JOURNAL OF BACTERIOLOGY, Apr. 1986, p. 224–229
0021-9193/86/040224-06$02.00/0

Vol. 166, No. 1

Role of Glutamine Synthetase Adenylylation in the Self-Protection of *Pseudomonas syringae* subsp. "*tabaci*" from Its Toxin, Tabtoxinine-β-Lactam

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Received 8 November 1985/Accepted 14 January 1986

Selected pathovars of *Pseudomonas syringae* produce an extracellular phytotoxin, tabtoxinine-β-lactam, that irreversibly inhibits its known physiological target, glutamine synthetase (GS). *Pseudomonas syringae* subsp. "*tabaci*" retains significant amounts of glutamine synthetase activity during toxin production in culture. As part of our investigation of the self-protection mechanism(s) used by these pathovars, we have determined that GS becomes adenylylated after toxin production is initiated and that the serine released from the zinc-activated hydrolysis of tabtoxin is a factor in the initiation of this adenylylation. The adenylylation state of this GS was estimated to range from $E_{5.0-7.5}$. The irreversible inactivation by tabtoxinine-β-lactam of unadenylylated and adenylylated glutamine synthetase purified from *P. syringae* subsp. "*tabaci*" was investigated. Adenylylated GS was inactivated by tabtoxinine-β-lactam at a slower rate than was unadenylylated enzyme. Adenylylated GS ($E_{7.5-10.5}$) was significantly protected from this inactivation in the presence of the enzyme effectors, AMP, Ala, Gly, His, and Ser. Thus, the combination of the adenylylation of GS after toxin production is initiated and the presence of the enzyme effectors in vivo could provide part of the self-protection mechanism used by subsp. "*tabaci*".

The mechanisms by which toxin-producing pathogens are resistant to their toxins, even though they contain a suitable target, are not well understood. The phytopathogenic bacterium *Pseudomonas syringae* subsp. "*tabaci*" and closely related pathovars produce the dipeptide pretoxin, tabtoxin, which is hydrolyzed to release the active form, tabtoxinine-β-lactam (T-β-L) [2-amino-4-(3-hydroxy-2-oxo-azacyclobutan-3-yl)-butanoic acid] and serine or threonine (3, 18). This hydrolysis is carried out by a zinc-activated aminopeptidase present in the periplasm (C. Levi and R.D. Durbin, Physiol. Plant Pathol., in press). The physiological target in plants, glutamine synthetase (GS) (EC 6.3.2.1.) (5, 6, 17) is irreversibly inhibited by T-β-L (6, 17). A preliminary study by Thomas and Durbin (16) showed that GS from subsp. "*tabaci*" was inactivated in vitro by T-β-L. Yet, pathogen growth in liquid culture is not inhibited and significant GS activity is detected during the production of T-β-L. This suggests that the GS of the pathogen may be insensitive to toxin action. Such a mechanism is employed by *P. syringae* pv. *phaseolicola* which produces phaseolotoxin, an inactivator of OCTase (ornithine carbamoyltransferase) (EC 2.1.3.3.) (10), an enzyme common to both pathogen and host. The pathogen protects itself by producing a toxin-resistant form of OCTase during toxin production (4).

GS is also common to both *P. syringae* subsp. "*tabaci*" and its host plant tobacco. Some pseudomonads regulate GS by an adenylylation cascade system (8), which is not found in plants. Adenylylated bacterial GS and plant GS have significantly different catalytic and regulatory properties, and these differences may be exploited by subsp. "*tabaci*" to protect its GS. Since the preliminary work of Thomas and Durbin (16) did not identify that GS in subsp. "*tabaci*" is regulated by the adenylylation cascade system, we established the existence of this system in subsp. "*tabaci*". Log-phase bacterial cultures growing with nitrate as the sole nitrogen source commonly have unadenylylated GS while cultures growing with ammonia as a nitrogen source have adenylylated GS (8, 14). We found that GS became adenylylated after toxin production was initiated in log phase, even though the culture was growing with nitrate as the sole nitrogen source. Thus, to more fully understand the self-protection mechanism employed by subsp. "*tabaci*", we estimated the adenylylation state of GS during toxin production and investigated the effects of adenylylation on the inactivation of purified subsp. "*tabaci*" GS.

