GLUCURONOXYLOMANNAN, THE MAJOR CAPSULAR POLYSACCHARIDE OF *CRYPTOCOCCUS NEOFORMANS*, IS RELEASED EXTRACELLULARLY IN SECRETORY VESICLES AND ASSEMBLED AT THE CELL SURFACE BY ION-DEPENDENT SELF AGGREGATION.

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Cryptococcus neoformans is the etiological agent of cryptococcosis and represents a unique model in cell biology studies since it is the only eukaryotic pathogen with a polysaccharide capsule. Glucuronoxylomannan (GXM) is its major polysaccharide component and is released extracellularly during infection, inducing a number of deleterious effects to the host. A central question in the biology of *C. neoformans* is the mechanism by which GXM, synthesized inside the cell, is exported to the extracellular environment and then assembled at the cell surface. In the present work, we describe that *C. neoformans* produces vesicles that are secreted across the cell wall. Supernatants of *C. neoformans* cultures contained vesicles with bilayered membranes enriched in key fungal lipids, such as glucosylceramide and sterols. GXM was detected inside these secreted compartments. During vesicle purification we observed that extracellular GXM coalesced into a polysaccharide film, indicating that the molecule is capable of self-aggregation. Measurement of microscopic fluid velocity fields based on dual beam optical tweezers revealed that the high polysaccharide viscosity decreases when the carboxyl groups of glucuronic acid (GlcA) are reduced. Na^+ and EDTA produced the same effect, but low concentrations of Ca^{2+} induced an increase in polysaccharide viscosity. These results suggest that the interaction of divalent ions with negatively charged GlcA residues in GXM fibers results in polysaccharide aggregation. We present here a novel mechanism for the release and assembly of the major virulence factor of C. neoformans, whereby polysaccharide packaged in lipid vesicles crosses the cell wall and the capsule network to reach the extracellular environment. According to this model, extracellular GXM is then released from secreted vesicles and assembled at the cell surface by ion-dependent self aggregation.

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