Proteinaceous α-amylase inhibitors: purification, detection methods, types and mechanisms

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ABSTRACT
α-Amylase is abundant in plants and animals. α-Amylase inhibitors can reduce endogenous α-amylase activity, playing an essential role in agricultural pest control, and preventing and treating human disease. In the agricultural field, α-Amylase inhibitors can restrict pest that relies on the starch of crops. Acarbose is an α-amylase inhibitor used to treat diabetes. Some α-amylase inhibitors are represented by antinutritional factors, while others are proteinaceous. Depending on their structures and sources, researchers have divided them into seven types: The knottin-like type, the γ-thionin-like type, the cereal type, the Kunitz type, the thaumatin-like type, and the lectin-like type. This paper introduces the methods for separating, purifying, and detecting proteinaceous α-amylase inhibitors while examining the structure and inhibition mechanism of several proteinaceous α-amylase inhibitors. Finally, it explores the potential applications of α-amylase inhibitors.

Introduction
Alpha-amylase (α-Amylase) is an enzyme that can hydrolyze α-D-(1,4)-glycosidic bonds in starch or polysaccharides. Starch and polysaccharides must be hydrolyzed into disaccharides and oligosaccharides which are then hydrolyzed by the α-glycosidase enzyme to monosaccharide molecules that are absorbed into the hepatic portal vena via the small intestine. α-Amylase inhibitors can impede amylase activity and are, therefore, also known as starch blockers. Researchers had discovered a type of flavone that can inhibit α-amylase, α-glycosidase, and aldose reductase. Over time, plants have developed amylase inhibitors as bio-defensins to protect them against diseases and pests, such as insects, which can cause extensive damage to agricultural crops. Almost one-fourth of global crops are destroyed by insects annually. Some larvae rely on the starch in crops, against which amylase inhibitors in plants provide some defense. Research has shown that the expression profiles of important DNA repair genes in bone marrow stem cells are negatively affected by the genotoxicity of pesticides. Vigna radiata plant defensin 1 has been reported to exhibit resistant activity against bruchids. Its structure forms a loop between structure β2 and β3 which can bind to the amylase site. Diabetes is a metabolic disorder, affecting more than 400 billion patients worldwide. This disease has many pathogenic mechanisms such as abnormal insulin secretion and an overactive polyol pathway. Researchers have found that some nonsteroidal drugs, calcium channel blockers, and pyrazolyl-thiazoles present useful inhibitive properties against aldose reductase or α-glycosidase. α-Amylase inhibitors could offer potential mitigation by inactivating the amylase in saliva or pancreatic digestive juice, thereby decreasing blood glucose and, consequently, alleviating diabetic
symptoms.\textsuperscript{[13]} Oolong tea polyphenols exhibit α-amylase activity in competitive pattern of half inhibitory concentration was 0.375 mg/mL.\textsuperscript{[14]}

Seven types of natural proteinaceous α-amylase inhibitors have been identified, six of which are extracted from plants, namely the knottin-like type,\textsuperscript{[15]} the γ-thionin-like type,\textsuperscript{[16]} the cereal type, the Kunitz type, the thauatin-like type, and the lectin-like type. The other is a kind of microbial metabolite, the microbial type. The molecular weights of these inhibitors range from 3 kDa to 23 kDa.\textsuperscript{[15]} The knottin-like type, the Kunitz type and the thauatin-like type are able to affect only the amylase in insects,\textsuperscript{[17]} however other types affect amylase in both insects and mammals. These inhibitors are of significant use in agriculture as well as medical treatments, such as diabetes. Research has shown that Amaranthus hypochondriacus α-amylase inhibitors (AAI) can combine with the active site triple amino acid Asp-Glu-Asp of amylase through electrostatic interaction generated by the positively charged amino acid and triple amino acid in amylase.\textsuperscript{[18]} The objective of this study was to overview proteinaceous α-amylase inhibitors purification methods, detection methods, types and mechanisms.

