A survey of IgA protease production among clinical isolates of *Proteaeae*

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Summary. A collection of 100 strains of *Proteaeae*, in which all species within the tribe were represented, was examined for IgA protease production. The strains were isolated from various clinical specimens from sick and healthy persons in several countries. IgA protease-producing strains were not found amongst species of *Providencia* and *Morganella* but were common in *Proteus* spp. All the strains of *P. mirabilis* and *P. penneri* and many of the strains of *P. vulgaris* examined produced an EDTA-sensitive protease that cleaved the IgA heavy chain outside the hinge region. The protease enzyme was different in this respect from the EDTA-sensitive, hinge-cutting proteases of other bacteria. The ability to produce IgA protease was unrelated to the O antigenicity, biotype or bacteriocin type of the strain. IgA protease production may be an important virulence mechanism for *Proteus* strains.

Introduction

Immunoglobulin A (IgA) in the form of secretory IgA, is the predominant immunoglobulin in mucous secretions. Its function is to protect mucous membranes and underlying tissue from damage by micro-organisms or their products. The function of serum IgA, which differs from the secreted form in being monomeric and lacking the secretory component, is less clear. It is believed to have a role in the regulation of the inflammatory response and in the disposal of antigens from the circulation. Of the many bacteria examined, only a few produce extracellular enzymes capable of degrading IgA (IgA proteases) (Kilian *et al.*, 1983a). These include major pathogens such as *Neisseria gonorrhoeae*, *N. meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae* and bacteria associated with periodontal disease and dental plaque formation (Plaut *et al.*, 1974, 1975; Blake and Swanson, 1978; Kilian *et al.*, 1979, 1983b; Male, 1979; Mulks *et al.*, 1980a,b; Kilian, 1981; Kilian and Holmgren, 1981); because other species of these genera, non-pathogenic for normal individuals, do not form IgA proteases, the production of these enzymes may be associated with virulence (Mulks and Plaut, 1978). IgA proteases act by cleaving the heavy chain of the human IgA1 isotype at specific sites within a 16-amino acid proline-rich polypeptide in the hinge

region. This sequence is not present in IgA2 which is, therefore, resistant to IgA protease cleavage (Plaut *et al.*, 1974, 1975; Kilian *et al.*, 1980; Mulks *et al.*, 1980b).

Among the *Proteaeae*, strains of *Proteus* spp., particularly *P. mirabilis*, are a common cause of chronic and recurrent urinary-tract infection and have been found more frequently than *Escherichia coli* in urinary-tract infections of the elderly (Senior, 1979) and young boys (Bergström, 1972; Hallet *et al.*, 1976; Khan *et al.*, 1978). Furthermore, the consequences of infection with *Proteus* spp. are often more serious because, whereas *E. coli* is usually confined to the bladder, *Proteus* strains have a special predilection for the upper urinary tract (Fairley *et al.*, 1971) where they may cause damage to the renal tubular epithelium (Braude *et al.*, 1960) and bring about conditions leading to the formation of renal stones (Griffith *et al.*, 1973). On the other hand, *Morganella morganii* and *Providencia* spp. are uncommonly associated with urinary-tract infections (Senior, 1979) but are more frequently associated with diarrhoea (Senior and Leslie, 1986). These differences arise through the operation of different pathogenic mechanisms in the different genera.

We have recently shown that *P. mirabilis* strains of diverse type, isolated from many different clinical situations, produce IgA protease. The enzyme from *P. mirabilis* strains, however, differed from that of other pathogenic bacteria by cleaving the IgA

heavy chain outside the hinge region (Senior *et al.*, 1987). This present investigation sought to determine whether, among the *Proteaeae*, IgA protease formation was restricted to *P. mirabilis*.

Materials and methods

Bacterial strains

A series of 100 strains presumed to belong to the tribe *Proteaeae* was isolated in pure cultures from clinical specimens of unrelated individuals and stored on nutrient-agar slopes in screw-capped bottles at 4°C. Most strains (83%) were isolated from patients resident in five British cities and the remainder (17%) were from patients in Canada, Germany, Hungary, Sweden and Turkey. The strains of *S. pneumoniae* and *N. gonorrhoeae* used for comparison were originally isolated from clinical specimens but had been maintained for several months by repeated subculture on heated-blood agar and modified New York City medium, respectively.

