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Immune evasion of the human pathogenic yeast *Candida albicans*: Pra1 is a Factor H, FHL-1 and plasminogen binding surface protein

Shanshan Luo[a], Sophia Poltermann[a], Anja Kunert[a], Steffen Rupp[b], Peter F. Zipfel[a,c,*]

[a] *Department of Infection Biology, Leibniz-Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Germany*
[b] *Fraunhofer Institute for Interfacial Engineering & Biotechnology, Stuttgart, Germany*
[c] *Friedrich-Schiller-University, Jena, Germany*

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ABSTRACT

The pathogenic yeast *Candida albicans* utilizes human complement regulators, like Factor H and Factor H like protein-1 (FHL-1) for immune evasion. By screening a *C. albicans* cDNA expression library, we identified the pH-regulated antigen 1 (Pra1) as a novel Factor H and FHL-1 binding protein. Consequently Pra1 was recombinantly expressed in *Pichia pastoris* and purified from culture supernatant. Recombinant Pra1 binds Factor H, FHL-1 and also plasminogen. Attached to Pra1, the three human proteins are functionally active. Factor H and FHL-1 inactivate complement and plasminogen can be activated to plasmin which then degrades the extra-cellular matrix component fibrinogen. Polyclonal Pra1 anti-serum was generated and used to localize Pra1 on the surface and also in the culture supernatant of both yeast cells and the hyphal form of *C. albicans*. Furthermore Pra1 expression was up-regulated upon induction of hyphal growth. Pra1, released by *Candida* cells binds back to the surface of Candida hyphae and in addition enhances the complement regulatory activity of Factor H in the fluid phase. A Pra1 overexpression strain, with about twofold higher levels of Pra1 on the surface binds more Factor H, and plasminogen. In summary, *C. albicans* Pra1 is a yeast immune evasion protein that binds host immune regulators and acts at different sites. As a surface protein, Pra1 acquires the two human complement regulators Factor H, FHL-1 and plasminogen, mediates complement evasion, as well as extra-cellular matrix interaction and/or degradation. As a released protein, Pra1 enhances complement control in direct vicinity of the yeast and thus generates an additional protective layer which controls host complement attack.

1. Introduction

In the last years, fungal infections have become a major economic and important health problem (de Berker, 2009; Richardson, 2005). *Candida albicans* is the most frequently fungal pathogen isolated from infected individuals and infections with Candida can range from superficial to systemic disorders (Tuomanen, 1996). Despite currently applied anti-fungal therapies, both mortality and morbidity mediated by human pathogens are still unacceptably high (Alonso-Valle et al., 2003; Gudlaugsson et al., 2003; Pappas et al., 2003). Therefore, new prophylactic and therapeutic strategies are urgently needed to prevent fungal infection. The identification of novel targets in form of yeast virulence factors that contribute to pathogenicity is necessary in defining new strategies to fight and interfere with *Candida* infections.

The complement system, which is a central part of the host innate immune defense forms a major and immediately acting barrier for invading microbes and pathogens (Walport, 2001a; Zipfel and Skerka, 2009). Complement is activated by three pathways which differ considerably in the initial steps. The alternative pathway (AP) which is initiated spontaneously and constantly generates C3b molecules which bind directly to any surface. The lectin pathway (LP) is activated upon binding of mannose-binding lectin to mannan-containing structures on microbial surfaces. The classical pathway (CP) is activated via antigen–antibody complexes. All three pathways activate C3 as the central component of the complement cascade, generating C3a and C3b (Walport, 2001a,b; Zipfel et al., 2007a). The cleavage product C3b, binds to microbial surfaces where it acts as opsonin and mediates recognition by host immune effector cells for phagocytosis (van Lookeren Campagne et al., 2007).

On the surface of host cells complement activation is controlled by multiple regulators which are distributed either in the fluid phase or on the surface. Factor H is the major fluid-phase complement regulator that controls alternative pathway activation at the level of C3. The 150-kDa Factor H protein is exclusively composed of 20 repetitive protein domains, termed short consensus repeats

* Corresponding author at: Department of Infection Biology, Leibniz-Institute for Natural Product Research and Infection Biology, Beutenbergstr. 11a, 07745 Jena, Germany. Tel.: +49 03641 532 1300; fax: +49 03641 532 0807.
E-mail address: peter.zipfel@hki-jena.de (P.F. Zipfel).

