

Chemical and Biological Characterization of a Gonococcal Growth Inhibitor Produced by *Staphylococcus haemolyticus* Isolated from Urogenital Flora

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The purified antigonococcal substance produced by *Staphylococcus haemolyticus* no. 7 has shown a broad antigonococcal spectrum and a narrow antibacterial spectrum. The inhibitor produced in vitro was also active in the guinea pig subcutaneous chamber. The inhibitor has shown hemolytic activity; the human, horse, and mouse erythrocytes were the most susceptible. Hemolytic and antigonococcal activities were inhibited in the presence of phosphatidylcholine. The amino acid composition of the antigonococcal substance was characterized by the absence of proline, tyrosine, histidine, cysteine, and tryptophan. The molecular weight was found to be 2,565, and the major isoelectric points were 4.8 and 4.9 in the presence of 8 M urea and 4.6 without urea. The inhibitor has some properties similar to those of the delta toxin of *Staphylococcus aureus*, although the two substances are different based mainly on their chemical characteristics. Also an antiserum directed against the gonococcal inhibitor did not give a precipitation line with the delta toxin, indicating that the two substances are antigenically unrelated.

It has been suggested that resistance to gonorrhea might be mediated by bacterial interference (11). Among the aerobic organisms present in the vaginal or cervical secretions, coagulase-negative staphylococci were the ones that most commonly interfered with the in vitro growth of *Neisseria gonorrhoeae* (5, 12, 23). Urethral colonization with *Neisseria meningitidis* (26) and *Staphylococcus epidermidis* (13) has been associated with the failure of men to become infected despite repeated exposures to women with gonococcal cervicitis. Saigh et al. (23) have also presented evidence that lactobacilli can produce a protective effect against gonococcal infection in humans. Ingraham et al. (11) and Lafond et al. (16, 17) were successful in obtaining soluble antigonococcal activity in a supernatant obtained by centrifuging frozen and thawed solid or semi-solid agar medium which had supported the growth of the staphylococci. The inhibitory activity detected by Lafond et al. (17) was differentiated into two types, bacteriostatic and bactericidal.

Beaudet et al. (2) have recently described the production and purification of the bactericidal substance produced by one of the active urogenital staphylococci (no. 7). The inhibitor was a lipoprotein or a lipid-associated protein in which the protein component was the active part. The protein component was a low-molecular-weight peptide which in the absence of a dissociating agent is present as large-molecular-weight complexes or aggregates. Bisailon et al. (J. G. Bisailon, R. Beaudet, and M. Sylvestre, unpublished data) have identified the coagulase-negative staphylococci as *Staphylococcus haemolyticus*.

The present work was carried out to further study the biological and chemical properties of the purified bactericidal substance. This substance was shown to have some properties similar to those of the delta toxin of *Staphylococcus aureus*.

MATERIALS AND METHODS

Bacterial strains. *S. haemolyticus* no. 7 was isolated from urogenital flora (5, 17) and was the producing strain selected for the purification of the inhibitor (2). The reference target strain was *N. gonorrhoeae* G-10. The *N. gonorrhoeae* strains and the aerobic and anaerobic bacterial strains have been previously described (4, 18). The gonococcal strains were laboratory strains or clinical specimens including T₁, different auxotypes, and penicillinase-producing strains. *S. aureus* NCTC 9393 and NCTC 9715 (National Collection of Type Cultures, London, England) are reference strains for the production of delta toxin. All these strains were kept lyophilized or as frozen suspensions at -80°C.

Culture media and conditions. The solid medium used for cultivation of the gonococcal target strains was GC agar base (GIBCO Diagnostics, Madison, Wis.) enriched with 1% (vol/vol) CVA (GIBCO) and 1.5% (vol/vol) lysed horse blood. These strains were grown in a 5% CO₂ atmosphere at 70% relative humidity and 37°C. The aerobic bacterial strains were grown on brain heart infusion agar plates (Difco Laboratories, Detroit, Mich.) under aerobic conditions at 37°C. The anaerobic bacterial strains were grown on pre-reduced brain heart infusion agar plates in an anaerobic jar at 37°C.

