subtilis glnA33* strain. The mutant enzyme was 28-fold less sensitive to DL-methionine sulfoximine and had a 13.0-fold-higher K_m for hydroxylamine and a 4.8-fold-higher K_m for glutamate than wild-type GS did.

Bacillus subtilis assimilates inorganic nitrogen by the concerted action of glutamine synthetase (GS) and glutamate synthase. GS catalyzes the ATP-dependent amidation of glutamate to form glutamine, a key metabolite that is not only necessary for protein biosynthesis but is also used as the nitrogen donor for the synthesis of most of the nitrogen-containing compounds in the organism. Thus, the formation of glutamine by GS may be considered the first step in a highly branched pathway, and the prominent role of GS in metabolism makes it a likely candidate for metabolic control.

In *B. subtilis*, GS specific activity is highest when the organism is grown in the presence of limiting ammonia or a poorly utilizable nitrogen source; growth in media containing excess nitrogen yields low enzyme levels (24, 26). This nitrogen-dependent response has been shown to be primarily due to regulation at the transcriptional level, and evidence for control of enzymatic activity by covalent modification has not been found (11, 14).

The structural gene for GS, *glnA*, lies in an operon called *glnRA* (32, 38). The *glnR* gene product, GlnR, is a repressor that interferes with transcription under conditions of nitrogen excess (3, 16, 32, 35). How GlnR activity is controlled is not known. However, genetic evidence has implicated a role for GS in regulation, and the enzyme may be utilized to signal information about nitrogen availability (6, 27, 32, 33, 36). Strains harboring *glnA* mutations that produce enzymes with altered kinetic and feedback inhibition properties were also found to express GS at high levels under repressing conditions (6, 27, 36). Since the mutations were found to lie within or near sequences contributing to the enzyme active site, it has been proposed that GS activity is necessary for regulation (42).

Mutants of *B. subtilis* that produce GS with altered regulatory properties either required glutamine for growth or grew very slowly in minimal medium in the absence of glutamine (a leaky phenotype) (5, 6, 12, 27, 36). The fact that both classes of mutants express enzymes unable to provide the organism with sufficient glutamine for growth has made it difficult to separate the catalytic and regulatory functions of GS. Thus, we were interested in examining characteristics of GS mutants that are glutamine prototrophs. *Salmonella typhimurium* mutants of this type have been isolated by selecting for resistance to the transition-state analog L-methionine-*SR*-sulfoximine (MSX) (21). MSX is a GS inhibitor that binds the enzyme irreversibly (19, 28, 29), and in *S. typhimurium* the resistance was due to a mutation that produced an enzyme with lowered affinity for glutamate and ammonia (21). Although the isolation of *B. subtilis* strains resistant to MSX has been reported, the strains were found to be defective in methionine transport and to exhibit pleiotropic effects on GS and glutamate synthase expression (10). We have isolated a *B. subtilis* GS mutant that displays tolerance to MSX, and we describe the effect of the *glnA* mutation on GS activity and regulation.

Plasmid pSF14 (Tet^r Cam^r *glnA*$^+$) (Fig. 1) contains the *B. subtilis glnRA* operon and, when introduced into *Escherichia coli* YMC11 [*endA1 thi-1 hsdR17 supE44 ΔlacU169 hutC*$_{Klebsiella}$ Δ(*glnA ntrBC*)*2000*] (2), will complement the glutamine auxotrophy of that host (11). We mutagenized plasmid pSF14 by passage through *E. coli* KD1067 (Su$^+$ *arg his mutD5*) (7) as described previously (17). After approximately 20 generations of growth in rich medium, plasmid DNA was isolated and used to transform strain YMC11 by the method of Cohen et al. (4). Transformants were plated onto minimal medium containing 50 μg of MSX per ml; this concentration was the minimal amount necessary for inhibiting the growth of strain YMC11(pSF14). One transformant, designated YMC11(pMSX), produced GS that was less sensitive to MSX than was wild-type GS (see below). GS levels in strain YMC11(pMSX) were approximately fourfold higher than those observed for the *glnA*$^+$ parent strain YMC11(pSF14) grown in the presence of excess nitrogen (glutamine plus ammonia) (Table 1). In addition, although GS levels were regulated 10.6-fold in strain YMC11(pSF14), only 3.4-fold regulation was found for strain YMC11(pMSX) (Table 1).

