Novel Structure, Properties and Inactivation of Glutamine Synthetase Cloned from *Bacteroides fragilis*

By JAMES A. SOUTHERN,¹ JOAN R. PARKER² AND
DAVID R. WOODS^{2*}

¹ State Vaccine Institute, Alexandra Road, Pinelands 7405, South Africa

² Department of Microbiology, University of Cape Town, Rondebosch 7700, South Africa

(Received 9 February 1987; revised 24 April 1987)

The cloned *Bacteroides fragilis* glutamine synthetase (GS) subunit produced in *Escherichia coli* had the same apparent M_r of approximately 75 000 as the GS subunit from *B. fragilis* cells. The *B. fragilis* GS enzyme had an apparent M_r of approximately 490 000 and it is concluded that the GS is a hexamer. The cloned GS did not appear to be regulated by adenylation and deadenylation and the cloned enzyme was inactivated by snake venom phosphodiesterase. The pH profiles of the cloned GS, assayed by the γ -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 *B. fragilis* cells but cell extracts showed no GGT activity. Cloned *B. fragilis* GS produced in *E. coli* was specifically and irreversibly inactivated by *B. fragilis* cell extracts.

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 archaeobacteria. GS enzymes of eukaryotic origin are octamers (Prusiner & Stadtman, 1973) whereas the GS enzymes of eubacteria and an archaeobacterium have been shown to be dodecamers with an M_r of about 600 000 composed of a single type of subunit whose M_r falls in the range 44 000–59 000 (Streicher & Tyler, 1980; Bhatnagar *et al.*, 1986). We recently reported that a cloned *Bacteroides fragilis* GS had an apparent subunit M_r of approximately 75 000 which is markedly larger than other bacterial GS subunits (Southern *et al.*, 1986). In view of the large M_r of the *B. fragilis* GS subunit we investigated the M_r , structure and properties of the GS enzyme.

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

Abbreviations: GGT, γ -glutamyl transferase; GS, glutamine synthetase; SVP, snake venom phosphodiesterase I.

MATERIALS AND METHODS

Bacterial strains, plasmids, media and growth conditions. *B. fragilis* strain Bf-1 was described by Mossie *et al.* (1979). Plasmid pJS139 [*glnA*⁺, ampicillin resistant (Ap^r)], is an *E. coli* recombinant plasmid which contains a *B. fragilis* Bf-1 *glnA* gene and enables *E. coli glnA* deletion mutants to utilize (NH₄)₂SO₄ as a sole source of nitrogen (Southern *et al.*, 1986). *E. coli* YMC-10 *glnA*⁺*ntrB*⁺*ntrC*⁺ and *E. coli* YMC-11 *glnA**ntrB**ntrC* were described by Backman *et al.* (1981).

B. fragilis was grown under anaerobic conditions in a complex medium which contained (l⁻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⁻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-(NH₄)₂SO₄ and reincubated until the cultures reached an OD₆₀₀ 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⁻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-(NH₄)₂SO₄ 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 μ mol γ -glutamyl hydroxamate formed (mg protein)⁻¹ min⁻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 phosphotungstate and examined in an electron microscope.

***M_r* determinations.** The *M_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 $\times$ 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⁻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_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 M-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⁻¹, sampled at various time intervals and immediately frozen at -70 °C. All the samples were assayed with and without 0.25 mM-Mn²⁺.

Inhibition of cloned GS by *B. fragilis* cell extracts. *B. fragilis* cells were collected by centrifugation, washed and resuspended in 0.01 M-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_r of *B. fragilis* GS

