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In Silico Inhibition of Essential *Candida albicans* Proteins by Arenicin, a Marine Antifungal Peptide

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Abstract

Fungal infections increased substantially in the last years, becoming a relevant public health problem. Many of these infections account for high rates of morbidity and mortality. The emergence of resistant fungal clinical isolates have also motivate studies to find new antifungal therapies. Candida albicans is an oportunistic pathogen and affects a great number of immunocompromised patients worldwide. The marine ecosystem has been considered a rich source of bioactive metabolites due to the complexity and originality of its structures. Proteins and peptides from marine organisms have been shown to have antiviral, anti-inflammatory, antimalarial, anticancer, antimicrobial and antifungal properties. Arenicins are antimicrobial peptides isolated from the marine lugworm Arenicola marina with 21 amino acid residues in a β-hairpin structure. Dihydrofolate reductase, exo-b-(1,3)-glucanase and sterol 14α-demethylase are essential C. albincas enzymes that take part in DNA, cell wall and membrane metabolism, respectively. The present study evaluates the interaction of arenicin with important enzymes of C. albicans related to cell wall, ergosterol and DNA metabolism in order to elucidate possible molecular targets. We showed through an in silico approach, that a single compound from a marine worm (A. marina), can bind to three C. albicans essential proteins. The interaction occurs in regions inside the active site or at least near, with amino acid residues evaluated as hot spots. Arenicin is a new promising antifugal drug. The next step is to investigate protein-protein interactions performed by DHFR, EBG and CYP51 and assess whether arenicin is able to disrupt essential interaction or not.

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Introduction

Fungal infections increased substantially in the last years, becoming a relevant public health problem. Many of these infections account for high rates of morbidity and mortality. Candidiasis, an opportunistic fungal infection is caused by fungi belonging to the *Candida* genus [1]. Although the antifungal agents used in clinical treatments of mycosis have sometimes proved efficient, they are restricted to few targets in fungal cells with high toxicity to humans [2]. In addition, the problem increases with the emergence of resistant clinical isolates [3,4], which makes the search for new antifungal therapies extremely relevant.

Since the discovery of spongouridine, molecular model of the first antiviral used as a therapeutic resource for Acquired Immunodeficiency Syndrome (AIDS), the biodiversity of the marine ecosystem has been considered a rich source of bioactive metabolites due to the complexity and originality of its structures [5]. In this scenario, peptides from marine organisms have been shown to have antiviral, anti-inflammatory, antimalarial, anticancer, antimicrobial and antifungal properties [6–8].

Arenicins are antimicrobial peptides isolated from the marine lugworm *Arenicola marina* with 21 amino acid residues in a β-hairpin structure (Figure 1). *In vitro* and *in vivo* studies have shown that arenicins exhibit a very potent bactericidal and antifungal effect against multi-resistant gram-negative bacteria and *Candida albicans,* respectively [9]. In bacterial, Arenicin interacts with membrane lipids promoting membrane permeabilization and detachment besides affecting cytoplasm metabolism [10]. However, the interaction of arenicin with important antifungal targets from Candida albicans is unknown.

Dihydrofolate reductase (DHFR) catalyzes the reduction of dihydrofolic acid to tetrahydrofolic acid [11]. The enzyme acts on the synthesis of purines, thymidylic acid, amino acids and the regulation of tetrahydrofolate levels in the cell, participating in proliferation and growth [12]. The protein exo-b-(1,3)-glucanase (EBG) takes part in cell expansion and differentiation through cell wall remodeling. EBG is also secreted during pathogenesis [13]. Finally, CYP51 (sterol 14α-demethylase) is a major drug target in the cytochrome P450 superfamily. This enzyme is related to ergosterol and membranes metabolism [14]. All the enzymes described above are important to survivability and pathogenesis of *Candida* species. The identification of novel therapeutics can drive a more efficient treatment of immunocompromised patients that are susceptible to fungal diseases.

