Gpm1p Is a Factor H-,-, FHL-1-, and Plasminogen-binding Surface Protein of Candida albicans*

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The human pathogenic yeast Candida albicans utilizes host complement regulators for immune evasion. Here we identify the first fungal protein that binds Factor H and FHL-1. By screening a protein array of 4088 proteins of Saccharomyces cerevisiae, phosphoglycerate mutase (ScGpm1p) was identified as a Factor H- and FHL-1-binding protein. The homologous C. albicans Gpm1p (CaGpm1p) was cloned and recombinantly expressed as a 36-kDa His-tagged protein. Purified CaGpm1p binds the host complement regulators Factor H and FHL-1, but not C4BP. The CaGpm1p binding regions in the host proteins were localized; FHL-1 binds via short consensus repeats (SCRs) 6 and 7, and Factor H utilizes two contact regions that are located in SCRs 6 and 7 and in SCRs 19 and 20. In addition, recombinant CaGpm1p binds plasminogen via lysine residues. CaGpm1p is a surface protein as demonstrated by immunostaining and flow cytometry. A C. albicans gpm1Δ/Δ mutant strain was generated that did not grow on glucose-supplemented but on ethanol- and glycerol-supplemented medium. Reduced binding of Factor H and plasminogen to the null mutant strain is in agreement with the presence of additional binding proteins. Attached to CaGpm1p, each of the three host plasma proteins is functionally active. Factor H and FHL-1 show cofactor activity for cleavage of C3b, and bound plasminogen is converted by urokinase-type plasminogen activator to proteolytically active plasmin. Thus, the surface-expressed CaGpm1p is a virulence factor that utilizes the host Factor H, FHL-1, and plasminogen for immune evasion and degradation of extracellular matrices.

Candida albicans is the most important human pathogenic yeast and causes disseminated infections (1, 2). The yeast form represents a common saprophyte in healthy individuals, which resides mainly on the skin, oral cavity, urogenital, and gastrointestinal tracts. In addition, C. albicans can cause systemic infections predominantly in immunocompromised or granulocytopenic patients (3). C. albicans systemic infections, which are difficult to diagnose and treat, can be lethal (4). Virulence of C. albicans is based on its ability to bind to host cells (5). Several secretory proteolytic enzymes are involved in tissue invasion of C. albicans (6). In addition, morphogenetic transition from yeast to hyphal forms correlates with the infection process and causes adherence to host cells and tissue penetration (7).

As a commensal, C. albicans has evolved highly effective strategies of immune evasion to survive in the host (8). The complement system represents an important part of host innate immunity. Four activation pathways have been described. The alternative pathway, which is initiated spontaneously, and by default, the lectin and classical pathway, which are triggered by antibodies or carbohydrates, are relevant for the recognition and elimination of microbes (9). The role of the recently described thrombin pathway (10) for immune defense needs to be worked out. C. albicans activates both the alternative and classical pathways of complement (11). C3b molecules bind directly to the C. albicans surface or via mannan-specific IgG antibodies, which occur naturally in human serum (12).

Upon entry into a human host, any microbe is attacked by the host complement system. However, pathogenic microbes control complement activation at their surface. This type of complement escape is mediated either by the acquisition of host regulators to the surface of the pathogen or by expression of endogenous complement regulators (13). An increasing number of pathogenic microbes utilize host complement regulators for immune evasion and for down-regulation of complement activation. For the yeast C. albicans, acquisition of the central fluid phase alternative pathway regulators Factor H and FHL-1 and the classical pathway regulator C4BP have been demonstrated (14, 15). Binding of host complement regulators such as Factor H, FHL-1, and C4BP was also shown for Gram-negative bacteria, such as Borrelia burgdorferi (16, 17), Neisseria gonorrhoeae (18), Neisseria meningitidis (19), Gram-positive bacteria, like Streptococcus pyogenes (20, 21), Streptococcus pneumoniae (22), and parasites including Onchocerca volvulus, Echinococcus granulosus (23), and the human immunodeficiency virus (24). In their bound configuration these host proteins maintain their regulatory activities and protect the microbes against complement-mediated phagocytosis and lysis. For several pathogens the ligands for the host regulators have been identified and include classical virulence factors like the M protein of S. pyogenes. Factor H- and FHL-1-binding proteins of C. albicans have been proposed (13), but so far these proteins have not been isolated. Here we identify the phosphoglycerate mutase Gpm1p of C. albicans (CaGpm1p) as the first fungal Factor H- and FHL-1-binding protein of yeast.
A Complement Regulator-binding Protein from \textit{C. albicans}

