Cloning of the Glutamine Synthetase Gene from Group B Streptococci

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The *glnA* gene from the human pathogen *Streptococcus agalactiae* was cloned from a genomic library prepared with the λ phage vector λ DASHII. A 4.6-kb DNA fragment of one of the recombinant phages was subcloned in pUC18. This *Escherichia coli* clone expressed a 52-kDa protein encoded by a 1,341-bp open reading frame. The nucleotide sequence of the open reading frame and the deduced amino acid sequence shared a significant degree of homology with the sequences of other glutamine synthetases (GS). The highest homology was between our deduced protein and GS of gram-positive bacteria such as *Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus*. Plasmids with the cloned streptococcal *glnA* were able to complement *E. coli glnA* mutants grown on minimal media. Rabbit antisera to streptococcal GS recombinant protein recognized not only the recombinant protein but also a similar-sized band in mutanolysin extracts of all group B streptococcal strains tested, regardless of polysaccharide type or surface protein profile. The amino acid sequence of the deduced protein had similarities to other streptococcal cell-surface-bound proteins. The possible functional role of the immunological features of streptococcal GS is discussed.

Group B streptococci (GBS) are responsible for a broad range of severe human diseases, predominantly neonatal sepsis and meningitis, with a high fatality rate. Two major classes of antigens on the cell surface of GBS have been described so far. Type-specific polysaccharide antigens impart resistance to phagocytosis and killing. Several other proteins such as c, R, and X proteins (8), and C5a peptidase are known to be present on the surface of the organism (6). The only protein proven to play a functional role in virulence is the c protein. The c protein, however, is not a single protein but a complex of distinct antigens (α , β , γ , and δ) (4). To date, two genes that encode proteins of the c protein family from GBS serotype Ib have been characterized. One gene encodes the β antigen that binds immunoglobulin A (IgA), and the other gene codes for α antigen (14, 16, 20). In the genomic library of GBS 78-471 (II/ α + β), we identified a clone expressing a protein which reacted with various sera directed against GBS. Unexpectedly, this protein was found to be highly homologous to glutamine synthetase (GS) and complemented *Escherichia coli* mutants with a defective GS.

MATERIALS AND METHODS

Bacterial strains and vectors. GBS 78-471 (II/ α + β) was the source of genomic DNA. Among the GBS strains used for Western immunoblot (WB) and Southern hybridization studies were 71-098 (Ia without c or R proteins), 75-341 (III without c or R proteins), 79-313 (Ib/ α + β), 80-236 (Ia/ α only), 81-316 (II/ α + β), 85-440 (Ia/ α + β), 89-022 (Ib/ α + β), and HIA-00008 (V/R1 + R4). Strains of other polysaccharide/c or R protein serotypes were also tested. All GBS strains, as well as the negative controls, *Staphylococcus aureus* and *Enterococcus faecium*, were clinical isolates from our laboratory collection. All had been stored at -70°C and were grown in Todd-Hewitt broth for use.

E. coli JM109 was used as a recipient for cloning and vectors pUC18, M13mp18 and M13mp19; *E. coli* strain LE392 was a recipient for construction of the phage library. The phage cloning vector λ DASHII was obtained from Stratagene, La Jolla, Calif. The packaging lambda DNA system was purchased from Promega, Madison, Wis. An *E. coli* GS-deficient strain M5004 [F^{-} *glnA3* *trpA9825* λ -IN (*rmD-rmE*)] was provided by Barbara J. Bachmann (*E. coli* Genetic Stock Center, Department of Biology, Yale University, New Haven, Conn.). Vector pUC18 without an insert was used as a negative control. The media and growth conditions for cultivating the *E. coli* bacterial strains and phages were described previously (6, 14).

