Novel Structure, Properties and Inactivation of Glutamine Synthetase Cloned from \textit{Bacteroides fragilis}

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The cloned \textit{Bacteroides fragilis} glutamine synthetase (GS) subunit produced in \textit{Escherichia coli} had the same apparent $M_r$ of approximately 75000 as the GS subunit from \textit{B. fragilis} cells. The \textit{B. fragilis} GS enzyme had an apparent $M_r$ of approximately 490000 and it is concluded that the GS is a hexamer. The cloned GS did not appear to be regulated by adenyllylation and deadenylylation and the cloned enzyme was inactivated by snake venom phosphodiesterase. The pH profiles of the cloned GS, assayed by the $\gamma$-glutamyl transferase (GGT) assay were similar for NH$_4^+$-shocked and unshocked cell extracts and an isoactivity point was not obtained from these curves. The cloned GS was subject to feedback inhibition by amino acids but not by AMP. The GGT activity of the cloned GS in NH$_4^+$-shocked and unshocked cell-free extracts was inhibited by Mg$^{2+}$. Mn$^{2+}$ stimulated the cloned GS GGT activity of NH$_4^+$-shocked cell-free extracts. Western blotting indicated that GS production was regulated by nitrogen in \textit{B. fragilis} cells but cell extracts showed no GGT activity. Cloned \textit{B. fragilis} GS produced in \textit{E. coli} was specifically and irreversibly inactivated by \textit{B. fragilis} cell extracts.

\section*{INTRODUCTION}

Glutamine synthetase (GS) (EC 6.3.1.2) plays a central role in nitrogen metabolism and the assimilation of ammonia in many prokaryotes, eukaryotes and archaebacteria. GS enzymes of eukaryotic origin are octamers (Prusiner & Stadtman, 1973) whereas the GS enzymes of eubacteria and an archaebacterium have been shown to be dodecamers with an $M_r$ of about 600000 composed of a single type of subunit whose $M_r$ falls in the range 44000–59000 (Streicher & Tyler, 1980; Bhatnagar \textit{et al.}, 1986). We recently reported that a cloned \textit{Bacteroides fragilis} GS had an apparent subunit $M_r$ of approximately 75000 which is markedly larger than other bacterial GS subunits (Southern \textit{et al.}, 1986). In view of the large $M_r$ of the \textit{B. fragilis} GS subunit we investigated the $M_r$, structure and properties of the GS enzyme.

Yamamoto \textit{et al.} (1984) investigated the pathway of ammonia assimilation in \textit{B. fragilis} and concluded that GS may not be important for ammonia incorporation into amino acids. They were unable to detect GS activity in \textit{B. fragilis} cell extracts by the $\gamma$-glutamyl transferase (GGT) assay. In contrast, we recently reported that the \textit{B. fragilis} GS cloned in \textit{Escherichia coli} is active in the GGT assay (Southern \textit{et al.}, 1986). We investigated the specific inhibition of \textit{B. fragilis} GS by \textit{B. fragilis} cell extracts and utilized the product of the cloned \textit{B. fragilis} gene to examine the presence of GS in \textit{B. fragilis} by Western blotting.

\textit{Abbreviations}: GGT, $\gamma$-glutamyl transferase; GS, glutamine synthetase; SVP, snake venom phosphodiesterase I.
Bacterial strains, plasmids, media and growth conditions. B. fragilis strain BF-1 was described by Mossie et al. (1979). Plasmid pJS139 [glnA\textsuperscript{+}], ampicillin resistant (Ap\textsuperscript{+}), is an E. coli recombinant plasmid which contains a B. fragilis BF-1 gln\textsuperscript{A} gene and enables E. coli glnA deletion mutants to utilize (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} as a sole source of nitrogen (Southern et al., 1986). E. coli YMC-10 gln\textsuperscript{A+ntrB+ntrC\textsuperscript{+}} and E. coli YMC-11 gln\textsuperscript{A+ntrB+ntrC} were described by Backman et al. (1981).

