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Article 10: The specificity of host-pathogen interactions with emphasis on the specific inhibitor enzyme synthesized in the invasion-defense process.

Author: Liu Huan (1983-), Master of Science (First Class Honours, 2009), The University of Auckland.

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Abstract

The immunology of enzyme inhibitors synthesized in the host cells are summarized in this article, with case studies in mammal species, insect species, plant species. However, the original viewpoints are presented in this article after each case study. Eventually, the enzyme inhibitors are critically discussed with comparison between immunology and bio-medicine solutions.

Key Words: Immunology, Specificity of host-pathogen interaction, Enzyme inhibitor, Protease inhibitor.

1. Introduction

Enzyme inhibitor is a kind of substance that is capable of inhibiting the specific enzyme activity related to disease in vivo, as immunology or curative medical solutions. So far, more than 100 kinds of enzyme inhibitors have been found, some of which have already been used in clinic. Protease inhibitors include gastric inhibitors, chymase inhibitors and so on. Different types of proteases correspond to specific enzyme inhibitors [15].

The active substances of enzyme inhibitors act on or affect the active center or essential group of an enzyme, resulting in the decrease or loss of enzyme activity to reduce the rate of enzymatic reaction. Enzyme inhibitors can be divided into reversible inhibitors and irreversible inhibitors, which shows relative specificity or selectivity for enzymes, inhibiting one or several kinds of enzymes[15].

Irreversible inhibitor binds to the essential group of the enzyme active center, which cannot be relieved by simple methods such as dilution or dialysis; In comparison, there are two kinds of reversible inhibitors, competitive and non competitive. Competitive inhibition is that inhibitors and substrates compete to

combine with enzymes, so that increasing substrate concentration weakens the inhibition function, whereas noncompetitive inhibitors play the role in inhibition by reducing enzyme activity[15].

Particularly, among the enzyme inhibitors, proteases are a kind of enzymes that hydrolyze proteins by cutting protein peptide bonds in organisms, which determine the size, composition, spatial conformation and final degradation of proteins. According to the International Federation of Biochemistry and Molecular Biology (Iub-MB), protease is classified as the 4th subclass of hydrolases.However, due to its own role and structural diversity, the nomenclature of protease is difficult to be consistent with the general nomenclature system[14].

2. Immunology of Enzyme Inhibition in Mammal Species

Staphylococcal aureus complement inhibitor (SCIN) of protein was discovered [1]. SCIN infects a broad range of animal species, including horses, humans, pigs, which is evolved into strong adaptiveness through genome modification (such as by gene communications with external genetic segments aiming to host-invasion interactions). For the human *S. aureus, a specific* variant stream was found to block human complement system; for the horse *S. aureus*, the specific equine variant of SCIN was indicated to inhibit the horse complement system. Accordingly, A specific Human monoclonal antibodies (humAbs), named as 6D4, was abstracted from B-cells screened randomly, which specifically binds the SCIN and C3 convertases as inhibitors against *S. aureus*[2].

In this article, it is further proposed that compared with *S. aureus* that evolves across host animal species with closer genetic distances, COVID-19 virus originates from the wild species with longer genetic distance to human, which is evolved into adaptation on human host by acquisition of human gene segments for self-modification of virus genome. This is one of the key factor to explain why COVID-19 is more epidemic than before.

3. Immunology of Enzyme Inhibition in Insects Species

The specificity of host–pathogen interactions in pathogenesis against melanin synthesized by the host cells of invertebrates, which is activated by prophenoloxidase pathway of metabolic process, is discussed [3]. Further more, there are totally six families of serine proteinase inhibitors, including Kazal, Kunitz, α-macroglobulin, Serpin and two recently reported families of low molecule weight [4], which functions in arthropod hemolymph immunology system to defense against a broad ranges of pathogens or parasites infections. Multiple families of inhibition protein protease are classified into two types according to the different functions played in the binding process: the active site inhibition protease families and a specific family of α2-macroglobulins[5]. The first type of inhibition protein protease directly bind and

inactivate the active functional group of the pathogenic protease molecules, whereas the second protein α2-macroglobulins bind protease of invasive pathogens by its specific metabolic process trapping molecules, in which the bound protease are converted into a endocytic system mediated by receptors, and subsequently this causes degradation in secondary lysosomes. The α2-macroglobulins are reported in a wide range of biological Kingdom, which is consequently considered as the expression of conserved chromosome arm in the 0.6 billion evolutionary process[5].

