Immunoglobulin A Proteases in Gram-Negative Bacteria Isolated from Human Urinary Tract Infections

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Several strains of gram-negative bacteria (seven genera, eight species) isolated from patients with urinary tract infections were found to hydrolyze myeloma immunoglobulin A (IgA) protein. Human IgG and IgM and colostrum IgA were not degraded by these organisms. Examination of cleavage digests showed two fragments of different electrophoretic mobilities, with antigenic reactivity and sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles consistent with their identification as Fc and Fab components. The immunoelectrophoresis patterns of cleavage digests suggested that the proteases responsible for this hydrolysis may be dissimilar in the specificity of their IgA cleavage sites.

It is now well established that several bacterial species elaborate an enzyme which specifically cleaves human immunoglobulin A (IgA) protein of the IgA1 subclass (1, 6, 9). This IgA protease has been reported in Neisseria meningitidis, Haemophilus influenzae, and Streptococcus pneumoniae, the three major etiological agents of bacterial meningitis (6, 8, 11), and in Neisseria gonorrhoeae, the causative agent of gonorrhea (13). In addition, the enzyme has been detected in some strains of Streptococcus sanguis and Streptococcus mitior, two organisms frequently associated with dental caries. More recently, species of Bacteroides and Capnocytophaga, suspected pathogens in peridontal disease, were reported to cleave IgA and In the latter case IgG as well (3).

It is significant that many closely related nonpathogenic species of these genera which frequent human mucous membranes have been found to be uniformly negative in regard to protease production (4, 6, 12). Such findings have prompted several authors to suggest that IgA proteases may be an important bacterial virulence factor (4, 6, 12).

In view of these data and the fact that human mucous membranes in the respiratory, alimentary, and urogenital tracts are characterized by an IgA-dominant secretory immune system, it is surprising that this enzyme has not been detected in those bacteria involved in human urinary tract infections.

We undertook to investigate this possibility, and the following report shows that several species of gram-negative organisms associated with human urinary tract infections produce an IgA protease and that some species previously reported as protease negative are protease positive when isolated from this environment.

MATERIALS AND METHODS

Bacterial strains. The bacteria used in this study were fresh clinical isolates obtained from the Kingston General and Hotel Dieu hospitals and the Regional Public Health Laboratory at Kingston, Ontario, Canada. The isolates were isolated from patients with urinary tract infections and checked for purity and identity by standard methods.

Detection of IgA protease activity. The IgA substrate used in this work was purified from sera of patients with multiple myeloma by hydrophobic affinity chromatography (2). The purity of this material was monitored by the Ouchterlony double diffusion technique with antisera of defined specificity for human serum proteins (Cappel Laboratories, Cochranville, Pa.). Commercial human serum IgA purified by affinity chromatography (Cappel) was used throughout this work as a standard.

Individual bacterial colonies from tryptic soy agar plates (Difco Laboratories, Detroit, Mich.) were suspended in 50 μl of a 5 mg/ml solution of IgA contained in 0.15 M Tris-hydrochloride buffer (pH 8.1) (10). After incubation for 12 to 15 h at 37°C, the bacteria were removed by centrifugation, and the supernatant was examined for cleavage products by immunoelectrophoresis in 2% agarose (Bio-Rad Laboratories, Richmond, Calif.) with 0.15 M Veronal buffer (pH 8.1) (10). Antiserum used for development of electrophoresis were unadsorbed rabbit anti-human IgA and monospecific anti-heavy (α) and anti-light (κ/λ) chain IgA preparations (Behring-Hoechst, Montreal, Quebec, Canada). Control digests were applied to test slides for proper comparison. Digests were also examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after disulfi de reduction in 0.125% 2-mercaptoethanol-0.125% SDS (13). Apparent molecular weights of fragments were calculated (14) with the following proteins as standards (Bio-Rad): bovine serum albumin (68,000), catalase (58,000), ovalbumin (43,000), and RNase (14,000).

RESULTS

IgA protease detection. The production of IgA protease by gram-negative bacteria freshly isolated from patients with urinary tract infections is shown in Table 1. The enzyme was detected in 16% (28 of 172) of the cultures representative of seven genera and eight species. It is important to note that most of these bacterial species isolated from other sources have been reported as protease negative (7, 10). Also, protease was not detected in strains of these species derived from our departmental stock culture collection.