MATERIALS AND METHODS

Bacteria and cell growth. Cultures of *P. syringae* subsp. "*tabaci*" Tox$^+$ (Pt113; University of Wisconsin), a virulent tabtoxin-producing strain, and *P. syringae* subsp. "*tabaci*" Tox$^-$ (Pa 45), a non-toxin-producing strain, were grown in shake culture at 25°C for 24 h in medium containing 30 mM K_2HPO_4, 7 mM KH_2PO_4, 10 mM NaCl, 20 mM KCl, 0.2 mM $CaCl_2$, 0.4 mM $MgSO_4$, and 0.002 mM $FeCl_2$ [pH 6.8]. Nitrogen was supplied as either 5 mM KNO_3 or 20 mM $(NH_4)_2SO_4$, and 18 mM glucose served as the carbon source. Cells were collected by centrifugation (14,500 × *g*, 10 min), washed once in the above medium and used for inoculation of 2-liter shake culture flasks containing 1 liter of the same medium to an initial cell density of 10^8 cells ml$^-$. Unadenylylated GS was obtained from cells cultured with NO_3 as the nitrogen source and harvested during the exponential growth phase. Adenylylated GS was obtained from cells cultured with $(NH_4)_2SO_4$ as the nitrogen source (which represses GS synthesis [21]) and supplemented with an additional 20 mM $(NH_4)_2SO_4$ (ammonia shock) during the stationary phase (1). Ammonia shock and harvesting were

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done during the stationary phase to maximize the adenylylation state.

Enzyme purification. The buffer used for the purification contained 10 mM imidazole hydrochloride (pH 7.5), 10 mM $MgCl_2$, and 1 mM dithiothreitol. All enzyme purification was done with *P. syringae* subsp. "*tabaci*" Tox^+.

(i) Crude extract. At 5 min before harvesting (4 liters of cells with 2.4 A_{500} ml^{-1}), CTAB (hexadecyltrimethylammonium bromide) was added to cultures (0.1 mg/ml) to prevent changes in adenylylation state (1). Cells were collected by centrifugation (14,500 × *g*, 10 min), washed once in imidazole hydrochloride buffer, and suspended in this buffer (1 g of cells per 3 ml of buffer). Cells were broken in a bead beater (Biospec Products, Bartlesville, Okla.) containing acid-washed sand by using three 1-min bursts, and the lysate was clarified by centrifugation (40,000 × *g*, 15 min).

(ii) Streptomycin sulfate precipitation. Streptomycin sulfate (10% [wt/vol]) was added (10% of initial volume) slowly to the cell extract. The mixture was stirred for 15 min and centrifuged (40,000 × *g*, 15 min).

(iii) Heat treatment. The supernatant from ii was placed in a water bath (63°C), allowed to reach 63°C (5 to 10 min), and then incubated for an additional 15 min with intermittent shaking. The mixture was centrifuged (40,000 × *g*, 15 min).

(iv) Ammonium sulfate precipitation. Solid ammonium sulfate was added to the supernatant from iii to 60% saturation and stirred for 30 min at 4°C. The mixture was centrifuged (40,000 × *g*, 15 min). The precipitate was suspended in 30 ml of imidazole hydrochloride buffer and dialyzed overnight against 2 liters of this buffer.

(v) Ion-exchange chromatography. The dialyzed fraction from step iv was applied to a column of DEAE-cellulose (1.5 by 30 cm), equilibrated with imidazole hydrochloride buffer, and washed with this buffer (400 ml), and GS was eluted with a linear gradient (600 ml) of 0 to 600 mM KCl in buffer. Fractions containing GS activity were pooled, protein was precipitated with $(NH_4)_2SO_4$ (70% saturation), the suspension was centrifuged, and the pellet was suspended in 2 ml of buffer without dithiothreitol and dialyzed against 2 liters of this buffer without dithiothreitol. Dithiothreitol was omitted from all remaining steps.

(vi) Gel filtration chromatography. The dialyzed fraction (2 ml) from v was applied to a column of Ultrogel AcA-22 (1 by 90 cm) equilibrated with imidazole buffer. Fractions containing GS activity were pooled. The enzyme was stored at 4°C for 3 to 5 weeks without loss of activity.

Glutamine synthetase assays. The γ-glutamyltransferase assay of Shapiro and Stadtman (12) was used to monitor enzyme purification and determine adenylylation state. One unit of enzyme activity is defined as 1 μmol of γ-glutamylhydroxamate formed per min at pH 7.5. GS activity from whole cell preparations was measured by the method of Bender et al. (1) by including CTAB (0.1 mg/ml) in the transferase assay. The spectrophotometric, coupled assay of Shapiro and Stadtman (12) was used for determining GS activity when the inactivation by T-β-L was studied. $MnCl_2$ (0.3 mM) replaced $MgCl_2$ in the assay when studying inactivation of adenylylated GS. Protein was determined by the method of Bradford (2), using bovine serum albumin as a standard.

