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Epitope mapping of *Burkholderia pseudomallei* serine metalloprotease: identification of serine protease epitope mimics

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Abstract

Filamentous phage random peptide libraries were used to identify the epitopes of *Burkholderia pseudomallei* protease by panning against IgG polyclonal sera that exhibited protease neutralizing properties. The isolated fusion peptides presented a consensus peptide sequence, **TKSMALSG**, which closely resembles part of the active site sequence, 435G**TSMAT**PHVA**G**445, of *B. pseudomallei* serine metalloprotease. By comparing the consensus sequence, TKSMALSG, with the predicted three-dimensional molecular model of *B. pseudomallei* serine metalloprotease, it appears that the potential antibody binding epitope was buried within the molecule. This active site was conformational whereby one continuous sub-region (SMA) was located between two discontinuous sub-regions, supplied by the flanking residues in the same polypeptide. All phages selected from the biopanning with IgG polyclonal sera showed good binding towards the polyclonal antibodies when compared to the negative control. In addition, these peptide-bearing phages showed competitive inhibition of *B. pseudomallei* serine metalloprotease binding to the polyclonal IgG.

Keywords: Epitope mapping; Phage display; *Burkholderia pseudomallei*; Molecular modeling

1. Introduction

Melioidosis is a tropical disease in humans and animals caused by *Burkholderia pseudomallei* that is endemic in Southeast Asia and Northern Australia [1]. The ability of *B. pseudomallei* to produce virulence factors such as exotoxin, phosphatase, hemolysin, lecithinase, lipase and protease [2–4] has been investigated by several groups, but the role of these factors remains unclear. Percheron et al. [5] have identified an exoprotease in *B. pseudomallei* that requires Zn^{2+} for optimal production. Sexton et al. [6] purified and characterized a protease with a molecular mass of 36000 Da from *B. pseudomallei* and the gene encoding this Zn-dependent metalloprotease (ZmpA) has recently been cloned [7]. A novel serine metalloprotease (MprA) from *B. pseudomallei* was also recently reported [8] whereby the protease production is modulated by the PmlI–PmlR quorum sensing system [9]. Sexton et al. [6] also reported that melioidosis patients produce antibodies to *B. pseudomallei* protease indicating that the protease is produced in vivo with the strain lacking the protease producing significantly less lung damage than the parental strain although Valade et al. [9] claim that the MprA protease only plays a minor role in *B. pseudomallei* virulence. Based on these numerous reports on the secretion of one or more proteases from *B. pseudomallei*,

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our group has undertaken to study the structure–function relationship of this protease. We have utilized anti-protease polyclonal antibodies as a tool for studying protease–antibody interactions.

Antibodies play a crucial role in the immune system, especially in defense against extracellular pathogens (such as encapsulated bacteria) and pathogens in extracellular phases of their life cycle (such as viral particles), and as such are a major immune component elicited by all vaccines currently in use for humans. Antibodies also have a variety of uses as biochemical and cellular markers, affinity reagents, diagnostics and therapeutics. Thus, our understanding of the principles governing antibody–antigen interactions is of great significance to a wide spectrum of research and applications.

Antibodies to protein antigens have been widely used in structural and functional analysis. Analyses of antibody–antigen complexes by X-ray crystallography [10,11], chemical modification [12] or deuterium exchange analysis by nuclear magnetic resonance [13] have provided a detailed picture of the amino acids that comprise a given epitope. Nevertheless, to date, few antibody–antigen complexes have been described in great detail because these methods require substantial effort and are technically difficult. Alternative techniques such as phage display have proven to be successful in the determination of continuous and discontinuous antigenic epitopes [14,15]. A library of random peptides displayed on the surface of filamentous phages can be screened for phages that bind to an antibody by a process known as biopanning [16]. This technique has been used in the mapping of critical residues involved at the interface of protein–protein complexes [17] although with limited structural information on the epitopes within the antigen and to identify short peptide mimetics of antigenic epitopes [14].

Polyclonal and monoclonal antibodies would allow for analysis of the structure–function relationship to understand the role of the protease in the pathogenesis of melioidosis. One way to achieve this would be by using the antibodies to epitope map the antigen. In our laboratory, we have generated polyclonal antibodies towards *B. pseudomallei* protease by rabbit immunization and armed with these antibodies, the current study was designed to define and localize the antigenic determinants of the *B. pseudomallei* protease. Determining its epitope(s) should lead to definition of the region within the protease molecule that is involved in the association with antibodies and this will allow for the design of peptides as diagnostic reagents or potential vaccine candidates.

As the three-dimensional (3-D) molecular structure of the *B. pseudomallei* protease has not been determined experimentally, we combined phage-display technology and molecular modeling in an attempt to characterize the molecular surface of the protease that interacts with antibodies. We screened a 12-mer linear peptide library displayed on filamentous phage with anti-protease polyclonal antibodies and three families of peptides that bind to polyclonal antibodies were identified.

2. Materials and methods

2.1. Preparation of antigens and antibodies

B. pseudomallei protease and IgG fractions of polyclonal antibodies specific against *B. pseudomallei* protease used in this study were prepared as described previously [18]. Briefly, protease was purified from brain–heart infusion (BHI, HispanLab, Cuba) broth cultures grown at 37 °C for seven days, following which, cultures were subjected to ammonium sulphate precipitation (70% saturation), DEAE-Cellulose Ion Exchange chromatography (pH 8) and CM-Cellulose Ion Exchange chromatography (pH 6). Chromatographic fractions demonstrating protease activity were pooled and dialyzed. Purified *B. pseudomallei* protease (200 μg) was mixed with 0.5 ml incomplete Freund's Adjuvant. Over a period of two to three months, two New Zealand White rabbits were treated with four subcutaneous injections of 1 ml emulsion samples and administered on days 0, 14, 28 and 42. Antibody titres were monitored by an enzyme-linked immunosorbent assay (ELISA) with peroxidase-conjugated goat anti-rabbit immunoglobulins as the secondary antibody.

2.2. Inhibition of protease activity by polyclonal antibodies

Inhibition of *B. pseudomallei* protease activity was performed according to Percheron et al. [5] with modifications. Sera (1 μg) was added to 1 μg protease and incubated for 30 min at 37 °C. Azocasein (2%) and Tris–HCl (87.5 mM) were added to the incubated mix and further incubated for 5-min intervals up to 30 min. The reactivity was terminated with the addition of tricholoroacetic acid and centrifuged (9000*g*/5 min). The supernatant (120 μl) was added to a microtiter plate well and incubated with 1 N NaOH briefly followed by measuring absorbency at 405 nm. Commercial proteases and protease inhibitors were utilized as controls.

2.3. Biopanning with polyclonal antibodies

The phage 12-mer linear peptide library was obtained from New England BioLabs Inc (USA) and biopanning was conducted essentially as described in the manufacturer's manual. In this library, random peptides are fused to the minor coat protein (pIII) of M13 phage and expressed at the N-terminus of pIII followed by the GGGS spacer. To identify the *B. pseudomallei*