**EXPERIMENTAL PROCEDURES**

*Strains and Growth Conditions of \textit{C. albicans}—The \textit{C. albicans} strains used in this study are listed in Table 1. Strains were grown in YPD medium (2% (w/v) glucose, 2% (w/v) peptone, 1% (w/v) yeast extract) or YPGE medium (3% (w/v) glucose, 2% ethanol (w/v), 2% (w/v) peptone, 1% (w/v) yeast extract) at 30 °C. Cells were counted with a hemocytometer (Fein-Optik, Bad Blankenburg, Germany). Hyphal growth was induced in liquid culture by a temperature shift from 30 to 37 °C.*

*Antibodies and Proteins—For specific detection of Factor H and FHL-1 in the protein array, monoclonal antibodies B22 and L20 (25) (directed against SCR 5 and SCR 19 of Factor H) were labeled with Alexa Fluor® 647 (Molecular Probes, Eugene, OR) according to the manufacturer’s instructions. Horseradish peroxidase-conjugated rabbit anti-goat antisera and horseradish peroxidase-conjugated rabbit anti-mouse antisera as well as horseradish peroxidase-conjugated swine anti-rabbit antisera were obtained from Dako (Glostrup, Denmark). Polyclonal goat anti-Factor H antisera (Calbiochem) and a polyclonal goat anti-plasminogen antisera (Acris, Hiddensen, Germany) was used for ELISA.3 Polyclonal antibodies against CaGpm1p were raised by immunizing rabbits with purified recombinant CaGpm1p. Alexa Fluor® 488-labeled goat anti-rabbit antisera (Molecular Probes), used for fluorescence microscopy, and fluorescein isothiocyanate-labeled swine anti-rabbit antisera (Dako, Glostrup, Denmark), used for flow cytometry, were obtained from Molecular Probes. A monoclonal mouse antibody directed against human γ tubulin (kindly provided by Peter Hemmerich, Jena, Germany) was used for detection of γ tubulin. For detection of C3b degrada
tions products, a polyclonal goat anti-C3 antiserum (Calbio-
chem) was used. Recombinant CaGpm1p was detected with PentaHis antisera (Qiagen, Hilden, Germany). Factor H, Factor I, and C3b were obtained from Calbiochem, uPA was from Chemicon (Hofheim, Germany), and plasminogen was from Chromogenix (Milano, Italy).

*Expression of Recombinant Proteins—The \textit{C. albicans} phosphoglycerate mutase gene \textit{CaGPM1} was amplified by PCR using genomic DNA from strain SC5314 and primers S1 (5'-GAAATTCTGTAGCAAGTTGTTTATG-3') and S2 (5'-TCTAGATATTTCTTTTGACCTTGAGCAG-3'). EcoRI and XbaI restriction sites are underlined. The resulting 760-bp DNA fragment contained the complete \textit{CaGPM1} coding region flanking an additional EcoRI and a XbaI restriction site. The DNA fragment was subcloned into \textit{Escherichia coli} cloning vector pCR4Blunt-TOPO (Invitrogen) and subsequently cloned into the EcorI and XbaI sites of the \textit{Pichia pastoris} vector pPICZαB (Invitrogen). CaGpm1p was recombinantly expressed as a His-tagged protein in \textit{P. pastoris} strain X33. Protein expression was induced with 1% methanol. After 3 days of expression the culture supernatant was harvested, and recombinant CaGpm1p was precipitated with 80% ammonium sulfate.*

