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Review

Fungal inhibitors of proteolytic enzymes: Classification, properties, possible biological roles, and perspectives for practical use

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ABSTRACT

Peptidase inhibitors are ubiquitous regulatory proteins controlling catalytic activity of proteolytic enzymes. Interest in these proteins increased substantially after it became clear that they can be used for therapy of various important diseases including cancer, malaria, and autoimmune and neurodegenerative diseases. In this review we summarize available data on peptidase inhibitors from fungi, emphasizing their properties, biological role, and possible practical applications of these proteins in the future. A number of fungal peptidase inhibitors with unique structure and specificity of action have no sequence homology with other classes of peptidase inhibitors, thus representing new and specific candidates for therapeutic use. The main classifications of inhibitors in current use are considered. Available data on structure, mechanisms and conditions of action, and diversity of functions of peptidase inhibitors of fungi are analyzed. It is mentioned that on one side the unique properties of some inhibitors can be used for selective inhibition of peptidases responsible for initiation and development of pathogenic processes. On the other side, general inhibitory activity of other inhibitors towards peptidases of various catalytic classes might be able to provide efficient defense of transgenic plants against insect pests by overcoming compensatory synthesis of new peptidases by these pests in response to introduction of a fungal inhibitor. Together, the data analyzed in this review reveal that fungal inhibitors extend the spectrum of known peptidase inhibitors potentially suitable for use in medicine and agriculture.

1. Introduction

Proteolytic enzymes participate in most important physiological processes on the cell level as well as on the level of the whole organism. These processes include digestion, blood coagulation, embryogenesis, immune response, etc. Cascades of rapid and often irreversible proteolytic reactions are also involved in processes whose control is an urgent component of cell functions. These reactions are regulated at different levels: transcription, translation, zymogen activation, specific degradation of mature enzymes, changes in environmental conditions (e.g., pH), and also by enzyme inhibitors. Most of the studied inhibitors are proteins or peptides, though there are also polysaccharides, polyphenols, glycerolipids, triterpenes, and some other low molecular mass non-proteinaceous compounds [1,2]. By regulating enzyme activity, inhibitors guide the flow of many reactions, playing important roles in inflammation, complement binding, and blood coagulation as well as control of intracellular proteolysis, transcription, apoptosis, immune response, and pathogenesis. Also, inhibitors play important roles in intracellular metabolism and in organization of defense systems protecting a host organism from attack and damage by various pathogens and pests, as well as protecting food sources and their ecotope from competitive organisms. There is already sufficient evidence for the participation of inhibitors in processes initiated by abiotic stress [3]. Many investigations on the use of peptidase inhibitors in agriculture for defense of plants against phytopathogens and insect pests, in medicine for treating inflammation, cancer, rheumatic arthritis, disseminated sclerosis, muscular dystrophy, and others diseases, and in pharmaceutical development are being performed. Thus, the search for investigation of new protein inhibitors from various sources is of special interest as it might reveal inhibitors with new specificities and promote new ideas. Fungi have proved to be a valuable source of new peptidase inhibitors having unique properties, and their potential use is still underestimated. In this review, we summarize available data on fungal peptidase inhibitors while emphasizing their properties, biological roles, and perspectives for practical use.

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2. Classification of peptidase inhibitors

Peptidase inhibitors are substances regulating the catalytic activity of proteolytic enzymes. There are three different classifications of peptidase inhibitors based on different principles – according to type of inhibited peptidase, according to mechanism of action, and according to amino acid sequence of the inhibitor.

2.1. Classification according to type of inhibited peptidase

Inhibitors of proteolytic enzymes are separated into two large groups – non-specific and class-specific.

Non-specific inhibitors regulate the activity of peptidases of different classes or even of all classes of peptidases. This group includes α-2-macroglobulin, a large protein with low specificity for the peptidases it inhibits. Possessing a wide activity spectrum, α-2-macroglobulin plays important roles in many physiological processes. α-2-Macroglobulin is found only in animals [4].

In contrast, class-specific inhibitors only inhibit peptidases of a corresponding class (for example, inhibitors of serine or cysteine peptidases). Inhibitors of this group have low molecular mass and high specificity in comparison to α-2-macroglobulin. Their high specificity is due to the position of the binding site inside the active center of the inhibitor.

It is noteworthy that this method of classification arose first and was widely used for a long time. However, it does not deal with such important features of the inhibitor as structure or mechanism of action, and this led to the creation of other more detailed classifications.

2.2. Classification according to mechanism of action

Most peptidase inhibitors function according to the principle of blockage of the active center. Classification according to mechanism of action is based on various types of binding and interaction of an inhibitor with a peptidase.