The cloned *B. fragilis* GS was shown to have an apparent subunit *M_r* of approximately 75000 (Southern *et al.*, 1986). The *M_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 M_r of approximately 490 000. The fraction from the S-400 column with an apparent M_r of approximately 490 000 showed GS activity (Fig. 1). This fraction gave a single band on pore gradient electrophoresis with an apparent M_r of approximately 490 000 (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 GGT activity. This decrease in activity was not due to an effect of pH and the pH profiles for GGT activity were similar for the shocked and unshocked cell extracts. The optimum pH for GGT 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 GGT activity of the GS whereas the other amino acids tested and (NH₄)₂SO₄ inhibited the GGT activity to varying extents. AMP did not inhibit GGT activity. L-Methionine-DL-sulphoximine, a glutamate analogue and noncompetitive inhibitor of GS in many bacteria (Meister, 1980) caused a 92% reduction in the GGT 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-MnCl₂ to extracts from NH₄⁺-shocked cells markedly increased the GGT activity, but had no effect on the GGT 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 GGT 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 GGT 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 GGT 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 decrease 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 M_r as the subunit of the purified, cloned GS (M_r approximately 75 000) (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. $\circ$, GGT activity; —, A_{254} . (b) M_r determination of GS holoenzyme. M_r markers: thyroglobulin, 670 000 (T); ferritin, 440 000 (F); catalase, 232 000 (C). The K_{av} of the GS is indicated by the dotted lines.

Fig. 2

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, 670 000 (T); ferritin, 440 000 (F); catalase, 232 000 (C); lactic dehydrogenase, 140 000 (L); bovine albumin, 67 000 (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

Fig. 3. Effect of pH on GGT activity in extracts from NH_4^+ -shocked ($\circ$) and unshocked ($\bullet$) *E. coli* YMC-11(pJS139) cells. GGT activity is expressed as $\mu\text{mol } \gamma\text{-glutamyl hydroxamate formed (mg protein)}^{-1} \text{min}^{-1}$. 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 [$2.4 \mu\text{mol } \gamma\text{-glutamyl hydroxamate formed (mg protein)}^{-1} \text{ min}^{-1}$]. SEM values were 5–10% of the values in the Table.

Modifier	Modifier concn (mM)	GGT activity (%)
L-Glutamine	50	110
AMP	20	104
L-Proline	50	89
L-Isoleucine	50	86
$(\text{NH}_4)_2\text{SO}_4$	50	82
L-Glycine	50	74
L-Glutamic acid	50	70
L-Alanine	50	68
L-Histidine	50	46
L-Arginine	50	38
L-Methionine-DL-sulphoximine	0.1	8

Fig. 4. Effect of Mn^{2+} and Mg^{2+} on the GGT activity of crude extracts of cloned *B. fragilis* GS from NH_4^+ -shocked (○) and unshocked (●) *E. coli* YMC-11(pJS139) cells. (a) GGT activity determined in the presence of MnCl_2 . (b) GGT activity determined in the presence of MgCl_2 . The results are expressed as a percentage of the GGT activity in cell extracts without added MnCl_2 or MgCl_2 respectively [1.00 and $0.76 \mu\text{mol } \gamma\text{-glutamyl hydroxamate formed (mg protein)}^{-1} \text{ min}^{-1}$ 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 NH_4^+ -shocked and unshocked *E. coli* YMC-11(pJS139) cells. (a) NH_4^+ -shocked extracts assayed in the presence (○) or absence (●) of MnCl_2 . (b) Unshocked extracts assayed in the presence (○) or absence (●) of MnCl_2 . 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 μ g protein); lane 2, purified GS (5 μ g protein). (b) *B. fragilis* crude extracts from nitrogen-limited (lane 1, 50 μ g protein) and nitrogen-repressed (lane 2, 50 μ g 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_r 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

Fig. 8

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 M_r values of approximately 75 000 (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 μ g protein) and -repressed (lane 2, 30 μ g protein) cells. *E. coli* YMC-11, crude extracts from nitrogen-limited (lane 1, 30 μ g protein) and -repressed (lane 2, 30 μ g protein) cells. *E. coli* YMC-11(pJS139), crude extracts from nitrogen-limited (lane 1, 5 μ g protein) and -repressed (lane 2, 5 μ g 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 μ g protein respectively); lanes 3 and 4, supernatant after immunoprecipitation of 1 and 2 μ g 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 μ mol 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.