DNA manipulations. GBS chromosomal DNA was isolated after lysis with mutanolysin (1 mg/ml) and lysozyme (10 mg/ml) for 30 min at 37°C following treatment with proteinase K (10 $\mu\text{g/ml}$; Bethesda Research Laboratories)—sodium dodecyl sulfate (10 mg/ml)—100 mM EDTA for 1 h at 60°C . After two phenol-chloroform extractions and ethanol precipitation, the DNA was resuspended in distilled water. Plasmids were isolated by the alkali lysis procedure described previously (19). DNA isolation and cloning procedures were performed as described by Maniatis et al. (19). DNA sequencing was carried out in both orientations after cloning inserts into M13 vectors or pUC18. Sequencing was performed by Microchemical Facility Laboratories (University of Minnesota Health Sciences Center) with a 370A DNA sequencer (Applied Biosystems, Foster City, Calif.) or manually using a *Taq* polymerase sequencing kit from Boehringer GmbH, Mannheim, Germany. Agarose gel electrophoresis and DNA-DNA hybridization conditions were as described previously (28). DNA sequences and translated protein sequences were compared to GenBank, EMBL, EMBL Update, Brookhaven Protein, SWISS-PROT, and Kabat-pro databases, which were available to the computer facilities of the Department of Microbiology and Immunology, University of Oklahoma, Oklahoma City, and Department of Biochemistry, Trinity College, Dublin, Ireland. Sequence analyses were performed with the PC GENE, SEQAID, PUSTELL, and DNA Strider 1.0 programs. PCR employing *Taq* polymerase were carried out on a thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.).

DNA primers were prepared by Microchemical Facility Laboratories (University of Minnesota Health Sciences Center). These primers, 5' GCGTCTCGCG AAGCT and 5' CGCCTGTGACGGTA, were designed from the DNA sequence of the *arp* gene of group A streptococci (11). PCR-generated DNA fragments were isolated from agarose, labeled with digoxigenin, and used as a probe for screening the phage library. These primers were chosen because our primary goal originally was to look for genes specifying IgA binding proteins, such as β protein, in GBS. The group A streptococcal IgA receptor gene sequence (*arp*) was used as a tool for primary screening by PCR.

Southern hybridization. Chromosomal DNA was prepared as previously described by us and others (7, 28), and 10 μg was digested with either 20 U of *Kpn*I (Boehringer Mannheim) or 20 U of *Sst*I (Bethesda Research Laboratories) followed by 20 U of *Sph*I (Bethesda Research Laboratories) as specified by the manufacturer.

Plasmid extraction and purification, and probe labeling. The plasmid used for the probe was extracted and purified by a modification of methods described previously (2). The random-primer labeling of the pR512 plasmid with digoxigenin-dUTP was performed with the Genius DNA labeling and detection kit as specified by the manufacturer (Boehringer Mannheim). After purification with phenol-chloroform and precipitation with absolute alcohol, the plasmid was dissolved in 10 mM Tris–1 mM EDTA with RNase (10 $\mu\text{g/ml}$).

The digested DNA was transferred to a nylon membrane (Boehringer Mannheim) by standard Southern transfer procedures and hybridized to the labeled pR512 probe.

Purification of GS and antiserum production. The *E. coli* transformant containing the pR512 plasmid was grown overnight in 2YT medium with ampicillin (20 $\mu\text{g/ml}$). The cells were centrifuged, washed with cold phosphate-buffered saline (PBS), incubated in cold 100 mM Tris–10% sucrose buffer (pH 8.0) with lysozyme (1 mg/ml), and then sonicated with a Sonifier cell disrupter (Ultrason-

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ics, Inc., Plainsview, N.Y.). The sonicate supernatant was concentrated, filtered, and run over a Sephacryl HR-200 (Pharmacia, Piscataway, N.J.) gel filtration column (0.05 M Tris plus 0.15 M NaCl elution buffer [pH 8.0]). Fractions were read at 280 nm and tested by WB with a rabbit serum with immunologic recognition of the cloned gene product. Fractions with strong GS reactivity by WB were pooled, concentrated, and stored at -20°C . Antiserum to semipurified GS was produced by immunizing a rabbit with this material and Ribi adjuvant system (Ribi ImmunoChem Research, Inc., Hamilton, Mont.) as specified in the product protocol. Antiserum with an excellent anti-GS titer was absorbed twice with a sonicate of the recipient strain to remove naturally occurring anti-*E. coli* antibodies. This absorbed serum was used for WB.

