INFECTION AND IMMUNITY, Apr. 1978, p. 228-234
0019-9567/78/0020-0228$02.00/0

Vol. 20, No. 1
*Printed in U.S.A.*

# Degradation of the Polysaccharide Component of Gonococcal Lipopolysaccharide by Gonococcal and Meningococcal Sonic Extracts

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Received for publication 21 December 1977

An extract made from the supernatant of *Neisseria gonorrhoeae* $Gc_2$ strain 1291 degraded the $Gc_2$ polysaccharide antigen. Chemical analysis of this polysaccharide indicated it contains glucose, galactose, glucosamine, galactosamine, glucosamine-6-phosphate, heptose, 2-keto-3-deoxyoctonate, and ethanolamine and is the polysaccharide component of gonococcal lipopolysaccharide. Degradation of the polysaccharide by sonic extracts resulted either in complete loss of antigenicity and immunogenicity or in partial degradation to subunits that could inhibit the $Gc_2$-specific hemagglutination inhibition. The factors responsible for degradation were destroyed by heating at 100°C for 5 min or by Pronase digestion, but were unaffected by ribonuclease, deoxyribonuclease, $Mg^{2+}$, $Ca^{2+}$, or ethylenediaminetetraacetic acid. The process was pH dependent, with optimal activity occurring at pH 7. Sonic extract supernatants from group B and C meningococcal strains contained degrading properties, whereas similar extracts produced from *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae,* and *Streptococcus pneumoniae* type II failed to degrade the $Gc_2$ polysaccharide.

A family of acidic polysaccharides, designated Gc antigens, have been isolated from the lipopolysaccharide (LPS) of *Neisseria gonorrhoeae* and used to serogroup gonococci (1, 2). These polysaccharides were purified by diethylaminoethyl chromatography from the alkaline digest of the phenol-water extract. In an effort to increase the yield of these polysaccharides, gonococcal broth supernatants have been studied as a source of crude antigen by methods similar to those used with a number of other bacterial polysaccharides (3, 5, 9). Gc antigens have been shown to be stable over the pH and temperature ranges encountered in broth cultures (1, 2), and significant amounts would be expected to be present in broth culture filtrates. However, studies of 100-fold concentrates of large volumes of gonococcal culture supernatants have failed to reveal these antigens. This suggests that biodegradation may be occurring and that the gonococcus might produce enzymes capable of digesting the polysaccharide component of its own LPS. In this paper, studies will be presented to support this concept.

## MATERIALS AND METHODS

**Organisms.** *N. gonorrhoeae* used in this study were obtained from our own collection. The prototype strain for the gonococcal $Gc_2$ serogroup, strain 1291, was used as a source of $Gc_2$ antigen and the $Gc_2$ antigen-degrading extracts. Strains of *N. meningitidis* group B were obtained from Harry Feldman of the State University of New York in Syracuse. *N. meningitidis* group A, C, and X, *Streptococcus pneumoniae* type III, *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus,* and *Salmonella typhosa* strains used in this study were selected from our own collection. All organisms were stored at −80°C in defibrinated rabbit blood.

**Antisera and antigens.** Antiserum to the $Gc_2$ serogroup was produced in rabbits by whole-organism immunization and made specific for the $Gc_2$ serogroup by absorption as previously described (1, 2). Pneumococcal polysaccharide type III was produced by the method of Kabat and Mayer (9), Vi antigen from *Salmonella typhosa* was produced by the method of Wong and Feeley (15), and meningococcal A, C, and X polysaccharides were produced by the method of Apicella and Robinson (3).

**Production of Gc antigen-degrading extracts.** Extracts for the study of antigen degradation were produced from *N. gonorrhoeae* cultured overnight on chocolate plates under an atmosphere containing 5% $CO_2$ at 35°C. Organisms were scraped from the plates with a glass slide and suspended in 2 ml of phosphate-buffered saline (PBS), pH 7.0. Approximately 1 g (wet weight) of organisms was used for each extract. This mixture was subjected to sonic disruption at 50 W for 8 min at 4°C using a microtip probe (Branson Sonic Power Co., Plainview, Long Island, N.Y.). The sonically treated organisms were centrifuged at 20,000 ×

*g* for 20 min. The supernatant was decanted and used as the antigen-degrading extract. The sediment was discarded. Extracts were also made from group B and C meningococci and from non-neisserial strains in an identical fashion, except that overnight growth was accomplished on tryptose blood agar base (Difco Laboratories, Detroit, Mich.).

