Neisseria gonorrhoeae IgA protease. Secretion and implications for pathogenesis

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Abstract. A cloned 5 kb DNA fragment from Neisseria gonorrhoeae strain MS11 promotes expression and excretion of IgA protease in E. coli and other Gram-negative hosts. DNA sequencing reveals a large open reading frame coding for a precursor molecule of 169 kd. The 106 kd mature IgA protease is released from the bacteria in conjunction with a 15 kd soluble precursor segment, the α-protein. In contrast, the carboxy terminal portion of the precursor, the β-protein (45 kd), remains associated with the outer bacterial membrane. The three proteins result form autoproteolytic cleavage at sites in the precursor which are similar to the target site in IgA1. Consensus sequences of the specific cleavage sites are found in a number of relevant human proteins. IgA protease may therefore have other natural substrates besides IgA1. The soluble α-protein as well as the membrane bound β-protein, both associated with IgA protease, may confer additional virulence functions to the gonococcus.

Introduction

IgA proteases are extracellularly secreted by pathogens of the genus Neisseria, Haemophilus, Streptococcus and others (Plaut et al. 1975; Kilian et al. 1979). Non-pathogenic species belonging to these genera do not exhibit IgA protease activity (Mulks et al. 1978). This exclusive association of IgA proteases with human pathogens, in conjunction with the specificity of IgA proteases for human IgA1, strongly suggests an involvement of these enzymes in bacterial virulence.

Several efforts have been made to clone and express IgA proteases of various species in E. coli (Koomey et al. 1982; Bricker et al. 1983; Fishman et al. 1985; Rahr et al. 1985). We also were able to isolate a single gene from N. gonorrhoeae MS11 which directs expression and excretion of IgA protease into the growth medium of the E. coli host (Halter et al. 1984). Sequence analysis of the cloned DNA fragment demonstrated that IgA protease is initially expressed as a precursor of 169 kd MW which is extensively processed in the course of its transport (Pohlner et al. 1987). The precursor can be dissected into three major domains (Fig. 1C), the amino terminal leader peptide of 27 amino acids, the IgA protease portion of 106 kd, and the carboxy terminal helper of about 60 kd in size. Our molecular analyses of the cloned iga gene led to a model for secretion of gonococcal IgA protease (Pohlner et al. 1987) and further allows some speculations on
While purified IgA protease was identified as a 106 kd entity (previously estimated as 105 kd, Halter et al. 1984), two additional forms of the enzyme of 121 kd, and 109 kd, could also be detected in crude culture supernatants of gonococci and E. coli (iga+). The high molecular weight forms are slowly converted into the 106 kd form. This observation led us to test if the precursor of IgA protease is processed autoproteolytically. We incubated a large fusion protein containing most of the enzyme’s precursor (fp170, Fig. 1B) with purified enzyme. This reaction yielded four specific cleavage products of about 95, 60, 45 and 15 kd (Fig. 2). By using specific antisera and monoclonal antibodies directed against distinct regions of the precursor we could identify three internal cleavage sites in the fusion protein fp170 (a, b, and c, Fig. 1C). The precise location of these sites (Fig. 3) was confirmed by amino terminal protein sequencing. Characteristic of these sequences are two proline residues at positions −1 and +2 with respect to the cleaved peptide bond, that are spaced by either a serine or a threonine (Fig. 3A). An exception is the internal target site (c) where an alanine residue separates the two prolines. This site, however, is less efficiently cleaved than sites a and b. Furthermore, every target sequence contains a third proline at position...