B. fragilis was grown under anaerobic conditions in a complex medium which contained (l\textsuperscript{-1}): Difco tryptic soy broth, 24 g; Difco yeast extract, 10 g; glucose, 1 g; L-cysteine hydrochloride, 0-5 g; haemin, 5 mg; and menadione, 0-5 mg (Southern et al., 1984). Growth under nitrogen-limiting conditions was achieved by incubation for 2 h following the addition of an equal volume of 0-01 m-potassium phosphate buffer pH 7-4, containing 0-15 m-NaCl and 2 g glucose l\textsuperscript{-1} to late exponential phase B. fragilis cultures growing in the complex medium. Alternatively brain heart infusion broth (Holdeman & Moore, 1972) cultures were diluted (1:40) into minimal medium (Varel & Bryant, 1974) which was nitrogen-free or contained 0-3 mM-(NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} and reincubated until the cultures reached an OD\textsubscript{560} value of 0-2.

After incubation at 37 °C the stabilizer of glutamine synthetase (GS), N-cetyl-N,N,N-trimethylammonium bromide (CTAB) (0-1 mg ml\textsuperscript{-1}, Merck), was added to the cultures and incubation was continued for 10 min. The cells were collected by centrifugation and resuspended to 1\% (v/v) of the original culture volume in extraction buffer (10 mm-imidazole/HCl pH 7-1, 100 mM-KCl) and disrupted by sonication. After centrifugation the supernatant was kept at 70 °C. The E. coli strains were grown in LB (Luria–Bertani) medium (Davis et al., 1980) or in CSH (Cold Spring Harbor) glucose minimal medium (Miller, 1972) supplemented as described in the text. Nitrogen-limiting conditions were achieved by the addition of an equal volume of nitrogen-free CSH minimal medium to late exponential CSH minimal medium cultures. After incubation CTAB was added and the cells were disrupted as described above. For ammonia-shocked cells, 15 mM-(NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} was added 5 min before the addition of CTAB.

GS assay. GS activity was assayed in crude cell extracts by the γ-glutamyl transferase (GGT) and forward transferase assays (Bender et al., 1977). Specific enzyme activity is expressed as μmol γ-glutamyl hydroxamate formed (mg protein)\textsuperscript{-1} min\textsuperscript{-1}.

Protein was determined using the dye-binding method of Bradford (1976).

Purification of GS. GS was extracted and purified from B. fragilis and E. coli YMC-11(pJS139) by differential polyethylene glycol (PEG) precipitation and gel filtration as described by Southern et al. (1986). Purified B. fragilis GS from E. coli YMC-11(pJS139) was stained with sodium phosphodiesterase and examined in an electron microscope.

M\textsubscript{r} determinations. The M\textsubscript{r} of the purified B. fragilis GS holoenzyme was determined by Sephacryl S-400 (Pharmacia) column chromatography and pore gradient electrophoresis. A Sephacryl S-400 column (10 × 400 mm) was equilibrated and eluted with column buffer (1\% w/v, KCl, 10 mM-imidazole/HCl pH 7-15) at 0-1 ml min\textsuperscript{-1} at 4 °C. Pore gradient electrophoresis was done in a 4–30\% (w/v) nondenaturing polyacrylamide gradient according to the manufacturer's instructions (Pharmacia). The electrophoresis buffer was 0-09 M-Tris/borate/EDTA (2 mM), pH 8-5. After electrophoresis the gel was divided in half and one half was stained with Coomassie brilliant blue. The other half was cut into 3 mm slices and assayed for GS activity. The M\textsubscript{r} of the GS subunits was determined by SDS-PAGE (Laemmli, 1970; O'Farrel, 1985; Southern et al., 1986).

Western blotting. Polypeptides resolved by SDS-PAGE (8\% w/v, acrylamide) were electroblotted onto nitrocellulose membranes (Towbin et al., 1979). The membranes were blocked with 10 mM-Tris/HCl pH 7-4, 0-15 mM-NaCl, 2\% (w/v) nonfat dried milk (Johnson et al., 1984) and 0-05\% (v/v) Tween 20 at room temperature for 18 h. B. fragilis GS antiserum was adsorbed with a sonicated cell extract of E. coli YMC-11 to remove non-specific E. coli antibodies. B. fragilis GS antibody binding (Southern et al., 1986) and the development of bands using a goat anti-rabbit serum conjugated to horseradish peroxidase were done as described by Rybicki & von Wechmar (1982), except that Tween 20 was used in place of NP-40.