The antisera to alpha (α) or beta (β) proteins were prepared in rabbits by immunization with formalin-killed whole bacterial strains. The antiserum used for the experiment shown in Fig. 2 was produced with strain 80-426 (NT/ α only), subsequently absorbed with cells of a type V/ β , R1 GBS strain, and *E. coli* JM109.

WB assay. Antigen preparations of GBS strains for polyacrylamide gel electrophoresis (PAGE) and WB were prepared by digesting log phase cells with mutanolysin (3,000 U/cells from 500 ml, 1 h at 37°C and pH 7.2) followed by disruption with a Mickle apparatus (H. Mickle, Gomshall, England) for 20 min at 4°C . Antigen preparations of the recipient and transformant pR512 were obtained by sonication. Antigen supernatants were boiled with 3% sodium dodecyl sulfate sample buffer–10% mercaptoethanol. PAGE on a 7.5% nondenaturing gel, transfer, and WB were done as described previously (10, 18) with the following modifications. PAGE and transfer were done at 4°C , transfer was onto Immobilon-P membrane (Millipore Corp., Bedford, Mass.), the primary antibody was rabbit anti-GS or anti- α serum, and the secondary antibody was alkaline phosphatase-labeled goat F(ab')₂ anti-rabbit IgG F(ab')₂ (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.).

Complementation studies. GS-deficient *E. coli* M5004 was used as a recipient for transformation with pR512. Transformants were selected from the minimal agar plates with glutamine (50 $\mu\text{g}/\text{ml}$) and ampicillin (50 $\mu\text{g}/\text{ml}$) and then plated on minimal agar with or without glutamine (50 $\mu\text{g}/\text{ml}$) (19).

Nucleotide sequence accession number. The GenBank accession number of the primary nucleotide sequence of the enzyme described above is U61271.

RESULTS

Cloning of the *glnA* gene fragment and its expression in *E. coli*. GBS 78-471 was the source of the DNA used in cloning. Chromosomal DNA from that strain was digested with *Sau*3a and ligated with *Bam*HI DNA fragments of λ DASHII phage vector. Approximately 1,000 recombinant phage clones were screened with a PCR-generated gene probe that was obtained after amplification of the GBS DNA by using *arp* primers.

FIG. 1. Restriction enzyme analysis and Southern hybridization of DNA from the transformant pR512 and from GBS strains of various serotypes double digested with *Sst*I and *Sph*I and hybridized with probe pR512: pR512 (lane 2), 78-471 (donor strain, II/ α + β) (lane 3), 71-098 (Ia/no c or R proteins) (lane 4), 79-313 (Ib/ α + β) (lane 5), 81-316 (II/ α + β) (lane 6), 75-341 (III/no c or R proteins) (lane 7), and H1A-00008 (V/R1 + R4 strain) (lane 8). Lane 1 contains DNA/*Hind*III-digested molecular size markers.

FIG. 2. WB of GS from the transformant pR512 and α protein from GBS strain 80-426 (NT/ α only) probed with rabbit anti- α serum. Pools from Sephacryl HR-S200 column purification of GS from transformant pR512 (lanes 1 to 3) and culture supernatant of strain 80-426 (lane 4) are shown. The positions of molecular mass standards are on the left.

Phage lysates from hybridization positive plaques were screened by WB analysis to detect the ability to react with sera against α and β antigens. One recombinant phage (λ 5) from plaques that expressed immunologic reactivity was selected and used for further experiments. In WB analysis, a major protein (52 kDa) that reacted with sera against both α and β antigens was detected (data not shown). The streptococcal DNA insert in this recombinant phage contained three *Eco*RI fragments of 4.6, 4.0, and 2.5 kb; each was separately cloned into pUC18. Strains carrying recombinant plasmids (pR512, pR517, and pR518) were analyzed for the expression of α and β antigens of c protein. One fragment (4.6 kb) expressed the reactive antigens. *E. coli* strains with this insert cloned in pUC18 in both orientations (pR512 and pR513) produced a protein that reacted with anti-c antibodies and monospecific α and β antisera, which was puzzling.