The production of delta toxin by *S. aureus* NCTC 9393 and NCTC 9715 was carried out on semi-solid brain heart infusion medium by the method used for the production of the gonococcal inhibitor (2). The presence of the delta toxin in the crude supernatant was determined by the presence of hemolytic activity at the characteristic electrophoretic mobility on agarose as described by Ali and Haque (1). No hemolytic activity was detected at the characteristic mobility of alpha and beta toxin.

Production and purification of the gonococcal inhibitor. The production and purification of the gonococcal inhibitor were carried out by methods previously described (2). The production was carried out on semisolid brain heart infusion

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TABLE 1. Antibacterial spectrum of the purified inhibitor produced by *S. haemolyticus* no. 7

Bacteria	Zone of inhibition (mm) ^a
Inhibited^b	
<i>N. gonorrhoeae</i>	
G-1 (NR)	2
G-5 (ND)	2
G-7 (Pro ⁻)	3.5
G-9 (Arg ⁻)	5
G-10 (Arg ⁻)	4
G-11 (Arg ⁻)	1
G-12 (Pro ⁻)	0
G-14 (NR)	3
G-17 (Pro ⁻)	2.5
G-20 (Arg ⁻)	2
18621 (ND)	0
PR77117 ^c (ND)	3
PR77112 ^c (ND)	3
9(T ₁) (ND)	4
27628 (NR)	2
27629 (Thi ⁻)	0
27630 (Pro ⁻ Hyx ⁻)	2.5
27631 (Pro ⁻ Met ⁻ Thy ⁻)	1
27632 (Arg ⁻ Met ⁻)	3.5
27633 (Arg ⁻ Hyx ⁻ Ura ⁻)	3
<i>N. meningitidis</i> Slaterus, serogroup Y	2.5
<i>Corynebacterium</i> 15927	1
Noninhibited	
<i>Staphylococcus epidermidis</i> ATCC 17917	
<i>Staphylococcus aureus</i> ATCC 6340	
<i>Staphylococcus coagulase</i> (-) 71	
<i>Staphylococcus coagulase</i> (-) 23	
<i>Streptococcus faecalis</i> ATCC 29200	
<i>Streptococcus</i> group D 12 and 70	
<i>Escherichia coli</i> 9980	
<i>Branhamella catarrhalis</i> 8176	
<i>Eubacterium lentum</i> E-28	
<i>Diplococcus pneumoniae</i> ATCC 6301	
<i>Peptococcus asaccharolyticus</i> B-1	
<i>Peptococcus magnus</i> Pc-8	
<i>Bacteroides vulgatus</i> B-4	
<i>Streptococcus morbillorum</i> Sc-13	
<i>Bacteroides ovatus</i> B-24	
<i>Peptococcus prevotii</i> Pb-8A	
<i>Propionibacterium jensenii</i> Pr-0	
<i>Veillonella parvula</i> V-12A	

^a *N. gonorrhoeae* strains were tested with the inhibitor at 55 AU per ml, and the other strains were tested at 200 AU per ml.

^b Letters within parentheses represent auxanographic groups: NR, not requiring; Pro⁻, proline; Arg⁻, arginine; Thi⁻, thiamine; Hyx⁻, hypoxanthine; Met⁻, methionine; Thy⁻, thiamine pyrophosphate; Ura⁻, uracil; ND, not determined.

^c PR, Pencillin resistant.

medium which had supported a 24-h growth of staphylococcus no. 7 strain at 37°C. Quantitative determination of the antigonococcal activity has already been described (16). The reciprocal of the dilution of a preparation yielding a 50% increase in absorbance of a gonococcal liquid culture with respect to the control (containing no inhibitor) was taken to be the activity in arbitrary units (AU) per milliliter.

Antibacterial spectrum. The antibacterial spectrum of the purified inhibitor was carried out on the solid growth medium used to cultivate the target strains as mentioned above. Each plate was seeded with 10⁶ to 10⁷ CFU from a standardized suspension of a fresh culture of the tested target organism. A well (diameter, 8 mm) in the solid medium was filled with 70 µl of the active preparation. The purified gonococcal inhibitor was used at a concentration of 55 AU

per ml when tested against *N. gonorrhoeae* strains and at 200 AU per ml against other bacterial species. The plates were incubated at 37°C for 24 h, and the distance between the well and the bacterial growth was determined.