GS	Inhibitor	Inhibitor protein concn (mg ml ⁻¹)	GGT activity (%)
Cloned GS	Untreated control	–	100
	Bf undialysed*	10	8
	Bf dialysed*	10	8
	Bf boiled*	3	17
	Complete medium	–	91
	Culture supernatant	–	95
<i>E. coli</i> GS	Untreated control	–	100
	Bf undialysed*	10	75
	Bf dialysed*	10	84

* *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 75000 and 490000 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 *Rhodospseudomonas 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-deadenylylation 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 archaeobacterium *Methanobacterium ivanovi* (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 NH_4^+ -shocked or unshocked cell extracts was inhibited to the same extent by Mg^{2+} . It is interesting that the GS from another anaerobe, *C. acetobutylicum*, was also inhibited by Mg^{2+} (Usdin *et al.*, 1986). The marked stimulation in GGT activity of the cloned GS from NH_4^+ -shocked cells by low concentrations of Mn^{2+} (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- Mn^{2+} .

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

- ALEF, K. & ZUMFT, W. G. (1981). Regulatory properties of glutamine synthetase from the nitrogen-fixing phototrophic bacterium *Rhodospseudomonas palustris*. *Zeitschrift für Naturforschung* **38c**, 784–789.
- BACKMAN, K., CHEN, Y.-M. & MAGASANIK, B. (1981). Physical and genetic characterization of the *glnA-glnG* region of the *Escherichia coli* chromosome. *Proceedings of the National Academy of Sciences of the United States of America* **78**, 3743–3747.
- BENDER, R. A., JANSSEN, K. A., RESNICK, A. D., BLUMENBERG, M., FOOR, F. & MAGASANIK, B. (1977). Biochemical parameters of glutamine synthetase from *Klebsiella aerogenes*. *Journal of Bacteriology* **129**, 1001–1009.
- BHATNAGAR, L., ZEIKUS, J. G. & AUBERT, J.-P. (1986). Purification and characterization of glutamine synthetase from the archaeobacterium *Methanobacterium ivanovi*. *Journal of Bacteriology* **165**, 638–643.
- BODASING, S. J., BRANDT, P. W., ROBB, F. T. & WOODS, D. R. (1985). Purification and regulation of glutamine synthetase in a collagenolytic *Vibrio alginolyticus* strain. *Archives of Microbiology* **140**, 369–374.
- BRADFORD, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254.
- DAVIS, R. W., BOTSTEIN, D. & ROTH, J. R. (1980). *Advanced Bacterial Genetics*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- DEUEL, T. F. & PRUSINER, S. (1974). Regulation of glutamine synthetase from *Bacillus subtilis* by divalent cations, feedback inhibitors and L-glutamine. *Journal of Biological Chemistry* **249**, 257–264.
- FALK, G., JOHANSSON, B. C. & NORLUND, S. (1982). The role of glutamine synthetase in the regulation of nitrogenase activity (switch off effect) in *Rhodospirillum rubrum*. *Archives of Microbiology* **132**, 251–253.
- FISHER, R., TULI, R. & HASELKORN, R. (1981). A cloned cyanobacterial gene for glutamine synthetase functions in *Escherichia coli*, but the enzyme is not adenylated. *Proceedings of the National Academy of Sciences of the United States of America* **78**, 3393–3397.
- FREY, T. D., EISENBERG, D. & EISERLING, F. A. (1975). Glutamine synthetase forms three- and seven-stranded cables. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 3402–3406.
- HOLDEMAN, L. V. & MOORE, W. E. C. (1972). *Anaerobe Laboratory Manual*. Blacksburg, USA: Virginia Polytechnic Institute and State University.
- JOHNSON, D. A., GAUTSCH, J. W., SPORTSMAN, J. R. & ELDER, J. H. (1984). Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. *Genetic Analytical Techniques* **1**, 3–8.
- KAULEN, H. & KLEMMER, J. H. (1983). No evidence of covalent modification of glutamine synthetase in the thermophilic phototrophic bacterium *Chloroflexus aurantiacus*. *FEMS Microbiology Letters* **20**, 75–79.
- KLEINER, D. (1979). Regulation of ammonium uptake and metabolism by nitrogen fixing bacteria. III. *Clostridium pasteurianum*. *Archives of Microbiology* **120**, 263–270.
- KRISHNAN, I. S., SINGHAL, R. K. & DUA, R. D. (1986). Purification and characterization of glutamine synthetase from *Clostridium pasteurianum*. *Biochemistry* **25**, 1589–1599.
- LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, London* **227**, 680–685.
- MANARAJ, R., ROBB, F. T. & WOODS, D. R. (1986). Temperature and oxygen regulated expression of a glutamine synthetase gene from *Vibrio alginolyticus*

