Molecular analysis of candida albicans Secreted Aspartyl Proteinase 3 (SAP3) Indonesian isolate

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Abstract. Secreted aspartyl proteinase 3 (SAP3) is one of the important virulence factor of Candida albicans, an opportunistic fungal pathogen responsible for superficial and life-threatening infections in humans. SAP3 also contribute to the pathogenicity of Candida albicans; therefore, become a target for therapy and vaccine development. However, data of the Candida albicans SAP3 protein from Indonesian isolate is limited. The present study aimed to molecular analysis the Candida albicans SAP3 cloned from Indonesian isolate. To study Candida albicans SAP3 protein from Indonesian isolate, a Candida albicans genomic DNA was isolated from a Javanese HIV/AIDS patient. A complete coding sequence of Candida albicans SAP3 gene was cloned and sequenced. The sequencing results were subjected to bioinformatics analysis. Physicochemical analysis revealed the molecular weight (Mw), estimated half-life, instability index, isoelectric point, aliphatic index, and hydrophilicity of the SAP3 protein. The antigenicity and epitope prediction also had been discussed. The results of this study would contribute information about Candida albicans SAP3 and benefits for further works willing to develop therapeutic and vaccine against the fungal.

1. Introduction

Candida albicans is a fungal frequently isolated from mucosal surfaces of a human. While normally found asymptomatic and benign, Candida albicans has the ability to cause superficial and systemic infections in the human host under optimal environmental conditions [1, 2]. Candida albicans infections often refractory to high morbidity and mortality [3]. Current therapeutic approaches for Candida albicans infections have limited effectiveness, especially in a systemic infection, due to the lack of an effective immune response [4]. The increased incidence of severe disseminated infections caused by the Candida albicans highlights the urgent need for research into the Candida albicans major virulence factors [5].

Candida albicans has the ability to produce and secrete hydrolytic enzymes namely aspartic proteases [6]. The pathogenesis of Candida albicans is multifactorial and different virulence attributes are important during the various stages of infection. The secreted aspartic proteases play a role in several infection stages [7]. The secreted aspartic proteases have several specialized functions during the infective process, which include the simple role of digesting molecules for nutrient acquisition, digesting or distorting host cell membranes to facilitate adhesion and tissue invasion, and digesting cells and molecules of the host immune system to avoid or resist antimicrobial attack by the host [8].
Therefore, the secreted aspartic proteases are potential targets for the development of novel anti-\textit{Candida albicans} drugs or the development of specific aspartic protease inhibitors \cite{9, 10}.

Secreted aspartic protease 3 (SAP3) is one of the important virulence factors during \textit{Candida albicans} mucosal or disseminated infections. Large amounts of SAP3 antigen is found within \textit{Candida albicans} yeast and hyphal cell walls, often predominantly in close contact with epithelial cells and contribute to tissue damage \cite{10}. SAP3 is correlated with oral disease and vaginal disease and has abilities to induce secretion of proinflammatory cytokines interleukin-1\(\beta\) (IL-1\(\beta\)) and tumor necrosis factor alpha (TNF-\(\alpha\)) by human monocytes, therefore, could be a potential target for immunotherapy against \textit{Candida albicans} \cite{11-13}. However, limited data is known about the \textit{Candida albicans} SAP3 biochemical and immunological properties including that of the \textit{Candida albicans} SAP3 protein from Indonesian isolate. The present study, therefore, aimed to perform molecular analysis (focusing on the biochemical and immunological properties) of the \textit{Candida albicans} SAP3 from Indonesian isolate.

2. Materials and methods
Our research group (A-IGIC/ A-Infection, Genomics, Immunology & Cancer) successfully isolated a \textit{Candida albicans} genome from a Javanese HIV patient in a molecular epidemiology study in Central Java, Indonesia \cite{14-19}. To study the \textit{Candida albicans} SAP3, the \textit{Candida albicans} SAP3 complete coding sequence (cds) was cloned from the \textit{Candida albicans} genomic DNA isolated from a Javanese HIV patient using by nested PCR using MyFi Mix (Bioline, London, UK) and KOD -Plus-Mutagenesis (Toyobo, Osaka, Japan). The PCR product was purified using Zymoclean Gel DNA Recovery (Zymo Research, Irvine, CA) then molecular sequenced three times for confirmation.

