Molecular basis of chemicals and odorants recognition in the insect model organism Tribolium castaneum by structural biology of chemoreceptor proteins.

Principal Investigator: Dr. Stefano Benini

Co-investigator: Dr. Sergio Angeli

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Abstract:

The olfactory and gustatory receptors in insects are a very sophisticated and evolved system that allows insect to adapt to many different environments (e.g., *via* pheromones to communicate fear, attraction, or induce aggregation, or to find food sources and to escape dangerous chemicals).

In order to gain a better understanding of the molecular basis of odour and taste recognition in insects a structural genomic study of odour binding proteins will be carried out on the model organism *Tribolium castaneum*.

Riassunto:

I recettori olfattivi e gustativi degli insetti sono altamente sofisticati ed evoluti. Essi permettono agli insetti di adattarsi a svariate condizioni ambientali (es.: tramite i feromoni per comunicare paura, attrazione o provocare aggregazione, o a trovare sorgenti di cibo e ad allontanarsi da composti chimici pericolosi).

Per capire meglio il meccanismo di riconoscimento degli odori e dei sapori negli insetti a livello molecolare verrà eseguito uno studio di genomica strutturale utilizzando l'organismo modello *Tribolium castaneum.*

Project description:

The red flour beetle *Tribolium castaneum* is an omnivorous insect and an important pest of stored agricultural products. It represents a powerful model organism for the study of insect development. Its genome has recently been sequenced and provides a promising source of genetic information which represents the ideal basis for a structural genomic approach. The information provided by this study could possibly be used for the development of more efficient pest control techniques by using limited amount of specific volatile chemicals that could be recognised by the target insects as attracting or repelling chemicals.

T. castaneum is a famous pest of stored grains and flours and has evolved the ability to interact with a diverse chemical environment thanks to very developed odorant and gustatory receptors. These characteristics make it an attractive system for developing selective "odour based" pests

control (as opposed to common pesticides) which could then be adapted to other insect parasites and pests (e.g., *Anopheles spp*., *Schistocerca gregaria*, etc.,).

In the recently published genome sequence 265 odorant receptors were identified and annotated, as well as 220 gustatory receptors.
It is believed that the increase in olfactory and gustatory chemoreceptors has largely compensated the decrease in opsin genes necessary for colour discrimination. This might be possibly due to adaptation to the low light condition found in stored foods. *T. castaneum* is attracted by damaged or deteriorated grains as it responds best to volatile compounds characteristic of damaged or fungus-infested grain than healthy grains.

In insects, the volatile compounds (odorants) interact first with an aqueous matrix, called sensillar lymph, surrounding the membranes of the sensory neurons. In the sensillar lymph a set of globular secreted proteins, called Odorant-Binding Proteins (OBPs) interact with the odorants and successively with the Odorant-Receptors (ORs).
OBPs are essential for correct odorant detection and olfactory behaviour *in vivo*. It is believed that OBPs shuttle odorants from the environment to the ORs, but the exact physiological function of these proteins is still unclear and might include partitioning hydrophobic odorant from air to aqueous phase, concentrating or sequestering it, transporting it to the OR, acting as conformation-specific OR-ligand, or even inactivating the odorant.

OBPs are a diverse protein family divided in Pheromone Binding Proteins (PBPs) and General Odorant-Binding Proteins (GOBPs), based on sequence similarity and functional data. Both are compact proteins of 110-140 aa, with high similarity and six conserved cysteines involved in disulfide bridges.

A second group of proteins, called Chemosensory Proteins (CSPs), was found to be expressed in the sensillar lymph of olfactory and gustatory sensilla. CSPs are usually smaller than OBPs (100-130 aa), featuring four conserved cysteines instead of six, and not sharing sequence similarity with OBPs.

A comprehensive genome analysis for genes coding for OBPs and the Chemosensory Proteins CSPs of *T. castaneum* has been carried out (*Tribolium* genome sequencing consortium, 2008; http://beetlebase.org/). 47 OBPs genes were identified, of which 26 genes code for the classic OBPs. The 47 OBPs of *Tribolium* share a low percentage of sequence identity (average 17% for classical OBPs). In addition, 19 genes were found to code for putative CSPs containing all 4 highly conserved cysteines. (Angeli et al., in preparation).
The wealth of information available will allow a systematic approach to elucidate the relationship of OBPs/CSPs with diverse odorants.

Aim of the project and expected results:

Aim of the project is the characterisation by X-ray protein crystallography of proteins involved in the first step in odour recognition in the model beetle *T. castaneum*. The genes of OBPs and CSPs of interest will be cloned, and the corresponding proteins overexpressed in *Escherichia coli*. After purification and biochemical characterisation the target proteins will be crystallised and the structure determined both in the native and in the odorant bound state thus providing useful information on the bound/unbound state sheding light onto the basis of odour detection

and discrimination.
The project will mainly focus on the biochemical and structural characterisation of complexes between proteins (OBPs and CSPs) and volatile compounds.
The results obtained with this project will represent a crucial starting point for an integrated study which could bring together different technologies and expertises (from genetics to structural biology, to biochemical characterisation of the interaction between the odorants and OBPs, moreover it could provide useful information on the binding mode of the molecules).
The results achieved will eventually guide structure based rationalisation of volatile compounds that could be then designed to become inhibitors or chemicals used as repellent or attractive to provide an efficient and environmentally friendly pest control. This project could then be extended to insects of forestal interest.

Materials and methods:

The genes of interest will be cloned by PCR using specific primers and *T. castaneum* genomic DNA. The resulting inserts will be ligated in expression vectors (pET28a or similar) and transformed into competent *E. Coli* cells for protein production (*E. Coli* strain BL21DE3 or similar). Protein purification will be carried out by standard liquid chromatography and FPLC where necessary. Protein purity will be assessed by SDS/PAGE. Screening for crystallisation conditions will exploit the availability of modern crystallisation plates and screens using the microbatch under oil technique. This technique will allow to explore many different crystallisation condition while saving on the quantity of protein and precipitants needed for the initial screening. Promising hits will be optimised both by microbatch under oil and hanging drop vapour diffusion.
X-ray diffraction data on the crystals obtained will be collected at the European Synchrotron Radiation Facility (ESRF) in Grenoble or at beamlines of the European Molecular Biology Laboratory outstation at the Hamburg synchrotron source (DESY, Deutsches Elektronen-Synchrotron).
As limited structural information is available for this class of proteins the anomalous signal of the naturally contained sulphurs (six and four cysteines involved in disulfide bridges in OBPs and CSPs respectively) will be exploited for structure solution either by SAD (Single-wavelength Anomalous Dispersion) or MAD (Multi-wavelength Anomalous Dispersion) methods.

Costs involved (consumables):

Enzymes and kits for cloning, cell culture media, chromatographic column for gel filtration Superdex 200 GEhealthcare (size exclusion chromatography), crystallisation plates for microbatch under oil and hanging drop, crystallisation screens: Index (Hampton Research), PACT, JCSG, CSSI, CSSII (Molecular Dimensions), salts, diverse precipitants (e.g., PEGs) and various buffers and additives.
Estimated cost 4.692,00 €.

Breakdown of expenses:
chromatographic column for gel filtration Superdex 200 GEhealthcare €1620
96-well IMP@CT Plate Clear (40 pack) €300
24-well XRL Plate (100) €180
Crystallisation kits €400
Enzymes and reagents €2192

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Dr. Stefano Benini