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Review article

Labelled animal toxins as selective molecular markers of ion channels: Applications in neurobiology and beyond

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ABSTRACT

Animal toxins are traditional and indispensible molecular tools that find application in different fields of biochemistry, neurobiology and pharmacology. These compounds possess several outstanding properties such as high affinity and selectivity with respect to particular molecular targets, most importantly ion channels and neuroreceptors, and stability. In addition to using toxins *per se*, a wide variety of labelled modifications have been obtained including radioactive and fluorescent derivatives. Here, we discuss the major types of labelled toxins, methods of their production and principal possibilities of application ranging from receptor localization and visualization to development of screening systems and diagnostic tools, and drug discovery.

1. Introduction

Natural toxins from venomous and poisonous animals are established powerful molecular tools that are used across different fields of biochemistry, neurobiology and applied medical science. They also possess considerable potential as therapeutic hits and diagnostic instruments [1]. The high interest in natural toxins is due to their unmatched ability to discriminate between thousands of molecular targets, most importantly ion channels and neuroreceptors [2]. It is intuitive therefore that effective labelling probes recognizing the respective targets can be produced based on the scaffold of natural toxins by attachment of reporter moieties.

Different techniques can be applied to obtain labelled toxin derivatives. One implicates incorporation of radioactive isotopes in the target molecule. For this purpose tritium (^{3}H) or iodine (^{125}I and ^{131}I) radionuclides are commonly used. Observation of the corresponding markers is realized due to the radioactive decay of the incorporated isotope [3,4]. Another approach utilizes fluorophores. Currently a long list of fluorescent dyes is available to researchers and for every specific case a dedicated probe can be produced [5]. Finally, fusion of fluorescent proteins (FP) and polypeptide toxins (Tx) has recently been reported [6]. The resulting FP-Tx chimeric proteins can serve good alternatives to conventional radioactive or fluorescently labelled toxins. Fluorophore-bearing toxins and FP-Tx chimeras can be detected by fluorescence microscopy (including super-resolution single-molecule techniques) or flow cytometry [7].

Labelled toxins as molecular markers have been successfully applied in dozens of studies. Pioneer investigations of structural and functional properties of the ionotropic acetylcholine receptors (AChR) as well as sodium (Na^+) and potassium (K^+) channels were carried out by dint of such ligands [8–10]. Usage of labelled toxins gave valuable results in screening approaches for novel ligand identification [11,12]. Significant progress was achieved in the field of receptor localization and visualization by using labelled toxins instead of or together with antibodies [13,14]. And finally, several of such probes demonstrated considerable potential as diagnostic tools [15–18].

2. Radiolabelled toxins

Radiolabelling is a special case of isotopic labelling in which substances are tagged by inclusion of radionuclides. This approach is applicable for a wide range of compounds – from simple inorganic and low-molecular-mass organic substances [19] to large macromolecular complexes [20]. It is not surprising that radiolabelling was also used for modification of natural toxins to create reporter tools [21].

Tritium (^{3}H) or iodine (^{125}I and ^{131}I) radionuclides are most commonly used to obtain radiolabelled derivatives of toxins [3,4]. For modification of small organic molecules, such as tetrodotoxin (TTX) and saxitoxin (STX), two classic Na^+ channel pore blockers initially isolated from the puffer fish and red bloom algae, incorporation of tritium is generally applied [3,22], whereas polypeptide toxins can also be marked by iodine [8,21]. ^{3}H can be introduced to toxins using