To test for antigen-degrading activity, the sonic extract supernatants were diluted 1:10 with PBS and mixed with an equal volume of $Gc_2$ polysaccharide at 1,000 μg/ml in PBS. After incubation, the sonic extract-polysaccharide mixture was placed in a boiling-water bath for 5 min to stop the degradation, then tested for $Gc_2$ polysaccharide by immunodiffusion analysis and hemagglutination inhibition (HAI). Controls consisted of incubation of polysaccharide with a sonic extract supernatant that had previously been boiled for 5 min. These were set up in parallel with unboiled sonic extracts and studied immunologically in the same manner.

**Immunological methods.** Immunodiffusion analysis was done by the method of Ouchterlony in 1.5% Noble agar in 0.1 M barbital (pH 8.3) (11). Hemagglutination and HAI determinations were performed in microtiter as previously described (1, 2). Analysis of the immunogenicity of the $Gc_2$ polysaccharide was done using a modification of the Jerne plaque assay technique (4) in BALB/c mice. Sheep erythrocytes used in these experiments were coated with 125 μg of $Gc_2$ polysaccharide per ml, and guinea pig serum was used as the source of complement (Grand Island Biological Co., Grand Island, N.Y).

**Enzyme methods.** Pronase (Calbiochem, San Diego, Calif.) digestions were performed in PBS at enzyme concentrations of 10 μg/ml. Ribonuclease and deoxyribonuclease (Worthington Biochemicals, Freehold, N.J.) digestions were performed in PBS at enzyme concentrations of 10 μg/ml. Proteolytic and nucleolytic enzyme digestions were performed at 37°C overnight.

**Chemical analysis.** Hydrolysis of $Gc_2$ polysaccharide for hexose, heptose, hexosamine, and 2-keto-3-deoxyoctonate analyses was performed under a wide range of conditions of hydrogen ion concentration, time, and temperature. The optimal conditions for hexose and heptose analysis by gas-liquid chromatography were 6 h of hydrolysis at 100°C in 1 N HCl; for hexosamine analysis, 100°C for 5 h in 3 N HCl; and for 2-keto-3-deoxyoctonate, 15 min at 100°C in 0.025 N $H_2SO_4$. Hydrolysates for hexose, heptose, and hexosamine analysis were flash evaporated in a rotary evaporator (Fisher Scientific, Rochester, N.Y.), reconstituted in distilled water, transferred quantitatively to 5-ml Reacti-vials (Pierce Chemical Corp., Rockford, Ill.), and lyophilized. Hydrolysates for hexose and heptose analysis were passed over Rexyn I-300 (H-OH) (Fisher Scientific) before lyophilization to remove amino sugars.

Quantitative and qualitative analyses of hexoses and heptoses were done using a Perkin-Elmer 990 gas chromatograph with a flame ionization detector. Chromatography was done on 10% SE-30 on 100–120 Gas-Chrom Q (Applied Sciences, State College, Pa.) in a 6-foot (ca. 1.83-m) glass column. An internal standard of α-mannoheptitol (Sigma Chemical, St. Louis, Mo.) was incorporated into each sample prior to hydrolysis for gas-liquid chromatography. Silylation of the 2- to 4-mg samples was achieved using 0.2 ml of Tri-Sil (Pierce Chemical Corp.). Injection volumes were 1.0 μl. Chromatographic conditions included programmed temperature increases from 160 to 240°C at 4°C per min after a 4-min initial period at 160°C. The carrier gas was nitrogen at 45 ml/min. Hexose, deoxyhexose, and pentose standards were obtained from Applied Sciences. Glucoheptose, obtained from Supelco, Bellefonte, Pa., was used as the heptose standard. In addition to gas-liquid chromatography, quantitative analysis for heptose was also done using the cysteine-hydrochloride method as modified by Osborn (10). Quantitative and qualitative analysis for hexosamines was achieved by ion-exchange chromatography using a Beckman 120C amino acid analyzer. Hydrolysates were reconstituted in pH 2.2 citrate buffer (Pierce Chemical Corp.). Approximately 0.1 to 0.2 mg of sample in 100 μl of buffer was applied to AA-15 resin in a column (60 by 1 cm). Buffer programming included 40 min of 0.24 M citrate buffer $Na^+$ (pH 3.49), 30 min of 0.44 M citrate $Na^+$ (pH 4.25), and 210 min of 1.04 M citrate $Na^+$ (pH 6.25). Buffer flow rate was 19.6 ml/min. Glucosamine, galactosamine, and glucosamine-6-phosphate standards were obtained from Schwarz/Mann, Orangeburg, N.Y.