Media

Nutrient broth was Nutrient Broth No. 2 (Oxoid CM 67), 2.5% w/v in distilled water. Nutrient agar (Oxoid CM3) and blood agar (BA) made up from Columbia Agar Base (Oxoid CM 331) with sterile horse blood (Oxoid SR 50) 5% v/v were prepared and sterilised according to the manufacturer's instructions. Heated-blood agar (HBA) was heated BA. Modified New York City medium (MNYC) was prepared according to the method of Young (1978) but with horse blood (Oxoid SR 50). Tryptophan deaminase agar (TDA), urea-indole medium, ornithine decarboxylase—H₂S medium and peptone-water sugar media were prepared and sterilised as previously described (Senior and Leslie, 1986).

Identification of strains

A nutrient-broth culture of each strain was inoculated as a spot on to a TDA plate and into urea-indole medium and mannose peptone water. After overnight incubation at 37°C, strains which formed tryptophan deaminase (Senior and Leslie, 1986) and urease but failed to form acid from mannose were identified as *Proteus* spp. Those which formed tryptophan deaminase and acid from mannose were identified as species of either *Morganella* or *Providencia*. Each strain was then speciated by its reaction in appropriate tests for the genus as shown in the table.

Bacteriocin typing of strains

Strains of *Proteus* spp. were typed for their ability to produce (P) bacteriocins (proticines) and for their sensitivity (S) to proticines—P/S typing—according to the method of Senior (1977) and Senior and Larsson

(1983). *M. morgani* strains were similarly typed for bacteriocin (morganocin) production and sensitivity (Senior, 1987).

Preparation of ¹²⁵I-labelled IgA

IgA was purified from normal human plasma as previously described (Senior *et al.*, 1987). The resulting pure IgA was labelled with ¹²⁵I by the chloramine-T method of Hunter and Greenwood (1962).

Preparation of IgA protease

Each strain was grown on dialysis-tubing membrane covering the surface of culture media plates—BA for *Proteaeae*, HBA for *S. pneumoniae* and MNYC for *N. gonorrhoeae*. The membrane and bacteria were subsequently washed in 5 mM Tris buffer (pH 7.2) and the supernate used as a source of IgA protease as previously described (Senior *et al.*, 1987).

Polyacrylamide gel electrophoresis and autoradiography

Purified ¹²⁵I-labelled IgA was digested with protease and the products of digestion analysed by SDS discontinuous gel electrophoresis on 5–22% gradient polyacrylamide gels and subsequent autoradiography according to methods previously described (Senior *et al.*, 1987).

Results

IgA protease production by *P. mirabilis* strains

A collection of 15 *P. mirabilis* strains of 14 distinct P/S types and at least 7 O-antigenic types was examined for IgA protease production. The strains included isolates from urine, faeces, sputum, renal and ureteric stones, and a brain abscess, from patients and from healthy individuals. Each strain produced IgA protease.

IgA protease production by *P. vulgaris* strains

A series of 24 strains of *P. vulgaris* of 15 different P/S types, 14 of biotype 2 and 10 of biotype 3, was examined. The strains were isolated from urine, faeces, a leg ulcer, a groin abscess, an infected ear and an infected burn. Only 12 (50%) of the strains—11 (79%) of the biotype-2 strains and 1 (10%) of the biotype-3 strains—produced IgA protease. There was no obvious association between protease production and source, biotype or P/S type of the strain. IgA protease producing and non-producing strains were found amongst those with the same biotype and P/S type. The low proportion of IgA

Table. The biochemical reactions of members of the tribe *Proteeae*

Species		Biochemical test and result												
		tryptophan deaminase	acid from mannose	urease	ornithine decarboxylase	H ₂ S	indole	acid from maltose	aesculin hydrolysis	gas from glucose	Acid from			
											inositol	adonitol	galactose	trehalose
<i>P. mirabilis</i>		+	-	+	+	+	-	-	-					
<i>P. vulgaris</i>	biotype 2	+	-	+	-	+	+	+	+					
	biotype 3	+	-	+	-	(+)	+	+	-			NT		
<i>P. penneri</i> *		+	-	+	-	(-)	-	+	-					
<i>M. morgani</i>		+	+	+	+	-	+							
<i>Prov. rettgeri</i>		+	+	+	-	-	+			(-)	+	+	+	(-)
<i>Prov. alcalifaciens</i>	biotype 1	+	+	-	-	-	+			+	-	+	-	-
	biotype 2	+	+	-	-	-	+			+	-	+	-	-
<i>Prov. rustigiani</i> †		+	+	-	-	-	+		NT	v	-	-	+	-
<i>Prov. stuarti</i>	biotype 4	+	+	+	-	-	+			-	-	-	+	+
	biotype 5	+	+	-	-	-	+			-	+	-	+	+
	biotype 6	+	+	-	-	-	+			-	+	+	+	+

+ = Production; - = no production; () = most strains; v = different strains give different results; NT = not tested.