Molecular anchoring is an important computational tool in drug design and targeting. The

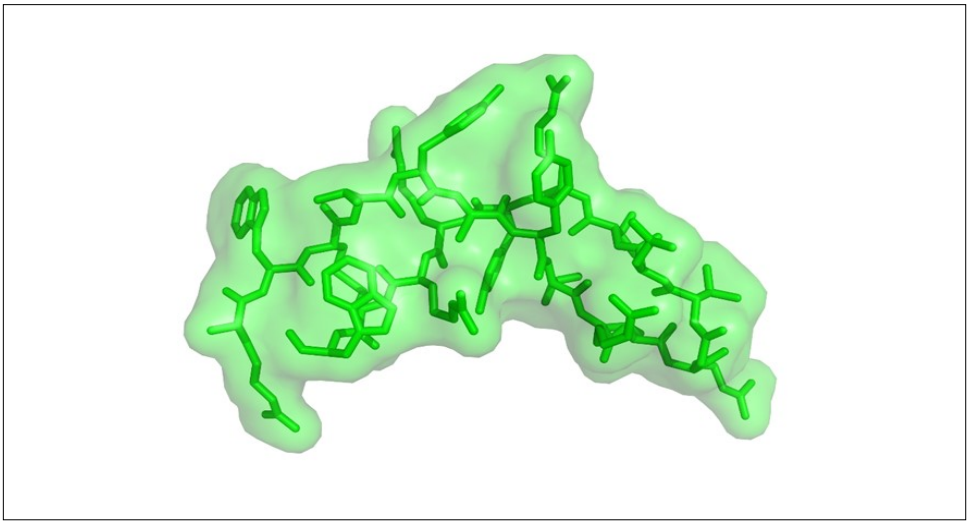

Figure 1. The arenicin structure. The conformational structure of the peptide folds into an antiparallel beta-sheet comprising two strands, which are important for its antimicrobial activity. Arenicin has a high affinity for lipids and disrupt fungal and other microorganisms membrane.

purpose of the ligand-protein anchor is to predict the best interaction orientation of a linker with a protein of known three-dimensional structure [15]. Thus, in this study we verified the interaction of arenicin with important enzymes of *C. albicans* related to cell wall, ergosterol and DNA metabolism in order to elucidate possible molecular targets.

Material and Methods

All the 3-D structures used in the analysis are available in the PDB (protein databank; https:// www.rcsb.org/). KBDOCK server were used in order to assess protein domains and possible interaction between protein domains [16]. The protein docking was performed by ClusPro [17]. We used PyMol (https://pymol.org) for the visualization of the interface of interaction, the visualization of hot spots and for creating the figures presented in this manuscript .The hot spots in the proteins under study were identified by KFC2. The server offers an automated analysis of a protein complex interface. The server analyses the structural environment around amino acid residues and checks for already known hot spots environments determined experimentally. The hot spot prediction is based on characteristics regarding conformation specificity (K-FADE) and biochemical features such as hydrophobicity (K-CON) [18,19].

Results and Discussion

The Arenicin Properties are Defined by its Amino Acid Composition

Arginine and valine account for 54% of the amino acid residues present in the arenicin structure (Figure 2) and they account for the properties presented by the peptide. The large amount of arginine residues has a role in maintaining the conformational state of the peptide and its overall charge [20–22]. Moreover, arginine residues may interact with active sites of proteins [23–25] that bind to phosphorylated substrates through hydrogen bonds. The large amount of valine residues helps the peptide folds into its β-hairpin structure (Figure 3) [26] and this is related to the presence of two non-hydrogen substituent attached to the valine C-beta carbon. In addition, valine plays a role in substrate recognition of hydrophobic ligands such as lipids [27,28], which could explain the high affinity the peptide has for membranes.

*Arenicin Binds to Residues in the Active Site of D*ihydrofolate Reductase

The main feature of the conformational structure of DHFR is a central region comprised by beta-pleated sheets [29]. The active site is surrounded by a domain that contain loops and regulate the interaction pattern with partner proteins and compounds [30]. An efficient strategy for inhibiting