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(SCRs) (Rodriguez de Cordoba et al., 2004). Factor H is a member of a protein family, which includes the Factor H like protein-1 (FHL-1) which is derived from an alternatively spliced transcript of the Factor H gene, and five Factor H related proteins (CFHRs) that are encoded by separate genes (Zipfel et al., 1999). Factor H and FHL-1 control complement activation by acting as cofactors for the serine protease Factor I, which cleaves C3b into iC3b. In addition, Factor H and FHL-1 compete with Factor B for C3b binding and accelerate the decay of a preformed alternative pathway C3 convertase. Thus, both Factor H and FHL-1 regulate the amplification of complement activation via the alternative pathway by dissociation of C3b from complexes and by displaying cofactor activity in the conversion of C3b to haemolytically inactive degradation products by factor I. Both proteins act in fluid phase in plasma and also on the surface of cells and pathogens (Zipfel et al., 1999; Whaley and Ruddy, 1976).

In order to survive and to establish an infection, pathogens need to inhibit the host complement attack. Apparently pathogens utilize multiple and distinct escape strategies. Human pathogenic fungi, like *C. albicans* and *Aspergillus fumigatus* acquire complement regulators Factor H, FHL-1 and C4BP from human plasma to their surface (Zipfel et al., 2007b; Meri et al., 2002, 2004; Behnsen et al., 2008). Bound to the surface, these human regulators retain complement regulatory functions and inhibit complement activation. Thus, acquisition of human regulators can mask the fungal surface and consequently inhibits and prevents immune attack (Meri et al., 2002, 2004). Furthermore, phosphoglycerate mutase (Gpm1), which is also termed *Candida* CRASP1 (Complement regulator acquiring surface protein) is the first fungal Factor H, FHL-1 binding surface protein identified from *C. albicans* (Poltermann et al., 2007).

Numerous pathogens bind Factor H, FHL-1 and C4BP and utilize these surface bound complement regulators for complement evasion. Such pathogens include Gram-negative bacteria, *Borrelia burgdorferi* (Alitalo et al., 2001; Kraiczy et al., 2001), *Pseudomonas aeruginosa* (Kunert et al., 2007), *Neisseria meningitidis* (Ram et al., 1999), Gram-positive bacteria, like *Streptococcus pyogenes* (Johnsson et al., 1998; Kotarsky et al., 1998), *Staphylococcus aureus* (Haupt et al., 2008), parasites such as *Onchocerca volvulus*, *Echinococcus granulosus* (Diaz et al., 1997), and human viruses like the immunodeficiency *virus* and *West nile virus* (Stoiber et al., 1997; Diamond et al., 2009). For some of these pathogens, the binding proteins for the human regulators have been identified, such as CRASPs of *B. burgdorferi* (Kraiczy et al., 2003; Hartmann et al., 2006; Cordes et al., 2006), Tuf of *P. aeruginosa* (Kunert et al., 2007), the M protein of *S. pyogenes* (Blackmore et al., 1998) and Sbi of *S. aureus* (Haupt et al., 2008).

The pH-regulated antigen 1 (Pra1) of *C. albicans* is composed of 299 amino acids and has a predicted molecular mass of 31 kDa (Sentandreu et al., 1998). Pra1 was originally identified as a fibrinogen binding protein (Casanova et al., 1992). Here we identify *Candida* Pra1 as a new Factor H, FHL-1 and plasminogen binding protein which functions at different sites. As a surface protein, Pra1 acquires the human proteins onto the yeast surface, masks the fungal surface and therefore aids in immune and complement evasion, as well as the degradation of the extra-cellular matrices. Pra1 is also released into the fluid phase where Pra1 enhances Factor H-mediated complement inactivation in direct surrounding of yeast cells.

