Origins of Recombinant Toxins

Subjects: Toxicology
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Toxins produced by various living organisms (bacteria, yeast, scorpions, snakes, spiders and other living organisms) are the main pathogenic factors causing severe diseases and poisoning of humans and animals. To date, recombinant forms of these toxins are widely used as antimicrobial agents, anticancer drugs, vaccines, etc. Various modifications, which in this case can be introduced into such recombinant proteins, can lead to a weakening of the toxic potency of the resulting toxins or, conversely, increase their toxicity. Thus, it is important to publicly discuss the situations and monitor the emergence of such developments.

protein recombinant toxin antivenom vaccines killer toxins enzymatic antidots

1. Introduction

To date, recombinant toxins from various biological sources (bacteria, yeast, scorpions, snakes, spiders and other living organisms) are widely used as: (i) antimicrobial agents for medical purposes, as well as antimicrobial additives for the food and biotechnological industries, (ii) groundwork for the creation of drugs with anticancer activity and the treatment of neurodegenerative diseases and (iii) the basis to develop vaccines, etc. Multiple works have been performed to study the mechanisms of action of genetically modified toxins and their applications [1][2][3][4][5][6] (**Figure 1**).

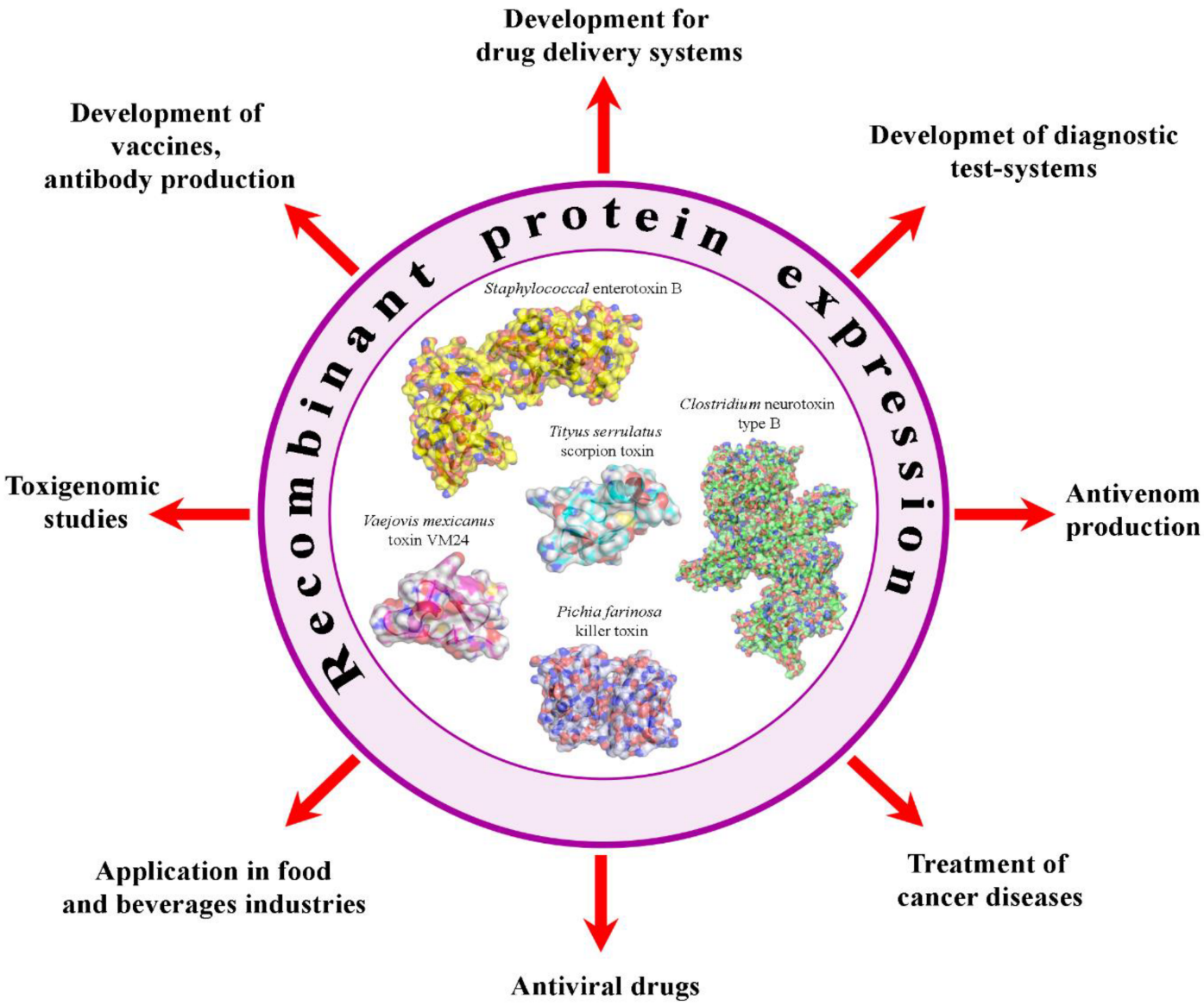

Figure 1. Various applications of recombinant toxins.

The protein/polypeptide nature of most of these natural toxins allows them to obtain their recombinant forms. The potential for developing these biomolecules in high enough quantities is the basis for further advancements in developing vaccines and drugs with reduced cost and their widespread use, on the one hand. On the other hand, the production of recombinant toxins avoids the need to work directly with the natural sources of these biomolecules (animals and microbial pathogens). Obtaining genetic constructs encoding the synthesis of recombinant toxins expands the possibilities of their synthesis in special modified forms. Like many recombinant proteins, recombinant toxins can be obtained in high yields using different expression systems, including extracellular secretion, and further isolated and finely purified using affine carriers [7][8].