2-Keto-3-deoxyoctonate analysis was performed colorimetrically by a modification of the method of Osborn, using 2-keto-3-deoxyoctonate (Sigma) as the standard (10). Ethanolamine content was determined by gas-liquid chromatography using conditions similar to those for hexoses, except the temperature program range was from 110 to 140°C. Ethanolamine-hydrochloride standard was obtained from Sigma.

Amino acid content of the $Gc_2$ polysaccharide was performed after hydrolysis in vacuo at 110°C in 6 N HCl for 48 h. Samples were flash evaporated, and analysis was performed on the Beckman 120C amino acid analyzer by the method of Spackman et al. (13). Analysis of the $Gc_2$ polysaccharide of strain 1291 for nitrogen and phosphorous was performed by Galbraith Analytical Laboratories, Knoxville, Tenn.

**Acrylamide gel electrophoresis.** Acrylamide gel electrophoresis was performed by the method of Hilborn and Anastassiadis (7) in 6% acrylamide tube gels (6.0 by 0.5 cm) in 0.05 M formate (pH 11.5) at 4 mA per gel for 3 h. Gels were stained with 0.2% alcian blue in 15% acetic acid. Gels were sliced into 2-mm segments for antigen elution into 0.2 M acetate (pH 4.0) buffer. The eluates were tested for the presence of antigen by both immunodiffusion and HAI analysis.

**Molecular sieve experiments.** Molecular sieve chromatography was performed over columns (30 by 0.9 cm) containing either P-10 or P-30 (Biorad Corp., Rockville Centre, N.Y) in PBS. Ultrafiltration of digested and control polysaccharide was performed using PM-10 and XM-50 membranes in a 25-cm concentrator (Amicon Corp., Lexington, Mass.).

## RESULTS

**Chemical analysis of the $Gc_2$ polysaccharide.** Table 1 demonstrates the results of chemical analyses of the $Gc_2$ polysaccharide of strain

TABLE 1. *Chemical analysis of strain 1291 $Gc_2$ antigen*

| Component | mg/100 mg of antigen | μmol/100 mg of antigen |
|---|---|---|
| Glucose | 10.7 | 66.1 |
| Galactose | 20.6 | 127.2 |
| Glucosamine | 21.2 | 131.7 |
| Galactosamine | 4.8 | 29.8 |
| Glucosamine-6-phosphate | 7.6 | 31.5 |
| Heptose[a] | 6.4 | 33.3 |
| 2-Keto-3-deoxyoctonate[a] | 8.9 | 36.9 |
| Ethanolamine | 6.0 | 139.5 |
| Nitrogen | 1.80 | |
| Phosphorus | 3.20 | |
| Amino acids | <1.0 | |

[a] Determined colorimetrically.

1291. Approximately 86% of the dry weight of the polysaccharide could be accounted for by these methods. Analysis of the gas-liquid chromatograms failed to reveal the presence of any pentoses, methyl pentoses, or deoxy hexoses. The glucose and galactose peaks were substantiated by co-chromatography with appropriate standards. For each micromole of heptose, there were approximately two of glucose, four each of galactose, ethanolamine, and glucosamine, and one each of galactosamine, glucosamine-6-phosphate, and 2-keto-3-deoxyoctonate. Phosphorus analysis indicated that a substantial portion of the sugars in the polysaccharide were phosphorylated prior to acid hydrolysis. Amino acid analysis revealed peaks compatible with alanine, glycine, and lysine, but the total content of amino acids relative to the dry weight of the polysaccharide was in all instances less than 1%.