Estimation of adenylylation state (E_n). Adenylylation state (E_n) was calculated by method C (in imidazole buffer) of Stadtman et al. (15). pH optimum, isoactivity point (7.5), and divalent cation requirements of GS from cells cultured with either NO_3 or NH_4 were determined using the gamma-glutamyltransferase assay. The pH optimum did not change when the transferase assay was supplemented with 60 mM $MgCl_2$, and therefore, the adenylylation state could be estimated from the isoactivity point (pH 7.5). Adenylylated GS was converted to the deadenylylated form by using snake venom phosphodiesterase (SVD) by the method of Michulski et al. (9). The low activity after treatment with SVD is consistent with the findings of Bender et al. (1) who also observed lowered GS activity after treatment with SVD. The [^{14}C]adenine moiety of ATP was incorporated into adenylylated GS by the method of Michulski et al. (9) after first NH_4-shocking toluene-treated cells cultured on NO_3.

TABLE 1. Purification of glutamine synthetase ($E_{0.0}$) from *P. syringae*

Step	Total protein (mg)	Total GS (U)	Sp act (U/mg)	% Yield
Crude extract	400	683	1.7	100
Streptomycin sulfate	395	675	1.7	99
Heat	95	500	5.2	73
Ammonium sulfate	54	332[a]	6.1	49
DEAE-cellulose	41	481	11.7	70
Ultrogel AcA-22	6.7	335	50.2	49

[a] Apparent yield.

Inactivation of GS. The inactivation of GS with T-β-L was done in 10 mM imidazole-hydrochloride (pH 7.5), 50 mM $MgCl_2$ for $E_{0.0}$ or 50 mM $MnCl_2$ for $E_{10.5}$ and 10 mM ATP. The inactivation was initiated at $t = 0$ by the addition of T-β-L. The rates of inactivation of GS by T-β-L were measured using at least a 500:1 molar excess of toxin to enzyme subunits. GS and T-β-L were combined at $t = 0$, the inactivation was stopped at $t = n$ by dilution of a sample (50 μl) at each time point into complete coupled assay mixture (1 ml) (pH 7.5), and the GS activity was measured. The reaction was monitored continuously spectrophotometrically and was linear for all the assays. Studies of the effects of the known end product effectors of GS on the inactivation were done using either the effectors individually (5 mM) or a mixture of all of the effectors (each 5 mM) in the buffer above without T-β-L for the initial incubations (30 min). The effect of the effectors on the GS activity was determined and the inactivation was then initiated by the addition of T-β-L. All inactivations were done at 25°C. T-β-L was obtained by the method of Thomas et al. (17). Attempts to recover GS activity by dialysis after inactivation were done by the methods of Maurizi and Ginsburg (7).

RESULTS

Purification of GS from *P. syringae* subsp. "*tabaci*". Purification methods with acid ammonium sulfate precipitation (8) or acetone precipitation (21) were not used because the enzyme was unstable with these treatments. A summary of the purification steps and typical results is presented in Table 1. The enzyme preparations were purified to homogeneity as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The subunit molecular weight was estimated as 60,000 by the method of Weber and Osborne (19), and the molecular weight of native enzyme was estimated as 700,000 by gel filtration. These results are consistent with those of Meyer and Stadtman (8) who found 12 subunits in native GS from pseudomonads. The specific activity (units per milligram of protein) of the several preparations of GS were 11.9 for $E_{10.5}$, 18.7 for $E_{7.5}$, 27.8 for $E_{3.2}$, and 50.2 for $E_{0.0}$.

Adenylylation of GS. The properties of GS obtained from

TABLE 2. Adenylylation state (E_n) of GS from *P. syringae* subsp. "*tabaci*"[a]

Nitrogen source	Harvest time (h)	GS activity (nmol/min/mg)		pH optimum	E_n
		Mn	Mg		
A. KNO_3, 5 mM	36	755	766	8.0	0.0
B. $(NH_4)_2SO_4$, 20 mM + NH_4-shock, 20 mM	43	220	47	6.8	9.5
C. B plus SVD	43	140	110	7.6	2.6