In vivo assay. The in vivo inhibitory activity of the purified substance was determined with the guinea pig model of gonococcal infection as described by Kraus et al. (14). Four perforated plastic tubes (Dynatech Laboratories, Inc., Alexandria, Va.) were implanted into the subcutaneous areas of each guinea pig (weight 350 to 400 g each) included in the experiment. Two weeks later the sterility of these chambers was verified. The sterile chambers were injected with suspensions of *N. gonorrhoeae* varying from 1 × 10³ to 5 × 10⁴ CFU/0.1 ml, and the purified antigonococcal substance (25 AU per 0.1 ml) in phosphate-buffered saline (PBS) was then injected into the chambers. Samples of fluid were taken from the chambers immediately after these injections and again 24 h later to determine the viable counts.

The following *N. gonorrhoeae* strains were used: G-10, G-11, 9, and 188. The last two strains were colonial type T₁. *N. gonorrhoeae* G-10 and G-11 were adapted, by three successive transfers in guinea pig subcutaneous chambers (21).

Amino acid analysis. Amino acid analysis was performed on a Beckman 119 CL amino acid analyzer, using norleucine internal standard (10 nmol per sample). Peaks were integrated by a Spectra-Physics computing integrator. Samples were hydrolyzed in sealed evacuated tubes with 6 N HCl for 24, 48, and 72 h at 110°C. Tryptophan was determined after hydrolysis on a sealed evacuated tube with 4 N methanesulfonic acid containing 0.2% 3-(2-aminoethyl indole) for 24 h at 115°C. Methionine and cysteine were determined on a sample oxidized with performic acid. Nearest-integer values for serine and threonine were obtained by extrapolation to zero time, whereas those for valine and leucine were taken as values obtained after 72 h of hydrolysis.

Isoelectric focusing. Isoelectric focusing in 7% polyacrylamide gels with or without the presence of 8 M urea was carried out by the procedure of Wrigley (28). Before staining, ampholytes were removed from the gels by repeated washing in 5% trichloroacetic acid. Gels were stained for proteins with 0.25% Coomassie blue in 50% methanol-5% acetic acid and destained in 7.5% acetic acid-5% methanol. pH gradient was determined with a pI marker protein kit (United States Biochemical Corp., Cleveland, Ohio).

Hemolytic activity. A modification of the method of Bernheimer and Schwartz (3) was used for the determination of hemolytic activity. Erythrocyte cells washed three times in 0.05 M PBS (pH 7.0) were adjusted to a concentration that gave an optical density of 0.500 at 545 nm when mixed with an equal volume of 0.05% saponin. The reaction mixture contained 1 ml of a two-fold serial dilution of the sample to be tested in PBS, to which was added 1 ml of the standardized erythrocytes suspension. The mixture was incubated at 37°C for 1 h and centrifuged for 10 min in a clinical centrifuge (model IEC 428; International Equipment Co., Div. of Damon Corp., Needham Heights, Mass.). The absorbance of each supernatant was determined at 545 nm. A hemolytic unit was defined as the reciprocal of the dilution of the sample that caused a 50% lysis as compared with the maximal hemolysis produced by 0.05% saponin. Rabbit erythrocytes were generally used, with the exception of the hemolytic spectrum experiment in which human, horse, mouse, bovine, and sheep erythrocytes were also utilized.

Inhibition of the hemolytic and antigonococcal activities by phosphatidylcholine. Samples of the purified inhibitor containing 40 AU per ml and 20 hemolytic units per ml and

TABLE 2. In vivo activity of the purified inhibitor in the guinea pig subcutaneous chamber model

Time after injection (h)	Substance injected into chamber	CFU of <i>N. gonorrhoeae</i> per ml of chamber fluid				
		G-10		G-11 adapted	Type T ₁ ^a	
		Laboratory	Adapted ^b		9	188
0	PBS	2.3 × 10 ^{4b}	2.8 × 10 ³	4.5 × 10 ⁴	1.2 × 10 ⁴	1.9 × 10 ³
	Inhibitor (25 AU)	1.0 × 10 ⁴	2.0 × 10 ³	3.6 × 10 ⁴	1.7 × 10 ⁴	4.0 × 10 ³
24	PBS	1.2 × 10 ⁴	1.0 × 10 ⁷	2.7 × 10 ⁶	1.2 × 10 ⁵	1.3 × 10 ⁵
	Inhibitor (25 AU)	10	2.7 × 10 ²	4.0 × 10 ³	5.0 × 10 ³	2.4 × 10 ⁴

^a Kellogg colony type.