Dideoxy sequencing (30) of the *glnA* DNA region of plasmid pMSX revealed a T · A-to-C · G transition mutation at position +1088, relative to the transcription start point.

* Corresponding author.
† Contribution 189 from the Center of Marine Biotechnology.
‡ Present address: Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322.

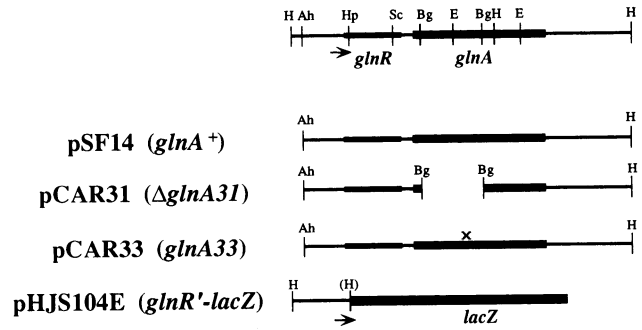

FIG. 1. Abbreviated restriction maps of plasmids used in this study. A 3.2-kb *Hin*dIII segment of *B. subtilis* DNA containing the *glnRA* operon is shown on the top line. Relative positions of GlnR (medium bar) and GS (heavy bar) coding regions are represented, as well as the location of the start point of transcription (arrow). Below are shown the DNA content of plasmids carrying portions of this region. Restriction sites: Ah, *Aha*III; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; Hp, *Hpa*I; Sc, *Sac*I. The alteration of the *Hpa*I site during cloning is indicated by parentheses. The approximate location of the mutation in plasmid pCAR33 is indicated by X.

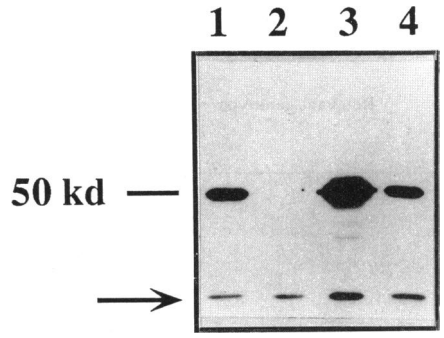

FIG. 2. GS antigen produced by wild-type and *glnA* derivatives. [^{35}S]methionine labeling of *B. subtilis* strains grown in the presence of TSS minimal medium (11) with glutamine as the nitrogen source was done as described previously (36). Approximately equal amounts of labeled crude extracts, as determined by trichloroacetic acid-precipitable radioactivity, were treated with antibody to GS (a gift from A. Aronson). Precipitated proteins were subjected to electrophoresis in a 10% polyacrylamide–sodium dodecyl sulfate gel, and labeled bands were visualized by autoradiography. Lanes: 1, strain SMY (*glnA*$^+$); 2, strain CAR2 (Δ*glnA31*); 3, strain CAR6 (*glnA33*); 4, strain CAR5 (*glnA*$^+$). Wild-type GS polypeptide has a molecular mass of approximately 50 kDa. The dye front is indicated by the arrow.

This mutation, designated *glnA33*, changed the valine at codon 190 to alanine. To determine whether the *glnA33* mutation was responsible for the observed characteristics, we introduced the 570-bp *Bgl*II fragment from plasmid pMSX, which contains the *glnA33* allele, into the *Bgl*II site of plasmid pCAR31 (Tet^r Cam^r Δ*glnA31*), creating pCAR33 (Fig. 1). Plasmid pCAR33 not only was capable of complementing the glutamine auxotrophy of strain YMC11 but also allowed for growth in minimal medium containing 50 μg of MSX per ml. Furthermore, GS levels in strain YMC11 (pCAR33) were similar to those observed for strain YMC11 (pMSX) (Table 1).

To construct a *B. subtilis* strain carrying the *glnA33* allele, we first introduced the *Bgl*II deletion harbored on plasmid pCAR31 (Δ*glnA31*) (Fig. 1) into the chromosome of strain SMY (*glnA*$^+$) by congression with chromosomal DNA from strain LS11(*rfm-500*) (37). Of the Rif^r transformants, approximately 1% acquired the Gln$^-$ phenotype. One transformant was chosen, and the absence of the 570-bp *Bgl*II *glnA* fragment was confirmed by Southern analysis (results not shown). The strain, named CAR2, did not exhibit detectable GS activity, and we could not detect any GS antigen when we probed [^{35}S]methionine-labeled extracts with antibody to GS (Fig. 2).

