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Biological assessment of neonicotinoids imidacloprid and its major metabolites for potentially human health using globular proteins as a model

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ABSTRACT

The assessment of biological activities of imidacloprid and its two major metabolites, namely 6-chloronicotinic acid and 2-imidazolidone for nontarget organism, by employing essentially functional biomacromolecules, albumin and hemoglobin as a potentially model with the use of circular dichroism (CD), fluorescence, extrinsic 8-anilino-1-naphthalenesulfonic acid (ANS) fluorescence as well as molecular modeling is the theme of this work. By dint of CD spectra and synchronous fluorescence, it was clear that the orderly weak interactions between amino acid residues within globular proteins were disturbed by imidacloprid, and this event led to marginally alterations or self-regulations of protein conformation so as to lodge imidacloprid more tightly. Both steady state and time-resolved fluorescence suggested that the fluorescence of Trp residues in proteins was quenched after the presence of imidacloprid, corresponding to noncovalent protein–imidacloprid complexes formation and, the reaction belongs to moderate association ($K = 1.888/1.614 \times 10^4\ M^{-1}$ for albumin/hemoglobin-imidacloprid, respectively), hydrogen bonds and π stacking performed a vital role in stabilizing the complexes, as derived from thermodynamic analysis and molecular modeling. With the aid of hydrophobic ANS experiments, subdomain IIA and $\alpha_1\beta_2$ interface of albumin and hemoglobin, respectively, were found to be preserved high-affinity for imidacloprid. These results ties in with the subsequently molecular modeling laying imidacloprid in the Sudlow's site I and close to Trp-213 residue on albumin, while settling down B/Trp-37 residue nearby in hemoglobin, and these conclusions further confirmed by site-directed mutagenesis and molecular dynamics simulation. But, at the same time, several crucial noncovalent bonds came from other amino acid residues, e.g. Arg-194 and Arg-198 (albumin) and B/Arg-40, B/Asp-99 and B/Asn-102 (hemoglobin) cannot be ignored completely. Based on the comparative studies of binding modes between imidacloprid and its two primary metabolites with globular proteins, it is evident to us that the noncovalent interactions of 6-chloronicotinic acid and 2-imidazolidone with biopolymers are not always to be decreased obviously as a result of the relatively small molecular structures of these metabolites, compared with parent compound imidacloprid. Conversely, this could probably strengthen the weak interactions existed in the macromolecules-metabolites conjugation, or rather, the metabolites such as 6-chloronicotinic acid and 2-imidazolidone contributed drastically to the overall toxicity of imidacloprid.

1. Introduction

The contamination of food by chemical hazards is an international public health anxiety and is also a possible inducement to human chronic diseases [1,2]. Contamination may arise by environmental pollution of the air, water and soil, such as the case with toxic metals, polychlorinated biphenyls and dioxins, or especially, through the purposeful use of diverse agrochemicals, e.g. pesticides [3]. Neonicotinoids are one of the most famous breakthroughs in the track of modern pesticides, and the most representative merchandise–imidacloprid (*N*-[1-[(6-chloropyridin-3-yl)methyl]-4,5-dihydroimidazol-2-yl]nitramide, structure shown in Fig. 1), was introduced to the market in 1991, and since then imidacloprid has become the fastest growing insecticide worldwide [4]. Imidacloprid acts on the central nervous system as

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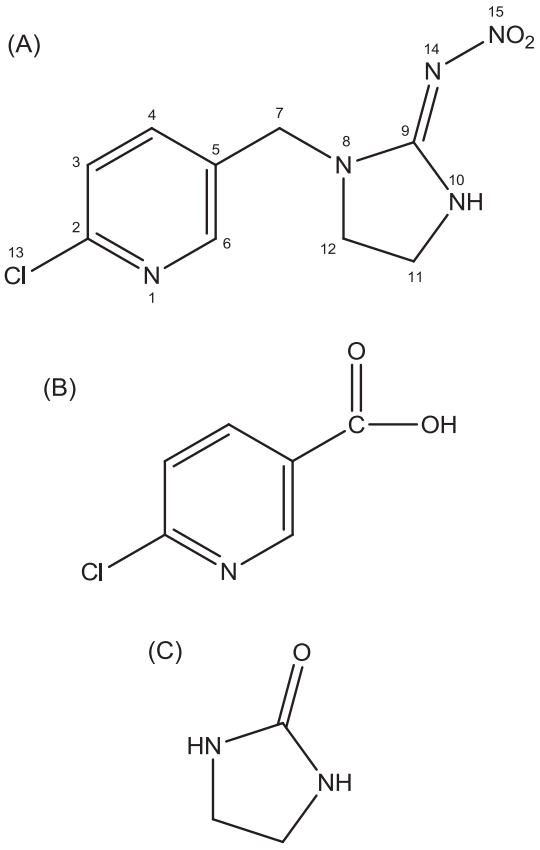

Fig. 1. Molecular structures of imidacloprid (A), 6-chloronicotinic acid (B) and 2-imidazolidone (C).