^b All adapted strains were transferred three times in guinea pig subcutaneous chambers.

various amounts of phosphatidylcholine (type V-E from egg yolk; Sigma Chemical Co., St. Louis, Mo.) were incubated at room temperature for 30 min. The hemolytic and antigonococcal activities were then evaluated as previously described.

Cells in tissue culture. The effect of the purified inhibitor on cells in tissue culture was determined with the established HeLa and WI 38 cell lines. They were maintained in medium 199 containing penicillin and streptomycin (25). Monolayers of the cells were utilized when they were ca. 75 to 80% confluent on cover slips placed in wells of tissue culture plates. Cover slips were then removed from the wells and repeatedly washed with antibiotic-free medium and overlaid with 2 ml of different concentrations of the purified inhibitor in PBS. Incubation in a 5% CO₂ atmosphere at 37°C proceeded for 10 min, after which the cover slips were vigorously washed with fresh medium 199. The monolayers were then fixed with 2% glutaraldehyde in 0.1 M sodium cacodylate and stained with 1% Giemsa for 30 min at room temperature. After the monolayers were washed with water, they were observed with a photomicroscope by putting the cover slips on microscope slides.

Immunological studies. Antiserum directed against the purified inhibitor was prepared in rabbits. The antigen was administered in a multiple emulsion of complete Freund adjuvant (Difco) with 1% Tween 80. Rabbits were initially inoculated in many footpad sites until a volume of 0.4 ml containing 1 mg of antigen was injected. The animals were boosted 1 month after the first injection by administering for 3 successive days a suspension containing 1 mg of antigen in 3 ml of PBS with 1% Tween 80. The intramuscular injection of 0.5 ml of the suspension on day 1 was followed by intravenous injections of 1.0 and 1.5 ml on days 2 and 3, respectively. The rabbits were bled 7 days after the last injection.

Immunodiffusion experiments were conducted by the Ouchterlony double-diffusion technique with 1% agarose in PBS containing 0.05% (wt/vol) sodium azide. Immunoelec-

trophoresis was carried out with 1% agarose in 0.1 M barbital buffer I (pH 8.6) (Bio-Rad Laboratories, Richmond, Calif.).

Chemicals. Agarose, acrylamide, *N,N'*-methylene-bis-acrylamide, and urea were electrophoresis purity reagent grade, purchased from Bio-Rad Laboratories, Mississauga, Ontario, Canada. Ampholines were products of LKB Instruments Inc., Rockville, Md. Other chemicals were reagent grade from Fisher Scientific Co., Montreal, Canada.

RESULTS

The antibacterial spectrum of the purified inhibitor (200 AU per ml) was determined against different species, most of which are representatives of the normal urogenital flora (Table 1). Only *Corynebacterium* 15927 and *N. meningitidis* serogroup Y were inhibited. When tested against *N. gonorrhoeae* strains with a preparation at 55 AU per ml, 17 of 20 strains were inhibited. The zone of inhibition varied from 1 to 5 mm. At the higher concentration of the inhibitor (200 AU per ml), all gonococcal strains were inhibited. The in vivo antigonococcal activity of the inhibitor was tested against different gonococcal strains in the guinea pig subcutaneous chamber model (Table 2). The inhibitor was tested against a laboratory strain (G-10), two adapted strains by

TABLE 3. Sensitivity of different erythrocytes to the purified inhibitor

Erythrocytes	Hemolytic units per mg of inhibitor
Human	44
Horse	45
Mouse	38
Rabbit	15
Bovine	11
Sheep	10