SVP treatment of GS. Cloned GS extracts were incubated at 37 °C with 0-5 mg snake venom phosphodiesterase I (SVP) ml\textsuperscript{-1}, sampled at various time intervals and immediately frozen at −70 °C. All the samples were assayed with and without 0-25 mM-Mn\textsuperscript{2+}.

Inhibition of cloned GS by B. fragilis cell extracts. B. fragilis cells were collected by centrifugation, washed and resuspended in 0-01 mM-Tris/HCl pH 7-9, followed by disruption by sonication. After centrifugation the supernatant was added to crude samples of the cloned B. fragilis GS.

RESULTS

M\textsubscript{r} of B. fragilis GS

The cloned B. fragilis GS was shown to have an apparent subunit M\textsubscript{r} of approximately 75 000 (Southern et al., 1986). The M\textsubscript{r} of the purified B. fragilis GS holoenzyme produced by pJS139 in
the E. coli YMC-11 GlnA^-NtrB^-NtrC^- deletion strain was determined by Sephacryl S-400 column chromatography (Fig. 1) and by pore gradient electrophoresis (Fig. 2). Both methods indicated that the cloned B. fragilis GS holoenzyme had an apparent Mr of approximately 490000. The fraction from the S-400 column with an apparent Mr of approximately 490000 showed GS activity (Fig. 1). This fraction gave a single band on pore gradient electrophoresis with an apparent Mr of approximately 490000 (Fig. 2). It was not possible to demonstrate GS activity in crushed 3 mm gel slices by the GGT assay.

Electron microscopy of negatively stained, purified, cloned B. fragilis GS revealed molecules with a central hole and hexagonal shape (data not shown). These features are characteristic of GS enzymes from other bacteria (Frey et al., 1975). However, tetragonal structures, which are indicative of side views of double-layered ring structures, were not observed.

Properties of the cloned B. fragilis GS

Crude cell extracts of E. coli YMC-11(pJS139) from NH4+-shocked and unshocked cultures were assayed for GGT activity between pH 6.0 and pH 8.0 (Fig. 3). NH4+-shocking caused a reduction in GS activity. This decrease in activity was not due to an effect of pH and the pH profiles for GS activity were similar for the shocked and unshocked cell extracts. The optimum pH for GS activity for both crude extracts was between pH 6.5 and 7.0. No GS isoactivity point was demonstrated. The optimum temperature for activity of the cloned GS in the GGT assay was 45 °C. The activity of the cloned B. fragilis GS in crude extracts from E. coli YMC-11(pJS139) was confirmed by the forward transferase assay.

The effect of feedback modifiers on the cloned B. fragilis GS was determined in crude extracts prepared from E. coli YMC-11(pJS139) cells grown under nitrogen-limiting conditions (Table 1). Glutamine, which is a substrate for the GGT assay, did not inhibit the GS activity of the GS whereas the other amino acids tested and (NH4)2SO4 inhibited the GS activity to varying extents. AMP did not inhibit GS activity. L-Methionine-DL-sulphoximine, a glutamate analogue and noncompetitive inhibitor of GS in many bacteria (Meister, 1980) caused a 92% reduction in the GS activity of the GS.

The effect of Mn2+ and Mg2+ on the GGT activity of crude extracts of cloned B. fragilis GS from NH4+-shocked and unshocked E. coli YMC-11(pJS139) cells was determined (Fig. 4). The addition of 0.25 mm MCl2 to extracts from NH4+-shocked cells markedly increased the GGT activity, but had no effect on the GS activity of GS from unshocked cells. At 0.5 mm MnCl2 the GS from shocked and unshocked cells had the same GGT activity which was similar to that when no MnCl2 was added. MnCl2 concentrations >0.5 mm inhibited the GS activity of GS from shocked and unshocked cells to the same extent. The GGT activity of extracts of GS from shocked and unshocked cells was inhibited by concentrations of MgCl2 >0.5 mm (Fig. 4).