The BLAST (Basic Local Alignment Search Tool), an algorithm for comparing primary biological sequence information, such as the nucleotides of DNA sequences and protein sequences, was performed to confirm the \textit{Candida albicans} SAP3 sequencing results. Multiple alignments of reference sequences were reconstructed using ClustalW as implemented in CLC Main Workbench 8.0.1 software. The \textit{Candida albicans} SAP3 predicted protein analysis was performed using CLC Main Workbench 8.0.1 software. The parameters for \textit{Candida albicans} SAP3 hydrophobicity plot was set by Kyte-Doolittle, Eisenberg, Engelman, Hopp-Woods, Janin, Rose, Cornette hydrophobicity scale, respectively, with several residues - must be odd as 11. The non-redundant protein sequences (nr) database with standard database genetic code was used for the blastp \textit{Candida albicans} SAP3 protein sequence analysis. Both Welling and Kolaskar-Tongaonkar antigenic scale was used to create the antigenicity plot of \textit{Candida albicans} SAP3, with a BLOSUM62 matrix with gap cost existence set as 11 and extension as 1 were used. The T Cell Epitope Prediction Tools from Immune Epitope Database (IEDB) Analysis Resource was used to predict the \textit{Candida albicans} SAP3 immunogenicity prediction and major histocompatibility complex-binding.

3. Results and discussion

3.1. Sequencing results
A complete coding sequence of \textit{Candida albicans} SAP3 gene was successfully cloned. The Basic Local Alignment Search Tool analysis was performed to confirm the \textit{Candida albicans} SAP3 gene sequences. Based on The Basic Local Alignment Search Tool analysis the clone was confirmed as \textit{Candida albicans} SAP3 gene and translated as \textit{Candida albicans} SAP3 with 398 amino acids (table 1). The \textit{Candida albicans} SAP3 gene had 100% homology (1197/ 1197 nucleotide base pairs) with strain WO-1 (GenBank Accession Number L22358), while the \textit{Candida albicans} SAP3 also had 100% homology (398/398 amino acids) with \textit{Candida albicans} SAP3 strain SC5314 (20-22), strain 12C (GenBank Accession Number KGT69638), strain P76055 (GenBank Accession Number KHC36851), and strain SS (UniProtKB/Swiss-Prot: P0CY28). Based on the sequencing results, the \textit{Candida albicans} SAP3 complete coding sequences was completely conserved, as reported previously \cite{20-22}. 
3.2. *Protein analysis results*

We performed the predicted protein analysis using CLC Main Workbench 8.0.1 software. The *Candida albicans* SAP3 protein has 398aa with 42.806 kDa of weight (table 1), consistent with previous results [20-22].

**Table 1. Candida albicans** SAP3 protein characteristics.

| Protein statistics | Characteristics            |
|--------------------|---------------------------|
| Length             | 398aa                     |
| Weight             | 42.806 kDa                |
| Isoelectric point  | 4.75                      |
| Aliphatic index    | 87.437                    |
| N-terminal Methionine Half-life |
| Half-life mammals  | 30 hours                  |
| Half-life yeast    | >20 hours                 |
| Half-life *E. coli*| >10 hours                 |
| Atomic composition |
| hydrogen (H)       | 0.493 (n= 2,941)          |
| carbon (C)         | 0.318 (n= 1,895)          |
| nitrogen (N)       | 0.083 (n= 493)            |
| oxygen (O)         | 0.105 (n= 626)            |
| sulfur (S)         | 0.001 (n= 5)              |
| Count of residues  |
| Hydrophobic (A,F,G,I,L,M,P,V,W) | 0.457 (n= 182)         |
| Hydrophilic (C,N,Q,S,T,Y)     | 0.379 (n= 151)           |
| Count of charged residues |
| Negatively Charged (D & E) | 0.101 (n= 40)            |
| Positively Charged (R & K)  | 0.055 (n= 22)            |
| Other              | 0.844 (n= 336)            |