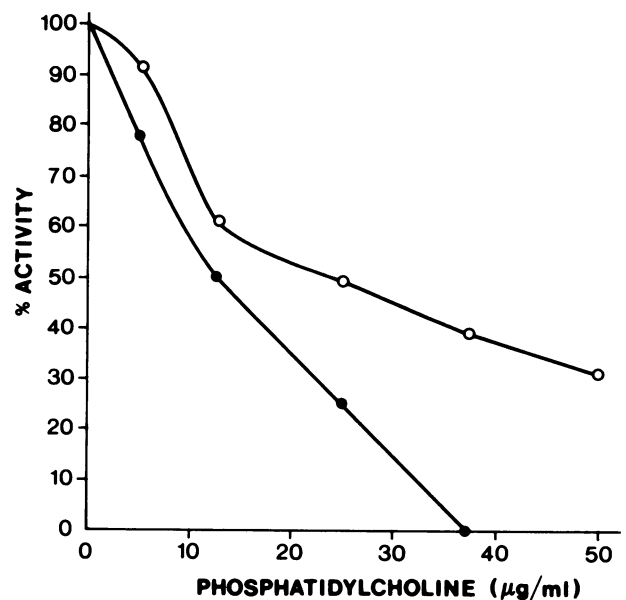


FIG. 1. Effect of various concentrations of phosphatidylcholine on the hemolytic (●) and antigonococcal (○) activities of the inhibitor.

three transfers in subcutaneous chambers, and two T₁ strains. Each of these strains, except G-10, was able to grow in the chambers, as determined by comparison of the CFU per milliliter injected with those recovered after 24 h. In all chambers containing the inhibitor, the number of CFU of each strain per milliliter was lower than the count obtained in the control chamber after 24 h; strain G-10 was the most susceptible.

The purified inhibitor showed hemolytic activity, and the sensitivity of different erythrocytes to the inhibitor is shown in Table 3. Human and horse erythrocytes are the most susceptible, whereas bovine and sheep erythrocytes are relatively less susceptible. Since the hemolytic pattern of the inhibitor is similar to that of the delta toxin, we also determined the inhibitory effect of phosphatidylcholine on the antigonococcal and hemolytic activities of the purified substance. Phosphatidylcholine is known to inhibit the hemolytic activity of the delta toxin. Complete inhibition of the hemolytic activity was obtained with 37 µg of phosphatidylcholine per ml (Fig. 1). At this concentration, 61% of the antigonococcal activity was inhibited, and complete inhibition was not attained in the concentrations used. The purified inhibitor at a concentration of 1 mg/ml also disrupted the cell structures of HeLa and WI 38 cells in 10 min.

The isoelectric point of the protein component of the gonococcal inhibitor was determined in polyacrylamide gel. In the presence of 8 M urea, two major bands were observed at pH 4.8 and 4.9 and two minor bands were observed at pH 7.1 and 4.4 (Fig. 2). Without the presence of urea, one major band was observed at pH 4.6 and one minor band was observed at pH 5.6.

The amino acid composition is presented in Table 4. The substance is characterized by the absence of proline, tyrosine, histidine, cysteine, arginine, and tryptophan. The hydrophobic nonpolar residues (glycine, alanine, leucine, isoleucine, valine, phenylalanine, and methionine) accounted for 60% of the total residues. The protein contains 25 amino acids, and the molecular weight was estimated to be 2,565.

The purified substance gave two lines of precipitation with the antiserum directed against the protein component when tested by immunoelectrophoresis (Fig. 3a). Those lines showed the same electrophoretic pattern. Two lines of precipitation were also observed with the crude supernatant

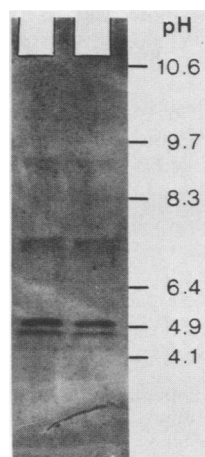


FIG. 2. Isoelectric focusing of the protein component of the inhibitor (75 µg) in 7% polyacrylamide gel in the presence of 8 M urea.