[a] The requirements of GS for divalent cations were determined by using the transferase assay at the isoactivity point (pH 7.5) containing either 0.3 mM $MnCl_2$ or 0.3 mM $MnCl_2$ and 60 mM $MgCl_2$. The pH optima were determined by the Mn^{2+}-dependent transferase assay. GS from NH_4-shocked cells was treated with SVD and incubated at pH 8.8 for 45 min at 37°C before measuring GS activity. The ratio of total protein to SVD was 10:1.

cells cultured on ammonium or nitrate were characteristic of adenylylated or unadenylylated GS, respectively (Table 2) (14). GS was shown to be adenylylated or unadenylylated by the effect of Mg^{2+} on the catalytic capability, different pH optima, and the deadenylylation of GS by SVD treatment (9). The specific activity of unadenylylated enzyme was four- to fivefold higher than that of adenylylated GS. The radioactive labeling of unadenylylated enzyme with the [^{14}C]adenine moiety of [^{14}C]ATP in vivo indicated adenylylation of subsp. "*tabaci*" GS. Areas of nondenaturing disk gels containing GS activity had 2,800 to 3,000 dpm/2-mm slice (background was 100 to 200 dpm/2-mm slice). The isoactivity point of the Mn^{2+}-dependent transferase reaction was 7.5 in 10 mM imidazole hydrochloride buffer. Transferase activity was measured at pH 7.5.

In vivo GS activity and T-β-L production. A cell suspension of subsp. "*tabaci*" growing in a tabtoxin-producing mode (3, 18) was shifted, by the addition of 25 μM $ZnCl_2$, to a state where the pretoxin, tabtoxin, was hydrolyzed, the active toxin, T-β-L, was produced, and serine and threonine were released (3, 19). The changes in total GS activity, state of adenylylation and T-β-L production accompanying this change are given in Table 3. T-β-L was produced and GS activity was partially adenylylated within 15 min after the zinc was added. T-β-L production did not lower the in vivo GS activity in these cells. This adenylylation of GS was investigated by testing the effect of 25 μM zinc on the adenylylation of GS in a tox$^-$ strain of *P. syringae* subsp. "*tabaci*" grown the same as the tox$^+$ strain. The adenylylation state of GS did not change from that of the untreated control Tox$^-$ strain after addition of zinc. The addition of 20 μM T-β-L to similar Tox$^-$ cultures did not alter the adenylylation state of GS either, but 66% of the GS activity was lost within 1 h. However, amino acids in the culture media influence adenylylation of GS (12), and since serine is released during tabtoxin hydrolysis and is a known effector of GS (22, 23), its effect on GS adenylylation was tested. Cells were grown in tabtoxin-producing mode (without zinc), and at 33 h, serine was added (t_0) to a final concentration of 0.25 μM. The adenylylation state (E_n) of GS was 0.0 at t_0, 7.2 at t = 0.25 h, 6.5 at t = 0.5 h, and 6.5 at t = 1 h in the serine-treated cultures. The adenylylation state of GS in untreated cells remained 0.0.

In vitro inactivation of GS. Purified GS obtained from cells cultured on either NO_3 ($E_{0.0}$) or NH_4 ($E_{10.5}$) was inactivated by T-β-L in vitro (Fig. 1). Unadenylylated GS was more

TABLE 3. GS activity and adenylylation state (E_n) in whole cells of subsp. "*tabaci*" during T-β-L production[a]

Incubation time (h)	GS activity (nmol/min/ml)		E_n	T-β-L (nmol/ml of culture filtrate)
	Mn	Mg		
Without zinc				
0	212	225	0.0	0.0
1	233	172	3.0	0.0
3	309	211	3.8	0.0
With zinc				
0	212	225	0.0	0.0
0.25	217	78	7.7	464
0.5	210	132	4.5	435
1	233	137	5.0	481
1.5	279	146	5.7	490
2	300	128	6.9	551
3	324	123	7.5	529

[a] Cells were cultured in two flasks with 5 mM KNO_3 without zinc for 36 h. The cell density of each flask was 10^8 cells per ml at t_0. These cells had been grown for 36 h when the experiment was begun (t_0). Zinc chloride (25 μM) was added at t_0 to one flask containing the cell suspension to induce T-β-L production.