**Materials and methods**

**Separation and purification of proteinaceous α-amylase inhibitors**

Purification of protein and peptide usually use chromatography method. Affinity chromatography, gel filtration chromatography and ion-exchange column chromatography are used frequently enzyme purification.\textsuperscript{[19–21]} Figure 1 schematically shows the process used to separate protein from plant. Depending on the particular characteristics of the raw material, various optimal methods are used. These chromatography methods are also used in proteinaceous enzyme inhibitors purification.\textsuperscript{[22,23]} Researchers have also reported the use of two different downstream process methods to purify a bifunctional amylase/trypsin inhibitor.\textsuperscript{[24,25]} Single-step affinity chromatography and two-step chromatography were performed by, first, ion exchange chromatography and then

![Figure 1. Process of separation and purification of protein α-amylase inhibitors from plants.](image-url)
gel chromatography. The purification of amylase and trypsin inhibitor determined by affinity chromatography was 6.59-fold, with a recovery rate of 81.48%, while the purification of amylase and trypsin inhibitor by ion exchange chromatography was 4.28-fold, with a recovery rate of 75.95%. The combination of gel chromatography with ion exchange chromatography improved the purity of amylase and trypsin inhibitors up to 6.67 times, with a recovery rate of 67.36%. These results indicate that, although the purity of the affinity chromatography was reduced during the purification process compared with the ion exchange following gel filtration chromatography, the former method obtained almost the same purity in a single step purification process but with a higher recovery rate. The industrial purification of amylase involves multiple steps, including clarification, the combination of downstream processes, and microfiltration. The properties of the specific combination of amylase/trypsin inhibitor with the amylase can contribute to the purity obtained, and can also expand the application of these inhibitors.\textsuperscript{[24]}

A new step in the purification of kidney bean (Phaseolus vulgaris) α-amylase inhibitor was developed based on the application of inorganic adsorbent zinc hydroxide (Zn(OH)\textsubscript{2}). The new method was found to be much faster than other purification methods. In kidney beans, 98% of the protein binds to precipitation at a Zn(OH)\textsubscript{2} concentration of between 1–4% (w/v), while the precipitation-bound kidney bean α-amylase inhibitor at a Zn(OH)\textsubscript{2} concentration of between 1–2% led to a significantly lower amount. The α-amylase inhibitor-rich fraction that was not combined with the Zn(OH)\textsubscript{2} was further purified by diethylaminoethyl (DEAE) chromatography and gel filtration. It was found that Zn(OH)\textsubscript{2} binds to most soluble kidney bean proteins while leaving the α-amylase inhibitor in the solution.\textsuperscript{[26]}

The purification of lectin-like type α-amylase inhibitor (α-AI) from wheat flour was performed using a novel aqueous two-phase system (ATPS) of polyethylene glycol (PEG)/fructose-1, 6-bisphosphate (FBP) trisodium salt. The system comprised the PEG molecular weight and PEG concentration, the concentration of sodium FBP, and the pH of the system, which influenced the distribution of α-AI. The inhibitors were prepared using PEG2000 with 11.7% (w/w) and 19% (w/w) with pH7.0 at the top phase. The purification factor was 3.2 and the recovery rate was 79%. In addition, the downstream process was easier. These results demonstrate the feasibility of α-AI purification by PEG2000/FBP ATPS. Furthermore, the partial purification of α-AI in an aqueous PEG2000/FBP two-phase system may influence future research of α-AI.\textsuperscript{[27]}

Proteinaceous α-amylase inhibitors also contain small peptides, which are separated and extracted mainly by proteolysis. Proteins can also be hydrolyzed by pepdistase and separated to produce hydrolyzate. Endopeptidases take effect first, followed by exopeptidases (mainly tripeptidyl and dipeptidyl peptidases, aminopeptidases and carboxypeptidases). The resulting peptides can be further hydrolyzed through gastrointestinal digestion to produce peptides of different sequences and lengths, some of which are related to biological activities.\textsuperscript{[28]} When the protein was extracted, first, by the alkali method, and then by isoelectric precipitation, the protein content of the isolate was reported to be between 90–95%. Bioactive peptide extraction was reported using different proteases hydrolysis of protein, and different peptide fractions were obtained by ultrafiltration membranes with different cutoff molecular weights, while the α-amylase inhibition rate was also determined.\textsuperscript{[29]} These methods are summarized in Table 1 separation section.

