CHAPTER 2
Plasminogen and Streptokinase

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A. Primary Structure of Human Plasminogen

The primary structure of human plasminogen (HPg), diagrammed in Fig. 1, has been deduced from the nucleotide sequence of the cDNA (Forsgren et al. 1987) and genomic DNA (Petersen et al. 1990) that encode this protein, and has been directly determined by amino acid sequence analysis (Wiman 1973; Wiman and Wallen 1975; Wiman 1977; Sottrup Jensen et al. 1978). HPg is synthesized as an 810-residue single polypeptide chain. A 19-residue leader peptide is excised during secretion, producing the mature form of HPg, which contains 791 amino acid residues (Forsgren et al. 1987). The only other known processing steps involved in production of plasma HPg are N- and O-linked glycosylation (Hayes and Castellino 1979a, b), and phosphorylation (Wang et al. 1997).

Plasmin (HPm) is formed from HPg as a result of activator-catalyzed cleavage of the Arg^561-Val^562 peptide bond in the zymogen (Robbins et al. 1967). The resulting Glu^1-Pm contains a heavy chain of 561 amino acid residues, originating from the amino-terminus of HPg, disulfide-linked to a light chain of 230 amino acid residues. This latter chain, containing the carboxyl-terminus of HPg, is homologous to serine proteases, such as trypsin and elastase. The heavy and light polypeptide chains of HPm are covalently linked by two disulfide bonds. One such bond bridges Cys^548 of the heavy chain and Cys^666 of the light chain, and another links Cys^558 of the heavy chain and Cys^566 of the light chain. A second functionally-significant hydrolytic reaction, catalyzed by HPm, occurs in HPg between residues Lys^77 and Lys^78, with additional assorted peptide bond cleavages within this 77-amino acid polypeptide, particularly at Lys^62 and Arg^68 (Horrevoets et al. 1995). Hydrolysis of this peptide bond from the amino-terminus of Glu^1-Pg, and/or the amino-terminus of the heavy chain of Glu^1-Pm, provides Lys^78-Pg and Lys^78-Pm, respectively (Violand and Castellino 1976).

The catalytic triad of amino acids that define serine proteases is entirely present within HPm, and involves His^63, Asp^64, and Ser^74. The crystal structure of the Ser741Ala mutant of the catalytic domain has been solved at 2.0 Å resolution. It revealed a deformed catalytic triad and a blocked S1
Fig. 1. Primary structure of human plasminogen. The cleavage sites of the signal peptide between residues -1 and 1, necessary for proper maturation of the plasma protein, and that between residues 77 and 78 required for release of the activation peptide (AP) resulting in the transformation of Glu1-plasminogen (Glu1-Pg) to Lys78 plasminogen (Lys78-Pg), respectively of Glu1-plasmin (Glu1-Pm) to Lys 8- plasmin (Lys8-Pm), and that between residues 561 and 562 required for activation of HPg to HPm (CS), are indicated by filled arrows. Positions of introns in the gene sequence are represented by unfilled arrows. The locations of the N-linked oligosaccharide at sequence position 289 and the O-linked glycan at position 346 are indicated by triangles. Members of the catalytic triad of plasmin consisting of His603, Asp646, and Ser741 are displayed (*). Disulfide bonds are depicted by heavy bars.

Specificity pocket (WANG et al. 2000). One consensus Asn-linked glycosylation sequence, Asn289-Arg-Thr, is present, which in human plasma contains biantennary, bisialylated glycan in approximately one-half of the HPg molecules (HAYES and CASTELLINO 1979a). HPg also contains one site containing O-linked glycan at Thr346 (HAYES and CASTELLINO 1979b) that is occupied on