To confirm that pR512 and pR513 carried a streptococcal DNA insert which was identical to that in the GBS chromosome, chromosomal DNA from strain 78-471 was digested with

FIG. 3. Restriction map of plasmid pR512 and its derivatives and the sequencing strategy. Plasmids pR21, pS98, pX35, and pX24 are derivatives of pR512 cloned into pUC18 by using the restriction sites indicated on the map.

FIG. 4. Nucleotide sequence and deduced amino acid sequence of the *glnA* gene from *Streptococcus agalactiae* 78-471. Putative promoter regions and ribosome-binding sites are indicated in boldface type and underlined. The ATG start codon and TAA stop codon are indicated in boldface type. The ATP binding region and adenylation region of GS are indicated in boldface type and underlined. The poly(T) termination region is underlined. The open reading frames are in capital letters.

restriction enzymes which cut the DNA within the insert (*Sst*I, *Kpn*I, and *Sph*I) and hybridized to the pR512 probe. With *Kpn*I digestion alone, the donor strain 78-471 and strain 89-022 hybridized at approximately 2.1 kb while the negative control recipient strain, *E. coli* JM109, did not hybridize (results not shown). As shown in Fig. 1, with *Sst*I and *Sph*I double digestion, a hybridizing fragment at approximately 3 kb was seen with digests of all the GBS strains tested (lanes 5 to 10), irrespective of the polysaccharide serotype or surface protein profile. The molecular sizes of the hybridizing fragments corresponded to the sizes of the restriction fragments from pR512, confirming that the clone was not an aberrant product of ligation of unlinked DNA fragments.

Analysis of the recombinant protein. Extracts from *E. coli* pR512 had 5- to 10-times higher expression than did those from plasmid pR513. A protein with the same molecular mass (52 kDa) as in the phage extract was detected after WB analysis (Fig. 2). As seen in this figure, the Sephacryl HR-5200-purified GS pools in lanes 1 to 3 had primarily bands of 52 kDa, recognized by the anti- α serum. Several weaker bands repre-

sented proteins with higher molecular mass (from 95 to 146 kDa) were also observed and were probably aggregates of the 52-kDa protein. Lane 4 contains a typical ladder effect of the α protein antigen. In contrast to these findings, clones representing the α and β proteins were not identified from the gene library.

The subclones that were described also produced the 146-kDa band, but weakly (not shown). This form converted to the 52-kDa form with mercaptoethanol reduction. When the 146-kDa band was cut from a PAGE gel, electroeluted, and reelectrophoresed, both the 146- and 52-kDa bands were observed.

PAGE-purified protein was extracted from both the 146- and 52-kDa bands and subjected to N-terminal sequence analysis, which made it possible to identify the first 25 amino acids. The sequence obtained in all the analyses was TITAEDIRRE VKEKNVTLRLMFTD. Plasmid pR512 was subjected to restriction endonuclease analysis, and different fragments were subcloned into pUC18. The restriction maps of the plasmid and derivatives obtained are presented in Fig. 3. Only two of the subclones, pR31 (4.0-kb fragment from *Sst*I to *Eco*RI) and

Protein			
M6	LPSTGETAN	PFFTAALTMATAGVAADV	KRKEE
GLNA	LPSTLENAL	KALEDEVVKAALGTHIYNFLD	KRIE
Bac	LPYTGVAEN	LVLEIMGLLGLIGTSFIAM	KRRKS
Pr. A	LPETGEENP	LIGTTVFGGLSLALGAALLAG	RRREL
BCA	LPATGENAT	PFFNVAALTIISVGLLSV	SKKKE
	LPXTGX	MEMBRANE	CHARGED
	CONSENSUS	SPANNING	TAIL
	REGION	REGION	