SVP digestion

Since the GS activity of cloned GS extracts from NH4+-shocked and unshocked cells was affected differently by 0.25 mm MnCl2, the effect of SVP on GS cell extracts was assayed in the presence or absence of 0.25 mm MnCl2 (Fig. 5). Treatment of cloned GS extracts from shocked and unshocked cells with SVP inhibited the GS activity in the presence or absence of 0.25 mm MnCl2. Similar results were obtained when SVP-treated extracts were assayed in the presence or absence of 10 mm-MgCl2 (data not shown). Control extracts incubated for 20 min without SVP treatment showed no increase in activity.

Detection of GS in B. fragilis cells by Western blotting

Western blotting with antiserum raised against the purified, cloned B. fragilis GS from E. coli cells showed that B. fragilis cells contained a polypeptide with the same Mr as the subunit of the purified, cloned GS (Mr, approximately 75000) (Fig. 6). Production of the GS subunit was induced by growth of B. fragilis cells in nitrogen-limited medium (Fig. 6b). SDS-PAGE fractionated proteins from E. coli YMC-10 GlnA^-NtrB^-NtrC^- and E. coli YMC-11 GlnA^-NtrB^-NtrC^- cells did not react with the B. fragilis GS antiserum (Fig. 7). The weak cross-reactivity, which is sometimes observed (Southern et al., 1986), was not detected. A strong
Fig. 1. Sephacryl S-400 column chromatography of the cloned B. fragilis GS from E. coli cells. (a) Elution profile of GS sample after precipitation by 6% (w/v) PEG. GGT activity; – A$_{280}$. (b) $M_r$ determination of GS holoenzyme. $M_r$ markers: thyroglobulin, 670000 (T); ferritin, 440000 (F); catalase, 232000 (C). The $K_m$ of the GS is indicated by the dotted lines.

Fig. 2. Pore gradient electrophoresis of the purified, cloned B. fragilis GS from E. coli cells. The gel contained a 4–30% (w/v) polyacrylamide gradient and was 0.5 mm thick. After electrophoresis as described in Methods it was stained with Coomassie brilliant blue. (a) $M_r$ determination. $M_r$ markers: thyroglobulin, 670000 (T); ferritin, 440000 (F); catalase, 232000 (C); lactic dehydrogenase, 140000 (L); bovine albumin, 67000 (A). The $R_f$ of the GS is indicated by the dotted line. (b) Stained gel. Lane 1, purified GS after S-400 column chromatography (5 µg); lane 2, $M_r$ markers.

Fig. 3. Effect of pH on GGT activity in extracts from NH$_4$-shocked (○) and unshocked (●) E. coli YMC-11(pJS139) cells. GGT activity is expressed as µmol γ-glutamyl hydroxamate formed (mg protein)$^{-1}$ min$^{-1}$. The SEM values were 5–10% of the values in the Figure.

reaction with the B. fragilis GS antiserum was detected in SDS-PAGE fractionated proteins from E. coli YMC-11(pJS139) cells. Treatment of the Western blot with antiserum to Vibrio alginolyticus GS, which cross-reacts with E. coli GS (Maharaj et al., 1986), revealed the E. coli GS subunit in SDS-PAGE fractionated proteins from E. coli YMC-10.
Table 1. Effect of feedback modifiers on cell-free activity of the cloned *B. fragilis* GS

The final concentrations of the modifiers added to the assay mixture were as shown. GGT activities are expressed as a percentage of the activity of the extract without added modifier [2.4 μmol γ-glutamyl hydroxamate formed (mg protein)⁻¹ min⁻¹]. SEM values were 5–10% of the values in the Table.

<table>
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<th>Modifier</th>
<th>Modifier concn (mM)</th>
<th>GGT activity (%)</th>
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<tbody>
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<td>L-Glutamine</td>
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<td>110</td>
</tr>
<tr>
<td>AMP</td>
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</tr>
<tr>
<td>L-Proline</td>
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</tr>
<tr>
<td>L-Isoleucine</td>
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</tr>
<tr>
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<td>82</td>
</tr>
<tr>
<td>L-Glycine</td>
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<td>L-Glutamic acid</td>
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<td>70</td>
</tr>
<tr>
<td>L-Alanine</td>
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</tr>
<tr>
<td>L-Histidine</td>
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</tr>
<tr>
<td>L-Arginine</td>
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</tr>
<tr>
<td>L-Methionine-DL-sulphoximine</td>
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</table>