* Data from Hickman *et al.* (1982).

† Data from Hickman-Brenner *et al.* (1983).

protease-producing strains of biotype 3 however was noted.

IgA protease production by P. penneri strains

Five strains of *P. penneri*, each with a unique P/S type, which had been isolated from faeces of three people with diarrhoea and two healthy people without diarrhoea, were examined. Each strain produced IgA protease.

Lack of detectable IgA protease formation by strains of Morganella and Providencia species

A total of 56 strains was examined for IgA protease production; 15 were *M. morgani* strains, each with a unique bacteriocine type, isolated from faeces, urine, CSF and wounds of patients with various clinical symptoms; 14 were strains of eight different O-antigenic types of *Prov. rettgeri* isolated from faeces and urine; 15 were strains of *Prov. alcalifaciens* isolated from faeces, seven being of biotype 1 and eight of biotype 2; five were strains of *Prov. rustigiani* isolated from faeces; seven were strains of *Prov. stuarti* biotype 5 isolated from

faeces, urine and a wound. Not one of these 56 strains produced IgA protease.

Characteristics of the IgA proteases of Proteus species

Polyacrylamide-gel analysis of the products of IgA cleavage by the enzyme from each of the 32 IgA protease producing *Proteus* strains showed that, regardless of the species, O antigenicity, biotype or bacteriocin type, the enzyme from each strain cleaved exclusively the IgA heavy chain, to give fragments of mol. wts (10^3) c. 47 and 34, and was inhibited by the presence of 5 mM EDTA. The cleavage products were quite different in size from the IgA heavy chain hinge-cutting proteases of *S. pneumoniae* (inhibited by 5 mM EDTA) and *N. gonorrhoeae* (resistant to 5 mM EDTA) (see figure).

Discussion

Although IgA protease production was not detected in any of the 56 diverse strains of *Morganella* and *Providencia* species examined, it was commonly found amongst strains of *Proteus*

Figure. Polyacrylamide gel analysis of ^{125}I -IgA digested with various microbial proteases. All the protease enzymes from strains of *Proteus* spp. were EDTA sensitive and cleaved the IgA heavy chain to fragments of mol. wts (10^3) 47 and 34. Left to right: 1. Control (untreated IgA); IgA treated with—2. *P. mirabilis* 64676 protease; 3. (2) with 5 mM EDTA; 4. *P. vulgaris* 60694/78 protease; 5. (4) with 5 mM EDTA; 6. *P. penneri* E1152 protease; 7. (6) with 5 mM EDTA; 8. *S. pneumoniae* protease; 9. (8) with 5 mM EDTA; 10. *N. gonorrhoeae* protease; 11. (10) with 5 mM EDTA. H=heavy chain; L=light chain.

spp. This was particularly true for strains of *P. mirabilis* and *P. penneri*—the enzyme being formed by every strain of these species examined—but true to a lesser extent for strains of *P. vulgaris* of which only 50% produced IgA protease. There was a significant difference in the frequency of IgA protease production by *P. vulgaris* strains of biotype 2 compared with those of biotype 3, but the reason for this is unclear. With this exception, IgA protease production did not appear to be associated with any other characteristic of the producing strain. It was detectable in strains of diverse O antigenic and bacteriocinogenic types, from various sources, in both sick and healthy individuals.

Our observation that the IgA protease of *N. gonorrhoeae* was more resistant to inhibition by EDTA than that of *S. pneumoniae* has also been reported by others (Kilian *et al.*, 1983a). This may indicate that the former enzyme is an alkaline metal-dependent protease, whereas the latter enzyme is a neutral metal-dependent protease. Although the IgA proteases of all the *Proteus* spp. were like the *S. pneumoniae* protease in being

inhibited by 5 mM EDTA, the size of the cleavage products of the IgA heavy chain were quite different from those resulting from cleavage with the IgA proteases of either *S. pneumoniae* or *N. gonorrhoeae*, which cut in the hinge region, and of a size indicating cleavage outside the hinge region. The action of IgA proteases of strains of *Proteus* spp. may, therefore, not be restricted, as are the IgA proteases of other bacteria, to the immunoglobulins of isotype IgA1. Such a wider activity would clearly be of advantage to an invading *Proteus* strain seeking to establish infection. We intend to purify the *Proteus* IgA protease, determine its precise site of cleavage and investigate the activity of the enzyme upon various purified immunoglobulins, including secretory IgA.

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