Wild-type and *glnA33* alleles were then introduced into strain CAR2 by transforming with linearized plasmids pSF14 (*glnA*$^+$) and pCAR33 (*glnA33*), producing strains CAR5 and CAR6, respectively, selected as Gln$^+$ transformants. Restoration of the 570-bp *Bgl*II fragment in both strains by a double-crossover event was confirmed by Southern analysis (results not shown).

In minimal medium containing glutamine, ammonia, or glutamate as the nitrogen source, strain CAR6 displayed generation times of approximately 50, 60, and 120 min, respectively, which were similar to those of the *glnA*$^+$ strains SMY and CAR5. In contrast to *E. coli* YMC11(pCAR33), strain CAR6 was not capable of growing in liquid or solid medium when MSX was present at a concentration as low as 1 μg/ml. The lack of resistance to MSX in *B. subtilis* may be due to special properties of the mutation in this organism or to the presence of other MSX-sensitive pathways.

We measured GS activities in permeabilized cells of strains CAR5 and CAR6 grown in the presence of glutamine and glutamate as nitrogen sources as described previously (33, 36); the results are shown in Table 2. Regardless of assay conditions, increased GS activities were observed in strain CAR6 compared with those in the wild-type strain. Furthermore, GS levels in strain CAR6 were only partially repressed under conditions of nitrogen excess (glutamine), and growth under nitrogen-limiting conditions (glutamate) resulted in only 1.6-fold derepression, compared with 5.1-fold for CAR5, as measured by the Mn^{2+}-dependent reverse transferase assay (Table 2). Similarly, when we examined crude extracts of strains labeled with [^{35}S]methionine, we found a qualitative three- to fivefold increase in the GS antigen level in strain CAR6 grown under repressing conditions (Fig. 2, lane 3) compared with that in wild-type strains SMY and CAR5 (Fig. 2, lanes 1 and 4, respectively). These

TABLE 1. Expression of *B. subtilis* GS in *E. coli* plasmid-carrying strains[a]

Plasmid	Sp act (mean ± SD)[b] of GS		Repression ratio[c]
	Glutamine + ammonia	Glutamine	
pSF14	2.9 ± 0.6	30.6 ± 6.1	10.6
pMSX	11.5 ± 2.0	38.7 ± 4.4	3.4
pCAR33	9.5 ± 1.3	42.5 ± 5.9	4.5

[a] GS activity was measured in *E. coli* YMC11 strains harboring the plasmids shown and grown in the presence of the indicated nitrogen sources as described previously (33).

[b] GS was measured by the Mn^{2+}-dependent reverse transferase assay (13), and activity is expressed as micromoles per minute per milligram of protein. Protein was determined by the method of Lowry et al. (18). Results are the averages of duplicate analyses of permeabilized cells prepared from at least two separate sets of cultures.

[c] Repression ratios were calculated by dividing the respective enzyme activities found in the glutamine cultures by the glutamine-plus-ammonia culture value.

TABLE 2. β-Galactosidase and GS activities of *B. subtilis* strains grown under conditions of excess and limiting nitrogen[a]

Strain	Relevant genotype	Assay	Sp act (mean ± SD)[b]			
			GS		β-Galactosidase	
			With glutamine	With glutamate	With glutamine	With glutamate
CAR5	*glnA*$^+$	Mn^{2+}-Rev.	377 ± 94	1,907 ± 95		
		Mn^{2+}-For.	27 ± 1	93 ± 2		
		Mg^{2+}-For.	25 ± 6	62 ± 6		
CAR6	*glnA33*	Mn^{2+}-Rev.	1,472 ± 162	2,365 ± 231		
		Mn^{2+}-For.	116 ± 1	195 ± 12		
		Mg^{2+}-For.	48 ± 5	75 ± 6		
CAR15	*glnA*$^+$ *glnop*::*lacZ*	Mn^{2+}-Rev.	376 ± 17	1,945 ± 11	1.2 ± 0.3	10.5 ± 0.4
CAR16	*glnA33 glnop*::*lacZ*	Mn^{2+}-Rev.	1,319 ± 250	2,791 ± 225	6.0 ± 1.2	14.0 ± 0.4

[a] GS and β-galactosidase activities were measured in *B. subtilis* strains grown in TSS minimal medium containing the indicated nitrogen source (each at a final concentration of 0.2%). GS activity in permeabilized cells was determined shortly after harvesting, using either the Mn^{2+}-dependent reverse transferase (Mn^{2+}-Rev.) (13) or the Mg^{2+}-dependent forward transferase (Mg^{2+}-For.) (biosynthetic) (6) assay. In cases when Mg^{2+} was substituted for Mn^{2+}, $MgCl_2$ was added at a final concentration of 40 mM. β-Galactosidase activity was measured as described previously (36).