The “trap” mechanism is characteristic of α-2-macroglobulin, according to which this inhibitor is able to inhibit all classes of peptidases. For all classes of peptidases, the peptidase binds to the same site of α-macroglobulin causing conformational changes in the inhibitor that block interaction of other proteins with the captured peptidase. The formed α-2-macroglobulin–peptidase complex is readily withdrawn by endocytosis into the endoplasmic reticulum. One α-2-macroglobulin molecule can bind and transfer up to two peptidase molecules [4].

The idea of a “standard” mechanism was introduced in 1980 [5]. This mechanism is based on high affinity of an inhibitor and the active center of a proteolytic enzyme. Such inhibitors have an indicative loop with “canonic” conformation noncovalently bound to the enzyme active center, generating spatial difficulty for further interaction of the enzyme with a substrate. Inhibitors acting by the standard mechanism are relatively small proteins (29–190 amino acids) [6].

The “suicidal” mechanism was named due to significant destruction of the inhibitor after irreversible covalent binding to the corresponding proteolytic enzyme. This mechanism is characteristic of endopeptidase inhibitors, since its initiation requires splitting of an internal peptide bond causing conformational changes in the structure of the inhibitor [6]. Thus, the active site of the inhibitor is strongly damaged by the peptidase. As result of changes in the inhibitor, it irreversibly binds to the enzyme, inactivating it [7]. The suicidal mechanism is characteristic of serpins, and a similar inhibition mechanism was found for some inhibitors of cysteine peptidases (papain-like proteases, metacaspases).

Another mechanism of inhibitor action is based on noncovalent binding of the inhibitor not with the active center of the enzyme, but with a closely situated site. Binding of the inhibitor to the enzyme in this way makes spatial difficulty for peptidase binding with the substrate and keeps the active center of the enzyme free. Such mechanism was found for cystatins and a number of families not related by any evolutionary or structural features [8].

It is noteworthy that mechanisms of action are studied for a relatively small number of inhibitors, and such data are constantly supplemented.

2.3. Classification according to amino acid sequence

Some problems of peptidase inhibitor nomenclature should also be mentioned. The name of an inhibitor of a proteolytic enzyme is formed from the name of the source of the inhibitor (organism or tissue) and the name of main inhibited peptidase, for example, subtilisin inhibitor from *Streptomyces* or trypsin inhibitor from potatoes. Thus, the name of an inhibitor does not carry information on its mechanism of action, and it may correspond to a few inhibitors simultaneously. In 1980, Laskowski and Kato suggested classifying peptidase inhibitors by homology of parts of their amino acid sequences, number and position of disulfide bonds, and reactive centers [5]. However, at that time there was not enough information for creation of such classification. Only in 2004, Rawlings et al. [7] attempted to work out the classification of inhibitors. They established 48 peptidase inhibitor families according to homology of parts of their amino acid sequences. Similarity of inhibitors from some families with known three-dimensional structures made it possible to separate 26 clans. The classification and nomenclature of all inhibitors of proteolytic enzymes with known sequences is presented in the virtual MEROPS database [http://www.merops.sanger.ac.uk/], which is constantly updated and now counts 76 families and 39 clans. At present, representatives of only 14 families of peptidase inhibitors have been revealed in the genomes of the studied fungi. Their classification and known amino acid sequences from MEROPS database are presented in Table 1. It is noteworthy that representatives of five families of inhibitors have been found so far in fungal genomes, and there are no data on their presence in cells as active protein molecules. Also, four families are represented exclusively by fungal inhibitors (I34, I48, I79, I85), and a fifth, I66, includes fungal proteins in the overwhelming majority of cases. Besides protein inhibitors, the database includes well studied and widely used low molecular mass inhibitors, both those synthesized and those isolated from bacteria and fungi [9].

3. Peptidase inhibitors found in fungi

In recent years, many new peptidase inhibitors have been found and characterized. A significant number have been discovered in microorganisms such as bacteria and fungi [10]. This is due first of all with the simplicity of work with microorganisms and the wide variety of inhibitors they secrete. Table 2 shows protein peptidase inhibitors from the MEROPS database [11] as well as some isolated and studied but still not included into the database. They include inhibitors from the fungi *Trametes versicolor* and *Abortiporus biennis* that cause white rot [35,36]. These inhibitors are acidic, thermostable proteins specifically inhibiting the first enzymes of the subtilisin family (involving proteinase K widely used in molecular biology for removing protein admixtures in nucleic acid preparations) and not active towards peptidases of the chymotrypsin family. There are also some inhibitors from white rot fungi that specifically inhibit chymotrypsin.

Thus, summarizing the available data, we conclude that at present only inhibitors of serine and cysteine proteases as well as a

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