**Kinetic studies of $Gc_2$ polysaccharide degradation.** Immunodiffusion analysis of the results of 37°C incubation at 6 and 24 h of the $Gc_2$ polysaccharide with the sonic extract supernatant of strain 1291 can be seen in Fig. 1. The loss in antigenicity appears to be progressive. At 6 h, the degraded polysaccharide showed a faint line of partial identity with its control. At 24 h, there was complete loss of precipitin line in the sonic extract supernatant-treated polysaccharide, while the control precipitin line remained intact.

To study the kinetics of degradation of the polysaccharide, HAI analysis of strain 1291 sonic extract supernatant and $Gc_2$ polysaccharide was serially performed over 24 h at 37°C incubation. The results of a typical study can be seen in Table 2. After 1 h of incubation, the HAI titer of the sonic extract supernatant-treated polysaccharide dropped 4-fold, and by 3 h it had fallen 16-fold. Under the same conditions, the inhibition of the boiled sonic extract supernatant and $Gc_2$ polysaccharide remained unchanged over 24 h.

In several instances, sonic extract degradation of the polysaccharide resulted in a 8- to 16-fold increase in the $Gc_2$ HAI titer over the 24-h period despite progressive loss of the precipitin line in immunodiffusion. Studies with these digests demonstrated that the control polysaccharides were retained by both PM-10 and XM-50 membranes, while the majority of the degraded polysaccharide could be detected by HAI in the eluate that passed through the PM-10 membrane. These studies suggest that a substantial reduction in the size of these polysaccharides had occurred in these digests but that these subunits were still antigenic. Absorption of $Gc_2$ antiserum with these digests resulted in an antiserum that could be used to establish an HAI system still capable of detecting control

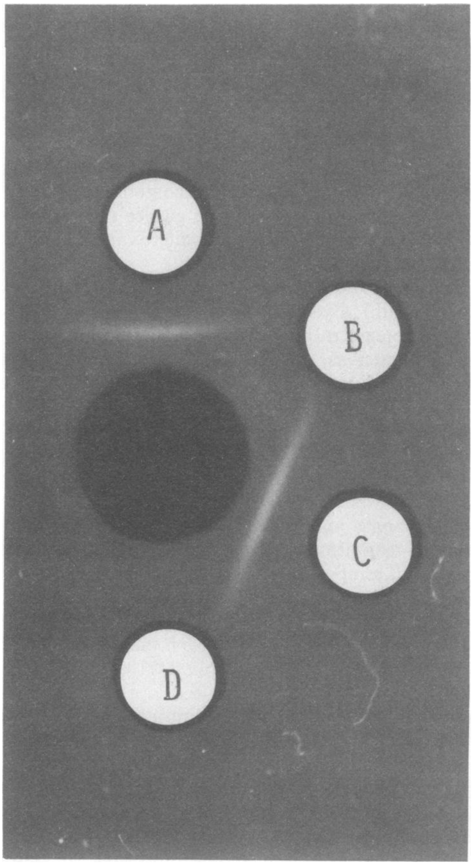


FIG. 1. *Immunodiffusion study demonstrating degradation of $Gc_2$ polysaccharide from strain 1291 at 6 and 24 h. Wells A and C contain boiled sonic extract-$Gc_2$ polysaccharide at 6 and 24 h. Wells B and D contain sonic extract-$Gc_2$ polysaccharide at 6 and 24 h. The concentration of the polysaccharide in each well is 500 μg/ml. The center well contains $Gc_2$-specific antiserum B-111.*

TABLE 2. *Degradation of $Gc_2$ polysaccharide over 24 h at 37°C*

| Time of incubation (h) | $Gc_2$ HAI antigen concn (μg/ml) | |
|---|---|---|
| | Control[a] | Experimental[b] |
| 0 | 31 | 31 |
| 1 | 31 | 125 |
| 3 | 31 | 250 |
| 6 | 31 | 500 |
| 24 | 31 | 500 |

[a] Sonic extract supernatant boiled for 5 min prior to incubation with 500 μg of $Gc_2$ polysaccharide per ml.

[b] Sonic extract supernatant plus 500 μg of $Gc_2$ polysaccharide per ml.

polysaccharide in the range of 31 to 63 μg/ml but failing to be inhibited by the absorbing digest in polysaccharide concentrations as high as 500 μg/ml. It would appear that the subunits of the $Gc_2$ antigen produced by these digestions retained a part of the $Gc_2$ polysaccharide specificity.