TABLE 4. Amino acid composition of the gonococcal inhibitor

Amino acid	mol per subunit ^a after time (h)			Nearest integer
	24	48	72	
Aspartic acid	2.50	2.46	2.46	2
Threonine	1.08	1.06	1.05	1
Serine	1.94	1.74	1.66	2
Glutamic acid	3.06	3.08	3.05	3
Proline	0	0	0	0
Glycine	3.84	3.80	3.81	4
Valine	2.02	2.24	2.38	2
Alanine	2.78	2.74	2.72	3
Isoleucine	2.10	2.34	2.48	2
Leucine	2.00	2.00	2.00	2
Tyrosine	0	0	0	0
Phenylalanine	1.16	1.18	1.16	1
Histidine	0.10	0.04	0.04	0
Lysine	2.22	2.24	2.24	2
Arginine	0	0	0	0
Methionine	0.64 ^b			1
Cysteine	0.24 ^b			0
Tryptophan	0.40			0

^a All molar ratios are based on leucine = 2.00.

^b Analysis after performic acid oxidation.

of *S. haemolyticus* no. 7 culture. No line of precipitation was observed when the serum was tested against crude supernatants of cultures of *S. aureus* NCTC 9393 and 9715, each containing delta toxin activity (Fig. 3b).

DISCUSSION

The characterization of the purified antigonococcal substance has confirmed the narrow antibacterial spectrum previously observed on solid medium (4) where most of the species tested except *N. gonorrhoeae* were resistant. To determine that the inhibitor was also active in an in vivo environment, the activity of the purified substance was tested against *N. gonorrhoeae* in guinea pig subcutaneous chambers. Antigonococcal activity was observed, and the variation between the strains tested could be due to their different susceptibility and to the conditions found with each chamber, i.e., amounts of blood and tissues present, physiological conditions of the host such as immunity, and affinity

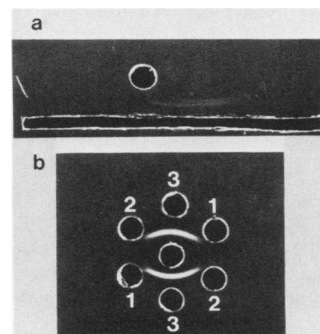


FIG. 3. (a) Immunoelectrophoresis of the protein component of the inhibitor. After electrophoresis, 100 µl of antiserum directed against the inhibitor was added. (b) Immunodiffusion experiment with crude supernatant of *S. aureus* culture containing delta toxin activity (well 1) strain NCTC 9393, (well 2) strain NCTC 9715, and (well 3) crude supernatant of *S. haemolyticus* no. 7 culture. The antiserum directed against the inhibitor was added in the center well.

of the inhibitor for erythrocytes and other host cells. The antagonococcal activity observed in vitro and in animal models is not necessarily representative of the complex series of interactions that occur in the urogenital tract. There is no animal model which reproduces the complexity of the urogenital tract with the normal urogenital flora and physiological conditions of the host, including the vaginal secretions, immunity, and the menstrual cycle. However, our results showed that under our conditions a staphylococcal strain isolated from the urogenital flora can produce an antagonococcal inhibitor.

The hemolytic spectrum of the inhibitor against erythrocytes of different animal species showed that human and horse erythrocytes were the most susceptible. This is a characteristic of staphylococcal delta toxin. In fact, it is differentiated from other staphylococcal hemolysins by its ability to lyse horse and human erythrocytes (6). The inhibitor disrupted tissue culture cells as reported for delta toxin (22, 27).

The hemolytic and antagonococcal activities of the purified substance were inhibited in the presence of phosphatidylcholine. The antagonococcal activity was only partially inhibited by the highest concentrations of phosphatidylcholine used, whereas the hemolytic activity was completely inhibited. This could mean that the mechanism or active part of the inhibitor is different for each activity. The inhibition of the hemolytic activity by phosphatidylcholine is also a property of the delta toxin of *S. aureus* (22, 27). The high content in hydrophobic residues of the gonococcal inhibitor, its inactivation by phosphatidylcholine, and the disruption of erythrocytes and cells in tissue culture suggest a possible surface-active effect on biological membrane. However, further work is needed to determine precisely the mode of action of this protein.