- cloned in *Escherichia coli*. *Archives of Microbiology* **146**, 30–34.
- MEISTER, A. (1980). Catalytic mechanism of glutamine synthetase, overview of glutamine metabolism. In *Glutamine Metabolism, Enzymology and Regulation*, pp. 1–40. Edited by J. Mora and R. Palacios. New York: Academic Press.
- MILLER, J. H. (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- MOSSIE, K. G. M., JONES, D. T., ROBB, F. T. & WOODS, D. R. (1979). Characterization and mode of action of a bacteriocin produced by a *Bacteroides fragilis* strain. *Antimicrobial Agents and Chemotherapy* **16**, 724–730.
- O'FARREL, P. H. (1985). High resolution two-dimensional electrophoresis of proteins. *Journal of Biological Chemistry* **260**, 4007–4021.
- PRUSINER, S. & STADTMAN, E. R. (Editors) (1973). *The Enzymes of Glutamine Metabolism*. New York: Academic Press.
- RYBICKI, E. P. & VON WECHMAR, M. B. (1982). Enzyme assisted immune detection of plant virus proteins electroblotted onto nitrocellulose paper. *Journal of Virological Methods* **5**, 267–278.
- SCHREIER, H. J., FISHER, S. H. & SONENSHEIN, A. L. (1985). Regulation of expression from the *glnA* promoter of *Bacillus subtilis* requires the *glnA* gene product. *Proceedings of the National Academy of Sciences of the United States of America* **82**, 3375–3379.
- SOUTHERN, J. A., KATZ, W. & WOODS, D. R. (1984). Purification and properties of a cell-bound bacteriocin from a *Bacteroides fragilis* strain. *Antimicrobial Agents and Chemotherapy* **25**, 253–257.
- SOUTHERN, J. A., PARKER, J. R. & WOODS, D. R. (1986). Expression and purification of glutamine synthetase from *Bacteroides fragilis*. *Journal of General Microbiology* **132**, 2827–2835.
- STADTMAN, E. R. & GINSBURG, A. (1974). The glutamine synthetase of *Escherichia coli*, structure and control. In *The Enzymes*, vol. 10, pp. 755–807. Edited by P. S. Boyer. New York: Academic Press.
- STADTMAN, E. R., GINSBURG, J. E., CIARDI, J. E., YEH, J., HENNING, S. B. & SHAPIRO, B. M. (1970). Multiple molecular forms of glutamine synthetase produced by enzyme catalysed adenylation and deadenylation reactions. *Advances in Enzyme Regulation* **8**, 99–118.
- STREICHER, S. L. & TYLER, B. (1980). Purification of glutamine synthetase from a variety of bacteria. *Journal of Bacteriology* **142**, 69–78.
- TOWBIN, H., STAHELIN, T. & GORDON, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of the National Academy of Sciences of the United States of America* **76**, 4350–4354.
- TRONICK, S. R., CIARDI, J. E. & STADTMAN, E. R. (1973). Comparative biochemical and immunological studies of bacterial glutamine synthetases. *Journal of Bacteriology* **115**, 858–868.
- USDIN, K. P., ZAPPE, H., JONES, D. T. & WOODS, D. R. (1986). Cloning, expression, and purification of glutamine synthetase from *Clostridium acetobutylicum*. *Applied and Environmental Microbiology* **52**, 413–419.
- VAREL, J. M. & BRYANT, M. P. (1974). Nutritional features of *Bacteroides fragilis* subspecies *fragilis*. *Applied Microbiology* **18**, 251–257.
- WOLHEUTER, R. M., SCHUTT, H. & HOLZER, H. (1973). Regulation of glutamine synthetase *in vivo* in *E. coli*. In *The Enzymes of Glutamine Metabolism*, pp. 45–66. Edited by S. Prusiner & E. R. Stadman. New York: Academic Press.
- YAMAMOTO, I., ABE, A., SAITO, H. & ISHIMOTO, M. (1984). The pathway of ammonia assimilation in *Bacteroides fragilis*. *Journal of General and Applied Microbiology* **30**, 499–508.