an agonist at the nicotinic acetylcholine receptor, and induces initial stimulation followed by fatigue of the agonized neurons and eventually meddles with the transmitting of neuronal impulses. The selective toxicity of imidacloprid to insects and relative sparing of mammals and other vertebrates befall through high idiosyncrasy for the nicotinic receptor subtypes found in insect tissues and by inferior permeation of the blood–brain barrier in mammals [5]. Accordingly, imidacloprid has mostly been regarded as low mammalian toxicity and categorized as a toxicity class II (moderately hazardous) pesticide by the World Health Organization [6]. Due to low toxicity to vertebrates, together with broad-spectrum of activity, imidacloprid has been registered in excess of 120 countries and is used on various crops, such as cereals, corn, cotton, potatoes, rice, sorghum and many vegetables, for mainly preventing different sucking insects, e.g. aphids, leafhoppers and planthoppers, thrips and whiteflies [4,5].

In recent years there have been continuous worries about the deleterious impacts of imidacloprid and its degradation products on the environment and finally the human health [7]. In view of imidacloprid has been largely used for more than 20 years, a huge amount of this neonicotinoid could probably be existed in the environment [8]. The residual imidacloprid go through many different routes, including transformation, sorption–desorption, volatilization, uptake by crops, runoff to surface waters, and transport to groundwater, so as to bioaccumulate in the food chain and eventually accumulate chiefly in the human diet [9]. There is forceful proof confirmed that the toxicity of plenty pesticides is most likely ascribed to the chronic accumulation rather than acute poisoning. And, these hurtful health effects contains acute and persistent hurt to the nervous system, lung damage, harm to the reproductive organs, and disfunction of the immune and endocrine systems, birth defects, and cancer [10,11]. As regards imidacloprid, toxicological evidences have plainly proved that genotoxicity, direct deoxyribonucleic acid strand breakages, and chromosome/genome mutation may be caused by this chemical, and it can also induce mutagenicity, oxidative stress, developmental immunotoxicity, and inflammation in the central nervous system and liver in nontarget organisms [12–14]. More importantly, the research testifies that long time and low dosage contact to imidacloprid could primarily be acted on the liver, thyroid and body weight (reduction), and low oral exposure has been connected with reproductive toxicity, developmental retardation and neurobehavioral deficits in several *in vivo* animal models such as rats and rabbits [15]. Nonetheless, no studies have so far been performed involving human subjects chronically exposed to imidacloprid, although an experiment conducted in Sprague–Dawley rats very recently suggests that the neonicotinoids, i.e. acetamiprid and imidacloprid, may negatively affect human health, particularly the developing brain when the concentrations larger than 1.0 μM [16].

Physiologically, a variety of low molecular weight substances such as bilirubin, colorants, drugs, hormones and metabolites are transported from their site of absorption to their location of action and excretion through the circulatory blood [17–20]. Even though some agents are simply dissolved in blood serum, many others are associated with blood components, e.g. albumin, erythrocytes, globulins, lipoproteins and polypeptides [21–23]. As Brodie [24] has indicated earlier, almost all ligands are reversibly conjugated with the proteins of the plasma or the tissues, and binding to proteins has physiological importance in transport, adjustment and deactivation of the chemicals and their metabolite activities. This event also undertakes a protective strategy in binding and in inactivating latent adverse substances to which the human body is exposed. As has been argued, although neonicotinoids are the largest group of insecticides in use today, the toxicity for human beings reserves immensely controversial. However, sparse systematic work has been done on the binding of neonicotinoids with several classically proteins. Investigations into the more delicate effects of the binding phenomena between neonicotinoids and proteins can supply significant perception for the general toxicity of these agrochemicals, as the protein–ligand of a compound in this manner may adjust its allocation in the body, and thereby influence both the dose–response connection and the speed of compound elimination, and ultimately the toxicological responses in the body [25–29]. The present investigation was schemed to delve the binding of imidacloprid, which is regarded as one of the typical neonicotinoids, with two representatively mammalian proteins, i.e. albumin and hemoglobin by means of circular dichroism, steady state and time-resolved fluorescence, extrinsic 8-anilino-1-naphthalenesulfonic acid (ANS) fluorescence and computer-aided molecular modeling. The conformation of proteins after complexation, reaction mechanism, binding affinity and binding region for different protein–imidacloprid complexes were all decoded in this study. In particular, two metabolites of imidacloprid, that is 6-chloronicotinic acid and 2-imidazolidone, were selected for further *in vitro* analyses based on molecular modeling, owing to these metabolites are deeply suspected to be contributed prominently to the apparently poisonous properties of imidacloprid.

2. Experimental

2.1. Materials

Albumin from bovine serum (A7030, lyophilized powder, fatty acid free, globulin free, ⩾98%), hemoglobin human (H7379, lyophilized powder), imidacloprid (37894, analytical standard) and 8-anilino-1-naphthalenesulfonic acid (A1028, ⩾97%) were