FIG. 5. Comparison of the C-terminal regions of the different surface-expressed proteins from gram-positive bacteria relative to GBS GS, M6, M protein gene from *S. pyogenes* (15); GLNA, GS from GBS described here; Bac, IgA receptor β gene from GBS (14, 16); Pr.A, protein A from *S. aureus* (27); BCA, c protein α antigen gene (20).

pSS8 (2.4-kb fragment from *SstI* to *SphI*), retained the immunological features of the 52-kDa protein. It appeared that the gene of interest was located between the *SstI* and *SphI* restriction sites. That portion of pR512 was subjected to DNA sequencing after subcloning into M13 phage vectors in both orientations. The DNA fragments from *SphI* to *XbaI*, from *XbaI* to *KpnI*, from *KpnI* to *SstI*, and from *SstI* to *EcoRI* were sequenced in both orientations.

DNA sequence analysis. Sequence analysis revealed an open reading frame which encoded a protein of 447 amino acids with molecular weight of 51,184 (Fig. 4). The predicted amino acid sequence corresponded well to that of the N-terminal amino acid sequence analysis of the 52-kDa protein band eluted from an SDS-containing gel. A potential ribosome-binding site, GG AGAC, and a putative -10 promoter sequence, AAATAT, were identified 43 bp upstream of an ATG codon. A possible -35 sequence, TTTCACG, was located 18 bp upstream from the -10 region. The open reading frame started with an ATG codon and stopped with a TAA codon, which repeated three times in a stretch of 20 nucleotides. Immediately downstream from the third stop codon were two inverted repeats. These repeats formed a potential hairpin sufficient to terminate transcription and were followed by a stretch of seven thymidines, typical of all known *E. coli* transcription terminators (21). There were eight repeat regions in the sequence, five inverted and three direct repeats. Interestingly, three of the inverted repeat regions were palindromes. The entire sequence was AT rich, which is typical for streptococcal genes; the overall codon usage in the sequence was characterized by a strong preference for T (42.4%) or A (32%) in the third position.

Analysis of the deduced amino acid sequence revealed that the N terminus of the protein had a 38-residue hydrophobic sequence with characteristics typical of a signal peptide. A potential cleavage site between Ala and Thr was in agreement with the -3 , -1 rule of von Heijne (30). At the C terminus of the protein, the amino acid sequence was similar to that of other cell surface proteins of gram-positive bacteria (9). This sequence included a charged hydrophilic tail, a membrane-spanning region, and the consensus sequence that is also represented in the surface proteins of many gram-positive bacteria (Fig. 5). Computer analysis with the TopPred II software package (5, 12) demonstrated that the putative GS protein had one distinct membrane-spanning region between amino acids 344 and 364. In contrast to other surface proteins, the C-terminal region of this protein was not highly proline rich. Only eight prolines were present in the 90-residue sequence. Prediction of the protein secondary structure by the method of Garnier revealed that 49.8% of the protein was α helical, including a 70-residue α -helical N terminus.

TABLE 1. Comparison of the deduced amino acid sequence of the GBS protein with GSs from different bacterial species following a computer search of GenBank database facilities

Bacterium	Score	Percentage	
		Identities	Positives
<i>Bacillus subtilis</i>	603	60	80
<i>Bacillus cereus</i>	594	58	77
<i>Clostridium acetobutylicum</i>	528	53	74
<i>Lactobacillus delbrueckii</i>	525	55	72
<i>Thermotoga maritima</i>	518	53	71
<i>Pyrococcus woesei</i>	424	47	64
<i>Methanococcus voltae</i>	505	51	72
<i>Sulfolobus solfataricus</i>	353	48	67
<i>Escherichia coli</i>	196	39	51

Homology search. Comparison of the open reading frame sequence with the Genbank database gave unexpected results. The DNA sequence and the protein sequence were both highly homologous to those of various GSs. The highest homology score of the deduced protein was for homology to GS of *Bacillus subtilis*, where 60% of their amino acids were identical and 80% were considered to be similar (positive). Homology of the GBS gene to other *glnA* genes was about 50%, but several blocks of the sequence were up to 70% identical to the sequence of the *glnA* gene from *Bacillus subtilis*. The similarity of the streptococcal protein to GS from *E. coli* was significantly lower. The deduced amino acid sequences showed less than 40% identities (Table 1). This finding led us to the conclusion that the cloned gene from group B streptococci was that for a streptococcal GS. There was no homology to published sequences of streptococcal α , β , or IgA binding genes.