2. Materials and methods

2.1. C. albicans strains and growth conditions

The *C. albicans* wild type strains SC5314 (Fonzi and Irwin, 1993) CA14 and a Pra1 overexpression strain on a CA14 background (manuscript in preparation) were cultivated in YPD medium (2% (w/v) glucose, 2% (w/v) peptone, 1% (w/v) yeast extract) at 30 °C. Hyphal growth was induced in RPMI 1640 liquid medium (BioWhittaker, Lonza) by temperature shift from 30 to 37 °C for 1.5 h. Yeast cells were collected by centrifugation and counted with a hemocytometer (Fein-Optik, Bad Blankenburg, Germany).

2.2. Antibodies and proteins

Polyclonal Pra1 anti-serum was raised by immunizing rabbits with purified recombinant Pra1. Alexa Fluor®-488 or 647 labeled goat anti-rabbit, Alexa Fluor®-647 labeled rabbit anti-goat and Alexa Fluor®-488 labeled rabbit anti-mouse (Molecular Probes) were used as secondary anti-sera for flow cytometry or confocal microscopy. Anti-sera against goat-, mouse-, and rabbit IgG that were raised in rabbits or swine (pigs) and conjugated with horseradish peroxidase-conjugated were obtained from Dako (Glostrup, Denmark). A monoclonal mouse His antibody was purchased from Qiagen (Hilden, Germany). Polyclonal goat anti-Factor H (Calbiochem) and a polyclonal goat anti-plasminogen (Acris, Hiddenhausen, Germany) were used for assaying Factor H and plasminogen binding. Factor H, Factor I and C3b were obtained from Calbiochem, uPA was purchased from Chemicon (Hofheim, Germany), plasminogen from Chromogenix (Milano, Italy) and BSA was obtained from Sigma.

2.3. Screening of cDNA library

A cDNA (λgt11) library generated from *C. albicans* cDNA was plated and screened for Factor H binding proteins (Cha et al., 1997). Two positive clones were identified and isolated. The inserts were amplified by PCR, the nucleotide sequence was determined.

2.4. Expression and purification of recombinant proteins

The *C. albicans PRA1* gene was amplified by PCR using genomic DNA from strain SC5314 and primers S1 (5′-GGGAATTCGGATGAATTATTTATTGTTTTGTT-3′) and S2 (5′-CGTCTAGAATACAGTGGACTCACCATCTGCA-3′). EcoRI and XbaI restriction sites are underlined. The resulting DNA fragment contained the complete *CaPRA1* coding region which was flanked by EcoRI and XbaI restriction sites. Following restriction digest, this DNA fragment was sub-cloned into *Escherichia coli* cloning vector pCR4Blunt-TOPO (Invitrogen), amplified, isolated and subsequently cloned into the EcoRI and XbaI sites of the *Pichia pastoris* vector pPICZαB (Invitrogen). Pra1 was recombinantly expressed as a His-tagged protein in *P. pastoris* strain X33. Protein expression was induced with 1% methanol. After 3 days of expression, the culture supernatant was harvested.

FHL-1 (SCRs 1–7) and recombinant deletion constructs of Factor H (SCRs 1–5, SCRs 1–6, SCRs 8–11, SCRs 11–15, SCRs 15–18 and SCRs 19–20) were expressed in the baculovirus system as described (Kuhn and Zipfel, 1995). All recombinant proteins were purified by nickel affinity chromatography using HisTrap columns in an Äkta FPLC system (GE Healthcare, Freiburg, Germany) and concentrated using Centricon® Plus-20 concentrators with a cut off of 10 kDa (Millipore).

2.5. ELISA

Pra1 or various Factor H deletion mutants (0.5 μg in carbonate-bicarbonate buffer) was immobilized onto a microtiter plate (MaxiSorb, Nunc) at 4 °C overnight. After washing, nonspecific binding sites were blocked with DPBS containing 1% BSA (Sigma) for 2 h at room temperature (RT). Then Factor H, FHL-1, plasminogen or Pra1 was added (1 μg/well) and the mixture was incubated for 1.5 h at RT. Wells were washed with DPBS-T buffer (DPBS contain-