FPI-F, one of the inhibitors found in the hemolymph and body wall of pure silkworm, has strong enzyme activity. So far it is confirmed that this protein inhibitors can eliminate the germination and bud tube growth of *Beauveria bassiana conidia* which shows strong pathogenicity to silkworm. The experiment results showed that FPI-F was a type of non-competitive inhibitor of fungal protease, with the inhibitor binding to fungal protease by the quantity ratio of 1:1. By comparing the inhibitory activities of six proteases, it was found that FPI-F actively inhibited microbial proteases, but resulted in significantly lower inhibitory effect on other non microbial proteases. By conducting the acid and heat treatment, it was reported that the activity of FPI-F inhibitor was stable under high temperature and acid conditions. The genetic diversity in FPI-F inhibitor family of various silkworm varieties was compared by electrophoresis experiment, indicating the richness in the genetic variants of FPI-F inhibitor family. These variants include thermally stable variants of lower molecular weights and thermally unstable variants of higher molecular weights, which are essential for insect defense against fungal invasion [11].

In this article, it is further suggested that the functional group triggering the same catalytic reaction of an isozyme family is expressed by the conserved genomes in the evolutionary process, so the functional groups of above protein protease families summarized becomes the indicators to synthesize the new primers used in isozyme technology, which can be also applicable on other biological species. In my previous article [16], the enzyme families of botanical species expressed under environmental stress is summarized in a table as systematic analysis of metabolomics study, which would be the linkage gene traits of immunology in host-pathogen interactions. However, this article more specifically targets the inhibition protein against invasive pathogens.

For the silkworm case study, my article further deduces that the results of FPI-F inhibitor does not only reveals the specificity of host-invasion interaction at molecular level, but also shows the environmental gradient of temperature along which gene expression on the specific inhibitor varies within this FPI-F protease family. The genetic diversity of FPI-F inhibitor reveals the relatively cold tolerance, further indicating that this silkworm insect species may evolve from the origin under cold temperatures.

4. Immunology of Enzyme Inhibition in Plant Species

The polygalacturonase-inhibiting protein (PGIP) derived from plants was studied on the interaction with endopolygalacturonases abstracted from fungi[6]. PGIP's plays a role in endopolygalacturonases which increase the synthesis of oligogalacturonides in which the active sites act as elicitors of phytoalexin (antibiotic) accumulation. This is important to other plant defense reactions.Specifically when oligogalacturonides reaches polymerization higher than nine degree are capable of eliciting phytoalexin synthesis of soybean cotyledons. In comparison, excessive PGIP was added to the digestion of polygalacturonic acid with the same amount of enzyme, the production rate of elicitor-active oligogalacturonides was significantly altered[6].

The xylem sap derived from cotton with infection by *V. dahliae* were tested by proteomics [7]. In total thousands of proteins species were identified as significant accumulation in ND601 inoculated by *V. dahliae*, which is resistant to disease, whereas approximately 1500 proteins species were significantly accumulated detected in a susceptible variety of CCRI8 inoculated with *V. dahliae*. It is drown the conclusion that the species of upregulated-protein were pathogenic protein species or relevant with cell-wall, but the majority of downregulated proteins shows relations to plant growth and development. There are six α-amylase inhibition types in total were summarized, mainly including lectin, knottin, cerea, Kunitz, γ-purothionin and thaumatin applied in the control of pest. The active sites of molecular structure in α-amylase inhibitors show significant differences in the diversity of inhibition modes, including proteinase inhibitory or chitinase activity [8]. Further more, the varieties of α-amylase inhibitors can be examined by isozyme technology [9]. Additionally, the not-specific gene transcriptional regulation for nitrogen oxide production is point out in many plant defense activities [10].

In this plant section, it is to characterize the specificity of enzyme/protease/proteinase on host-pathogen interactions over the whole biological kingdoms: firstly, compared with the not-specific gene expression of physiological defense (such as synthesis of nitrogen oxide production in plant), the specific host enzyme species correspond to the specific strain of pathogens due to the inter-dependent evolution in the long-term between both parties; secondly, this specificity of protease synthesis is sometimes coupled with the inoculation of third party microbes as to trigger the active immunological enzyme production discussed above; thirdly, the dose of enzyme production is specific as well; finally, in comparison to the proteomics test of not-selective proteins which detects and examines a large number of protein species above, the enzyme species test more specifically targets the specific disease infection or pest invasion, all of which use the same catalyzing pathway identified by isozyme technology developed in my study [16], and consequently understand the pathways of immunological process. Please note: the same isozyme family is not identical to the same enzyme/protease/proteinase family in definitions, and none of them are identical to the protein definition as well.