Figure 1 shows the cleavage of myeloma IgA into two fragments of different electrophoretic mobilities by representative gram-negative bacteria. The immunoelectrophoretic patterns and antigenic reactivity appeared similar and were consistent with the hydrolysis of IgA into faster-moving Fab and slower-moving Fc fragments, respectively. This was supported by the immunoelectrophoretic patterns (Fig. 2) with Proteus mirabilis as a representative organism. The faster-moving fragment reacted with both anti-heavy (α) chain and anti-light (κ/λ) chain antisera, and the slower-
TABLE 1. Production of IgA protease by bacteria isolated from patients with urinary tract infections

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. examined</th>
<th>No. IgA protease positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>73</td>
<td>10</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>A. calcoaceticus var. Lwoffi</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>A. calcoaceticus var. anitatus</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Moraxella species</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

moving fragment, which had a mobility similar to that of undigested protein, reacted only with the anti-heavy (α) chain antiserum. SDS-PAGE of these digests (reduced) from P. mirabilis-IgA incubations showed (Fig. 3) the presence of two bands corresponding to heavy chain pieces of Fc and Fd size, with apparent molecular weights of approximately 30,000 to 35,000 and 25,000 to 28,000, respectively.

Figure 4 shows the resemblance in the immunoelectrophoretic patterns of IgA digested by Proteus vulgaris and P. mirabilis. IgA digested by the Escherchia coli strains gave a mutually similar immunoelectrophoretic pattern, which differed, however, from that observed with the Proteus species. No cleavage products were detected when these organisms were incubated with IgG, IgM, and serum albumin.

**DISCUSSION**

This study demonstrated that several species of gram-negative bacteria isolated from patients with urinary tract infections were capable of hydrolyzing IgA myeloma protein. These organisms cleaved IgA into two fragments which gave reactions of nonidentity by immunoelectrophoresis and showed antigenic reactions with anti-heavy and anti-light chain sera and SDS-PAGE electrophoresis patterns consistent with reported properties of Fc and Fab components.

This clearly establishes that some of the bacteria which frequent the urinary tract, as well as the respiratory and alimentary tracts, produce this type of enzyme. It should be noted that Kornfeld and Plaut (7) have suggested that, since this activity was originally detected in human feces, it would be likely that eventually some enteric organism should be reported as protease positive.

The observation that the immunoelectrophoresis patterns of the Fc pieces released by the action of Proteus and Escherichia sp. strains were not identical suggests a similarity to the findings with Haemophilus and Streptococcus species (6). It was reported that the difference in the immunoelectrophoretic patterns of Fc fragments produced by the action of Haemophilus and Streptococcus species were probably due to a difference in the substrate specificity of their proteases (6). Subsequently this was confirmed by sequence analysis (5, 10). Until the sequence of cleavage fragments resulting from the action of urinary tract organisms is established, the relationship between the enzymes of these organisms will remain unknown.

In other aspects of this work (data not shown) we found that there was no obvious correlation between protease production and antibiotic and heavy-metal sensitivity of these bacteria. Repeated attempts to associate this activity with the presence or absence of a specific plasmid were uniformly negative. Also, even though some strains lost the capacity to produce the enzyme in stock culture, there was no apparent influence of a variety of nutritional factors, i.e.,

![FIG. 1. Immuno electrophoresis of IgA myeloma protein incubated with suspensions of representative bacterial strains isolated from patients with urinary tract infections. A. Buffer control; B. P. mirabilis; C. Klebsiella pneumoniae; D. E. coli; E. Acinetobacter calcoaceticus var. Lwoffi. The antiserum used was rabbit anti-human IgA (unadsorbed). The anode is to the left.](image)

![FIG. 2. Immuno electrophoresis of IgA myeloma protein incubated with P. mirabilis isolated from a patient with a urinary tract infection. The antiserum in the trough was rabbit anti-human IgA. A. Unadsorbed B, anti-light chain; C. anti-heavy chain. Wells: 1. buffer control; 2 and 3. P. mirabilis. The anode is to the right.](image)

![FIG. 3. SDS-PAGE patterns of IgA myeloma protein. Gels: A. IgA protein after incubation with P. mirabilis; B. IgA control; C. IgA protein incubated with P. mirabilis (protease negative). H. IgA heavy chain; L. IgA light chain.](image)
carbon-nitrogen source on the production (induction-repression) of protease by these bacteria.

An interesting aspect of this investigation was the finding that not all organisms associated with urinary tract disease produced protease under the experimental conditions. However, since the clinical isolates were not specifically documented regarding infection in the patient, it was not possible to determine whether all isolates were considered to be the causative agents of urinary tract disease or were simply present in the urine as contaminants. More detailed examination of this point is warranted before any support can be given to a positive role for IgA protease as a virulence factor in gram-negative urinary tract disease.

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LITERATURE CITED