**Effect of proteolytic enzymes, divalent cations, and pH on antigen-degrading activity.** Treatment of the strain 1291 sonic extract supernatant with Pronase prior to $Gc_2$ polysaccharide incubation completely destroyed the ability of the supernatant to degrade $Gc_2$ polysaccharide. Treatment with ribonuclease and deoxyribonuclease had no effect on polysaccharide-degrading activity. Incorporation of $CaCl_2$ and $MgCl_2$ 0.01 and 0.001 M solutions in 0.1 M tris(hydroxymethyl)aminomethane-sodium chloride (pH 7.0) failed to enhance or inhibit antigen-degrading activity as measured by $Gc_2$-specific HAI analysis. Ethylenediaminetetraacetic acid at 0.01 and 0.001 M, incorporated into the tris(hydroxymethyl)aminomethane-sodium chloride buffer, also failed to inhibit or enhance activity.

To determine the optimal pH for antigen-degrading activity, sonic extracts were prepared in a series of buffers from pH 4.5 to pH 10 and incubated with $Gc_2$ polysaccharide for 24 h. Controls were tested at each pH level. The results of HAI analysis can be seen in Table 3. No activity was present below pH 5 or at pH 9 or above. The optimal pH value was 7.

The possibility that the loss in $Gc_2$ polysaccharide antigenicity was due to protein binding to the polysaccharide-occluding antigen sites was studied. Treatments designed to destroy any potential protein binders, while releasing the polysaccharide intact, were employed. Sonic extract-$Gc_2$ polysaccharide mixtures, which had been previously incubated and shown to contain no polysaccharide by immunodiffusion, were subjected to Pronase digestion overnight or to incubation at 37°C in 0.1 N NaOH. Neither treatment resulted in return of $Gc_2$ antigen in HAI or immunodiffusion. Sonic extract controls studied under the same conditions demonstrated that the $Gc_2$ antigen was unaltered by similar proteolytic enzyme or alkali treatment.

The $Gc_2$ antigen-degrading effect of the sonic extract supernatants was labile at 4°C. Activity was present for approximately 72 h after the extract was produced. Addition of glutathione or mercaptoethanol had no detectable stabilizing effect. Antigen-degrading activity was present in the resulting precipitate after the sonic extract supernatant was made up to 50% saturation with $NH_4SO_4$. Activity remained in this precipitate when stored at 4°C for up to 3 months. Conjugation of the strain 1291 sonic extract supernatant to cyanogen-bromide-activated Sepharose 4B resulted in retention of $Gc_2$ polysaccharide-degrading activity and stabilization of this activity for up to 14 days. Figure 2 shows the results in acrylamide gel electrophoresis of the treatment of $Gc_2$ polysaccharide with sonic extract supernatant linked to Sepharose 4B. A single alcian blue-staining band is present in the gel, containing polysaccharide treated with the control (boiled) Sepharose-sonic extract. Elution of 2-mm segments of acrylamide demonstrated by both immunodiffusion and HAI that the region of gel associated with this band contained the $Gc_2$ polysaccharide. After degradation of the $Gc_2$ polysaccharide by treatment with the active (unboiled) Sepharose-sonic extract, no band was seen. Gel elution studies failed to detect $Gc_2$ polysaccharide by immunodiffusion or HAI.

**Effect of antigen degradation on immunogenicity of the $Gc_2$ polysaccharide.** To study the effect of degradation of the polysaccharide on immunogenicity, BALB/c mice were immunized with $Gc_2$ polysaccharide degraded by

TABLE 3. *$Gc_2$-specific HAI analysis to determine optimal pH of degrading activity after 24 h at 37°C*

| pH | $Gc_2$ HAI antigen concn (μg/ml) | |
|---|---|---|
| | Control[a] | Experimental[b] |
| 4.5 | 31 | 31 |
| 5.0 | 31 | 31 |
| 6.0 | 31 | 125 |
| 7.0 | 31 | NI[c] |
| 8.0 | 31 | 125 |
| 9.0 | 31 | 31 |
| 10.0 | 31 | 31 |

[a] Boiled $Gc_2$ sonic extract supernatant and 500 μg of $Gc_2$ polysaccharide per ml.

[b] $Gc_2$ sonic extract supernatant and 500 μg of $Gc_2$ polysaccharide per ml.