Although some similarities exist between the purified inhibitor and the delta toxin, we believe that the substances are different, based mainly on their chemical characteristics. The delta toxin of *S. aureus* was described as a low-molecular-weight peptide present as complexes or aggregates of molecular weight ranging from 68,000 to 200,000. In the presence of nonionic detergents, the toxin was dissociated into five subunits weighing ca. 21,000 each (22, 27). The antagonococcal substance is also a low-molecular-weight peptide estimated at 2,565, present as complexes or aggregates which are dissociated in subunits estimated at 15,900 in the presence of 4 M urea. Both inhibitors differed in their isoelectric points. The pI of the major component of the delta toxin is in the pH range of 9.0 to 9.6 (22, 27). In the absence of urea, the pI of the major band of the gonococcal inhibitor was estimated at pH 4.6. No band was observed in the basic pH range.

Both substances are characterized by the absence of proline, tyrosine, histidine, arginine, and cysteine. However, several differences are observed when the amino acid composition of the antagonococcal substance is compared with that of the delta toxin (8). Moreover, no line of precipitation was observed when the antiserum directed against the inhibitor was tested with crude supernatants from *S. aureus* cultures containing delta toxin activity. These results suggest that the antagonococcal substance is chemically different from the delta toxin, although many properties are shared by both substances. Preliminary results obtained from the amino acid sequence determination of the antagonococcal substance have confirmed that it is completely different from the delta toxin produced by *S. aureus* (unpublished data).

Based mainly on the biological and chemical characterization, our gonococcal inhibitor is different from the staphylococci Bac R₁ (19), C55 (7), 414 (9), IYS2 (20), and A (15), the lysostaphin (24), the epidermidins, (10), and the other known toxins of *S. aureus* (22, 27). Staphylococci Bac R₁, C55, and A are proteins which are partially purified and have shown wide bacterial spectrum against gram-positive strains. Staphylococcin 414 is a lipoprotein-carbohydrate complex larger than 200,000 daltons composed of 12,500-dalton units. Staphylococcin IYS2 is a 5,000-dalton protein which differs from our gonococcal inhibitor by its amino acid composition and its bacterial spectrum. Lysostaphin is a 32,000-dalton peptidase lytic for staphylococci. The epidermidins are cyclic peptides composed of 11 amino acids, active on gram-positive microorganisms, and inactive on gram-negative strains. Alpha, beta, gamma, and epsilon toxins differed from our gonococcal inhibitor by their structural properties, hemolytic spectrum, and many other biological properties.

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LITERATURE CITED

1. Ali, M. I., and R. Haque. 1972. Identification of staphylococcal epsilon hemolysin. *Can. J. Microbiol.* **18**:535-536.
2. Beaudet, R., J. G. Bisailon, S. A. Saheb, and M. Sylvestre. 1982. Production, purification, and preliminary characterization of a gonococcal growth inhibitor produced by a coagulase-negative staphylococcus isolated from the urogenital flora. *Antimicrob. Agents Chemother.* **22**:277-283.
3. Bernheimer, A. W., and L. L. Schwartz. 1963. Isolation and composition of staphylococcal alpha toxin. *J. Gen. Microbiol.* **30**:455-468.
4. Bisailon, J. G., R. Beaudet, L. Lafond, S. A. Saheb, and M. Sylvestre. 1981. Antigonococcal and antibacterial spectra of some bacterial isolates of the urogenital flora. *Rev. Can. Biol.* **40**:215-227.
5. Bisailon, J. G., R. Beaudet, L. Lafond, S. A. Saheb, and M. Sylvestre. 1981. Interference of *Neisseria gonorrhoeae* growth by aerobic bacterial representatives of the urogenital flora. *Rev. Can. Biol.* **39**:201-208.
6. Chow, A. W., M. J. Gribble, and K. H. Bartlett. 1983. Characterization of the hemolytic activity of *Staphylococcus aureus* strains associated with toxic shock syndrome. *J. Clin. Microbiol.* **17**:524-528.
7. Dajani, A. S., and L. W. Wannamaker. 1969. Demonstration of a bactericidal substance against β -hemolytic streptococci in supernatant fluids of staphylococcal cultures. *J. Bacteriol.* **97**:985-991.
8. Fitton, J. E., A. Dell, and W. V. Shaw. 1980. The amino acid sequence of the delta hemolysin of *Staphylococcus aureus*. *FEBS Lett.* **115**:209-212.
9. Gagliano, V. J., and R. D. Hinsdill. 1970. Characterization of a *Staphylococcus aureus* bacteriocin. *J. Bacteriol.* **104**:117-125.
10. Hsu, C.-Y., and G. M. Wiseman. 1972. The nature of epidermidins, new antibiotics from staphylococci. *Can. J. Microbiol.* **18**:121-125.
11. Ingraham, L. S., S. J. Kraus, and G. F. Brooks. 1978. Microbial inhibition of *Neisseria gonorrhoeae*, p. 350-355. In G. F. Brooke, E. C. Gotschlick, K. K. Holmes, W. D. Sawyer, and F. E. Young (ed.), *Immunobiology of Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D.C.
12. Kaye, D., and M. E. Levison. 1977. *In vitro* inhibition of growth of *Neisseria gonorrhoeae* by genital microorganisms. *Sex.*

