Brief Definitive Report

IgA PROTEASES OF TWO DISTINCT SPECIFICITIES ARE RELEASED BY *NEISSERIA MENINGITIDIS**

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IgA proteases are metal chelator-sensitive neutral proteolytic enzymes elaborated into the extracellular environment by bacteria that cause human infections. Enzyme-positive bacteria that have been identified are *Neisseria gonorrhoeae* (1), *Neisseria meningitidis* (1), *Hemophilus influenzae* (2–4), *Streptococcus pneumoniae* (2–4), and *Streptococcus sanguis* (5, 6). The IgA proteases each cleave a single peptide bond in the unusual duplicated octapeptide Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser that is found in the human IgA1 heavy-chain hinge region (1, 7); proteins of the IgA2 subclass, having a hinge-region deletion that includes a large portion of the duplicated segment (8), are resistant to IgA protease cleavage (9), as are all other proteins yet examined. The peptide bonds cleaved by the proteases of these bacteria are shown diagramatically in Fig. 1, where it can be seen that a proline residue contributes the carboxyl group in each susceptible bond. Each enzyme catalyzes the hydrolysis of a single specific peptide linkage in the alpha chain, despite the abundance of pro-R linkages (R denoting threonyl or seryl residues) throughout the hinge-region segment; because cleavage is at a single peptide bond, the products of hydrolysis are intact Fabα and Fcα fragments.

While studying the IgA proteases of *N. meningitidis*, it was noted that the IgA1 cleavage products differed in size and electrophoretic migration among *N. meningitidis* isolates. We have now shown that this species releases two types of enzymes, each bacterial isolate elaborating one or the other enzyme but not both. Limited amino-terminal sequence analysis of the Fcα digestion products identified the peptide linkages cleaved in the alpha chain by each enzyme, and showed that the susceptible bonds are separated by only two amino acid residues.

Materials and Methods

N. meningitidis strains were clinical isolates of human origin grown in pure culture on chocolate agar plates (Baltimore Biological Laboratories, Cockeysville, Md.) at 37°C under 5% CO_2. Two selected isolates were grown overnight at 37°C in brain-heart infusion (Difco Laboratories, Detroit, Mich.) that contained 1% Isovitalex (Baltimore Biological Laboratories), and IgA protease was purified from the spent culture medium by sequential salt precipitation and column chromatography, as previously described (1, 5).

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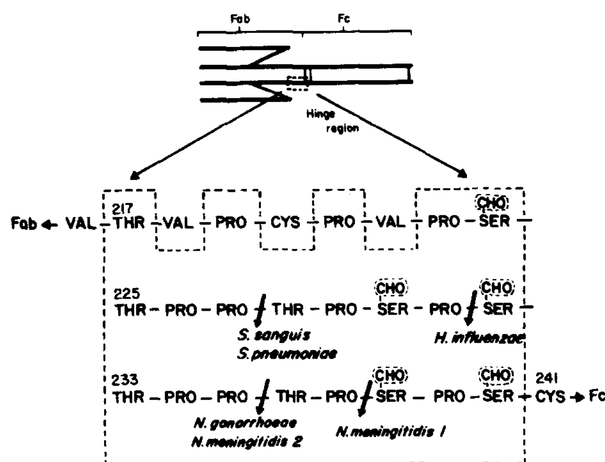

FIG. 1. The hinge region of human IgA1, showing the location of the peptide bonds cleaved by the two types of meningococcal IgA protease. *N. meningitidis* Type 1 cleaves the Pro-Ser bond at position 237–238; *N. meningitidis* Type 2 cleaves the Pro-Thr bond at 235–236. The peptide bonds cleaved by *N. gonorrhoeae* (1), *S. sanguis* (1), *S. pneumoniae* (7), and *H. influenzae* (7) are shown for comparison. The hinge sequence is arranged to illustrate our interpretation that a triplication of an eight amino acid segment is found in this region of the human IgA1 heavy chain. Other interpretations involving more numerous copies of a shorter sequence are also possible.