[c] NI, No inhibition at 500 μg of $Gc_2$ polysaccharide per ml.

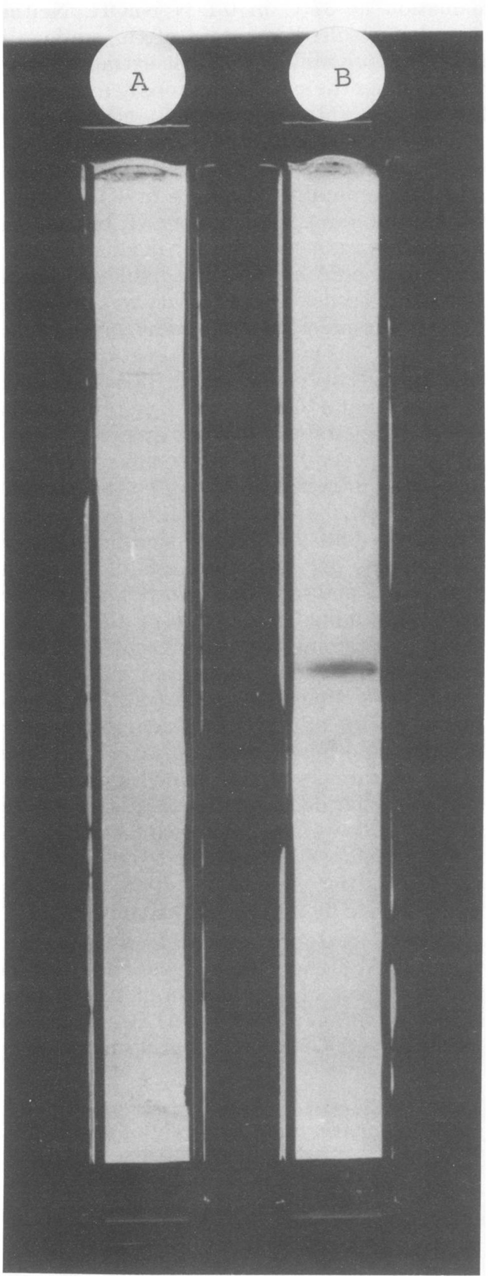


FIG. 2. *Acrylamide gel electrophoresis of* $Gc_2$ *polysaccharide after treatment with (A) Sepharose-4B-linked sonic extract and (B) Sepharose-4B-linked sonic extract that had previously been boiled. Electrophoresis time was 3 h at 4°C.*

Sepharose 4B-linked sonic extract supernatant. The $Gc_2$ polysaccharide was incubated with the bound sonic extract for 24 h at 37°C. The Sepharose beads were separated from the supernatant by centrifugation, and the supernatant was tested for antigenicity and then used as immunogen in the BALB/c mice. Control $Gc_2$ immunogens were prepared by incubating $Gc_2$ polysaccharide with Sepharose-linked sonic extract supernatant that had previously been boiled for 5 min. Table 4 demonstrates that immunization of BALB/c mice with degraded $Gc_2$ polysaccharide resulted in a 10-fold decrease in plaque-forming cells. The immunogenicity of the $Gc_2$ polysaccharide, as well as its antigenicity, was degraded by treatment with the strain 1291 sonic extract supernatant.

**Effects of sonic extracts of other microbial species on $Gc_2$ polysaccharide degradation.** Sonic extract supernatants were made to *E. coli, Klebsiella, N. meningitidis* B and C, *Streptococcus pneumoniae* type III, and *Staphylococcus aureus* strains. Only the *N. meningitidis* B and C produced sonic extracts that demonstrated the ability to degrade $Gc_2$ polysaccharide. Studies with 10 $Gc_2$ strains showed that 9 had antigen-degrading activity in their sonic extract supernatants.

**Effect of strain 1291 sonic extract supernatant on various microbial polysaccharides.** Group A, $C_{1+}$, $C_{1-}$ and X meningococcal capsular polysaccharides, pneumococcal type III polysaccharide, and Vi antigen from *Salmonella typhosa* were treated with the strain 1291 sonic extract supernatant at 37°C for 24 and 48 h. Immunodiffusion analysis of the respective mixtures failed to demonstrate any loss in antigenicity of these polysaccharides when compared with control incubations.