- Transm. Dis. 4:1-3.
13. Kraus, S. J., and N. Ellison. 1974. Resistance to gonorrhea possibly mediated by bacterial interference. *Appl. Microbiol.* 27:1014-1016.
 14. Kraus, S. J., R. C. Geller, G. H. Perkins, and D. L. Rhoden. 1976. Interference of *Neisseria gonorrhoeae* growth by other bacterial species. *J. Clin. Microbiol.* 4:288-295.
 15. Lachowicz, T. 1965. Investigations on staphylococcus. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig.* 196:340-351.
 16. Lafond, L., J. G. Bisaillon, S. A. Saheb, R. Beaudet, and M. Sylvestre. 1981. *In vitro* antigonococcal activity of urogenital coagulase-negative staphylococci. *Can. J. Microbiol.* 27:138-141.
 17. Lafond, L., J. G. Bisaillon, S. A. Saheb, R. Beaudet, and M. Sylvestre. 1982. Bactericidal and bacteriostatic antigonococcal activities produced by urogenital staphylococci. *Microbios* 33:27-34.
 18. Morin, A., S. A. Saheb, J.-G. Bisaillon, R. Beaudet, and M. Sylvestre. 1980. *In vitro* inhibition of *Neisseria gonorrhoeae* growth by strict anaerobes. *Infect. Immun.* 28:766-770.
 19. Morris, D. M., J. W. Lawson, and M. Rogolsky. 1978. Effect of a staphylococcal on *Neisseria gonorrhoeae*. *Antimicrob. Agents Chemother.* 14:218-223.
 20. Nakamura, T., N. Yamazaki, H. Taniguchi, and S. Fujimura. 1983. Production, purification and properties of a bacteriocin from *Staphylococcus aureus* isolated from saliva. *Infect. Immun.* 39:609-614.
 21. Penn, C. W., D. Sen, D. R. Veale, N. J. Parsons, and H. Smith. 1976. Morphological, biological and antigenic properties of *Neisseria gonorrhoeae* adapted to growth in guinea-pig subcutaneous chambers. *J. Gen. Microbiol.* 97:35-43.
 22. Rogolsky, M. 1979. Nonenteric toxins of *Staphylococcus aureus*. *Microbiol. Rev.* 43:320-360.
 23. Saigh, J. H., C. C. Sanders, and W. E. Sanders, Jr. 1978. Inhibition of *Neisseria gonorrhoeae* by aerobic and facultatively anaerobic components of the endocervical flora: evidence for a protective effect against infection. *Infect. Immun.* 19:704-710.
 24. Schindler, C. A., and V. T. Schuhardt. 1964. Lysostaphin: a new bacteriolytic agent for the *Staphylococcus*. *Proc. Natl. Acad. Sci. U.S.A.* 51:414-421.
 25. Swanson, J. 1973. Studies on gonococcus infection. IV. Pili: their role in attachment of gonococci to tissue culture cells. *J. Exp. Med.* 137:571-589.
 26. Volk, J., and S. J. Kraus. 1973. A symptomatic meningococcal urethritis—possible protective value against gonococcal infection by bacteriocin production. *Br. J. Vener. Dis.* 49:511-512.
 27. Wiseman, G. M. 1975. The hemolysins of *Staphylococcus aureus*. *Bacteriol. Rev.* 39:317-344.
 28. Wrigley, C. 1968. Gel electrofocusing. A technique for analyzing multiple protein samples by isoelectric focusing. *Sci. Tools* 15:17-22.