Expression of GS in clinical GBS isolates. By using rabbit antiserum produced to semipurified GS, WB analysis (Fig. 6) revealed a band of approximately 52 kDa in the transformant pR512 (lane 6) and in all GBS strains tested (lanes 3 to 5, 7 and 8). This protein was the size of the deduced GS. In reduced antigen preparations, the GS band appeared to have a slightly lower molecular mass (approximately 48 kDa). This GS band

FIG. 6. WB analysis of semipurified GS from the transformant pR512 and GS from crude mutanolysin extracts from five GBS strains of various serotypes. The arrow points to the GS band present in GBS 80-236 (Ia/ α only) (lane 3), 75-341 (III/no c or R proteins) (lane 4), and 85-440 (Ia/ α + β) (lane 5), and GS semipurified from pR512 (lane 6), donor strain 78-471 (II/ α + β) (lane 7), and 71-098 (Ia/no c or R proteins) (lane 8). Negative controls were the recipient strain, *E. coli* JM109 (lane 1), and *Enterococcus faecium* (lane 2). The positions of the molecular mass markers are shown on the left. Rabbit anti-GS was the primary antibody.

FIG. 7. Transformation of the *glnA* *E. coli* mutant with plasmid pR512. Sections 1 to 7 represent different *E. coli* transformants; section C contains control *E. coli* 5004 carrying pUC18. (A) Minimal agar with glutamine. (B) minimal agar without glutamine.

was more intense in some GBS strains than in others (lanes 4, 5, and 7) but was present in all strains regardless of polysaccharide serotype or surface protein (α , β , or R) composition. It was found also in GBS strains without any of these proteins. Additional evidence for the ubiquity of GS in GBS was the fact that antibody to GS was detected in rabbit polyclonal antisera to α and β proteins, produced with a variety of GBS serotypes (results not shown). In contrast, *Enterococcus faecium* (lane 2) was not blotted by anti-GS. The recipient *E. coli* strain (lane 1) and *S. aureus* (results not shown) had a faint band of a higher molecular weight. Antigen preparations from other GBS strains also were recognized by the anti-GS serum (data not shown).

The lower-molecular-weight bands observed in the lanes containing group B streptococcal extracts probably represent degradation products of GS produced during the mutanolysin extraction followed by vigorous sonication. These were not seen from *E. coli*, since those cells required brief sonication to release GS and the *E. coli* GS was column purified.

Biological test for GS activity. To prove that the GBS gene fragment (*glnA*) did in fact encode GS, an *E. coli* GS-deficient strain, M5004, was transformed with plasmid pR512 and its derivatives. Plasmid-carrying *E. coli* transformants could grow without glutamine, providing direct evidence for expression of the streptococcal enzyme in *E. coli* (Fig. 7). Plasmids pR512, pR513, pR31, and pSS8, the shortest derivative of pR512 which contained the entire GS gene, complemented the GS defect in strain M5004. Other derivatives failed to complement M5004 for GS activity.

DISCUSSION

GS is one of the key bacterial enzymes responsible for ammonia assimilation, catalyzing the only known biosynthetic route for the synthesis of glutamine (1). Since GS is one of the oldest functional existing genes known, homology between different GS genes from prokaryotes and eukaryotes is often used for molecular evolutionary studies (17, 26). Synthesis of GS in *E. coli* is regulated both at the transcriptional level and by means of posttranslational modification of the enzyme. At least six different genes are involved in such a global system of regulation (1). The purified GS is a dodecamer of identical 55,000-Da subunits, and each subunit can be inhibited separately by adenylation of tyrosine residues. The adenylated enzyme is completely inactive in the presence of 60 mM Mg^{2+} . Several lines of evidence supported the conclusion that we have cloned a gene encoding group B streptococcal GS. This fact was strongly supported by a significant degree of homology of the cloned DNA sequence and the deduced amino acid