Human IgA1 myeloma paraprotein, digested with both types of *N. meningitidis* protease and the prototype enzymes of *N. gonorrhoeae* and *S. sanguis*, was examined on 9% polyacrylamide slab gels that contained 0.1% sodium dodecyl sulfate (SDS) after disulfide reduction in 0.125% B-mercaptoethanol and 1.25% SDS (10). Gels were fixed and stained with 0.05% Coomassie blue in 25% isopropanol-10% acetic acid. Molecular weight markers were purchased from Bio-Rad Laboratories (Richmond, Calif.) as a standard mixture including lysozyme (14,300 dalton), soybean trypsin inhibitor (21,000 dalton), carbonic anhydrase (30,000 dalton), ovalbumin (43,000 dalton), bovine serum albumin (68,000 dalton), and phosphorylase B (94,000 dalton).

For amino acid sequence studies, 30 mg of IgA1 protein, purified by hydrophobic affinity chromatography (11), were digested with each meningococcal enzyme for 48 h at 37°C and Fcα fragments purified from these digestion mixtures by molecular sieve chromatography on Sephadex G-150. Residual uncleaved IgA was removed from Fc material by affinity chromatography on Sepharose 4B-bearing anti-light chain antisera. Fcα material found free of other fragments and contaminating intact IgA protein by Ouchterlony analysis was lyophilized, completely reduced and alkylated (12), and subjected to automated amino acid sequence analysis in a Beckman 890C sequencer (Beckman Instruments, Inc., Fullerton, Calif.) using 0.1 M Quadrol buffer. Identification of phenylthiohydantoin amino acids was generally done by amino acid analysis after back hydrolysis in 0.2 ml 6 N HCl that contained 5 μl 5% $SnCl_2$ *in vacuo* (13), and high pressure liquid chromatography using a Waters HPLC model ALC/GPC-204 equipped with a 3.9 mm × 30 cm μ Bondapack C18 column (Waters Associates, Inc., Milford, Mass.). The position of carboxymethyl cysteine was confirmed by measuring radioactivity (14).

Results

SDS-polyacrylamide gel analysis (Fig. 2) revealed two distinct IgA protease cleavage patterns among *N. meningitidis* isolates; we have termed these Type 1 and 2. Type 1 cleavage yielded an Fd fragment of the alpha chain of 28,500 dalton and an Fc fragment of 30,500 daltons, whereas Type 2 cleavage was indistinguishable from the pattern observed with *N. gonorrhoeae* enzyme, yielding Fdα and Fcα fragments of 28,000 and 31,000 daltons, respectively. No *N. meningitidis* isolate yielded both cleavage