The *Candida albicans* SAP3 protein has methionine N-terminal half-life in yeast more than 20 hours. The extinction coefficient of *Candida albicans* SAP3 at 280 nm for non-reduced cysteines was 34,660 (absorption at 280nm 0.1% = 0.81) while for reduced cysteines was 34,420 (absorption at 280nm 0.1% = 0.804). The *Candida albicans* SAP3 beta strand secondary structures were frequently found (n= 38) with only had three alpha helix secondary structures at position 281-293, 383-386, 394-396. The motif of the SAP-like region, a pepsin-like protease secreted from pathogens to degrade host proteins cd05474, was found at amino acids 70-386 of *Candida albicans* SAP3. The Asp motif, a Eukaryotic aspartyl protease, was found at amino acids 71-386. Taken all data together, the *Candida albicans* SAP3 confirmed to belong to the endopeptidase family with aspartic proteases activities, characterized by the conserved sequence Asp-Gly-Thr at the active site, as reported previously [20-23].
The inhibitor binding sites were found in *Candida albicans* SAP3 at position 71, 137, 140, 145, 151, 186, 355, and 359, respectively. The catalytic motifs in *Candida albicans* SAP3 were found at position 90-93 and 274-277, while the catalytic residues were found at position 90 and 274, respectively. The active site flap motifs, the beta-hairpin located over the active site cleft, opens to allow substrate access to the active site trench, closes on substrate binding and opens again to allow products to leave, were found at position 138-143 and 145-149 in *Candida albicans* SAP3. All described positions herein were consistent with previous results [20-22]. Due to the continuing increase of drug-resistant strains, the *Candida albicans* SAP3 is also currently considered as promising drug target candidates, by targeting the respected binding sites and motifs [24].

3.3. Protein analysis results

We performed the immunogenicity prediction and major histocompatibility complex-binding for the *Candida albicans* SAP3 using the T Cell Epitope Prediction Tools from Immune Epitope Database (IEDB) Analysis Resource. In total, 14 epitopes were found had a high affinity (percentile rank 0.01) for Major histocompatibility complex II-binding (table 2), consistent with the antigenicity plot.

| Allele                              | Position |
|-------------------------------------|----------|
| HLA-DPA1*02:01/DPB1*01:01          | 1-15     |
| HLA-DPA1*03:01/DPB1*04:02          | 1-15     |
| HLA-DPA1*02:01/DPB1*01:01          | 2-16     |
| HLA-DPA1*03:01/DPB1*04:02          | 2-16     |
| HLA-DPA1*03:01/DPB1*04:02          | 3-17     |
| HLA-DPA1*03:01/DPB1*04:02          | 4-18     |
| HLA-DPA1*03:01/DPB1*04:02          | 5-19     |
| HLA-DRB3*01:01                     | 314-328  |
| HLA-DRB3*01:01                     | 315-329  |
| HLA-DRB3*01:01                     | 316-330  |
| HLA-DRB3*01:01                     | 317-331  |
| HLA-DRB3*01:01                     | 318-332  |
| HLA-DRB3*01:01                     | 319-333  |
| HLA-DRB3*01:01                     | 320-334  |

The transition from round budding cells to long hyphal forms and production of secreted aspartic proteases are considered virulence-associated factors of *Candida albicans* [25]. The fungal is directly attacked by the host innate immune system upon infection, such as complement proteins. However, the *Candida albicans* SAP3 degrade host complement components and inhibit terminal complement complex (TCC) formation since the *Candida albicans* SAP3 expression of pseudohyphae and true hyphae in serum were intensive [25, 26]. The *Candida albicans* SAP3 reported effectively cleaved the kininogens, one of the major homeostatic systems in human hosts, with the highest hydrolytic activity toward the low-molecular-mass form (LK) [27]. In the present study, we found the amino acids at position 1 until 19 and 314-334 had a high affinity for Major histocompatibility complex II-binding with the high antigenic property, therefore, may be potential as a target to improving diagnostic, therapeutic, or vaccine efficacy.
4. Conclusions
The present study will contribute information about Candida albicans SAP3 especially that of the Candida albicans SAP3 protein from Indonesian isolate and benefits for further works willing to develop a diagnostic kit, immunotherapeutic, or vaccine strategies against Candida albicans.

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