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Topic: Physicochemical Characterization of Als proteins from *Candida albicans.*

Abstract

C. albicans, which is a medically important fungus, has a number of GPI anchored proteins on its cell surface, many of which are responsible for its virulence. The Als family of proteins is one such family of GPI anchored proteins in *C. albicans*, which confers upon these cells the ability to aggregate and to adhere to a variety of ligands on the host cell surface. Thus, these proteins are critical determinants of infection and virulence in this pathogenic fungus. There have been very few *in vitro* studies on adhesins done previously, and one of the main reasons for this is the difficulty in purifying these large-sized, aggregation-prone proteins. We began by trying to clone and express 3 different Als proteins (Als2, Als4, and Als5) from *C. albicans*. Thus, our studies focused on the physicochemical and functional characterization of the Als5 protein. As with other Als proteins, the full length Als5 protein has so far not been characterized *in vitro*. However, many different *in vitro* studies have been done on isolated domains of Als5 that give us a hint on its functionality. We expressed Als5 as a GST-fusion protein in bacteria and successfully purified it using glutathione-affinity chromatography. We then went on to demonstrate that the purified protein was functional; it could bind to collagen type IV as well as to specific peptide ligands *in vitro* as assessed by collagen binding assays and fluorescence spectroscopy. Further, TEM studies indicated that it could form self-aggregates as expected of an adhesin. Conformationally, when studied by CD spectroscopy, this protein appeared to have a significant amount of intrinsically disordered regions and adopted a fold typical of intrinsic pre-

molten globules. Mutational analysis of key residues involved in peptide-ligand binding pocket also supported the hypothesis that the plasticity of the ligand-binding pocket stems from the fact that there are multiple residues involved in the interaction and no single residue could solely determine the affinity of the interaction. The main question we have tried to address in this study, is whether the GPI anchor attachment signal sequence (SS) of Als5 acts merely as a flag for GPI anchor attachment or whether it also determines the folding and functionality of the protein. For this purpose, we expressed Als5-SS, the full length Als5 protein containing the C-terminal SS, as a GST-fusion and purified it in a manner similar to GST-Als5. We then compared the folding and functionality of GST-Als5-SS with that of GST-Als5. In comparison to Als, Als5-SS showed lesser adhesion to collagen type-IV and showed no tendency to aggregate. We could associate these differences in functionality to a very significant difference in conformation between the two proteins. The CD spectrum of Als5-SS, suggested that the protein was α-helix-rich,. The altered folding also appeared to perturb the peptide binding pocket of the protein, altering its ligand binding characteristics. Thus, it would appear that the SS not only directs the attachment of the GPI anchor but also holds Als5 in an α-helical conformation with reduced functionality. Using homology modeling and docking studies, we showed that the C-terminal of SS could interact with the Als5 N-terminal domain, and propose a model wherein the torsion induced in Als5-SS due to such an interaction is responsible for the altered conformation and function of the protein. Thus, we propose a model of how the Als5 protein matures in the ER lumen upon GPI anchor attachment: Cleavage of the C-terminal SS by the GPI-transamidase converts Als5 to the mature β-sheet-rich form of the protein, which is functional and aggregation prone and is attached to the ER membrane by the newly formed GPI anchor.