BINDING OF TENEBROID MOLITOR PERITROPHIN CBDS TO COLLOIDAL CHITIN

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INTRODUCTION
Peritrophic membrane (PM) is a structure composed of chitin and proteins. It separates ingested food from the midgut epithelium of most insects and is involved in increasing the digestive process efficiency. PM structural proteins are called peritrophins and may contain from 1 to 19 chitin binding domains (CBDs or CBM14). The 3D structure of a CBD has been yet unresolved, but inspection of homolog models suggests that its binding may occur through interaction of chitin with hydrophobic residues brought together by disulfide bridges between conserved cysteines present in the domain.

OBJECTIVES
We aim to gather information on CBD structure and on its interaction with chitin and acetylchitooligosaccharides.

MATERIALS AND METHODS
Four recombinant proteins with one to three CBDs of a Tenebroid molitor peritrophin were produced in E. coli strain BL21(DE3). The proteins contained N-terminal His-tag and SUMO fusion protein, which enhances recombinant protein solubility. After purification by affinity chromatography, the proteins were incubated with colloidal chitin prepared by acidic treatment of commercial chitin powder. The unbound fraction was collected after brief centrifugation and the colloid was washed twice with 10mM Tris-HCl 100mM NaCl pH 8. The bound proteins were washed out with a 30% acetid acid solution. Total protein of each fraction was determined either by Bradford assay or fluorescence emission.

DISCUSSION AND RESULTS
The fractions contained respectively approximately 30%, 20%, 5% and 30% of the initial protein, while the negative control (egg albumin) distribution was approximately 50%, 35%, 7% and 4%. Treatment of the proteins with DTT, a strong reducing agent, lead to the loss of binding ability. After SUMO fusion protein and His-tag removal with a specific commercial peptidase, the resulting recombinant proteins maintained the binding capacity.

CONCLUSIONS
The results indicate specific interaction of the recombinant proteins with colloidal chitin and suggest that the oxidized cysteines are essential for maintaining a 3D structure necessary for binding.

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Keywords: chitin binding domain, peritrophin, peritrophic membrane.