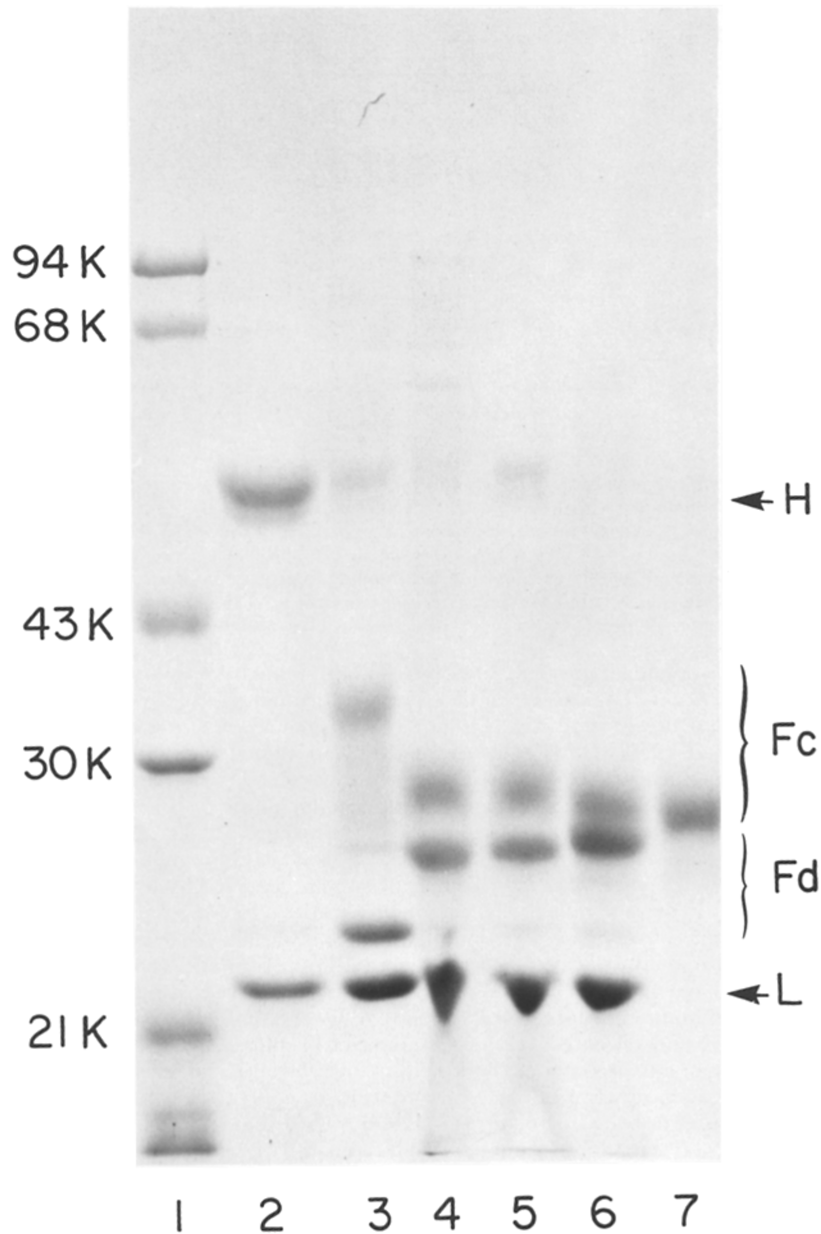

FIG. 2. Meningococcal IgA protease digests of human myeloma IgA1 examined by SDS-polyacrylamide gel electrophoresis. Lane 1, molecular weight standards; 2, IgA1 control, no enzyme added; 3, *S. sanguis* enzyme; 4, *N. gonorrhoeae*; 5, *N. meningitidis* Type 2; 6, *N. meningitidis* Type 1; 7, purified Fcα fragment produced by *N. meningitidis* Type 1 protease. H, IgA1 heavy chain; Fc, Fcα fragment; Fd, heavy chain component of the Fabα fragment; L, light chain. The cleavage pattern of *N. meningitidis* Type 2 protease (lane 5) is indistinguishable from that of *N. gonorrhoeae* (lane 4). In contrast, *N. meningitidis* Type 1 protease (lane 6) yields a larger Fdα and a smaller Fcα than does Type 2. In comparison, *S. sanguis* (lane 3) yields the largest Fcα and the smallest Fdα fragment of all the known IgA proteases. These patterns are consistent with the location of the peptide bonds in the heavy-chain hinge region cleaved by each enzyme.

patterns, and fragments produced by one enzyme did not change size when they were incubated with the other. The Type 1 and 2 patterns were observed with three different IgA preparations and, thus, were independent of the substrate used.

Because Type 1 Fdα was larger and Fcα smaller than Type 2, we concluded that these enzymes cleaved different peptide bonds closely spaced in the IgA1 hinge region. This was confirmed by the limited amino-terminal sequence of the Fcα fragment of Type 1 cleavage, which was -Ser-Pro-Ser-Cys-Cys-His-Pro-Arg-Leu-Ser-Leu-His-Arg-Pro-Ala-Leu-Glu-Asp-Leu-Leu-Leu-Gly-Ser-Glu. The corresponding sequence of the Type 2 Fcα fragment was: Thr-Pro-Ser-Pro-Ser-Cys-Cys-His-Pro-Arg-Leu-Ser-Leu-His-Arg-Pro-Ala-Leu-Glu-Asp-Leu-Leu-Leu. When aligned for complete homology with the IgA1 hinge sequence determined earlier (7, 15, 16), Type 2 cleavage was found to be at the same prolyl-threonyl peptide bond attacked by *N. gonorrhoeae* protease in the right half of the duplicated octapeptide, whereas Type 1 enzyme cleaved at the first prolyl-seryl bond two residues to the right of the Type 2 cleavage site (Fig. 1).

Among 100 *N. meningitidis* strains examined in our laboratory, 98 had IgA protease activity. The cleavage pattern of IgA could be unambiguously assigned as Type 1 in 54% and Type 2 in 46% of the positive strains with both types represented among isolates from cerebrospinal fluid, septicemic blood, and nasopharyngeal carrier strains. As shown in Table I, both Type 1 and 2 protease were found in most serogroups, but Group A organisms yielded only Type 1 and Groups X and Y Type 2 cleavage patterns. During repeated laboratory passage of these strains, we did not observe conversion of one enzyme type to the other. The relationship between both serogroups and serotypes and the type of IgA protease released is being examined in more detail in an ongoing prospective study of meningococcal infection.

