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Diversity of Odorant-binding Proteins and Chemosensory Proteins in Insects

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Odour and pheromone perception occurs through a complex series of events, many aspects of which have been elucidated in the most recent years. The discovery of membrane-bound olfactory receptors, both in vertebrates and in insects, and their functional expression in heterologous systems have shown that they can be directly activated by odorant and pheromone molecules.

Although a pathway of signal transduction from the peripheral olfactory receptors to the areas of the central nervous system can explain how olfactory messages are translated into behavioural responses, the role of soluble proteins at the periphery of the olfactory system remains elusive.

On one hand, messenger molecules (odorants and pheromones) are able to directly activate olfactory receptors, but on the other hand, the very high concentration of soluble proteins around olfactory dendrites indicates an important, yet unknown, role. In fact, the great amount of energy involved in their synthesis and turnover cannot be justified without a great benefit for the individual or for the species. This is particularly true for insects, which often live on a very critical energy balance.

In vertebrates, odorant-binding proteins (OBPs) represent the soluble proteins present in the perireceptor area (Pelosi *et al.*, 1982; Pelosi, 2001), in insects two classes of soluble proteins have been so far identified in the lymph of chemosensilla, odorant-binding proteins (OBPs) and chemosensory proteins (CSPs) (Vogt and Riddiford, 1981; Pelosi, 1998; Steinbrecht, 1998; Vogt *et al.*, 1999; Jacquin-Joly *et al.*, 2001; Calvello *et al.*, 2003). These three classes of polypeptides share a small size (12–18 kDa), very high solubility and a capacity of reversibly binding small molecules, such as odorants and pheromones. However, they are structurally very different. Vertebrates' OBPs belong to the lipocalin family and are folded in a β-barrel motif, made mostly of antiparallel β-strands. By contrast, insects' OBPs and CSPs mainly contain α-helical domains, but folded in two different patterns (Sandler *et al.*, 2000; Lartigue *et al.*, 2002; Tegoni *et al.*, 2004). There is no similarity also in their amino acid sequences. OBPs are generally divergent across species and within the same species, with percent of conserved residues as low in some cases as 8%. Their signature is represented by a pattern of six cysteines in conserved positions, that are connected in the native protein by three interlocking disulphide bridges. CSPs are better conserved with often >50% of identical residues even between members of phylogenetically distant species. Their signature is constituted by four cysteines connected by disulphide links between adjacent residues, resulting in the formation of two small loops of eight and four amino acids.

Several pieces of evidence indicate that OBPs and CSPs of insects represent two classes of proteins performing similar roles, despite their different structures. In fact, both classes of proteins reversibly bind small ligands with dissociation constants in the micromolar range. In some cases complexes with ligands have been crystallized and their structures resolved. Also, both OBPs and CSPs are secreted in the lymph of chemosensilla, where their concentration can reach millimolar levels. Depending on the species, they can be expressed in one-pore contact sensilla as well as in multipore olfactory hairs (Angeli *et al.*, 1999).

Originally OBPs were considered as the proteins active in perireceptor events of chemoreception, while CSPs were regarded as involved in general, yet undefined, functions. This view was also supported by the observation that OBPs were antennal specific, while CSPs had been identified in several chemosensory as well as non-chemosensory tissues. However, the antennal specificity of OBPs, first observed in Lepidoptera and other orders of insects, is not verified in other cases. In the Argentine ant, for instance, a CSP has been recently reported as antennal specific (Ishida *et al.*, 2002). At about the same time we showed that in the paper wasp *Polistes dominulus* CSP is antenna-specific, while OBP is also expressed in legs and wings (Calvello *et al.*, 2003). The same distribution has also been found in another wasp, *Vespa crabro*, indicating that different species may have chosen OBPs or CSPs as the 'important proteins' during evolution.