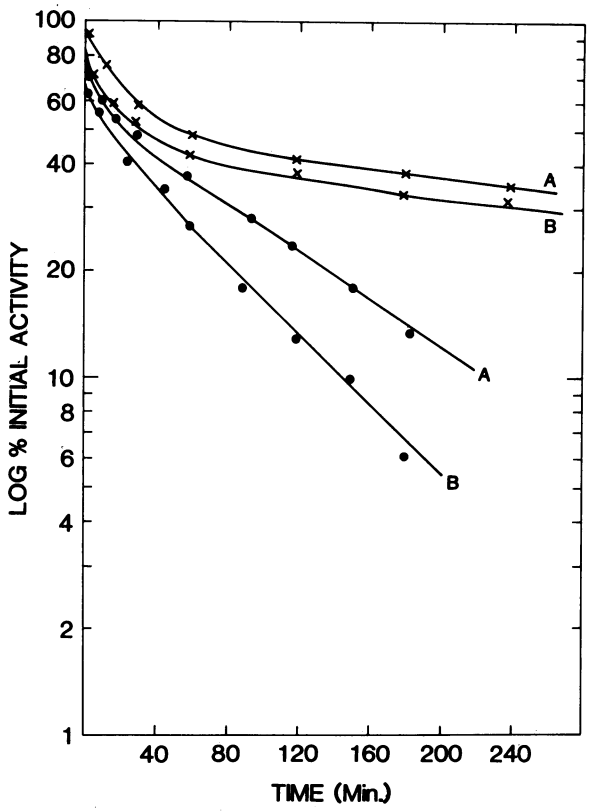

FIG. 1. Inactivation of GS from subsp. "*tabaci*" T-β-L. Unadenylylated, $E_{0.0}$ (●), and adenylylated, $E_{10.5}$ (×), GS were inactivated using (A) 10 μM T-β-L and (B) 50 μM T-β-L. Inactivation was done in 10 mM imidazole hydrochloride buffer (pH 7.5) containing T-β-L, 10 mM $MgCl_2$ for ($E_{0.0}$) or 10 mM $MnCl_2$ for ($E_{10.5}$) and 10 mM ATP. The coupled assay for unadenylylated GS contained 50 mM imidazole (pH 7.5), 50 mM $MgCl_2$, 10 mM NH_4Cl, 10 mM glutamate, 10 mM ATP, and the coupling system. The coupled assay for adenylylated GS contained 50 mM imidazole (pH 7.5), 50 mM $MnCl_2$, 50 mM NH_4Cl, 50 mM glutamate, 10 mM ATP, and the coupling system. The GS and T-β-L mixture (50-μl samples) was diluted into the assay solution.

sensitive to T-β-L inactivation than adenylylated GS. Inactivation rates ($t_{1/2}$) for both enzyme forms reached a maximum at their respective pH optima for their biosynthetic activities (Fig. 2). Inactivation of both forms of GS was biphasic, with a rapid initial inactivation rate, followed by a second slower rate (Fig. 1). Inactivation of GS by T-β-L was irreversible, and enzyme activity could not be recovered after inactivation by dialysis against (i) imidazole buffer, (ii) synthetase assay mixture containing glutamate, or (iii) imidazole buffer after ammonium sulfate acid precipitation of GS (data not shown [7]).

The K_m for glutamate increased as adenylylation state increased with GS of $E_{0.0}$ having a K_m of 1 mM, $E_{3.5}$ of 1.4 mM, $E_{9.5}$ of 3.6 mM, and $E_{10.5}$ of 4.5 mM. However, the K_m for glutamate (1.4 mM) did not change as GS ($E_{3.2}$) was inactivated with T-β-L (100, 75, and 35% initial activity). Glutamate concentrations greater than 15 mM inhibited GS and also protected GS from inactivation by T-β-L (Fig. 3). To assure that T-β-L was not being depleted during inactivation, a sample was withdrawn at 50% initial activity of GS, ($E_{10.5}$) and the initial concentration of T-β-L (10 μM) was doubled in the new sample. The rates of inactivation were monitored in both samples (Fig. 4). The initial rate of inactivation was not reestablished after the increase in T-β-L concentration.

Effects of enzyme effectors on T-β-L inactivation. Cumulative inhibition of adenylylated and unadenylylated GS was observed when the enzymes were incubated with a mixture of known feedback inhibitors of GS (22, 23). Alanine, serine, glycine, histidine, and AMP (5 mM each) were used since all are end products of glutamine metabolism. The concentration chosen for the effectors (5 mM) was the same as that