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3 The abbreviations used are: ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; RT, room temperature; BSA, bovine serum albumin; SCRs, short consensus repeats; uPA, urokinase-type plasminogen activator; eACA, ε-aminocaproic acid.
bicans cells were cultivated in Soll’s medium (27), pH 4.5, at
30 °C overnight followed by growth in Soll’s medium, pH 6.5, at
37 °C for 45 min. The cells were harvested by centrifugation and
stored at −20 °C. After resuspending the pellet in PBS supple-
mented with 25 mM DTT and 12.5 mM phenylmethylsulfonyl
fluoride (final concentrations), the cells were lysed by glass
beads in a Mini-BeadBeater (Biospec Products) followed by
three freeze and thaw cycles. For the isolation of cytoplasmic
proteins, cell lysate of the C. albicans null mutant and the wild
type strain was centrifuged, and the supernatant was collected.
Cell debris was resuspended in PBS and again centrifuged. This
step was repeated 10 times. Proteins derived from cell wall pel-
let were extracted by reducing loading buffer Roti-Load1 (Roth,
Karlsruhe, Germany) incubated at 99 °C for 5 min and then
separated by SDS-PAGE.

Cofactor Assay—Cofactor activity of CaGpm1p-bound com-
plement regulators Factor H and FHL-1 was performed as
described (15). CaGpm1p was coated to the surface of a micro-
titer plate (half-area plate, Corning), and Factor H (0.2 μg/well)
or FHL-1 (0.2 μg/well) was bound to this matrix. After exten-
sive washing with PBS, C3b (0.4 μg/well) and Factor I (0.8 μg/well) were added. After the incubation at 37 °C the super-
natant was separated by SDS-PAGE, and C3 degradation prod-
ucts were analyzed by Western blot using an anti-C3 antisemur
and a horseradish peroxidase-conjugated anti-mouse serum as
secondary antibody.

Disruption and Reintegration of CaGPM1 Gene—The
CaGPM1 gene was disrupted using the hisg-URA3-hisG cassette (28). The 5′ region of CaGPM1 was amplified by PCR using chromosomal DNA of C. albicans SC5314 as a
template and primers SP1-Sacl (5′-ATATATCGAAGACTG-
TTAGATCACCTTATCTCCTCAAG-3′, position −354 to −330) and SP2-KpnI (5′-ATATATCGCGT GTAATGGTTAGTTGCT-3′, position +868 to +891) and primer SP7 PstI (5′-ATATAATG
ACTGCAATAATGGTTAGCTAATATACC-3′, position +1200 to +1176) were used to amplify the downstream
region. This resulting 332-bp product was digested with Sall
and PstI and cloned into plasmid pG2, yielding pG3. CaGPM1
was reintroduced into the null mutant strain CAP4 by transfor-
mation with the 5.6-kilobase Sacl/PstI insert of pG3 that har-
bors the CaGPM1 gene, upstream as well as downstream
regions for homologous recombination, and the URA3 gene as
a selectable marker, and yielded CAP5.

