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## **SOLUBLE LIPID BINDING PROTEINS FROM RELEVANT PARASITIC HELMINTHS: FROM STRUCTURE TO FUNCTION**

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### **Introducción**

Parasitic helminths cause serious and difficult to treat diseases in humans, animals and plants. A high percentage of the earth's population, mainly in developing countries, suffers from helminth infections. Helminth parasites have a restricted lipid metabolism and must acquire simple and complex lipids from their hosts for energy metabolism, membrane construction, and lipid-based signaling, the latter possibly also encompassing modifications of the host's immune and inflammatory defense systems. These organisms produce and release an unexpectedly wide range of lipid binding proteins (LBPs) types structurally distinct from those of their hosts. Some will be associated with specialized external functions, including acquisition and distribution of nutrients. Yet others will be involved in modulation of the host's local tissue environment, and its innate and acquired immune systems by secreted lipids and carrier proteins. It is important to note that antihelmintic drugs are partially hydrophobic and may require parasite's own carrier proteins for reaching their site of action.

### **Objetivos**

We have selected four important types of LBPs from pathogenic helminth parasites that affect populations of South America and worldwide with the aim of solve their three dimensional structures and *in vitro* functions: a) a novel class of fatty acid and retinol binding protein with a structure that has no known counterpart, b) relatives of the fatty acid binding protein family, including members that are structurally modified in ways that are unique to nematodes, c) Antigen B, a member of a new family of ligand binding proteins present in cestodes, and d) nematode polyprotein allergens/antigens (NPA).

### **Materiales y Métodos**

Protein purification and sample preparation for NMR. The proteins were over-expressed as N-terminal His-tag fusion or GST-tag fusion and purified using corresponding affinity columns, followed by gel filtration chromatography. In order to strip E. coli ligands from the protein, reverse phase – HPLC (RP-HPLC) was used, employing an increasing acetonitrile gradient.

NMR spectroscopy. All NMR experiments were carried out on a Bruker 600 MHz NMR spectrometer at 298K. All data were processed using Azara and analyzed using CCPN Analysis.

Structure Calculation. Structures are being calculated based on distance restraints derived from <sup>15</sup>N- and <sup>13</sup>C NOESY experiments, using ARIA.

ITC. Calorimetric titrations (Microcal) were used for binding parameters determination and functional analysis of fatty acid binding protein (As-p18) with oleic acid at 25°C.

Fluorescence. Fluorimetric titrations were performed employing fluorescent ligands, recording changes in ligand emission, and employing nonfluorescent ligands, following changes in protein intrinsic fluorescence.

Protein membrane interaction. Binding of cytochrome c to acidic membranes was monitored employing a resonance energy transfer assay in which the dansyl fluorescence of DPE-labelled SUV is quenched upon binding of cytochrome c, which contains the heme moiety quencher. An inhibition of cytochrome c-dependent quenching is interpreted as evidence for protein interaction with the SUV and prevention of subsequent cytochrome c interaction with the bilayer.

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## **Resultados**

The atomic structures of some of these proteins are under analysis employing NMR spectroscopy, for which we already have obtained high quality data and full structure determination is in progress. Characterization of ligand-bound states has shown evidence of conformational changes between the apo form (absence of ligand) and the holo form (presence of ligand). We are also analyzing their ligand-binding properties employing fluorescence-based systems and ITC. Additionally, protein-membrane interaction assays are being performed in vitro in order to characterize LBPs' possible functions.

## **Conclusiones**

As a whole this studies confirm that LBPs are small soluble proteins and bind natural ligands and fluorescent analogues with high affinity. Structural and functional studies will enhance our understanding of the unique features of helminth LBPs that may be related to the survival of the organisms and could be used as potential drug targets.