## DISCUSSION

Chemical analyses of the $Gc_2$ antigen of strain 1291 indicate that it is the polysaccharide component of gonococcal LPS. Wiseman and Caird (14) demonstrated similar carbohydrate components in 38 gonococcal LPS specimens analyzed. Pentoses were not found in these preparations, and rhamnose was found only in LPS isolated from $T_3$, $T_4$, and $T_5$ colonial forms. Perry and co-workers (12) also had similar chemical results, but found xylose and fucose as well as rhamnose in LPS from some $T_1$ forms. In addition, this group identified ethanolamine in the core portion of the polysaccharide component of gonococcal LPS. Previous immunological studies (1, 2) with the Gc polysaccharides have indicated that they contain common core determinants as well as serogroup-specific antigens. The combined chemical and immunological data now support the hypothesis that the Gc antigens are the O-specific side chains and LPS core antigens of the gonococcus.

TABLE 4. *Effect of degradation on immunogenicity of strain 1291 Gc polysaccharide*

| Antigen dose | Treatment | Immunodiffusion | HAI inhibition (μg/ml) | No. of animals | PFC response[a] |
|---|---|---|---|---|---|
| 100 μg of $Gc_2$ antigen of strain 1291 | Heat-inactivated sonic extract bound to Sepharose 4B | + | 31.25 | 5 | $26.4 \times 10^3$ ($12 \times 10^3$–$43 \times 10^3$) |
| 100 μg of $Gc_2$ antigen of strain 1291 | Sonic extract bound to Sepharose 4B | − | NI[b] | 5 | $2.3 \times 10^3$ (0–$5.8 \times 10^3$) |

[a] Plaque-forming cells (PFC) in BALB/c mouse spleens.
[b] NI, No inhibition at 500 μg/ml.

The results of this paper have also shown that $Gc_2$ gonococci produce substances that are capable of degrading the polysaccharide components of their own endotoxins. Kinetic studies indicate that the process of degradation is progressive, resulting either in complete loss of antigenicity in both immunodiffusion and HAI or in the release of smaller subunits that are antigenic in HAI but do not precipitate with antibody in immunodiffusion systems. Absorption studies indicate that these subunits retain only a portion of the determinants of the original polysaccharide. The substances responsible for this degradation are protein in nature, as demonstrated by their susceptibility to Pronase digestion. Due to the chemical complexity of the $Gc_2$ polysaccharide and the progressive nature of the degradation process, more than one type of glycolytic enzyme may be involved in the process. The degradation to subunits suggests that depolymerization or debranching may be at least one part of the process. The sonic extract is a crude mixture, and, until the activity can be stabilized and purified and the products of degradation clearly identified, any considerations on the mechanisms of degradation must be speculative. It is of interest that *N. meningitidis* group B and C strains also produced extracts capable of degrading the $Gc_2$ polysaccharide. Studies with gonococcal sonic extracts from other Gc serogroup strains are still in progress, but it appears that some heterologous degradation of Gc polysaccharides does occur. The identification of endogenous enzymes capable of degrading the gonococcal cell wall components is not unique. Hebeler and Young (6) demonstrated that the autolysis of gonococci is associated with the action of endogenously produced *N*-acetylmuramylalanine amidase against the peptidoglycan layer of the cell wall.

At present, the biological significance of these enzymatic processes is unknown. It is interesting, however, to speculate on the potential advantages such enzyme systems could confer on the survival and propagation of the organism in the infected host. If the organism has the capability of degrading major antigen components of its cell wall, recognition of these constituents by the host's immune system could be significantly impaired. In addition, if, during the process of degradation, non-immunogenic hapten-like subunits are released into the milieu, neutralization of protective antibodies could occur and reduce both complement-mediated and opsonic-mediated killing of organisms. Further studies are necessary for substantiation of these speculations.


## ACKNOWLEDGMENTS

This study was supported by Public Health Service grants 2-RO1-AI-10236 and 2-RO1-AI-11873 from the National Institute of Allergy and Infectious Diseases. J.F.B. was the recipient of Public Health Service postdoctoral fellowship 5-F32-AI-05182, and M.A.A. was supported under Public Health Service research career development award KO4-AI-70747, both from the National Institute of Allergy and Infectious Diseases.

Appreciation for expert technical assistance is expressed to Maureen Mills, Debra Flierl, and Phyllis Rosenberg.


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