Candida ELISA—For Candida ELISAs, C. albicans yeast
cells from an overnight culture were washed with PBS, diluted
into carbonate-bicarbonate buffer (Sigma) to 1 × 107 cells/ml
and immobilized onto a microtiter plate (MaxiSorb, Nunc) at
4 °C overnight. After washing with PBS-T buffer once, nonspec-
ific binding sites were blocked with PBS containing 0.05% Tween 20 (Block-T) and
immobilized onto a microtiter plate (MaxiSorb, Nunc) at
4 °C overnight. After washing with PBS-T buffer once, nonspec-
ific binding sites were blocked with PBS containing 2% BSA, plasminogen (0.6
μg/well) in 0.32M Tris-HCl, 1.77 M NaCl, pH 7.5, were
used to amplify the 3′ region. The 334-bp product was digested with Sall and PstI and
cloned into plasmid pG0, resulting in pG1. For gene disruption,
plasmid pG1 was treated with Sacl and PstI. The 3.3-kilobase
Sacl/PstI fragment of plasmid pG1 containing the URA1-blaster
flanked by short sequences from the 5′ and 3′ ends of CaGPM1
and portions of the promoter and terminator, respectively, was
used to transform C. albicans Ura− strain CAI-4. Transforma-
tion was performed in the presence of lithium acetate. After
selection on Sabouraud (1% (w/v) peptone (casein), 2% (w/v)
glucose) medium, the resulting Ura− transformants were ex-
amined for gene replacement by PCR and Southern analysis.
In the first step one allele of CaGPM1 was replaced by the hisg-
URA3-hisG cassette (CAP1). Strain CAP1 was plated on 5-fluo-
roorotic acid-containing medium for isolation of Ura− seg-
regants (CAP2). A second transformation with the same
disruption construct led to the isolation of a CaGpm1 null
mutant (CAP3) on Sabouraud-GE (1% (w/v) peptone (casein),
3% (w/v) glycerol, 2% (w/v) ethanol) plates. Again, Ura− seg-
regants were selected (CAP4). For homologous reintegration of
the CaGPM1 gene, CaGPM1 was amplified by PCR with primer
SP1-Sacl (5′-ATATATCGAAGCTGTTAGATCACCTTT-
TACCTCAAG-3′, position −354 to −330) and SP5-PstI (5′-
ATATATCGCGT GTAATGGTTAGTTGCT-3′, position +868 to +891) and primer SP7 PstI (5′-ATATAATG
ACTGCAATAATGGTTAGCTAATATACC-3′, position +1200 to +1176) were used to amplify the downstream
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mation with the 5.6-kilobase Sacl/PstI insert of pG3 that har-
bors the CaGPM1 gene, upstream as well as downstream regions for homologous recombination, and the URA3 gene as
a selectable marker, and yielded CAP5.

Activation of Plasminogen and Assaying Plasmin Activity—
Plasmin activity was measured as described (29). Briefly,
recombinant CaGpm1p (0.25–2 μg) was immobilized onto the
surface of a microtiter plate (half-area plate, Corning).
After blocking with PBS containing 2% BSA, plasminogen (0.6
μg/well) was added for 2 h at RT. Unbound plasminogen was
removed by washing with PBS/substrate buffer. Plasminogen
activator uPA (4 ng/well) and chromogenic substrate S-2251
(o-tyl-tycyl-lysyl-nitroanilide dihydrochloride, Sigma; 150
μg/well) in 0.32 M Tris-Cl, 1.77 M NaCl, pH 7.5, were
added. Plasmin activity was measured at 37 °C in intervals of 30
min by recording the absorbance at 405 nm in an ELISA plate
reader (SpektraMax 190, Molecular Devices).

RESULTS

Identification of ScGpm1p as a Factor H- and FHL-1-binding
Protein—We have previously shown that host complement reg-
ulators Factor H and FHL-1 bind to the pathogenic yeast C. al-
bicans (15). To identify yeast proteins that bind Factor H and
FHL-1, a protein microarray representing 4088 recombinant proteins of the related yeast *S. cerevisiae* was used, and four Factor H-binding (YDR047W, YKL152C, YGR191W, YBL024W) and three FHL-1-binding (YDR047W, YKL152C, YBL024W) proteins were identified. All three FHL-1-binding proteins do also bind Factor H, and YGR191W binds specifically Factor H but not FHL-1. Spot YKL152C, which bound both Factor H and FHL-1, represents the phosphoglycerate mutase ScGpm1p (Fig. 1A). The *C. albicans* homologue CaGpm1p showed the highest homology to the *S. cerevisiae* protein (78%). Because this protein was recently identified as a plasminogen-binding protein (30), it was selected for further analyses.

To confirm this interaction, the Gpm1p of *C. albicans* was cloned and recombinantly expressed in the yeast *P. pastoris*. The culture supernatant and various fractions obtained by nickel chelate chromatography were separated by SDS-PAGE and analyzed by silver staining (Fig. 1B). Two prominent bands of 36 and 31 kDa were detected in the supernatant and the elute fraction. Western blotting with α-His antisera identified the 36-kDa band as the recombinant CaGpm1p protein (Fig. 1B, lane 5), suggesting that the second 31-kDa band represents a degradation product that lacks the His tag.

