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Imidacloprid and thiacloprid neonicotinoids bind more favourably to cockroach than to honeybee α6 nicotinic acetylcholine receptor: Insights from computational studies

Balaji Selvam[a,**], Jérôme Graton[a], Adèle D. Laurent[a], Zakaria Alamiddine[a], Monique Mathé-Allainmat[a], Jacques Lebreton[a], Olivier Coqueret[b], Christophe Olivier[b], Steeve H. Thany[c], Jean-Yves Le Questel[a,*]

[a] Université de Nantes, UMR CNRS 6230, Chimie Et Interdisciplinarité: Synthèse, Analyse, Modélisation (CEISAM), UFR des Sciences et des Techniques, 2, rue de la Houssinière, BP 92208, 44322 Nantes Cedex 3, France
[b] Université d'Angers, Récepteurs et Canaux Ioniques Membranaires, UPRES EA 2647, USC INRA 2023, UFR Sciences, 2 boulevard Lavoisier, 49045 Angers Cedex, France
[c] Centre de Recherche en Cancérologie Nantes-Angers, UMR_S 892 – C 6299, Institut de Recherche en Santé IRS Université de Nantes, Unité INSERM 892 – CNRS 6299 Centre de Recherche en Cancérologie Nantes-Angers, 8 quai Moncousu, BP70721, 44007 Nantes Cedex, France

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ABSTRACT

The binding interactions of two neonicotinoids, imidacloprid (IMI) and thiacloprid (THI) with the extracellular domains of cockroach and honeybee α6 nicotinic acetylcholine receptor (nAChR) subunits in an homomeric receptor have been studied through docking and molecular dynamics (MD) simulations. The binding mode predicted for the two neonicotinoids is validated through the good agreement observed between the theoretical results with the crystal structures of the corresponding complexes with *Ac*-AChBP, the recognized structural surrogate for insects nAChR extracellular ligand binding domain. The binding site of the two insect α6 receptors differs by only one residue of loop D, a serine residue (Ser83) in cockroach being replaced by a lysine residue (Lys108) in honeybee. The docking results show very close interactions for the two neonicotinoids with both α6 nAChR models, in correspondence to the trends observed in the experimental neonicotinoid-*Ac*-AChBP complexes. However, the docking parameters (scores and energies) are not significantly different between the two insect α6 nAChRs to draw clear conclusions. The MD results bring distinct trends. The analysis of the average interaction energies in the two insects α6 nAChRs shows indeed better affinity of neonicotinoids bound to α6 cockroach compared to honeybee nAChR. This preference is explained by tighter contacts with aromatic residues (Trp and Tyr) of the binding pocket. Interestingly, the non-conserved residue Lys108 of loop D of α6 honeybee nAChR interacts through van der Waals contacts with neonicotinoids, which appear more favourable than the direct or water mediated hydrogen-bond interaction between the OH group of Ser83 of α6 cockroach nAChR and the electronegative terminal group of the two neonicotinoids (nitro in IMI and cyano in THI). Finally, in both insects nAChRs, THI is consistently found to bind more favourably than IMI.

1. Introduction

The growing world population and the resulting continuously rising demand in food supply are facing serious threat of crops damages by invertebrate pests. In this context, an important area of sustainable agriculture is the design of potent, selective, efficient and safe insecticides. The modern insecticides should be highly selective of insects over the mammals as well as selective of pests over the beneficial insects such as honeybees. nicotinic acetylcholine receptors (nAChRs) have shown growing interest for many years and have been recognized as insecticide molecular targets,

Abbreviations: nAChR, nicotinic acetylcholine receptor; *Ac*-AChBP, *Aplysia californica*-acetylcholine binding protein; LGICs, ligand-gated ion channels; GABA, gamma-aminobutyric acid; 5-HT3, 5-hydroxytryptamine receptor subtype 3; CHARMM, Chemistry at Harvard Molecular Mechanics; IMI, imidacloprid; THI, thiacloprid.

* Corresponding author. Tel.: +33 (0)2 51 12 55 63; fax: +33 (0)2 51 12 55 67.
** Corresponding author. Tel.: +33 (0)2 76 64 51 70; fax: +33 (0)2 51 12 55 67.
E-mail addresses: balaji.panneerselvam@univ-nantes.fr (B. Selvam), jean-yves.le-questel@univ-nantes.fr (J.-Y. Le Questel).

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especially for the design of neonicotinoids, classified as agonists of these receptors [1]. Indeed, the role of nAChRs in mediating fast excitatory synaptic transmission in the central nervous system of insects has made them one of the most attractive target for exploration in insecticide discovery [2].nAChRs belong to the Cys-loop ligand-gated ion channels (LGICs) superfamily, which also includes the GABA, glycine, and 5-HT3 receptors [3,4]. nAChRs are pentameric LGICs, the five subunits being symmetrically or pseudosymmetrically arranged around a central ion-conducting pore, forming homo- or heteropentamers of related subunits [5,6]. The functional organization of nAChRs, as well as their diversity in terms of subunit composition and stoichiometries, is much better known in vertebrates than in insects [7–9]. The agonist binding site of nAChRs is localized at interfacial regions between subunits and consists of several discontinuous loops (A–F), specific subunit combinations conferring differences in sensitivity to ACh and in pharmacological profiles [5,10]. Rational design of more efficient and selective insecticides would be greatly helped by a high resolution structure of nAChRs. Today, the highest resolution (4.0 Å) structural information on nAChRs comes from electron diffraction of helical tubular crystals of nAChRs from *Torpedo marmorata* [11,12]. In the last years, the determination of the X-ray crystallographic structures of the bacterial transmembrane proteins GLIC (*Gloeobacter violaceus* pentameric ligand-gated ion channel homologue) and ELIC (a bacterial homologue from *Erwinia chrysanthemi*), which are distant homologues of the nAChRs, have revealed many structural features of these membrane proteins, notably the architecture of the pore, including its gate and its selectivity filter [3,13–15]. However, these structures do not allow a comprehensive description of the ligand–nAChRs interactions.