Discussion

This study showed that among *N. meningitidis* isolates, there are two forms of IgA protease that were identified by the peptide bonds cleaved in the IgA hinge region. The unambiguous amino-terminal sequence analysis with each Fcα fragment indicates that Type 1 and 2 enzymes each cleave a single and specific peptide bond, and as has been the case with all other studies of IgA protease specificity, proline contributes the

TABLE I

Distribution of IgA Protease Types among Serogroups of N. meningitidis

Serogroup	Number examined	Protease +		Protease −
		Type 1	Type 2	
A	10	10	0	0
B	10	7	3	0
C	10	8	2	0
D	2	2	0	0
X	8	0	7	1
Y	10	0	9	1
Z	10	3	7	0
W-135	10	8	2	0
29-E	10	5	5	0
Non-groupable*	10	5	5	0
Saline Agglutinable‡	10	4	6	0
Total	100	52	46	2

* Non-groupable, organism does not react with any of the known grouping antisera.

‡ Saline agglutinable, organism agglutinates in saline and normal rabbit serum and thus cannot be serogrouped; these are usually isolated from carriers.

carboxyl group to the susceptible linkage. Type 1 and 2 enzymes are mutually exclusive among the *N. meningitidis* strains we have examined because no strain elaborated both enzymes; segregation of enzyme type within certain serogroups is suggested by preliminary data in Table I. Although variability of IgA protease specificity within a species has not been previously documented, a similar situation may also exist in *H. influenzae* strains where three distinct patterns of IgA cleavage by *H. influenzae* proteases have been noted, and these patterns also show a pronounced correlation with serotype (17). The peptide bonds cleaved by each *N. meningitidis* enzyme being two amino acids apart again attests to the extraordinary specificity of the IgA proteases for cleavage of highly restricted regions within the IgA1 hinge segment.

The peptide bonds cleaved by most of the IgA proteases are now known, and in every case the bond is located in the unique IgA1 subclass duplicated octapeptide sequence Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser. It is interesting to note, as shown in Fig. 1, that the eight residues (217–224) preceding the duplication (225–232; 233–240) in the IgA1 alpha chain are Thr-Val-Pro-Cys-Pro-Val-Pro-Ser; therefore, five of the eight amino acids in this sequence are identical to those in the immediately following duplicated octapeptide. Moreover, the serine residue in this segment also bears an *O*-linked oligosaccharide side chain, as do the serines in the duplicated segment. For these reasons, we propose that the IgA hinge region should properly be thought of as triplicated (or alternatively as a four residue fragment duplicated six times) with all known IgA proteases cleaving within the hexadecapeptide 225–240. The sequence stretch 217–224, also present in part in proteins of the IgA2 subclass, is clearly resistant to cleavage, possibly owing to its valine or cystine residues, the relative paucity of carbohydrate, or its proximity to the heavy-chain constant domain. The genetic origin of the hinge region repeating octapeptide subunit in human IgA is unknown. Data on internally deleted human proteins (18) and cloned genes for the murine IgG heavy chain reveal that noncoded DNA segments probably occur on both sides of the hinge region, suggesting that this region is under separate genetic control (19, 20). The specificity of IgA proteases for the human IgA1 hinge peptide, the association of these enzymes with human pathogens within a genus (2–4, 21), and the prominent role of IgA in mucosal immunity at portals of entry for these organisms, continue to provide circumstantial evidence that these enzymes have a role in the infectious process.

Summary

Strains of *Neisseria meningitidis* produce two distinct extracellular IgA proteases that cleave the human IgA1 heavy chain at different points within the hinge region. Type 1 protease cleaves the prolyl-seryl peptide bond at position 237–238; type 2 protease cleaves the prolyl-threonyl bond two residues amino terminal to that bond attacked by type 1 enzyme. Each meningococcal isolate elaborates only one of these two enzymes, and the type of protease produced correlates with certain serogroups: group A yielding only type 1, and groups X and Y only type 2 enzyme. In addition, analysis of amino acid sequences of human alpha-chain proteins reveals that the repeating octapeptide characteristic of the IgA1 hinge region is actually triplicated.

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