**CaGpm1p Binds Human Plasma Proteins**—Binding of Factor H and FHL-1 to recombinant CaGpm1p was assayed by ELISA. CaGpm1p was immobilized to the microtiter plate, and purified Factor H and FHL-1 were added. Both immune regulators bound to CaGpm1p. CaGpm1p was recently identified as a plasminogen-binding protein (30), and this interaction was confirmed for the recombinant yeast protein. The classical pathway regulator C4BP did not bind to immobilized CaGpm1p (Fig. 2). Factor H and FHL-1 as well as plasminogen bound to recombinant immobilized CaGpm1p.

**Mapping of the Binding Regions in Factor H and FHL-1**—To localize binding sites for CaGpm1p within the two host regulators, recombinant deletion constructs of FHL-1 and of Factor H representing SCRs 1–5, SCRs 1–6, SCRs 1–7/FHL-1, SCRs 8–11, SCRs 11–15, SCRs 15–18, and SCRs 19 and 20 (26) were tested for CaGpm1p binding. FHL-1 (SCRs 1–7) and SCRs 1–6 did bind to immobilized CaGpm1p, but SCRs 1–5 showed rather weak binding. In addition, recombinant deletion constructs of Factor H SCRs 19 and 20 did clearly bind, whereas SCRs 15–18 bound to a smaller extent. Constructs SCRs 8–11 and SCRs 11–15 did not bind (Fig. 3A). Based on these results it is concluded that FHL-1 has one interacting region with CaGpm1p, located in SCRs 6 and 7 (region I). Factor H has two binding regions, one shared with FHL-1 and a second region (region II) located in the C terminus, i.e. SCRs 19 and 20 (Fig. 3B). In the ELISA assays region I binds with higher intensity than region II.

**Lysine-dependent Binding of Plasminogen to CaGpm1p**—To characterize the CaGpm1p plasminogen interaction in more detail we analyzed the role of the lysine analogue εACA. εACA inhibited plasminogen binding to CaGpm1p in a dose-dependent manner. Inhibition of about 20% was observed with 0.1 mM εACA, and maximal inhibition (>90%) was observed with 10 mM of the inhibitor (Fig. 4). Thus,
binding of plasminogen to CaGpm1p is mediated by lysine residues.

CaGpm1p Is a Surface Protein of C. albicans—To confirm CaGpm1p expression on the surface of intact C. albicans, an antiserum was raised against the recombinant yeast protein. Confocal laser scanning microscopy showed expression of CaGpm1p on the surface of intact C. albicans yeast cells (Fig. 5A, upper left panel). Staining was particularly prominent on the tip of the hyphae (Fig. 5A, lower left panel). In addition, surface localization of CaGpm1p is demonstrated by flow cytometry (Fig. 5B). Furthermore, the native 27.5-kDa CaGpm1p protein was identified in the cytoplasmic fraction and also in a cell wall extract by Western blotting using the specific antiserum (Fig. 5C, lanes 1 and 2). Preimmune serum showed no reactivity (data not shown). To exclude contamination of the cell wall fraction with intracellular proteins, the presence of the intracellular protein γ-tubulin was assayed. Staining for the 53-kDa γ-tubulin is detected in the cytoplasmic but not in the cell wall fraction (Fig. 5C, bottom panel), thus, demonstrating that the cell wall extract is free of major cytoplasmic contaminants. In summary, three independent methods show expression of CaGpm1p at the surface of C. albicans.