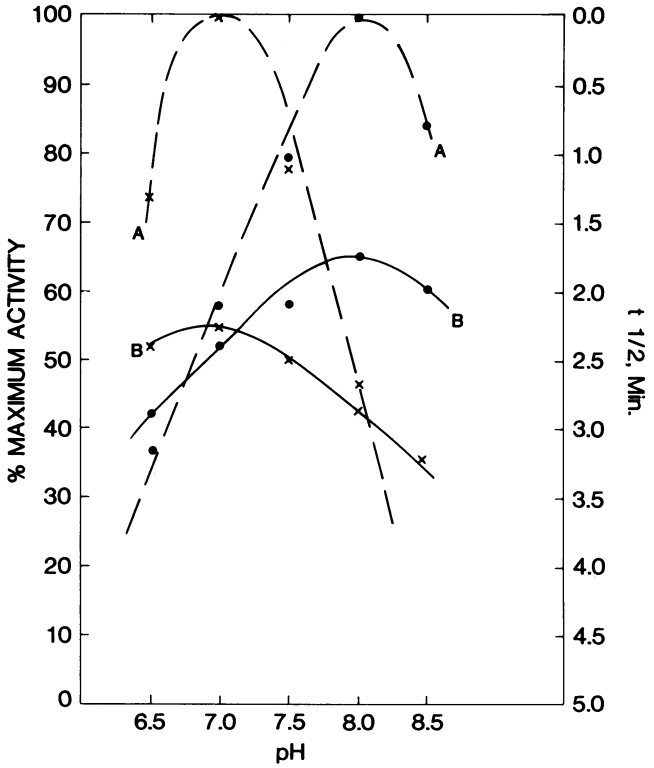

FIG. 2. Effect of pH on the rates of enzyme catalysis and inactivation. (A) The pH optima for GS catalysis and (B) the effect of pH on rates of inactivation ($t_{1/2}$) were determined for unadenylylated, $E_{0.0}$ (●), and adenylylated, $E_{9.5}$ (×), GS. T-β-L (0.5 mM) was used in the inactivation, and the assay was as described in the legend to Fig. 1, except the pH was adjusted with HCl to the appropriate values.

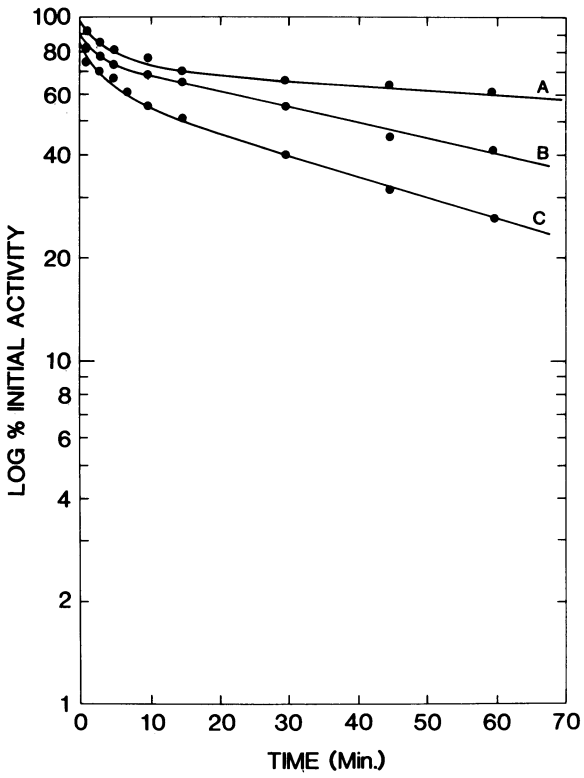

FIG. 3. Effect of glutamate on the rate of inactivation of GS by T-β-L. GS ($E_{0.0}$) was inactivated by using 50 μM T-β-L as described in the legend to Fig. 1, with the addition of various concentrations of glutamate: (A) 100 mM, (B) 25 mM, and (C) 0 mM. The assay was done as described in the legend to Fig. 1.