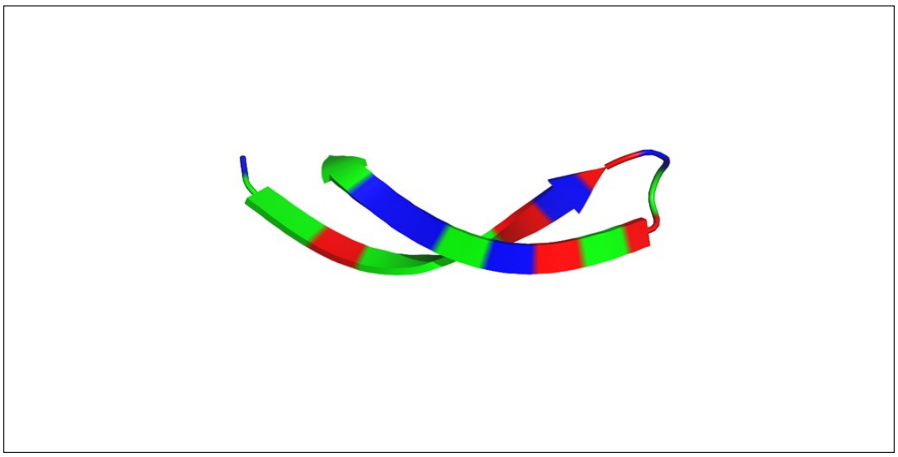

Figure 2. The arenicin arginine and valine residues. Arginine and valine amino acids correspond to 54% of residues in the arenicin structure. They contribute to the conformational state of the peptide, the overall charge and affinity for ligands. Red – arginine; blue – valine; green – other amino acid residues.

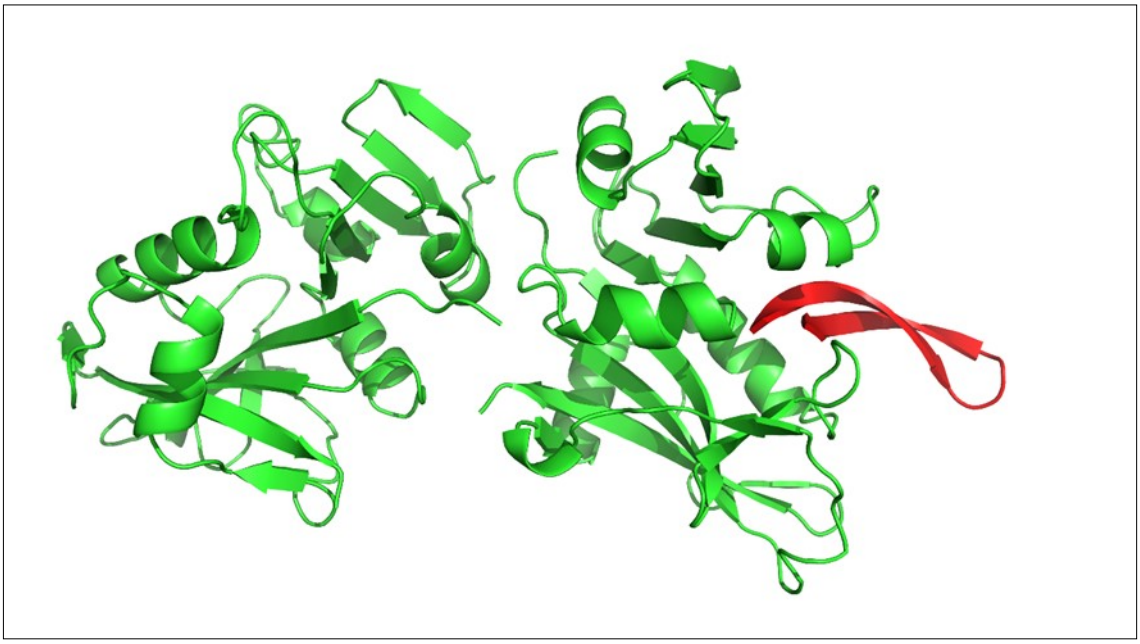

Figure 3. Dihydrofolate reductase (DHFR) interaction with arenicin. DHFR is a homodimeric protein and the inhibitor marine compound arenicin binds to a loop within the active site of the protein. Arenicin anchors inside the active site and alter slightly the conformational structure of DHFR. Green – DHFR; red – arenicin.

DHFR from *C. albicans* is to target essential amino acid residues at the active site of the enzyme [31–33]. Arenicin interacts with a loop inside the active site of DHFR (Figure 4). There are five main amino acid residues (Ile-Pro-Gln-Lys-Phe) that contribute considerably for the stability of the interaction between DHFR and the arenicin peptide and they have been shown to link to other inhibitor compounds as well [33].