In this context, the discovery and crystallization of ligand-free and ligand-bound structures of acetylcholine binding protein (AChBP) have allowed to gain deep insights into the details of the binding site and its relation to function [16–22]. In the field of insecticides, AChBP extracted from *Aplysia californica* (*Ac*-AChBP) has been used as a plausible structural surrogate of insects nAChRs because it has been shown to be pharmacology reminiscent for the insect nAChR subtypes, that is, to present high neonicotinoid sensitivity [23]. These data have provided the structural basis for the design of homology models for extracellular domains of specific nAChRs subunits combinations and the investigation of the binding of neonicotinoids on the corresponding receptor–ligand binding interfaces [24]. Considering the fact that the exact composition of the sensitive nAChRs is still unknown, various nAChRs isotypes from different insect species have been already considered: *e.g.* α2β1, α1β2 from peach potato aphid (*Myzus persicae*) and honeybee (*Apis mellifera*) [2,22,24–27].

From an experimental point of view, several studies combining binding assays, molecular biology and electrophysiology have investigated the sensitivity of various nAChRs subtypes to neonicotinoid insecticides [27–32]. However, these studies have been hindered by difficulties encountered in expressing recombinant insect nicotinic receptors leading to functional recombinant nAChRs. A number of strategies was developed to overcome such difficulties and, only experimental data have been obtained for *Drosophila* nAChR subunits Dα5 and Dα7 [32] and Nlα1 and Nlα2 subunit of brown planthopper *Nilaparvata lugens* [33]. Within this framework, the expression of functional recombinant nAChRs has been reported for several insect nAChR α subunits and co-expressed with vertebrate β subunits [33,34].

In the present work, we have investigated through complementary molecular modelling methods the binding of imidacloprid (IMI) and thiacloprid (THI), two important neonicotinoids, to homomeric α6 nAChRs of cockroach (*P. Americana*) and honeybee (*A. mellifera*). It is worth noticing that we have limited the modelling of nAChRs to the isolated extracellular domains (ECD) rather than considering the full-length nicotinic acetylcholine receptor. We have selected the α6 subunits because they have strong sequence similarity with α5 and α7 nAChRs subunits of the *Drosophila* insect model that have been proven to form functional homomeric and heteromeric ion channels [33]. The consideration of homomeric species obviously simplifies the pentameric nAChR model. By selecting cockroach and honeybee nAChRs, our objectives are (i) to rationalize at the atomic level the binding of IMI and THI in the targeted nAChRs and (ii) to draw guidelines for the development of new compounds selective of pests with respect to honeybees. Actually, IMI and THI neonicotinoids are the only two representatives of this class of insecticides for which crystallographic data are available and accurately describe the specific interactions with *Ac*-AChBP at the atomic level [35]. Therefore, these structural data allows us to check *a posteriori* the validity of the receptor–ligand complexes built from the homology models. After a discussion on the homology models, the docking results are presented and compared for the neonicotinoids in each targeted nAChRs (α6 cockroach and α6 honeybee). A comparison of the binding interactions observed for the two insecticides in the two insect species nAChRs is carried out from these results. Significant differences in terms of the nature of the interactions and their geometric features are obtained. Molecular dynamics simulations have then been realized to complete these data. These analyses provide key elements rationalizing the behaviour of both neonicotinoids with respect to the targeted nAChRs and could be used for the rational design of new compounds with a better efficiency and selectivity.

2. Methods

2.1. Homology modelling

The amino acid sequences of the cockroach *P. americana* and honeybee *A. mellifera* α6 subunits were extracted from the Uniprot server (www.expasy.org) [36]. The closest homology of the target sequences were identified using the BLAST programme [37]. The crystal structure of the acetylcholine binding protein (*Ac*-AChBP) extracted from *A. californica* (*Ac*) (PDB ID: 3C79) [24] was selected as a template to build the three-dimensional (3D) model of cockroach and honeybee nAChRs. Indeed, *Ac*-AChBP is the recognized surrogate for the ligand binding domain of the extracellular domain of insect nAChRs [38]. The crystal structures of the *Ac*-AChBP–neonicotinoid complexes (PDB ID: 3C79 and PDB ID: 3C84 for IMI and THI ligands, respectively) were downloaded from the Protein Data Bank (www.pdb.org) [39]. The pairwise sequence alignments were performed to align the target and the template sequence. The 3D homology models were built using the Prime v3.6 [40] module of the Schrodinger suite 2014-1 [41]. The rotamers of the conserved amino acid residues are preserved in the homology model such that the final 3D model does not significantly deviate from the template structure. The stereochemical quality of the model was verified using MolProbity (Figs. S1 and S2) [42].

2.2. Docking

The chemical structures of IMI and THI neonicotinoids are shown in Fig. 1. The structures have been converted to 3D at pH 7.0 ± 0.2 using the LigPrep v3.0 [43] module of the Schrodinger suite 2014-1 [10]. The 3D ligand molecules were then subjected to the confgen [44] programme to retrieve the lowest energy conformer for docking. The docking was performed using the Glide v6.3 [45] programme of the Schrodinger suite 2014-1 [10]. The residues around 6 Å of the ligand were defined as the active site and were selected for the receptor grid generation. The extra-precision (XP) [46] mode of the docking algorithm was employed to dock IMI and