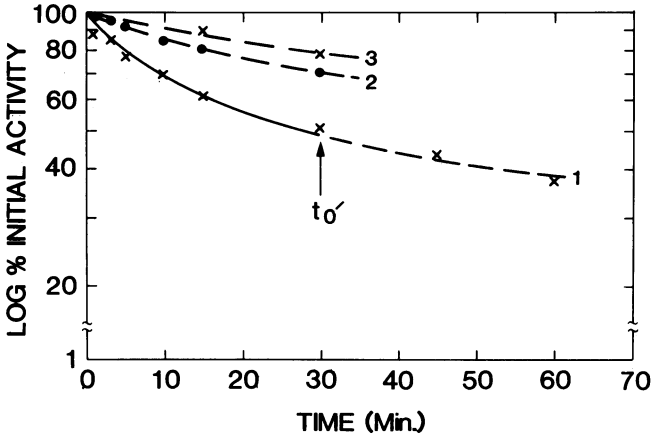

FIG. 4. Effect of addition of T-β-L during inactivation of GS ($E_{10.5}$). GS ($E_{10.5}$) was incubated with 10 μM T-β-L, and GS activity was assayed as described in the legend to Fig. 1, except at t = 30 min the sample was divided into two samples, and additional T-β-L was added to the second sample to give 20 μM T-β-L. This time was defined as t_0, and was used to replot the loss of GS activity in the two samples (curves 2 and 3). The initial phase of inactivation is denoted by (——) and the second phase is denoted by (-----). Inactivation was with 10 μM T-β-L (×) 20 μM T-β-L (●).

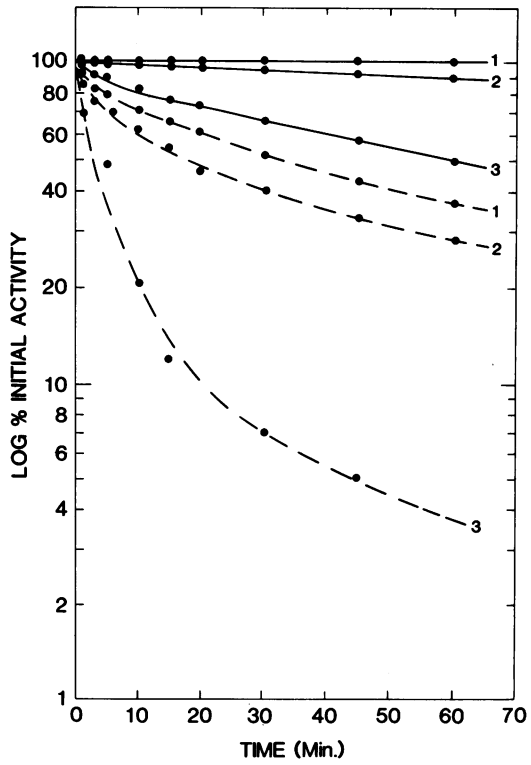

FIG. 5. Inactivation of unadenylylated or adenylylated GS in the presence of a mixture of enzyme effectors. The effectors Ser, Gly, Ala, His, and AMP were used (——) and compared with inactivation without effectors (-----). GS of adenylylation states of (curve 1) $E_{10.5}$, (curve 2) $E_{7.5}$, and (curve 3) $E_{0.0}$ was used. GS was incubated with 10 mM imidazole buffer–10 mM $MgCl_2$–10 mM ATP–5 mM enzyme effectors for 15 min before the addition of 0.5 mM T-β-L. Inactivation and assay conditions were as described in the legend to Fig. 1.

used by Woolfolk and Stadtman (22, 23) in their studies of the regulation of *Escherichia coli* GS. Adenylylated GS ($E_{10.5}$) retained 31% of initial activity and unadenylylated GS ($E_{0.0}$) retained 43% of initial activity after incubation with a mixture of all of these enzyme effectors (each 5 mM) and without T-β-L. Dialysis of the GS-enzyme effector mixture allowed recovery of 100% of the initial activity of adenylylated and unadenylylated GS. Adenylylated GS ($E_{7.5}$ and $E_{10.5}$) was well protected against inactivation by T-β-L when first incubated with the enzyme effector mixture (Fig. 5). The adenylylated GS ($E_{10.5}$) lost none of its activity in the presence of T-β-L when first incubated with the mixture of enzyme effectors (Fig. 5). However, unadenylylated GS ($E_{0.0}$) was only partially protected by incubation with the mixture of enzyme effectors (Fig. 5 and 6).

DISCUSSION

GS from *P. syringae* subsp. "*tabaci*" is regulated by an adenylylation system in which the GS is unadenylylated when the culture is growing with nitrate as the sole nitrogen source and is adenylylated when the culture is supplied ammonia as a nitrogen source. The GS activity is inhibited by end products of glutamine. Thus, GS from subsp. "*tabaci*" is regulated in a manner similiar to that in *E. coli* and other gram-negative bacteria (8, 14, 22, 23). However, the GS of subsp. "*tabaci*" becomes partially adenylylated when T-β-L production is initiated. Woolfolk and Stadtman (23) suggested that cultures with fully adenylylated GS employ an alternative mechanism(s) for assimilation of ammonia and utilize the residual GS activity for glutamine production needed for protein synthesis. However, the subsp. "*tabaci*" GS is only partially adenylylated during T-β-L production, and a significant amount of GS activity remains available to the bacterium (Table 3) for continued assimilation of ammonia and glutamine production. Investigation of this adenylylation showed that zinc and T-β-L did not directly effect the adenylylation state of GS. However, addition of serine was immediately followed by partial adenylylation of GS in the Tox^+ strain when growing in the tabtoxin-producing mode. This observation is consistent with the adenylylation of GS that results when amino acids are supplied to cultures of *E. coli* (12). These results strongly suggest that the serine, and probably also the threonine, released upon the zinc-activated hydrolysis of tabtoxin causes the partial adenylylation of GS in T-β-L-producing cultures.