Generation of a C. albicans gpm1 Null Mutant Strain—To verify the role of CaGpm1p in the binding of Factor H, FHL-1, and plasminogen to C. albicans, a Cagpm1p knockout mutant was generated using the Ura-blaster method (Table 1). The knock-out strain did not grow on glucose-supplemented YP medium but did grow when both ethanol and glycerol were applied as carbon sources. In addition, Southern blotting (data not shown) and cytoplasmic and cell wall fractions were generated from heterozygous (CAP1), homozygous mutants (CAP3), and a revertant strain (CAP5). Staining of these preparations for CaGpm1p revealed reduced protein levels in the heterozygote and the revertant strains and the complete absence in the knock-out strain (Fig. 6A).
A Complement Regulator-binding Protein from C. albicans

TABLE 1

| Strain or plasmid | Genotype or description | Ref. or source |
|------------------|-------------------------|---------------|
| SC5314           | Wild-type               | 28            |
| CAI-4            | Δura3::imm434/Δura3::imm434, isogenic to SC5314 | 28            |
| CAP1             | Δgpm1::hisG-URA3::hisG/GPM1, Δura3::imm434/Δura3::imm434 | This work     |
| CAP2             | Δgpm1::hisG/GPM1, Δura3::imm434/Δura3::imm434 | This work     |
| CAP3             | Δgpm1::hisG/GPM1:hisG-URA3::hisG, Δura3::imm434/Δura3::imm434 | This work     |
| CAP4             | Δgpm1::hisG/GPM1:hisG, Δura3::imm434/Δura3::imm434 | This work     |
| CAP5             | Δgpm1::hisG/GPM1::hisG-URA3::hisG, Δura3::imm434/Δura3::imm434 | This work     |
| pMB7             | C. albicans gene disruption vector | 28            |

Figure 6. Binding of Factor H and plasminogen to C. albicans wild type strain SC5314 and CaGPM1 mutant strains. A, CaGpm1p levels in C. albicans mutant strains. Cytoplasmic (CYT) and cell wall (CW) extracts derived from the wild type (lanes 1 and 2), the heterozygous mutant (CAP1) (lanes 3 and 4), the homozygous mutant (CAP3) (lanes 5 and 6), and the revertant strain (CAP5) (lanes 7 and 8) were separated by SDS-PAGE and transferred to a membrane, and the presence of CaGpm1p was analyzed with the CaGpm1p specific antiserum. The wild type and indicated C. albicans mutant strains were immobilized on to the surface of a microtiter plate and incubated with NHS. After washing bound Factor H (B) or plasminogen (C) was identified using specific antisera. The bars represent the mean values of three independent experiments, and S.D. are indicated. OD, optical density.

DISCUSSION

The human pathogenic yeast C. albicans utilizes host complement regulators for immune evasion. Here, we identify the first fungal protein that binds the host immune regulators Factor H and FHL-1. By screening a protein array of S. cerevisiae, phosphoglycerate mutase (ScGpm1p) was identified as a Factor H- and FHL-1-binding protein. The homologous C. albicans Gpm1p was cloned and recombinantly expressed. The purified CaGpm1p binds specifically Factor H and FHL-1 but not C4BP. The CaGpm1p binding sites were identified in the two host proteins. FHL-1 binds to CaGpm1p via SCRs 6 and 7, and Factor H utilizes two binding regions that are located in SCRs 6 and 7, and in SCRs 19 and 20. In addition, recombinant CaGpm1p binds plasminogen via lysine residues. CaGpm1p is a surface protein and was identified in the cell wall fraction by Western blotting and by immunostaining of intact C. albicans yeast cells and hyphae using a specific antiserum. Attached to CaGpm1p, each of the three host plasma proteins is functionally active. Factor H and FHL-1 show cofactor activity for cleavage of C3b, and α’43 kDa (Fig. 7, lanes 1 and 3). The same cleavage products were generated when Factor H was directly coated and used as cofactor for C3b degradation (Fig. 7, lane 5). These results show that Factor H and FHL-1 attached to Gpm1p of C. albicans maintain complement regulatory function and mediate cofactor activity.

Plasminogen Bound to CaGpm1p Is Converted to Proteolytically Active Plasmin—In addition, we assayed if plasminogen bound to CaGpm1p is converted to the functional protease plasmin. Plasminogen was attached to CaGpm1p and treated with the activator uPA, and the capacity of the activated plasmin to cleave the chromogenic substrate S-2251 was assayed. This activity was dose-dependent and correlated with the amount of immobilized CaGpm1p (Fig. 8). Thus, plasminogen attached to CaGpm1p is accessible for the activator uPA, and the activated plasmin retains its proteolytic activity.