2. Spectrum of Recombinant Toxins and Their Origins

Most of proteinaceous toxins well-studied to date are produced by various bacteria. However, toxins that are found in yeast, snake, scorpion and spider venoms and other living organisms are also actively studied by various scientific groups today. Recombinant toxins obtained from various origins and purposes of their obtaining are presented in **Table 1** [9][10][11][12][13][14][15][16][17][18][19][20][21][22][23][24][25][26][27][28][29][30][31][32][33][34][35][36][37][38][39][40][41][42][43][44][45][46][47][48][49][50][51][52][53][54][55][56][57][58][59][60][61][62][63][64].

Table 1. Recombinant toxins from various origins and the purposes of their obtaining.

Protein	Origin	Reference
Production		
BoNT	Bacteria *Clostridium botulinum*	[9]
Killer toxins K1, K28, K1L	Yeast *Saccharomyces paradoxus*	[10][11][12]
Killer toxin Kpkt	Yeast *Tetrapisispora phaffii*	[13][14]
ppa1, Tppa2, Tce3, Cbi1	Scorpions of the genus *Tityus* and *Centruroides*	[15]
β/δ agatoxin-1	Spider *Agelena orientalis*	[16]
Purotoxin-1	Spiders of the genus *Geolycosa* sp.	[17]
Azemiopsin, Three-Finger Toxins	Viper *Azemipos feae*	[18][19]
$MdumPLA_2$	Coral snake *Micrurus dumerilii*	[20]
APHC3, HCRG21	Sea anemone *Heteractis crispa*	[21][22]
Toxicity assays		
C3bot, $C3bot_{E174Q}$, C2IIa	Bacteria *Clostridium botulinum*	[23][24][25]
LeTx	Bacteria *Bacillus anthracis*	[26]
HlyII	Bacteria *Bacillus cereus*	[27][28]
Cry1Ia	Bacteria *Bacillus thuringiensis*	[29]
BFT	Bacteria *Bacterioides fragilis*	[30]
EGFP-SbB, translocation domain (TD) of the diphtheria toxin	Bacteria *Corynebacterium diphtheriae*	[31][32]
In1B	Bacteria *Listeria monocytogenes*	[33]
LcrV	Bacteria *Yersinia pestis*	[34]
AtaT2	Bacteria *Escherichia coli*	[35]