Arenicin Binds to Hot Spots Residues Near the Active Site of EBG

EBG folds into 8-barrel structure with both alfa and beta sheets, a typical conformation presented by the glycosyl hydrolase family. The active site is in a pocket within the protein structure [34]. The pocket is energetically maintained by hydrogen-bonded interactions [35]. Figure 5 shows the surface of EBG and the place where arenicin invades EBG towards the active site. We found seven hot spot residues on the EBG surface. They energetically contribute to the binding between the protein and the marine inhibitor. The main hot spot residues from EBG interacting with the inhibitor peptide are near residues belonging to the active site (figure 6). Arenicin bind to hot spot residues and changes the conformational structure of amino acids surrounding the active site of the protein, consequently inhibiting its activity.

Arenicin is Surround by Hot Spots Residues in a Cleft of CYP51

CYP51 is required for ergosterol biosynthesis [36]. It has been extensively investigated as the main target for azole antimicrobial drugs [37–39]. We found 11 hot spot residues on the *C. albicans* CYP51 interface of interaction with the peptide arenicin (Figure 7). The necessity of finding new target to fungal diseases is real, since azoles, although having the ability to inhibit CYP51, are toxic to mammals cells and lead to severe side effects in patients [40–43]. The marine peptide arenicin is held near the active site of the enzyme by the hydrophobicity in that area. Inhibition of CYP51 leads to disruption of the membrane and cell death. The marine peptide fits in a cleft of the protein, at the entrance of the active site, and is surrounded by hot spot residues, interacting with them via hydrogen bonds.

Concluding Remarks

The number of deaths of immunocompromised patients caused by opportunistic fungi, including *C. albicans*, has increased significantly [44]. The

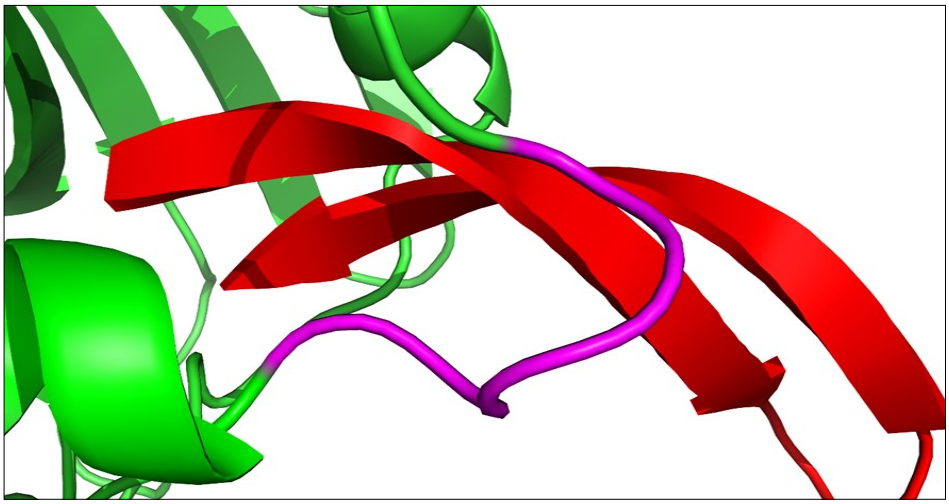

Figure 4. Arenicin anchors inside the active site of dihydrofolate reductase. The loop in the active site of DHFR (pink) contains the five amino acid residues Ile-Pro-Gln-Lys-Phe. The binding of the inhibitor compound arenicin within the active site of the enzyme might hinder the efficiency of interaction between DHFR and partner proteins and consequently reduces its activity