[b] GS and β-galactosidase specific activities are expressed as micromoles per minute per milligram of protein and nanomoles per minute per milligram of protein, respectively. Results are the averages of duplicate analyses of permeabilized cells prepared from at least two separate sets of cultures.

results indicated that the *glnA33* mutation influenced both GS specific activity and GS antigen levels.

To determine whether the *glnA33* allele affected *glnRA* expression, we constructed strains CAR15 and CAR16 from CAR5 and CAR6, respectively, by transforming with linearized plasmid pHJS104E (Fig. 1) (32) and selection for Cam^r by plating on tryptose blood agar base plates (Difco) containing chloramphenicol (2.5 μg/ml). Plasmid pHJS104E (Amp^r Cam^r Lac$^+$) contains a *glnR'-lacZ* transcriptional fusion and is a derivative of the *B. subtilis* integrative vector pAF1 (15). β-Galactosidase expression is driven by a 700-bp *Hin*dIII fragment containing the *glnRA* promoter and regulatory sequences and the first 83 bases of *glnRA* mRNA (32). Integration of the linearized plasmid at the chromosomal *amyE* locus occurs by homologous recombination via a double-crossover event and results in an Amy$^-$ phenotype (32), which was assayed on starch agar plates as described by Nicholson and Chambliss (23). Strains CAR15 and CAR16 are both Amy$^-$, and Southern analysis showed that integration did not occur within the *glnRA* DNA region (results not shown). Furthermore, both strains exhibited similar chloramphenicol acetyltransferase activities when measured in crude extracts of glutamine-grown cells (34), indicating that the copy number of the integrated fusion gene was equivalent in the two strains.

β-Galactosidase activities expressed from the *glnRA* promoter in both fusion-containing strains are shown in Table 2. For strain CAR15, the activity varied 8.8-fold, being regulated in the same manner as GS. On the other hand, in glutamine-grown cultures, β-galactosidase activity was only partially repressed in strain CAR16, being regulated only 2.3-fold. Thus, the *glnA33* mutation altered the ability of GS to regulate transcription in *trans* under conditions of nitrogen excess.

We partially purified GS from late-exponential cultures of strain CAR6 and *E. coli* YMC11(pCAR33) and compared its kinetic properties with those of GS prepared from wild-type strains. Partial purification was done by the procedure of Deuel et al. (9) up to and including ammonium sulfate fractionation. The enzyme isolated from the *E. coli* host was used for the results reported here since yield and purity were severalfold higher than those of the enzyme prepared from *B. subtilis* strains (not shown). Under derepressing conditions, GS makes up 10 to 20% of the protein in crude extracts of strain YMC11(pSF14) (31) and the product obtained after ammonium sulfate fractionation is approximately 50 to 60% purified. The properties observed for the enzyme from *E. coli* YMC11(pCAR33) were, in all cases, identical to those found for the CAR6 GS (data not shown).

The effect of MSX on GS activity from strains YMC11 (pSF14) and YMC11(pCAR33) was examined. MSX concentrations in excess of 50 μM inhibited GS activity by more than 95% from both strains; however, the GS from YMC11 (pCAR33) was more tolerant to the inhibitor than was the wild-type enzyme. $I_{0.5}$ values derived from the reciprocal plots of fractional inhibition versus MSX concentration (Fig. 3) were 8.3 and 0.29 μM for the *glnA33* and wild-type enzymes, respectively. The single-amino-acid change at codon 190 was responsible for altering some aspect of the catalytic properties of the enzyme, making it 28-fold less sensitive to MSX.

To examine the parameters that were altered, we determined saturation curves for glutamate, ATP, and the ammonia analog hydroxylamine. The ATP saturation curves for both wild-type and *glnA33* enzymes (results not shown) yielded similar apparent K_ms of 0.4 and 0.5 mM, respectively. On the other hand, the enzyme from YMC11 (pCAR33) showed decreased affinity for hydroxylamine and glutamate compared with that of the enzyme from the wild-type strain. For hydroxylamine, reciprocal plots (Fig. 4) indicated apparent K_ms for the *glnA*$^+$ and *glnA33* enzymes to be 0.59 and 7.7 mM, respectively. The reciprocal plots for glutamate (Fig. 5) revealed apparent K_ms of 0.46 mM for the *glnA*$^+$ enzyme and 2.2 mM for the *glnA33* enzyme and were characteristically nonlinear at high substrate concentrations (8).