Protein	Origin	Reference
Killer toxin Kpkt	Yeast *Tetrapisispora phaffii*	[36]
MeICT, KTx	Scorpion *Mesobuthus eupeus*	[37][38][39]
Tbo-IT2	Spider *Tibellus oblongus*	[40]
α-conotoxins, α-cobratoxin	Marine snail and snake venom	[41]
Three-Finger Toxins	Viper *Azemipos feae*	[42]
α-neurotoxins	Cobra *Naja melanoleuca*	[43]
Hct-S3	Sea anemone *Heteractis crispa*	[44]
Immunology assays		
BoNT	Bacteria *Clostridium botulinum*	[45]
Beta and epsilon toxins	Bacteria *Clostridium perfringens*	[46][47]
Cholera toxin subunit B (CTB)	Bacteria *Vibrio cholerae*	[48]
Ancrod, batroxobin, RVV-V	Snakes *Calloselasma rhodostoma*, *Bothrops atrox*, *Daboia russelii*	[49][50]
Modifications		
BoNT/B-MY, C2IN-C3lim	Bacteria *Clostridium botulinum*	[51][52]
DT389-YP7, s-DAB-IL-2(V6A), DT2219	Bacteria *Corynebacterium diphtheriae*	[53][54][55]
rPA83m + plant virus spherical particles (SPs)	Bacteria *Bacillus anthracis*	[56][57][58]
SElP + Zn	Bacteria *Staphylococcus aureus*	[59]
PE38 + AgNP	Bacteria *Pseudomonas aeruginosa*	[60]
CTB-KDEL	Bacteria *Vibrio cholerae*	[61]
GFP-L2-AgTx2	Scorpions *Mesobuthus eupeus* and *Orthochirus scrobiculosus*	[62]
LgRec1ALP1	Spiders of the genus *Loxosceles*	[63]
Ms 9a-1 fragments and homologues	Sea anemone *Metridium senile*	[64]

Finding ways of obtaining effective antibodies and the development of vaccines against recombinant toxins is one of the main goals today [65][66]. For maximal quality and efficiency of immunologic medications, initial toxins should be highly purified, be in sufficient quantities and stimulate selective immune response. Recombinant toxins' production solves the first two issues, though vaccines can still have cross-specificity.

4. Prediction of Toxicity of Synthetic Recombinant Proteins

The majority of the publications of recent years emphasize the importance of using bioinformatics methods to identify new variants of toxins and clarify the mechanisms of their toxic effects. Molecular modeling facilitates the understanding of the interaction of toxins with their receptors and/or targets, especially when these compounds are bound to the membrane, and biochemical approaches to the study of these processes are complex [67]. Due to advances in synthetic biology, the cost and time required for the development and synthesis of individual recombinant products are steadily decreasing. Many research laboratories regularly create genetically modified proteins as a part of their research activities. However, manipulations of amino acid sequences in proteins can lead to the unintended production of protein toxins. Therefore, the ability to determine the toxicity of a protein before its synthesis reduces the risk of the potential danger of synthetic production of protein toxins. For this purpose, various methods based on machine learning are being developed to predict the toxicity of proteins in silico based on a number of initial data (**Figure 2**).