Purified GS from subsp. "*tabaci*" is inhibited irreversibly in vitro by T-β-L and the inactivation is slower in the presence of glutamate, suggesting that the inactivation is active site directed. The most rapid rate of inactivation is observed at the pH optimum for the biosynthetic activity of the enzyme and factors affecting the rate of catalysis, including adenylylation, also affect the rate of inactivation, suggesting that the enzyme catalyzes a covalent change in T-β-L during inactivation. The inactivation of subsp. "*tabaci*" GS is not linear, i.e., not first order, and is probably similar to the nonlinear inactivation of *E. coli* GS

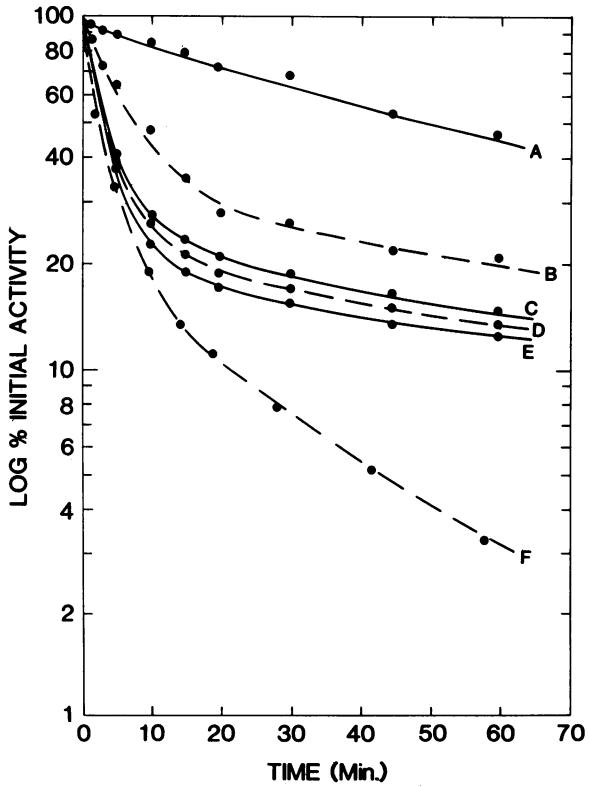

FIG. 6. Inactivation of unadenylylated GS ($E_{0.0}$) by T-β-L in the presence of enzyme effectors. (A) Ser, Gly, Ala, His, and AMP; (B) AMP; (C) Ser; (D) Gly; (E) Ala; and (F) no effectors. The incubations, inactivations, and assays were done as described in the legend to Fig. 5, using 0.5 mM T-β-L.

by methionine sulfoximine. Inactivation of *E. coli* GS by methionine sulfoximine induces a conformational change in the enzyme subunits and reduces the binding affinity for methionine sulfoximine in the remaining active subunits (11, 13, 20). This mechanism is consistent with our observation that addition of a second sample of T-β-L to partially inactivated GS did not restore the initial rate of inactivation (Fig. 4). Adenylylation also induces changes in the binding of glutamate (14) and methionine sulfoximine (20). Increasing the adenylylation state of subsp. "*tabaci*" GS slowed the rate of the inactivation. In the presence of the mixture of the effectors, fully adenylyated GS was completely protected from T-β-L and partially adenylylated GS was inactivated very slowly. Thus, the partially adenylylated GS present in subsp. "*tabaci*" during T-β-L production is less susceptible to inactivation by T-β-L than is the unadenylylated GS found in the culture before toxin production was initiated. These findings suggest that adenylylation of GS during toxin production provides partial protection of GS, and the presence of the well-established end product effectors of GS can increase this protection.

ACKNOWLEDGMENTS

This work was supported by the U.S. Department of Agriculture (competitive research grant 83-CRCR-1-1201), the University of Wisconsin, and the U.S. Department of Energy.

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