To our knowledge, this is the first report of a *B. subtilis* GS mutant with altered sensitivity to MSX. The kinetic parameters obtained for the *glnA33* GS indicated that its partial resistance to MSX was due to decreased affinity for both glutamate and hydroxylamine (ammonia). Similar variations were found for the GS from *S. typhimurium* containing the *glnA292* mutation, which was shown to be unaffected by MSX when the inhibitor was present at a concentration as high as 500 μM (21). Because the location of the *glnA292* allele was not characterized, the active-site domain modified by the mutation is not known. However, since MSX binding is competitive with glutamate binding (28) and since the

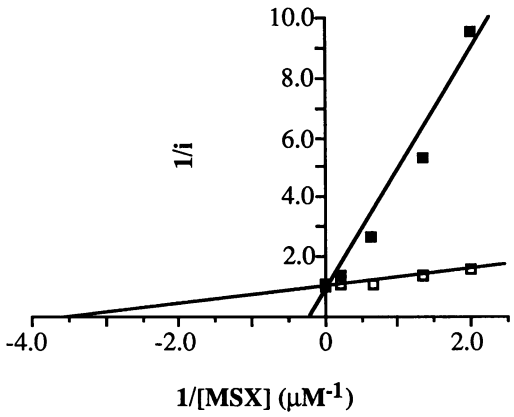

FIG. 3. Double-reciprocal plot for fractional inhibition of enzyme activity by various concentrations of MSX. Fractional inhibition, i, is defined as $(V_0 - V)/V_0$, where V_0 and V represent the reaction velocities at zero and finite levels of inhibitor, respectively (40). The y intercept is $-1/I_{0.5}$, which is used to determine the value of $I_{0.5}$. Samples of partially purified GS from strains YMC11(pSF14) (*glnA*$^+$) (□) and YMC11(pCAR33) (*glnA33*) (■) were assayed by the Mn^{2+}-dependent forward transferase assay (13). Enzyme was preincubated for 5 min at 37°C in the presence of NH_2OH, ATP (final concentrations, 40 and 10 mM, respectively), and various concentrations of MSX. Glutamate (final concentration, 100 mM) was then added to start the reaction. For these experiments, the L isomer of MSX was used. Specific activities of GS preparations from YMC11(pSF14) and YMC11(pCAR33) were 0.28 and 4.0 μmol/min/mg of protein, respectively.

S-methyl group of the sulfoximine moiety of the analog appears to occupy the ammonia-binding portion of the active site (20), it is likely that the alteration in the *glnA292* mutant affected a residue involved in the formation of both ammonia and glutamate domains. For *glnA33* in *B. subtilis*, partial resistance to MSX was due to the substitution of Ala for Val at codon 190, which lies within a stretch of amino acids that is conserved among 17 different GSs (25). Codon 190 is adjacent to a glutamate residue (Glu-189) used as a ligand for metal binding and is part of the β-strand-forming region (region II) of the active site in the C-terminal domain, as defined for the *S. typhimurium* enzyme (1, 41). Photolabeling experiments with the *B. subtilis* enzyme have implicated this region in ATP binding, since nearby His-187 and His-188 residues are modified by the ATP analog 8-azidoadenosine 5′-triphosphate (39). However, the *glnA33* mutation does not appear to significantly influence ATP binding. Thus, the alteration in hydroxylamine and glutamate affinities may be due to slight changes in the active-site domains of these substrates as a consequence of the subtle Val-to-Ala substitution, which would be consistent with the way MSX binds to the enzyme active site. Further clarification will require detailed physical studies of the *glnA33* and similar mutant enzymes.