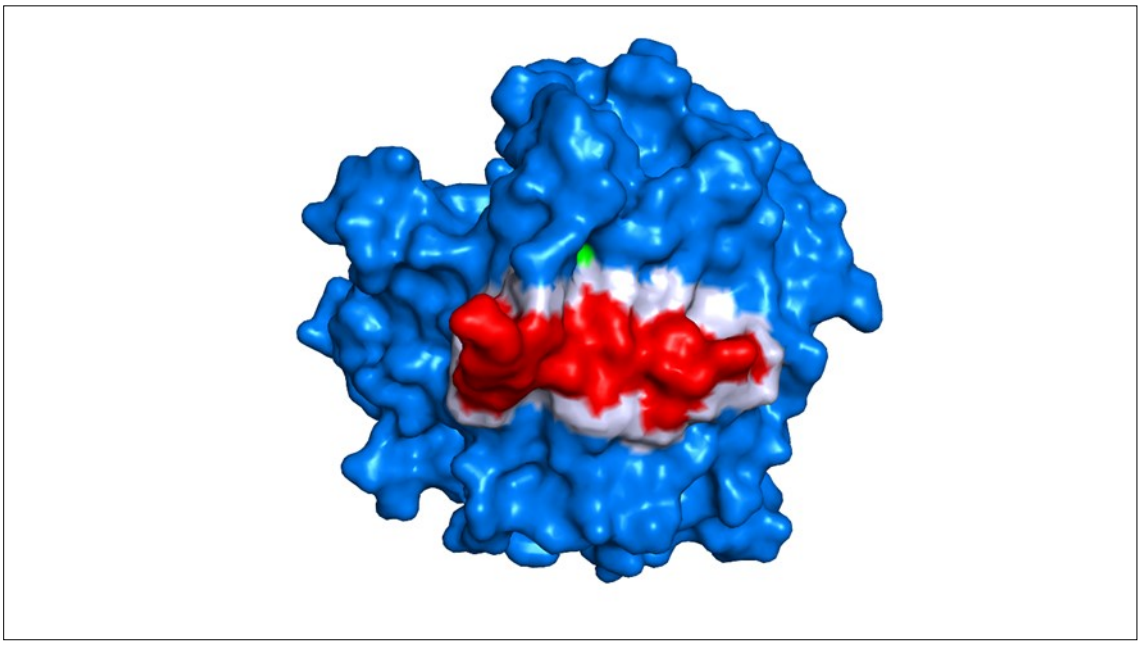

Figure 5. Surface overview of EBG and arenicin interaction. Arenicin (red) binds to EBG and invades a pocket region of the protein that has amino acid residues belonging to the active site. Blue – EBG; red – arenicin; white – interface of interaction between the inhibitor peptide arenicin and EBG; green – hot spot residue.