Binding of Factor H and Plasminogen from Normal Human Serum to C. albicans SC5314 and GPM1 Mutant Strains—When the mutant strains were analyzed for Factor H and plasminogen binding, no significant reduction in attachment was observed for both plasma proteins in the knock-out strain (CAP3) (Fig. 6, B and C). The reduction but not the complete loss of binding by ~6% (Factor H) and 10% (plasminogen) is explained by the presence of additional Factor H- and plasminogen-binding surface proteins.

Factor H and FHL-1 Bound to CaGpm1p Maintain Complement Regulatory Activity—To assess whether Factor H and FHL-1 bound to CaGpm1p maintain complement regulatory function, cofactor activity of the CaGpm1p-bound regulators was analyzed. Factor H and FHL-1 were attached to immobilized CaGpm1p and C3b together with factor I were added. After incubation for 60 min, the supernatants were separated by SDS-PAGE, and C3b degradation products were identified by Western blotting. Cofactor activity of the bound regulator is revealed by the appearance of cleavage products of α’68, α’46 and α’36 kDa (Fig. 7, lanes 1 and 3). The same cleavage products were generated when Factor H was directly coated and used as cofactor for C3b degradation (Fig. 7, lane 5). These results show that Factor H and FHL-1 attached to Gpm1p of C. albicans maintain complement regulatory function and mediate cofactor activity.
A Complement Regulator-binding Protein from C. albicans

The phosphoglycerate mutase Gpm1p was initially identified as a cytoplasmic glycolytic protein regulating the catalytic conversion of 3-phosphoglycerate to 2-phosphoglycerate during glycolysis and the reverse reaction in gluconeogenesis. The phosphoglycerate mutase of C. albicans has a predicted molecular mass of 27.5 kDa and lacks apparent signal and transport sequences. C. albicans Gpm1p is located in the cytoplasm and bound plasminogen is converted by uPA to proteolytically active plasmin. Thus, surface-expressed CaGpm1p acquires the host complement regulators Factor H, FHL-1, and the protease precursor plasminogen. This acquisition and surface decoration leads to immune evasion and degradation of extracellular matrices.

The dose-dependent inhibition with the lysine analogue eACA suggests that lysine residues are relevant for the interaction between plasminogen and CaGpm1p (Fig. 4). At present several plasminogen-binding surface proteins, all secreted by non-conventional routes, have been identified for C. albicans such as glyceraldehyde-3-phosphate dehydrogenase, alcohol dehydrogenase, catalase, and thioredoxin peroxidase (39).

Plasminogen bound to CaGpm1p is accessible for the activator uPA, is converted to the active serine protease plasmin, and bound plasminogen is converted by uPA to proteolytically active plasmin. Thus, surface-expressed CaGpm1p acquires the host complement regulators Factor H, FHL-1, and the protease precursor plasminogen. This acquisition and surface decoration leads to immune evasion and degradation of extracellular matrices.

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which in its bound form cleaves the chromogenic substrate S-2251 (Fig. 8). The serine protease plasmin is a key enzyme of intravascular fibrinolysis and displays extravascular functions in the degradation of extracellular matrix components such as laminin, vitronectin, and fibronectin (40). Thus, pathogens bind plasminogen to their surface and activate plasmin to degrade host extracellular matrix. Because endothelial cells secrete the plasminogen activator tissue plasminogen activator, the direct contact with these host cells results in the activation of attached plasminogen at the fungal surface (40).

Apparently, *C. albicans* utilizes host plasma proteins for multiple purposes. Attached to the surface of *C. albicans*, host complement regulators such as Factor H and FHL-1 control complement activation and inhibit the generation of toxic C3 activation products. In addition, surface-attached plasminogen can be converted to proteolytic active plasmin. Thus, CaGpm1p represents one single multifunctional virulence factor of *C. albicans* that mediates immune evasion (inhibition of complement activation) and degradation of extracellular matrix components. This combination reveals a strategy of how the human pathogenic yeast survives in the human host and how it mediates tissue invasion.

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