Insect Biochemistry and Molecular Biology 66 (2015) 31–41

Contents lists available at ScienceDirect

Insect Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/ibmb

Review

Towards an understanding of the structural basis for insect olfaction by odorant receptors

Colm Carraher [a], Julie Dalziel [b], Melissa D. Jordan [a], David L. Christie [c], Richard D. Newcomb [a, c], Andrew V. Kralicek [a, *]

[a] The New Zealand Institute for Plant & Food Research Limited, Private Bag 92169, Auckland 1142, New Zealand
[b] Food Nutrition & Health Team, Food & Bio-based Products Group, AgResearch Private Bag 11008, Palmerston North 4442, New Zealand
[c] School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

ARTICLE INFO

Article history:
Received 10 May 2015
Received in revised form
16 September 2015
Accepted 16 September 2015
Available online 28 September 2015

Keywords:
Insect olfaction
Odorant receptor
Orco
Structure
Function

ABSTRACT

Insects have co-opted a unique family of seven transmembrane proteins for odour sensing. Odorant receptors are believed to have evolved from gustatory receptors somewhere at the base of the Hexapoda and have expanded substantially to become the dominant class of odour recognition elements within the Insecta. These odorant receptors comprise an obligate co-receptor, Orco, and one of a family of highly divergent odorant "tuning" receptors. The two subunits are thought to come together at some as-yet unknown stoichiometry to form a functional complex that is capable of both ionotropic and metabotropic signalling. While there are still no 3D structures for these proteins, site-directed mutagenesis, resonance energy transfer, and structural modelling efforts, all mainly on *Drosophila* odorant receptors, are beginning to inform hypotheses of their structures and how such complexes function in odour detection. Some of the loops, especially the second extracellular loop that has been suggested to form a lid over the binding pocket, and the extracellular regions of some transmembrane helices, especially the third and to a less extent the sixth and seventh, have been implicated in ligand recognition in tuning receptors. The possible interaction between Orco and tuning receptor subunits through the final intracellular loop and the adjacent transmembrane helices is thought to be important for transducing ligand binding into receptor activation. Potential phosphorylation sites and a calmodulin binding site in the second intracellular loop of Orco are also thought to be involved in regulating channel gating. A number of new methods have recently been developed to express and purify insect odorant receptor subunits in recombinant expression systems. These approaches are enabling high throughput screening of receptors for agonists and antagonists in cell-based formats, as well as producing protein for the application of biophysical methods to resolve the 3D structure of the subunits and their complexes.

Contents

* Corresponding author.
E-mail address: andrew.kralicek@plantandfood.co.nz (A.V. Kralicek).

http://dx.doi.org/10.1016/j.ibmb.2015.09.010
0965-1748/

1. Introduction

Olfaction is the most important of the senses for insects, being critical for feeding, oviposition, mate recognition and predator avoidance (Carey and Carlson, 2011). Insects detect odours using an array of receptors that fall into two major classes. Ionotropic Receptors (IRs) are ligand-gated ion channels that are sensitive to acid and amine odours (Rytz et al., 2013). Odorant Receptors (ORs) are a much larger class that are also ligand-gated ion channels (Kaupp, 2010) and are related to insect Gustatory Receptors (GRs) (Montell, 2009, 2013). The ORs are capable of discriminating amongst thousands of volatiles (Kaupp, 2010), detecting many compounds with great sensitivity (Angioy et al., 2003). These receptors are integral membrane proteins found in the dendritic membranes of olfactory sensory neurons (OSNs), housed within sensilla (often fine hair-like structures) located on the insect's antennae. Odorant molecules are thought to diffuse through pores in the walls of sensilla and enter a lymph, where they are transported by odorant binding proteins (OBPs) to membrane-bound ORs (Leal, 2013). Odorant binding by these receptors results in OSN depolarisation and a neuronal signal that is decoded by the insect brain, informing behavioural response decisions. In this review we will focus on the ORs and in particular on what is currently known about their structure and function.

Insect OR-mediated olfaction requires the co-expression of two OR genes in each OSN: a co-receptor *Orco*, previously known as *Or83b* (Vosshall and Hansson, 2011), which is broadly expressed across OSNs (Larsson et al., 2004), and an odorant-binding subunit (*OrX*) that is expressed in a specific subset of OSNs (Carey et al., 2010; Hallem et al., 2004; Wang et al., 2010). Orco protein interacts with OrXs early in the endomembrane system in OSNs, is necessary for correct trafficking of the complex to the dendritic membrane, and is essential to maintain the OR complex within the sensory cilia (Benton et al., 2006). However, Orco has not been found to have any olfactory function without the presence of an OrX (Elmore et al., 2003). The caveat to this assertion is the discovery of some allosteric agonists and antagonists for Orco that are proving useful in structure/function studies of the co-receptor (Jones et al., 2011, 2012; Kumar et al., 2013; Taylor et al., 2012). Orco orthologues from different species can rescue function in null mutants of *Drosophila melanogaster*, indicating a conserved functional role across insects (Jones et al., 2005), and that Orcos from different species have little impact on the tuning of the OrX partner (Nichols et al., 2011).