The presence of CSPs or OBPs in non-sensory organs, such as the wings, has suggested an additional role for these soluble proteins, in analogy to what is known in some vertebrates. The male mouse, for instance, secretes in the urine large amounts of lipocalins, structurally very similar or even identical to their nasal OBPs. These proteins, which are void of ligands when purified from the nasal mucosa, are associated to specific pheromones in the urine. We have demonstrated that in some insect species CSPs (in the locusts) or OBPs (in the wasps), when purified from the wings, are complexed with different small compounds that may be involved in chemical communication (Ban *et al.*, 2003; Calvello *et al.*, 2003). Therefore, like lipocalins in vertebrates, both OBPs and CSPs in insects could be endowed with a dual role, detecting as well as releasing chemical messengers.

In addition, the families of OBPs and CSPs could include members bearing no relationship to chemical communication, as in the case of lipocalins, that include proteins of passive transport across the blood stream and even enzymes. The genome projects have revealed the presence of 51 genes encoding OBP-like proteins in *Drosophila melanogaster* and 72 in *Anopheles gambiae*. It is likely that only a subgroup of these genes encode proteins relevant for olfaction or chemical communication.

Another element supporting an important role for OBPs and CSPs is their number in the same species. Generally, several proteins of these families are actually expressed in chemosensory organs, often structurally different from one another, so that subclasses can be identified. The diversity of soluble proteins within the same species suggests a discriminating role, although clear evidence supporting such hypothesis has not been provided.

Finally, the study of the three-dimensional structures of OBPs and CSPs in association with their ligands have uncovered major conformational changes associated with their binding. In the pheromone-binding protein of *Bombyx mori* the C-terminal region, which has no definite structure at neutral pH, folds into an α-helical segment at low pH and fits into the pheromone-binding cavity (Horst *et al.*, 2001). This mechanism has been proposed to occur in the proximity

of the dendritic membrane to release the pheromone molecule and handle it to the receptor. The CSP of *Mamestra brassicae* also changes its conformation in the presence of ligands (Campanacci *et al.*, 2003), swelling its binding pocket to accommodate up to three molecules of bromododecanol.

These pieces of information suggest that OBPs and CSPs perform similar roles in chemical communication of insects and that an important, although still undiscovered, function is associated with these soluble polypeptides.

To get insights into the function of these proteins, the study of mutants where their absence is associated with a modified behaviour is extremely informative. So far only two such phenotypes have been reported.

In *D. melanogaster* the knock-out of the gene encoding the odorant-binding protein LUSH has been reported to modify the flies' behaviour to ethanol (Kim *et al.*, 1998). In fire ants it has been observed that colonies making more than one queen do not express one of their OBPs (Krieger and Ross, 2002). In the latter case, being such OBP normally produced in the thorax, it is difficult to conceive that this protein could mediate perception of pheromonal compounds. More likely, this OBP could be a pheromone carrier, as in the case of wasps, and therefore its absence might prevent the pheromone being released in the environment.

Recently we investigated the role of LUSH in more detail by measuring the binding of bacterial expressed protein to several compounds. Unexpectedly, we were unable to detect any binding to ethanol or lower alcohols. On the contrary, the best ligands of LUSH were aromatic molecules of medium size, such as the fluorescent probe used in our assay (*N*-phenyl-1-naphthylamine) and some dialkyl phthalates (Zhou *et al.*, 2004).

In the crystal structure of LUSH, the C-terminus is folded back into the core of the protein, in a fashion similar to what observed in the acidic form of *B. mori* pheromone-binding protein (Kruse *et al.*, 2003). Such structure would not allow the entrance of a large ligand into the binding cavity without a major conformational change, involving flipping of the C-terminal region outside the protein. This hypothesis is supported by the fact that in our experiments the binding of aromatic molecules is not associated with quenching of fluorescence of the only tryptophan residue, located in the C-terminal segment and well inside the binding pocket in the published structure.

Conformational changes associated with ligand binding could be a general phenomenon for OBPs and CSPs and suggest some further interactions of these proteins with other elements of the signal transduction chain.

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