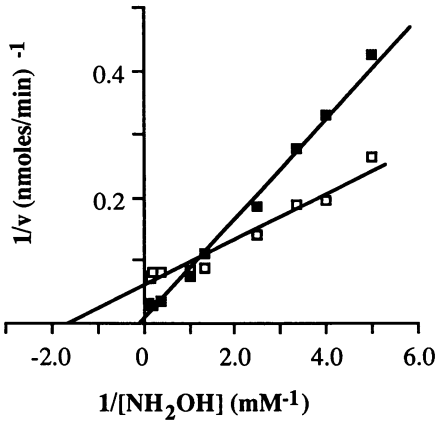

FIG. 4. Effect of NH_2OH concentration on reaction velocity for GS from *glnA*$^+$ and *glnA33* strains. Assays were done under standard conditions measuring the Mn^{2+}-dependent forward transferase activity (13), except that the NH_2OH concentration was varied and the concentrations of $MnCl_2$, ATP, and glutamate were 10, 10, and 100 mM, respectively. Reactions were initiated by the addition of partially purified GS at a final concentration of 40 and 48 μg/ml from *glnA*$^+$ (□) and *glnA33* (■) strains, respectively.

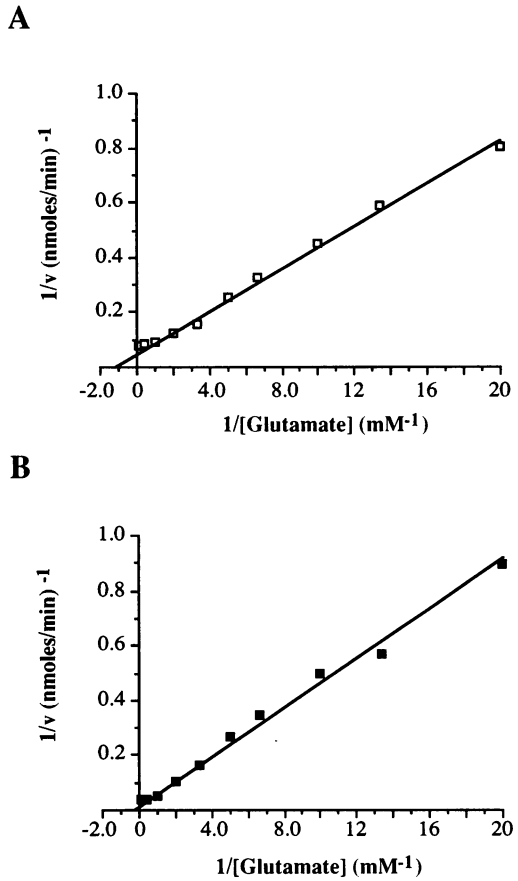

FIG. 5. Effect of glutamate concentration on reaction velocity for GS from YMC11(pSF14) (A) and YMC11(pCAR33) (B). Conditions were as described in the legend to Fig. 4, except that the glutamate concentration was varied and the concentration of NH_2OH was 50 mM.

The altered regulation observed for GS levels in *glnA33*-containing strains supports the model that GS catalytic activity is involved in regulation. However, the *glnA33* GS is unlike all previous mutants in that it is not defective in enzyme activity at saturating substrate concentrations in vitro and causes no growth defects in glutamine-free medium. If the role of GS in regulation is to provide or maintain the supply of a certain metabolite(s) used in modulating GlnR activity, the *glnA33* protein may be impaired in its

ability to support such a pool without affecting other metabolic processes. The decreased affinities for hydroxylamine and glutamate suggest that the mutant enzyme may not be capable of assimilating ammonia in the same manner as the wild type, perhaps falsely signaling nitrogen limitation under growth conditions of nitrogen excess. Evidence for such a model may be obtained by comparing the intracellular pool concentration of glutamine and other nitrogen metabolites in the *glnA33* and *glnA*$^+$ strains under different physiological conditions. On the other hand, if GS protein itself directly influences GlnR activity, as has been suggested by recent in vitro studies (3, 22), the mutant protein may not be capable of achieving the correct conformation necessary for it to interact in this capacity. Such a scenario would require that catalytic and regulatory domains overlap, a possibility that cannot be ruled out from available information. Further studies with the *glnA33* enzyme and similar *glnA* mutants will be helpful in elucidating the role of GS in its own regulation.

We thank Bob Belas for useful discussions, Linc Sonenshein for critical comments on the manuscript, and Art Aronson for his generous gift of anti-GS antibody. We also thank C.-M. Lin for oligonucleotide synthesis.

This work was supported by Public Health Service grant GM39541 to H.J.S. from the National Institutes of Health.

