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Plasmin cleaves fibrinogen and the human complement proteins C3b and C5 in the presence of *Leptospira interrogans* proteins: A new role of LigA and LigB in invasion and complement immune evasion

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ABSTRACT

Plasminogen is a single-chain glycoprotein found in human plasma as the inactive precursor of plasmin. When converted to proteolytically active plasmin, plasmin(ogen) regulates both complement and coagulation cascades, thus representing an important target for pathogenic microorganisms. *Leptospira interrogans* binds plasminogen, which is converted to active plasmin. Leptospiral immunoglobulin-like (Lig) proteins are surface exposed molecules that interact with extracellular matrix components and complement regulators, including proteins of the FH family and C4BP. In this work, we demonstrate that these multifunctional molecules also bind plasminogen through both N- and C-terminal domains. These interactions are dependent on lysine residues and are affected by ionic strength. Competition assays suggest that plasminogen does not share binding sites with C4BP or FH on Lig proteins at physiological molar ratios. Plasminogen bound to Lig proteins is converted to proteolytic active plasmin in the presence of urokinase-type plasminogen activator (uPA). Lig-bound plasmin is able to cleave the physiological substrates fibrinogen and the complement proteins C3b and C5. Taken together, our data point to a new role of LigA and LigB in leptospiral invasion and complement immune evasion. Plasmin(ogen) acquisition by these versatile proteins may contribute to *Leptospira* infection, favoring bacterial survival and dissemination inside the host.

1. Introduction

Plasminogen is a single-chain glycoprotein synthesized as an inactive proenzyme. It is found in plasma and extracellular fluids at concentrations of approximately 180–200 μg/ml (Danø et al., 1985). Proteolytic processing of plasminogen by human urokinase-type (uPA) or tissue-type plasminogen activators generates the trypsin-like serine protease plasmin (Cesarman-Maus and Hajjar, 2005). Bacterial activators, such as staphylokinase and streptokinase can also activate plasminogen (Lähteenmäki et al., 2001, 2005).

Plasmin is a key enzyme in fibrinolysis and homeostasis, degrading extracellular matrix components (ECM) including fibrinogen, vitronectin and laminin (Kost et al., 1996; Lähteenmäki et al., 2000). Plasmin degrades fibrin clots and cleaves the complement proteins C3b and C5, linking two important effector cascades: the coagulation and the complement systems (Pillermer et al., 1953; Seya et al., 1985; Amara et al., 2008; Barthel et al., 2012a).

Several human pathogens acquire plasminogen on their surfaces and use the proteolytic active plasmin to disseminate in host tissues and to evade the immune system. Among these pathogenic microorganisms are *Neisseria meningitidis* (Knaust et al., 2007), *Yersinia pestis* (Hallström et al., 2010), *Staphylococcus aureus* (Kuusela and Saksela, 1990; Koch et al., 2012), *Pseudomonas aeruginosa* (Kunert et al., 2007), *Haemophilus influenzae* (Barthel et al., 2012b), *Candida albicans* (Luo et al., 2009; Poltermann et al., 2007), *Borrelia burgdorferi* (Hallström et al., 2010; Cordes et al.,