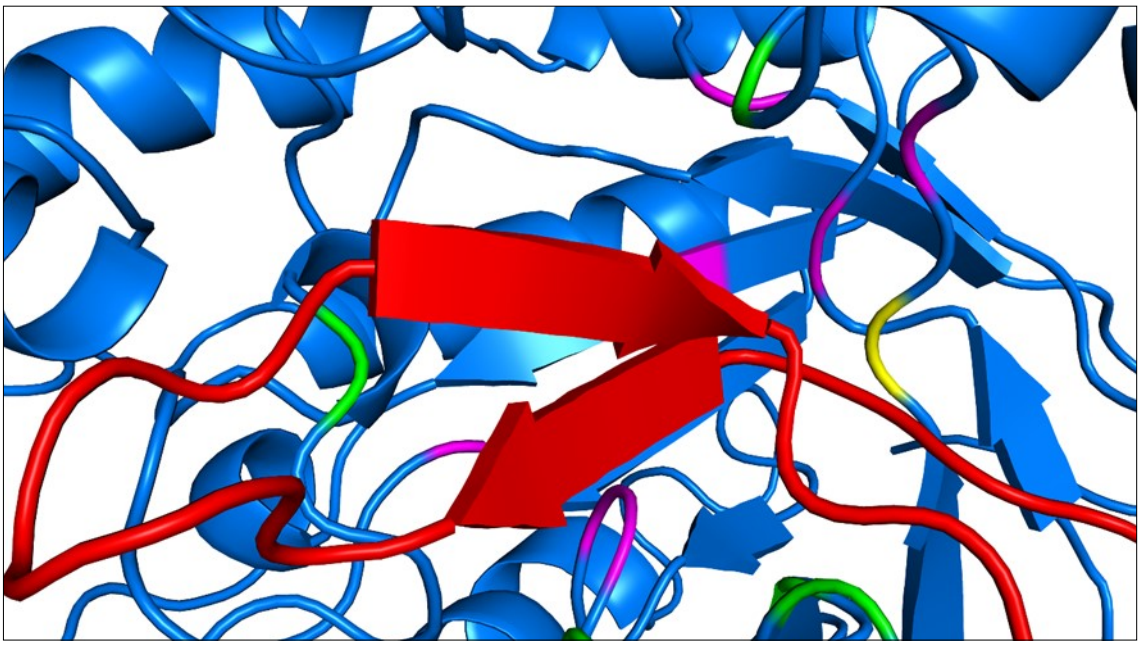

Figure 6. Arenicin and the pocket region of EBG. Arenicin (red) binds to EBG and invades a pocket region of the protein that has amino acid residues belonging to the active site (pink). Green amino acids are hot spots on EBG and the yellow amino acid residue was classified as a hot spot and belongs to the active site of EBG, an important residue for inhibiting assays.

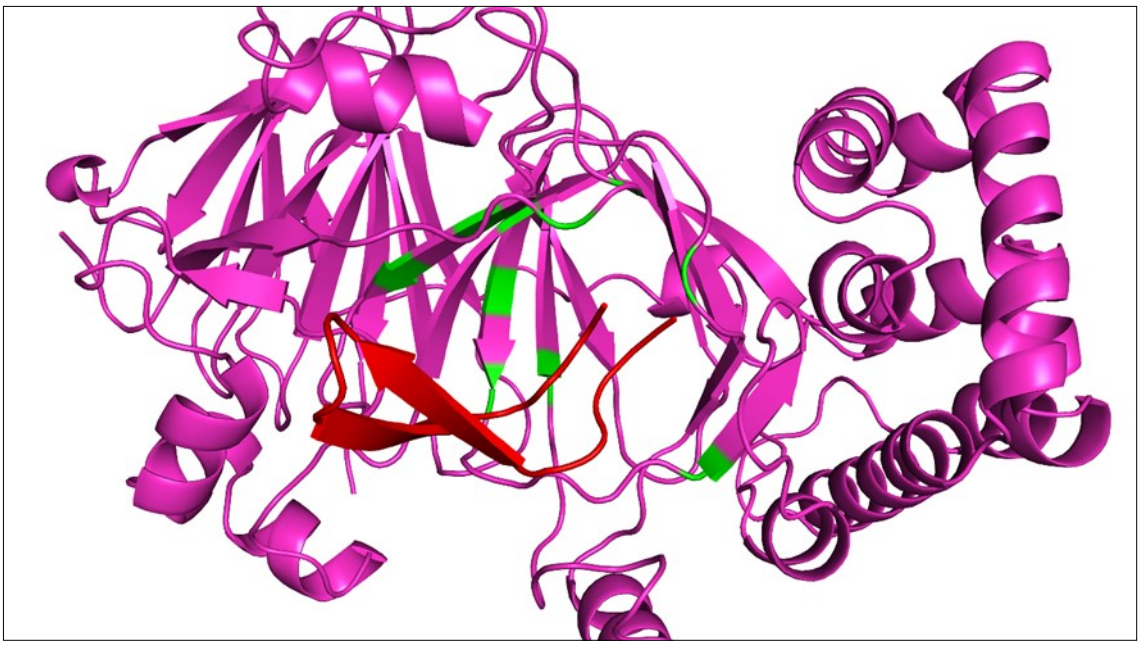

Figure 7. Arenicin surrounded by hot spot residues on CYP51 structure. Arenicin (red) binds to CYP51 (pink) and interacts with hot spot residues. Green amino acids are hot spots on CYP51, the conformation of the region of interaction changes, affecting the ability of the protein to perform its function.

therapeutics used against such infections are mostly based on azoles and other drugs that develop severe side effects in those patients. Here, we showed through an *in silico* approach, that a single compound from a marine worm (*A. marina)*, can bind to three *C. albicans* essential proteins. The interaction occurs in regions inside the active site or at least near, with amino acid residues evaluated as hot spots. Arenicin is a new promising antifugal drug. The next step is to investigate protein-protein interactions performed by DHFR, EBG and CYP51 and assess whether arenicin is able to disrupt essential interaction or not.

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