REFERENCES

1. **Almassy, R. J., C. A. Janson, R. Hamlin, N.-H. Xuong, and D. Eisenberg.** 1986. Novel subunit-subunit interactions in the structure of glutamine synthetase. Nature (London) **323:**304–309.
2. **Backman, K., Y.-M. Chen, and B. Magasanik.** 1981. Physical and genetic characterization of the *glnA-glnG* region of the *Escherichia coli* chromosome. Proc. Natl. Acad. Sci. USA **78:**3743–3747.
3. **Brown, S. W., and A. L. Sonenshein.** Autogenous regulation of the *Bacillus subtilis glnRA* operon by GlnR and glutamine synthetase. Submitted for publication.
4. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *E. coli* by R-factor DNA. Proc. Natl. Acad. Sci. USA **69:**2110–2114.
5. **Dean, D. R., and A. I. Aronson.** 1980. Selection of *Bacillus subtilis* mutants impaired in ammonia assimilation. J. Bacteriol. **141:**985–988.
6. **Dean, D. R., J. A. Hoch, and A. I. Aronson.** 1977. Alteration of the *Bacillus subtilis* glutamine synthetase results in overproduction of the enzyme. J. Bacteriol. **131:**981–987.
7. **Degnen, G. E., and E. C. Cox.** 1974. Conditional mutator genes in *Escherichia coli*: isolation, mapping, and effector studies. J. Bacteriol. **117:**477–487.
8. **Deuel, T., and E. R. Stadtman.** 1970. Some kinetic properties of *Bacillus subtilis* glutamine synthetase. J. Biol. Chem. **245:**5206–5213.
9. **Deuel, T. F., A. Ginsberg, J. Yeh, E. Shelton, and E. R. Stadtman.** 1970. *Bacillus subtilis* glutamine synthetase. J. Biol. Chem. **245:**5195–5205.
10. **Fischer, R. S., and J. F. Kane.** 1982. Regulation of glutamate synthase in *Bacillus subtilis*, p. 50–57. *In* A. T. Ganesan, S. Chang, and J. A. Hoch (ed.), Molecular cloning and gene regulation in bacilli. Academic Press, Inc., New York.
11. **Fisher, S. H., M. S. Rosenkrantz, and A. L. Sonenshein.** 1984. Glutamine synthetase gene of *Bacillus subtilis*. Gene **32:**427–438.
12. **Fisher, S. H., and A. L. Sonenshein.** 1977. Glutamine requiring mutants of *Bacillus subtilis*. Biochem. Biophys. Res. Commun. **79:**987–996.
13. **Fisher, S. H., and A. L. Sonenshein.** 1984. *Bacillus subtilis* glutamine synthetase mutants pleiotropically altered in glucose catabolite repression. J. Bacteriol. **157:**612–621.
14. **Fisher, S. H., and A. L. Sonenshein.** 1991. Control of carbon and nitrogen metabolism in *Bacillus subtilis*. Annu. Rev. Microbiol. **45:**107–135.
15. **Fouet, A., and A. L. Sonenshein.** 1990. A target for carbon source-dependent negative regulation of the *citB* promoter of *Bacillus subtilis*. J. Bacteriol. **172:**835–844.
16. **Gutowski, J. C., and H. J. Schreier.** 1992. Interaction of the *Bacillus subtilis glnRA* repressor with operator and promoter regions in vivo. J. Bacteriol. **174:**671–681.
17. **Henkin, T. M., and A. L. Sonenshein.** 1987. Mutations of the *Escherichia coli lacUV5* promoter resulting in increased expression in *Bacillus subtilis*. Mol. Gen. Genet. **209:**467–474.
18. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein determination with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
19. **Manning, J., S. Moore, W. Rowe, and A. Meister.** 1969. Identification of L-methionine-S-sulfoximine as the diastereomer of L-methionine-SR-sulfoximine that inhibits glutamine synthetase. Biochemistry **8:**2681–2685.
20. **Meister, A.** 1980. Catalytic mechanism of glutamine synthetase: overview of glutamine metabolism, p. 1–40. *In* J. Mora and R. Palacios (ed.), Glutamine: metabolism, enzymology, and regulation. Academic Press, Inc., New York.
21. **Miller, E. S., and J. E. Brenchley.** 1981. L-Methionine *SR*-sulfoximine-resistant glutamine synthetase from mutants of *Salmonella typhimurium*. J. Biol. Chem. **256:**11307–11312.
22. **Nakano, Y., and K. Kimura.** 1991. Purification and characterization of a repressor for the *Bacillus cereus glnRA* operon. J. Biochem. **109:**223–228.
23. **Nicholson, W. L., and G. H. Chambliss.** 1985. Isolation and characterization of a *cis*-acting mutation conferring catabolite repression resistance to α-amylase synthesis in *Bacillus subtilis*. J. Bacteriol. **161:**875–881.
24. **Pan, F. L., and J. G. Coote.** 1979. Glutamine synthetase and glutamate synthase activities during growth and sporulation of *Bacillus subtilis*. J. Gen. Microbiol. **112:**373–377.
25. **Pesole, G., M. P. Bozzetti, C. Lanave, G. Preparata, and C. Saccone.** 1991. Glutamine synthetase gene evolution: a good molecular clock. Proc. Natl. Acad. Sci. USA **88:**522–526.
26. **Rebello, J. L., and N. Strauss.** 1969. Regulation of glutamine synthase in *Bacillus subtilis*. J. Bacteriol. **148:**653–658.
27. **Reysset, G.** 1981. New class of *Bacillus subtilis* glutamine-requiring mutants. J. Bacteriol. **148:**653–658.
28. **Ronzio, R., W. Rowe, and A. Meister.** 1969. Studies on the mechanism of inhibition of glutamine synthetase by methionine sulfoximine. Biochemistry **8:**1066–1075.
29. **Rowe, B., R. Ronzio, and A. Meister.** 1969. Inhibition of glutamine synthetase by methionine sulfoximine. Studies on methionine sulfoximine phosphate. Biochemistry **8:**2674–2680.
30. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
31. **Schreier, H. J.** Unpublished data.
32. **Schreier, H. J., S. W. Brown, K. D. Hirschi, J. F. Nomellini, and A. L. Sonenshein.** 1989. Regulation of *Bacillus subtilis* glutamine synthetase gene expression by the product of the *glnR* gene. J. Mol. Biol. **210:**51–63.
33. **Schreier, H. J., S. H. Fisher, and A. L. Sonenshein.** 1985. Regulation of expression from the *glnA* promoter of *Bacillus subtilis* requires the *glnA* gene product. Proc. Natl. Acad. Sci. USA **82:**3375–3379.
34. **Schreier, H. J., K. D. Hirschi, and C. A. Rostkowski.** 1990. *cis*-acting sequences regulating *glnRA* expression in *Bacillus subtilis*, p. 81–87. *In* J. A. Hoch and A. T. Ganesan (ed.), Genetics and biotechnology of bacilli. Academic Press, Inc., New York.
35. **Schreier, H. J., C. A. Rostkowski, J. F. Nomellini, and K. D. Hirschi.** 1991. Identification of DNA sequences involved in regulating *Bacillus subtilis glnRA* expression by the nitrogen source. J. Mol. Biol. **220:**241–253.
36. **Schreier, H. J., and A. L. Sonenshein.** 1986. Altered regulation of the *glnA* gene in glutamine synthetase mutants of *Bacillus subtilis*. J. Bacteriol. **167:**35–43.
37. **Sonenshein, A. L., B. Cami, J. Brevet, and R. Cote.** 1974.