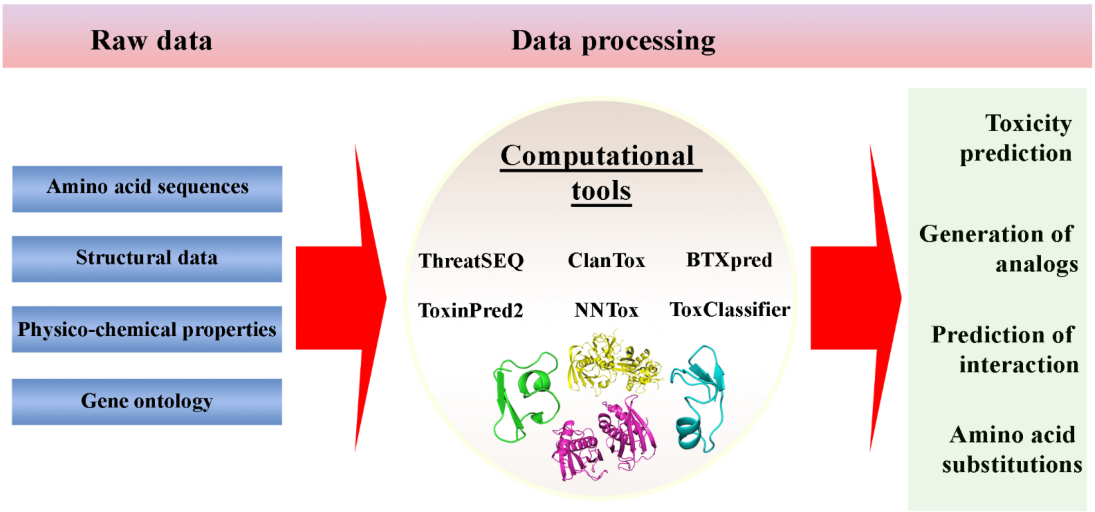

Figure 2. Machine-learning methods based tools for protein toxicity prediction.

5. Potential Enzymatic Antidotes for Recombinant Toxins

Due to the wide variety of toxins known to date and differences in the mechanisms of their action, there is an urgent need to create antidotes that both have a specific effect and are active against a wide range of toxins. The main directions of antidote development today are either the creation of various inhibitors capable of blocking the sites of binding of toxins to targets or the production of proteins (usually antibodies) capable of acting as bioscavengers via binding directly to the toxins themselves, thereby limiting their interactions with targets [68]. However, the search and development of new antidotes based on other principals, namely using molecules capable of detoxifying toxins by their enzymatic transformation into less toxic or nontoxic molecules, may become a promising alternative to existing solutions. To date, several enzymes are known that can act as antitoxins against various bacterial toxic substances, as well as enzymes that exhibit hydrolytic activity against PrP (**Table 2**, [69][70][71][72][73][74][75][76][77]).

Table 2. Enzymes as antidotes for toxic and prion proteins.

Protein	Enzyme	Mechanism of Action	Reference
Guanylyltransferase TglT from *Mycobacterium tuberculosis*	Serine protein kinase TakA	Specifically, phosphorylates the cognate toxin at residue S78, thereby neutralizing toxicity	[69]
HepT toxin from *Shewanella oneidensis*	Minimal nucleotidyltransferase (MNT)	MNT acts as an adenylyltransferase and mediates the transfer of three AMPs to a tyrosine residue next to the RNase domain of HepT	[70]
Bacterial GhoT toxin	Endoribonuclease	GhoS is a sequence-specific endoribonuclease that cleaves mRNA encoding GhoT, preventing its translation	[71]
Hha toxin from *Escherichia coli*	An oxygen-dependent antitoxin TomB	Inactivation of the Hha by oxidation with molecular oxygen mediated by the TomB	[72]
Mycobacterium tuberculosis toxin DarT	DarG—DNA ADP-ribosyl glycohydrolase	DarG could reverse the DNA ADP-ribosylation by DarT	[73]
PrP	Commercially available subtilisin enzyme, Prionzyme	Proteolytic inactivation/degradation	[74]
PrP	Subtilisin 309 and Subtilisin 309-v	Proteolytic inactivation/degradation	[75]
PrP	Nattokinase (NK, also known as subtilisin NAT) produced by *Bacillus subtilis natto*	NK is capable of decreasing amyloid structure of recombinant human PrP fibrils	[76]
PrP	Keratinase KerA from *B. licheniformis* PWD-1	Proteolytic inactivation/degradation	[77]

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