Fig. 4. Effect of Mn²⁺ and Mg²⁺ on the GGT activity of crude extracts of cloned *B. fragilis* GS from NH₄⁺-shocked (○) and unshocked (●) *E. coli* YMC-11(pJS139) cells. (a) GGT activity determined in the presence of MnCl₂. (b) GGT activity determined in the presence of MgCl₂. The results are expressed as a percentage of the GGT activity in cell extracts without added MnCl₂ or MgCl₂ respectively [1.00 and 0.76 μmol γ-glutamyl hydroxamate formed (mg protein)⁻¹ min⁻¹ respectively for shocked and unshocked cells]. The SEM values were 5–10% of the values in the Figure.

Fig. 5. The effect of length of SVP treatment on GGT activity of crude extracts of cloned *B. fragilis* GS from NH₂⁺-shocked and unshocked *E. coli* YMC-11(pJS139) cells. (a) NH₂⁺-shocked extracts assayed in the presence (○) or absence (●) of MnCl₂. (b) Unshocked extracts assayed in the presence (○) or absence (●) of MnCl₂. The results are expressed as a percentage of the activity of the starting material (see Fig. 4 for absolute values). The SEM values were 5–10% of the values in the Figure.
Fig. 6. Western blots of purified, cloned GS and crude extracts from *E. coli* YMC-11(pJS139) and *B. fragilis* cells. (a) *E. coli* YMC-11(pJS139): lane 1, crude extract (5 μg protein); lane 2, purified GS (5 μg protein). (b) *B. fragilis* crude extracts from nitrogen-limited (lane 1, 50 μg protein) and nitrogen-repressed (lane 2, 50 μg protein) cultures.

**Immunoprecipitation of the cloned *B. fragilis* GS from *E. coli***

A partially purified preparation of the cloned *B. fragilis* GS from *E. coli* was assayed before and after immunoprecipitation with the antiserum. Total loss of GGT activity was observed after immunoprecipitation. The immunoprecipitate and supernatant lacking GS activity were fractionated by SDS-PAGE. The presence of the *M*. *75000* GS polypeptide was demonstrated by Western blotting in the purified GS preparation before immunoprecipitation and in the immunoprecipitate, but this band was absent in the supernatant (Fig. 8). The non-specific bands in the supernatant were due to the high concentration of antiserum proteins in the supernatant.

**GS activity in *B. fragilis* cell extracts**

Crude extracts from *B. fragilis* cells grown under conditions of limiting nitrogen, which induced the presence of GS detected by Western blotting, were assayed for GGT activity. No GGT activity was detected.

Attempts at purification of GS from *B. fragilis* cell extracts by differential PEG precipitation and gel filtration (Southern et al., 1986), which had proved successful for the purification of the cloned GS, did not yield active GS. It was not possible to purify GS from washed or osmotically shocked *B. fragilis* cells.

**Inhibition of cloned *B. fragilis* GS by *B. fragilis* cell extracts**

The effect of *B. fragilis* cell extracts on the GGT activity of cloned *B. fragilis* GS from *E. coli* and *E. coli* GS was investigated (Table 2). The GGT activity of the cloned *B. fragilis* GS from
Fig. 7. Western blot of crude extracts from *E. coli* YMC-10 GlnA⁺ NtrB⁺ NtrC⁺ (a), *E. coli* YMC-11 GlnA⁻ NtrB⁻ NtrC⁻ (b) and *E. coli* YMC-11(pJS139) (c) cells. The SDS-PAGE-fractionated proteins were transferred to nitrocellulose paper and reacted initially with antiserum prepared against purified samples of the cloned *B. fragilis* GS. This reaction only revealed the bands with apparent *Mₗ* values of approximately 75000 (A). Subsequent reaction of the blot with an antiserum prepared against purified samples of *V. alginolyticus* GS, which cross-reacts with *E. coli* GS (B), revealed the other bands (C). *E. coli* YMC-10, crude extracts from nitrogen-limited (lane 1, 30 µg protein) and -repressed (lane 2, 30 µg protein) cells. *E. coli* YMC-11, crude extracts from nitrogen-limited (lane 1, 30 µg protein) and -repressed (lane 2, 30 µg protein) cells. *E. coli* YMC-11(pJS139), crude extracts from nitrogen-limited (lane 1, 5 µg protein) and -repressed (lane 2, 5 µg protein) cells.