Isolation and characterization of rifampin-resistant mutants of *Bacillus subtilis*. J. Bacteriol. **120:**253–265.

38. **Strauch, M. A., A. I. Aronson, S. W. Brown, H. J. Schreier, and A. L. Sonenshein.** 1988. Sequence of the *Bacillus subtilis* glutamine synthetase gene region. Gene **71:**257–265.
39. **Tanaka, E., and K. Kimura.** 1991. Identification of amino acid residues modified by two ATP analogs in *Bacillus subtilis* glutamine synthetase. J. Biochem. **110:**780–784.
40. **Wedler, F. C., and P. D. Boyer.** 1973. Mechanisms of enzyme control as probed by equilibrium exchange rates: patterns of modifier effects with a two substrate, two product system. J. Theor. Biol. **38:**539–558.
41. **Yamashita, M. M., R. J. Almassy, C. A. Janson, D. Cascio, and D. Eisenberg.** 1989. Refined atomic model of glutamine synthetase at 3.5 Å resolution. J. Biol. Chem. **264:**17681–17690.
42. **Zhang, J., M. Strauch, and A. I. Aronson.** 1989. Glutamine auxotrophs of *Bacillus subtilis* that overproduce glutamine synthetase antigen have altered conserved amino acids in or near the active site. J. Bacteriol. **171:**3572–3574.