Cotton pathogens *Xanthomonas campestris pv.malvacearum* produces extra-cellular protease activity in the presence of casein (skimmed milk), which contains at least three proteases with apparent molecular weights of 29 (named as protease-1), 38 and 43 KD respectively. The results showed that protease-1 was purified and its optimal active PH environment was between 5.5 and 7.5. Inhibition studies showed that protease-1 could be inhibited by several inhibitors, including phosphoramidone, EDTA and 1,10-phenanthroline, but after this it could be reactivated by zinc ion incubation, indicating that it is a metalloproteinase. However, more specifically, it was also found that protease - 1 cleaved peptide chains with the exclusive specificity of peptide bond[12].

However, the inhibitors used to control cotton pathogens *Xanthomonas campestris pv.malvacearum* above is the artificial chemicals (rather than enzyme inhibitors) which may be applicable on the pesticides as chemical control solution. My article here suggests that the specific enzyme inhibitor synthesized by cotton plant cells itself should be the focus of inhibition study as plant immunological pathways against pathogens, which is more sustainable and environmental friendly.

5. Protease Inhibitor Production for Bio-Medicine

Serratia protease is classified into the M10B subfamily of zinc metalloproteinases, which is an extracellular endopeptidase widely existing in Gram-negative bacteria. It is commonly found in pathogen bacteria *Serratia*, *Erwinia chrysanthemum*, *Ehrlichia Freund* and *Pseudomonas aeruginosa*. The active segment of zinc metalloproteinases molecules is composed of Zn2+, which activates water molecules and makes water nucleophilic molecules bind to the carbonyl of peptide bonds. The pathological characters caused by *Serratia* protease include meningitis, endocarditis, nephritis, plague, dermatitis, soft tissue infection, septicemia, Pseudomonas, pneumonia and other respiratory and urinary tract infections, and even causing bacteremia and various systemic infections[13]. Below there are four commonly used inhibitors summarized by Zhang et al.(2018).

An alkaline protease APR and inhibitor APRin from *Pseudomonas aeruginosa* are the effective inhibitors of *Serratia* proteases. The crystal diffraction structure analysis of protease APR and inhibitor APRin shows that there are two spatial bindings: one is inhibitor β- folding the loop ring between IV and V, which is in contact with the methionine corner of the protease; and the other is the Trunk structure composed of five N-terminal residues, which is capable of inserting into the protease as a straight line β- Barrel structure, occupying the active segments of the enzyme molecules[13] .

Serratia metalloproteinase inhibitor (SMAPI) is a type of precursor inhibitors, producing inhibition function by cleaving peptide signals of 26 amino acid residues, with reversible denaturation, which persist in boiling water for up to 30 Mins[13].

Erwinia chrysanthemi, a plant pathogen, can produce *Serratia* protease inhibitor INH. INH is a kind of protein which is very thermally stable (T1 / 2 > 30 min at 95 ℃) with an isoelectric point of about 8.5. Inhibitor INH is synthesized as 12 kDa protein in cells, including 19 residues of peptide signals. Unlike SMAPI, INH is capable of inhibiting several species of Serratia proteases[13] .

There is an inhibitor gene LupI downstream of the low-temperature protease MP gene from marine bacterial strain ys-80-122. The expression product LupI of this gene can completely inhibit the activity of protease MP in vitro. The inhibition constant of LupI on its target metalloproteinase MP is 0.64 μ mol / L; It is heat-resistant at high temperature, and its inhibitory activity remained the effectiveness of 35.6% ~ 90.7% even after heat treatment at 100 ℃ for 1 ~ 60 min [13].

Compared with the host cells itself producing inhibitors, the summarized protease inhibitors are abstracted from microbial engineering products as bio-medicines. It can be seen that when the host cells itself may generate very limited inhibitors as immunology, the inhibitors of bio-medicine abstracted from microbial products are abundant to be chosen as remedy solution rather than immunology. However, the developed resistance in pathogens against the bio-medicine inhibitors may eliminate the immunology potential in host cells, which is less recommended by my article.

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