TABLE 2. Adenylation sites of different GSs

Bacterium	Position in GS sequence	Sequence
GBS	376	NIYAM
<i>Bacillus cereus</i>	373	NIYVM
<i>Bacillus subtilis</i>	373	NIYVM
<i>Clostridium acetobutylicum</i>	392	NLYEAV
<i>Escherichia coli</i>	393	NLYDLP

sequence to the sequences of various GSs. The size of the deduced protein (52 kDa) and the size of the open reading frame (1.5 kb) were also similar to those of other known GSs. Comparison of adenylation and catalytic domains between different GSs with our deduced streptococcal GS protein sequence demonstrated a significant degree of homology. An ATP binding site at positions 238 to 243, an adenylation site at positions 374 to 378, and a conserved N-terminal tetrapeptide DGSS at positions 54 to 57 of the streptococcal GS protein (Table 2) were very similar to those in GSs from other bacteria. The presence of these three conserved motifs in the GS amino acid sequence indicated that this protein most probably belonged to the family of class I GSs (17).

Analysis of the DNA sequence upstream of the *glnA* open reading frame revealed the presence of *glnR*, a repressor gene which is present in other gram-positive bacteria. An open reading frame 360 bp upstream of the structural gene of *glnA* was preceded by a typical ribosome-binding site GGAGG and a -10 promoter region ATATAT and was related to repressor genes from GS operons in *B. subtilis*, *B. cereus*, and *S. aureus* (13, 23, 29). Complementation studies to correct the glutamine deficiency of an *E. coli* strain confirmed that the streptococcal *glnA* gene functioned in *E. coli*.

Surprisingly, the group B streptococcal GS possessed several features of cell surface proteins of bacteria. This conclusion was reached on the basis of sequence analysis of the N and C termini of the deduced GS protein, which were similar to those of the other streptococcal surface proteins (3, 24). The 52-kDa protein extracted from the polyacrylamide gel had an N-terminal amino acid sequence that completely matched the deduced sequence of streptococcal GS. However, we could not find significant sequence homology between the streptococcal *glnA* and genes coding for α and β proteins that have been sequenced by others (14, 16, 20).

Not surprising were the initial observations of immunologic recognition of the cloned protein by anti- α and anti- β sera. Based on the WB findings that mutanolysin extractions of GBS of various serotypes, with or without α , β , or R protein expression, were blotted by the anti-GS serum, we concluded that the earlier findings were due to the unknown presence of anti-GS antibodies in these diverse polyclonal sera. Since the GS may be cell surface associated, the GBS whole bacterial cells, expressing the α or β antigens, may have exposed the GS antigen to immune processing cells. It is also likely that the enzyme is highly immunogenic. The WB results were substantiated by the hybridization assays, confirming that the *glnA* gene was present in all of the GBS strains studied.

We do not have direct evidence of the presence of streptococcal GS on the bacterial surface; however, several functional enzymes on the cell surface of other pathogenic bacteria have been described. Pancholi and Fischetti (25) demonstrated a major surface protein from group A streptococci that has glyceraldehyde-3-phosphate-dehydrogenase enzymatic activity and binding capacity for a variety of proteins. Munson and Sasaki

(22) showed that glycerophosphodiester phosphodiesterase from *Haemophilus influenzae* is responsible for Ig D binding (Ig D receptor). The relationship of GS to cell wall synthesis by *S. aureus* was demonstrated in a recent study (13), whereby a mutation in the GS operon (*femC*) reduced the resistance of staphylococci to methicillin. The possible role of GS in enhancing the pathogenicity and virulence of GBS in human infections requires further study, but one can speculate that because of its apparent cellular location, it may mimic other bacterial enzymes contributing to these processes. It may also function in cell wall synthesis vis à vis its catalyzing the biosynthetic route for glutamine synthesis, necessary for the cross-linking of peptidoglycan. Further study is necessary to define its niche in the biology of GBS.

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