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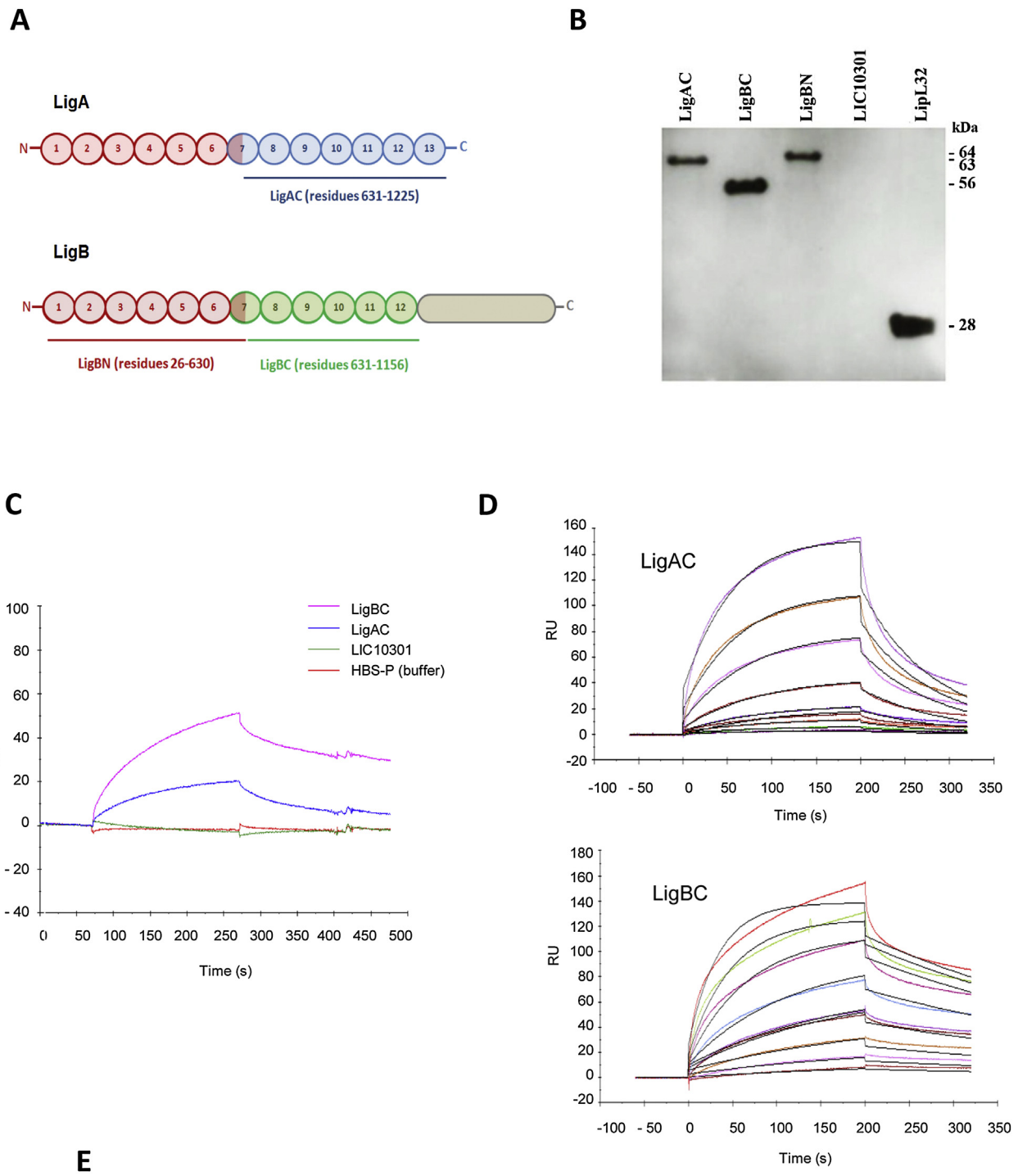

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Analyte	ka (1/Ms)	SE (ka)	kd (1/s)	SE (kd)	K_D (M)
LigAC	1.927×10^4	2.8×10^2	0.02285	3.3×10^{-4}	1.186×10^{-6}
LigBC	3×10^4	67	0.002856	1.5×10^{-5}	9.521×10^{-8}

Fig. 1. Lig proteins interact with plasminogen. (A) Schematic diagram of LigA and LigB proteins with 13 and 12 Big domain repeats respectively. LigAC comprises half of Big domain 7 and Big domains 8–13 of LigA (residues 631–1225); LigBN comprises Big domains 1–6 and half of Big domain 7 of LigB (residues 26–630); LigBC comprises half of Big domain 7 and Big domains 8–12 (residues 631–1156). (B) Purified recombinant proteins LigAC, LigBC and LigBN were subjected to SDS–PAGE and transferred to a nitrocellulose membrane. LipL32 and LIC10301 were also included as positive and negative controls, respectively (Wolff et al., 2013). Purified human plasminogen (10 μg/ml) was added to the membranes and, after washes, bound plasminogen was detected with anti-human plasminogen. (C) Analysis of the interaction with plasminogen by surface plasmon resonance (SPR) using BIAcore T100 system. LigAC, LigBC and LIC10301 (100 nM in HBS-P buffer) were individually injected over