Fig. 8. Western blots of a partially purified preparation of the cloned *B. fragilis* GS from *E. coli* before and after immunoprecipitation with antiserum prepared against purified samples of the cloned *B. fragilis* GS. Lanes 1 and 2, active partially purified GS (1 and 2 µg protein respectively); lanes 3 and 4, supernatant after immunoprecipitation of 1 and 2 µg partially purified GS protein respectively; lanes 5 and 6, immunoprecipitates corresponding to the preparations in lanes 3 and 4 respectively.

Table 2. Inhibition of cloned *B. fragilis* GS from *E. coli* by *B. fragilis* cell extracts

GGT activities are expressed as a percentage of the activity of untreated cell extracts [2-03 and 0-85 µmol γ-glutamyl hydroxamate formed (mg protein)⁻¹ min⁻¹ for cloned GS and *E. coli* GS respectively]. SEM values were 5-10% of the values in the Table.

<table>
<thead>
<tr>
<th>GS</th>
<th>Inhibitor</th>
<th>Inhibitor protein concn (mg ml⁻¹)</th>
<th>GGT activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloned GS</td>
<td>Untreated control</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Bf undialysed*</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Bf dialysed*</td>
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<td>8</td>
</tr>
<tr>
<td></td>
<td>Bf boiled*</td>
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</tr>
<tr>
<td></td>
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<tr>
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<td>75</td>
</tr>
<tr>
<td></td>
<td>Bf dialysed*</td>
<td>10</td>
<td>84</td>
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</table>

* *B. fragilis* cell extracts.
E. coli YMC-11(pJS139) was inhibited by > 90\% by dialysed and non-dialysed crude extracts from B. fragilis cells. Boiled B. fragilis cell extracts inhibited the cloned GS by > 80\%. In contrast, GS from E. coli YMC-10 cells was not markedly affected by dialysed and non-dialysed B. fragilis cell extracts (approximately 16–25\% inhibition). B. fragilis complete medium and B. fragilis culture supernatants did not inhibit the cloned B. fragilis GS.

Inhibition of the cloned B. fragilis GS from E. coli cells by B. fragilis cell extracts was irreversible and attempts to purify the treated GS did not reactivate the GS. Previously, we reported an increase in total GS activity and an apparent recovery of 214\% of GS activity during the purification of the cloned B. fragilis GS from E. coli YMC-11(pJS139) cell extracts (Southern et al., 1986). This increase was due to the removal of an inhibitor during the purification. Addition of E. coli YMC-11 crude extracts to purified cloned B. fragilis GS caused an 80\% decrease in GGT activity.

**DISCUSSION**

All prokaryote GS enzymes studied have been shown to consist of identical subunits. The apparent GS subunit and holoenzyme $M_r$ values of approximately 75,000 and 490,000 respectively indicate that the B. fragilis GS appears to be composed of six identical subunits. The GS from the anaerobe B. fragilis differs from other prokaryote GS enzymes which normally consist of 12 subunits (Prusiner & Stadtman, 1973; Streicher & Tyler, 1980). The GS from another anaerobe, Clostridium pasteurianum, has also been shown to be different in that it appears to consist of 20 subunits (Krishnan et al., 1986).

NH$_4^+$-shocking of crude cell extracts containing the cloned B. fragilis GS resulted in a loss of GGT activity which was not due to a shift in the pH optimum and an isoactivity point could not be determined. In E. coli the optimum pH for the GGT assay is pH 6-9 for shocked preparations and 7-9 for unshocked preparations (Stadtman & Ginsburg, 1974). A NH$_4^+$-dependent increase in GGT activity and the inability to determine an isoactivity point has been reported for Rhodopseudomonas palustris (Alef & Zumft, 1981), Rhodospirillum rubrum (Falk et al., 1982), Chloroflexus aurantiacus (Kaulen & Klemme, 1983), and V. alginolyticus (Bodasing et al., 1985).