Vertebrate ORs are seven transmembrane helix (TMH) G protein-coupled receptors (GPCRs) (Kato and Touhara, 2009). Insect ORs are also seven TMH proteins; however, membrane topology analysis of the insect OR subunits both *in vivo* and expressed in cell lines revealed that they have the opposite orientation in the membrane compared with GPCRs, with an intracellular N-terminus and an extracellular C-terminus (Benton et al., 2006; Jordan et al., 2009; Lundin et al., 2007; Smart et al., 2008; Tsitoura et al., 2010). Furthermore, it is generally accepted that insect OrXs and Orco form a greatly expanded phylogenetic lineage that seems to be derived from insect GRs (Missbach et al., 2014; Robertson, 2009; Robertson et al., 2003) and are not related to GPCRs (Benton et al., 2006).

A number of lines of evidence support a stable heteromeric complex being formed between Orco and OrX subunits, including *in vivo* protein fragment complementation assays (PCA), resonance energy transfer (RET), and co-immunoprecipitation (Benton et al., 2006; German et al., 2013; Neuhaus et al., 2005; Tsitoura et al., 2010). None of these studies, however, has provided information on the stoichiometry of the receptor subunits required for these interactions or addressed Orco's ability to couple promiscuously with a large number of highly divergent OrX subunits (61–341 depending on the insect species) (Touhara and Vosshall, 2009). There is some evidence that this interaction is mediated through contacts between the third intracellular loops (ICL3s) of the subunits or the proximate TMH regions (Benton et al., 2006). However, sequence analysis of Orco and OrX subunits has failed to identify common oligomerisation motifs, despite the higher degrees of conservation found around these regions in other proteins (Clyne et al., 1999; Miller and Tu, 2008; Ray et al., 2014; Vosshall, 2003).

The Orco/OrX complex is believed to form an odorant-gated non-selective cation channel with ionic permeability for Ca^{2+}, Na^{+} and K^{+} (Nakagawa et al., 2012; Sato et al., 2008; Smart et al., 2008; Wicher et al., 2008; Yao and Carlson, 2010). Odorant binding induces ionotropic signalling by the complex. There is however, some additional *in vitro* evidence for the existence of a metabotropic signalling pathway, supporting a role for G protein/secondary messenger regulation of ORs (Chatterjee et al., 2009; Deng et al., 2011; Getahun et al., 2013; Jones et al., 2011; Kaupp, 2010; Raja et al., 2014; Sargsyan et al., 2011; Wicher, 2013; Wicher et al., 2008) (Fig. 1). A G protein-binding site, however, is yet to be definitively identified in either Orco or any OrX. For an overview and discussion concerning the mechanisms and relative importance of these different signalling pathways, please see the following review articles (Kaupp, 2010; Nakagawa and Vosshall, 2009; Silbering and Benton, 2010; Stengl and Funk, 2013; Wicher, 2013).

What little is known of the mechanism of activation and structural organisation of the insect Orco/OrX complex has so far come from studies on insect Orco and OrX subunits expressed in heterologous cell lines, *Xenopus* oocytes, and transgenic flies. However, knowledge on this topic remains poor in comparison to understanding of other membrane protein receptors, particularly for examples where structural data are available (Corringer et al., 2012; Kumar and Mayer, 2013; Moreira, 2014; Vaidehi et al., 2014; Zhang et al., 2013). The lack of a crystal structure from closely related receptor/channel families has also negated the use of homology modelling approaches on insect ORs. However, a recent analysis of amino acid covariation across insect Orcos and OrXs has been used to *de novo* construct the first 3D models of the *D. melanogaster* odorant receptors, DmOrco and DmOr85b (Hopf

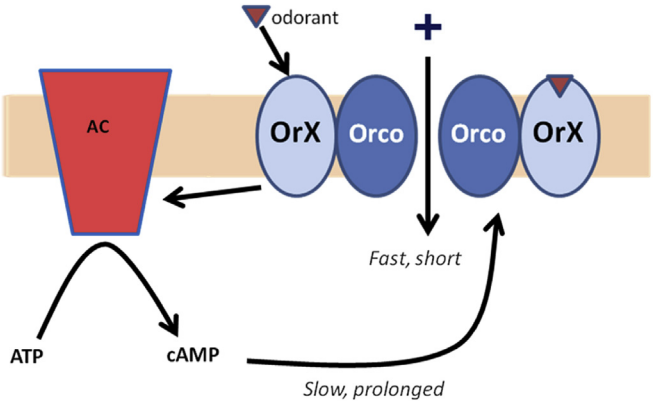

Fig. 1. Overview of insect odorant receptor signal transduction. Odorant binding to the OrX subunit in the Orco/OrX complex activates two signalling pathways, a fast short ionotropic pathway and a slow prolonged metabotropic pathway. The ionotropic pathway involves the direct odour activated opening of the ion channel pore (Sato et al., 2008). The metabotropic pathway involves the indirect opening of the channel pore, as odorant binding to the OrX subunit activates an adenylyl cyclise (AC) that causes cAMP production (Wicher et al., 2008). The increased concentrations of cAMP prolongs the opening of the channel pore (Sargsyan et al., 2011). Figure adapted from Kaupp (2010).