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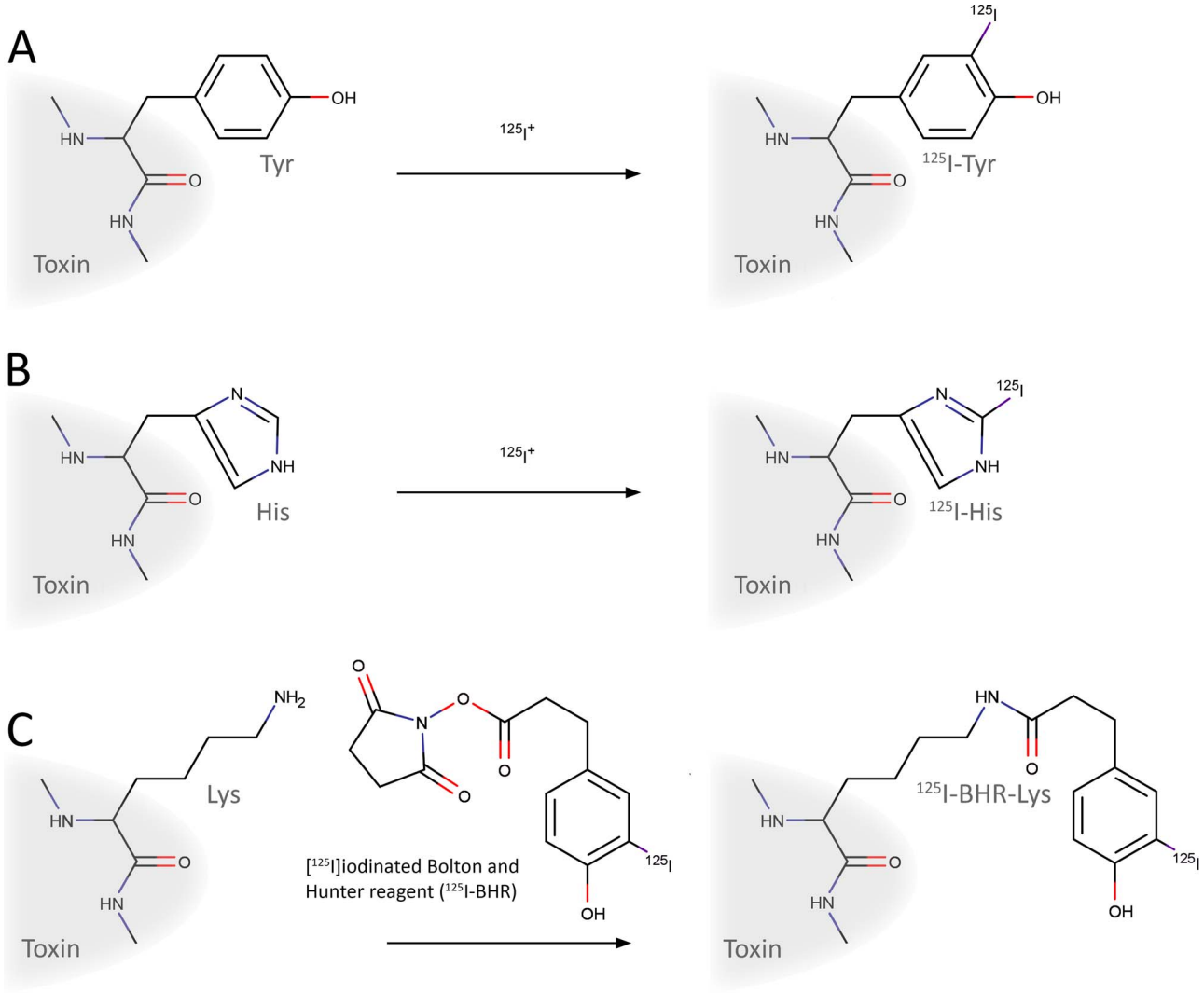

Fig. 1. A and **B,** Direct iodination of tyrosine and histidine residues of polypeptide toxin. To obtain $^{125}I^+$, solution of $Na^{125}I$ is treated by oxidizing agents. **C,** Iodination of lysine residues using the Bolton and Hunter reagent.

different approaches, for example, tritium exchange with carrier-free ^3H_2O [22], a procedure that combines the Pfitzner-Moffatt oxidation of the hydroxyl group, Schiff base formation with tritiated amines and reduction of the imino group [3], and labelling by N-succinimidyl-[2,3-^{3}H]propionate (^{3}H-NSP) in case of polypeptides [23].

Production of iodinated toxins is generally performed by using one of the following two ways. The first is direct labelling through incorporation of iodine (for example, ^{125}I) into tyrosine or histidine residues of the polypeptide. In this approach, reagents such as chloramine-T [24], lactoperoxidase/hydrogen peroxide [25], and 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril (Iodo-gen) [26] are used to oxidize $^{125}I^-$ to $^{125}I^+$ in $Na^{125}I$ solution. The $^{125}I^+$ cation is able to interact with the aromatic ring of tyrosine/histidine residues by electrophilic substitution (Fig. 1**A, B**). The second way is conjugation of radioiodinated Bolton and Hunter reagent (N-succinimidyl-3[4-hydroxy-5-[^{125}I]iodophenyl]propionate) with ε-amino groups of lysine residues, N-terminal amino groups, or thiol groups of cysteine residues (Fig. 1**C**) [27]. This second technique of radiolabelling is helpful when it is necessary to avoid modification of tyrosine and histidine residues [28]. Chromatographic methods are commonly used for purification of modified toxin derivatives and tag localization is established by a combination of Edman degradation, mass spectrometry, and selective proteolysis.

There are two major advantages to the use of ^{125}I tracer over ^{3}H. The first is necessity to apply small amounts of radioiodinated substance due to high values of specific activity of the ^{125}I tracer. And the second is possibility to use iodine labels *in vivo*, because they show lower radiation damage on biological objects compared to ^{3}H. However, modification by ^{125}I is not free of limitations. A comparatively large iodine atom can influence the toxin molecular properties and may even be able to inhibit the interaction of the modified ligand with its receptor [28]. For example, for many toxins the importance of certain amino acid residues has been shown [29], and iodation of these residues reduces the receptor-binding activity [8,30].

Radiolabelled toxins were utilized most extensively from the 1980s to 2000s prior to the rapid development of cell-based functional assays and fluorescent techniques. However, a significant number of highly important neurobiological, biochemical and physiological studies were performed just by using toxins labelled with radioactive tracers [8,10,31,32].

Perhaps most impressively look those studies, where tritiated and iodinated toxins served as tools that allowed purifying ionotropic (nicotinic) AChR [33]. α-Bungarotoxin (α-BTX, from the venom of the many-banded krait *Bungarus multicinctus*) with ^{3}H and ^{131}I tracers was used for identification of binding sites and distribution of AChR in the rat brain. Important kinetic studies were also performed using [^{3}H] and [^{125}I]α-BTX [9,34]. Currently, radioligand-binding assay with application of [^{125}I]α-BTX is routinely utilized for the development and