The GS enzymes from Gram-negative bacteria have been shown to be covalently modified by adenylylation, whereas there is no evidence for GS regulation by adenylylation in Gram-positive bacteria (Tronick et al., 1973; Wolhueter et al., 1973). Alteration of GS activity by SVP treatment is utilized as presumptive evidence for regulation by the adenylylation–de-adenylylation system (Stadtman et al., 1970). GGT activity of adenylylated or NH$_4^+$-shocked E. coli GS measured in the presence of 0.3 mM-Mn$^{2+}$ is unchanged after SVP treatment, whereas activity in the presence both of 0.3 mM-Mn$^{2+}$ and of 60 mM-Mg$^{2+}$ is greatly increased. There is no change in the GGT activity of GS from the Gram-positive bacteria Bacillus subtilis (Schreier et al., 1985), C. pasteurianum (Kleiner, 1979) and Clostridium acetobutylicum (Usdin et al., 1986) and the archaebacterium Methanobacterium iwanovi (Bhatnagar et al., 1986) in the presence of Mn$^{2+}$ or Mg$^{2+}$ or Mn$^{2+}$ plus Mg$^{2+}$. In contrast, SVP treatment of the cloned B. fragilis GS resulted in the inactivation of GS from NH$_4^+$-shocked and unshocked cells and Mn$^{2+}$ and Mg$^{2+}$ did not affect this inactivation by SVP. The activity of the cloned B. fragilis GS is not inhibited by AMP, whereas in other bacteria AMP acts as a feedback inhibitor of GS (Deuel & Prusiner, 1974). In most Gram-negative bacteria the GS is subjected to the adenylylation control mechanism and is less affected by feedback inhibitors than GS from Gram-positive bacteria. The cloned B. fragilis GS appears to be sensitive to feedback inhibition by amino acids. Arginine, histidine, alanine, glutamic acid and glycine inhibited GGT activity significantly (> 20\%).

The sensitivity of the cloned B. fragilis GS to SVP and the apparent lack of adenylylation in E. coli cells is interesting since E. coli is known to adenylylate other cloned GS genes from Gram-negative bacteria. The Anabaena GS cloned in E. coli is also not subject to adenylylation (Fisher et al., 1981).

In E. coli and other Gram-negative bacteria the addition of Mg$^{2+}$ to NH$_4^+$-shocked or unshocked cells increased or inhibited the GGT activity respectively. The GGT activity of the
cloned *B. fragilis* GS from NH₃⁺-shocked or unshocked cell extracts was inhibited to the same extent by Mg²⁺. It is interesting that the GS from another anaerobe, *C. acetobutylicum*, was also inhibited by Mg²⁺ (Usdin *et al.*, 1986). The marked stimulation in GGT activity of the cloned GS from NH₃⁺-shocked cells by low concentrations of Mn²⁺ (0.25 mM) appears to be a unique feature of the cloned *B. fragilis* GS. GGT activity of the cloned *B. fragilis* and the *E. coli* GS (Stadtman & Ginsburg, 1974) was inactivated by >0.5 mM-Mn²⁺.

Western blotting indicated that the *B. fragilis* *glnA* gene appeared to be cloned on pJS139 and similar GS subunits were produced in *E. coli* YMC-11(pJS139) and *B. fragilis*. Previously, we showed that GS production by the cloned *B. fragilis* *glnA* gene in *E. coli* was regulated by nitrogen. Although it was not possible to assay GS activity in *B. fragilis* cell extracts, Western blotting indicated that GS production was regulated by nitrogen.

The importance of GS for ammonia incorporation into amino acids in *B. fragilis* has been questioned (Yamamoto *et al.*, 1985). A problem in assessing the role of GS in *B. fragilis* is the inability to assay the enzyme in *B. fragilis* cell extracts. We have shown that the lack of GGT activity in *B. fragilis* cell extracts is not due to very low levels of enzyme but is the result of the inactivation of the GS by *B. fragilis* cell extracts. This inactivation appears to be relatively specific since *E. coli* GS was not inactivated to the same extent. The relatively small amount of inactivation of the *E. coli* GS by *B. fragilis* cell extract was presumably due to feedback inhibition. The inactivation of the cloned *B. fragilis* GS by *B. fragilis* cell extracts was irreversible, but is unlikely to involve an enzyme reaction as boiled cell extracts inhibited the GS.

**REFERENCES**


cloned in *Escherichia coli*. Archives of Microbiology 146, 30-34.