The method used in the extraction of proteinaceous α-amylase inhibitors in plants is similar to that used in the extraction of plant proteins. In both instances, separation and purification are performed according to the characteristics of protein solubility and isoelectric points. In the purification process, the amylase inhibitory activity is used as one of the reference indicators for separation and purification to obtain the target protein.

Methods and models for studying α-amylase inhibitor activity

Colorimetric method: α-Amylase catalyzes the hydrolysis of starch to produce reducing sugars, which can be measured in vitro by starch consumption or by reducing sugar production to characterize the
activity of α-amylase. To further calculate, the inhibitory effect of the inhibitors, the starch content can be determined using the iodine-starch method. The reducing sugar can be determined by 3,5-dinitrosalicylic acid (DNS). DNS reacts with reducing sugar under alkaline conditions to produce 3-amino-5-nitro salicylic acid. This reaction appears as a brown-red color under boiling conditions, and the content of reducing sugar in the system can be determined by colorimetry at 540 nm.  

**Quick determination method**

The colorimetric method is only suitable for samples with a single system, however, for complex systems, accurate methods can be used. High performance anion exchange chromatography combined with pulsed amperometric detection (HPAEC-PAD), for example, was used to detect maltose produced by α-amylase catalysis. The detection limit of glucose and maltose was found to be up to 4.5 ppm with ion-exchange resin as the stationary phase and sodium hydroxide or sodium hydroxide and acetic acid as the mobile phase. HPAEC-PAD was reported to be a simple, sensitive and less intrusive method for the determination of amylase inhibitors in food supplements and raw materials. Capillary electrophoresis also plays an important role in detecting the interaction of various substances with proteins or enzymes during drug screening. After incubating the test subject with amylase for a period of time, comparative analysis can be performed with blank capillary electrophoresis to determine the binding of amylase to the test subject.

**Silicon method**

Molecular docking is a method used to predict the binding site and affinity of small ligand molecules at receptor macromolecular binding sites. The docking process mainly involves spatial and energy matching between the ligand and the receptor to obtain the best conformation, and focuses on their compatibility. Molecular docking can also be used in the screening of α-amylase inhibitors to determine whether the small inhibitors have an effect on amylase. For example, the inhibitory effect of carotene on α-amylase was studied and the binding compared with that of positive control acarbose by computer simulation. The binding kinetics of the inhibitors to amylase can also be digitally simulated. These detection methods are also summarized in Table 1 detection section.

**Cell experiments**

In vitro cell experiments are performed to detect the inhibitory effect of the α-amylase inhibitor. The AR42J cells from rat pancreatic acinar carcinoma were used in one study to detect the inhibitory effect of five peptides from pinto protein extract, and the addition of polypeptide in the cell culture medium

### Table 1. Different types of α-amylase inhibitors separation and detection methods.

| Kinds | Technical principle | Properties |
|-------|---------------------|------------|
| Separation | Affinity chromatography | Molecular binding | High purity, High recovery |
| | Ion-exchange chromatography | Charge intensity | Simple |
| | Ion-exchange chromatography + gel chromatography | Charge intensity molecular weight | Highest |
| | Zn(OH)₂ adsorbent + DEAE ion-exchange chromatography | Inorganic reaction | Removes many impurities |
| | Polyelectrolyte glycol/fructose-1,6-bisphosphate two-phase system | Solubility | Easier |
| Detection | High performance anion exchange chromatography- pulsed amperometric detection | Enzyme digestion | Peptide purification |
| | Capillary electrophoresis | Colorimetric | simple |
| | Silicon method | Pulsed amperometric detection | Simple, Sensitive, Less intrusive |
| | Lamarckian Genetic Algorithm | Various substances | |
was compared with that of a normal medium. Using the DNS method, the amount of α-amylase was measured to characterize the effect of polypeptides on the secretion of cell amylase, and the cell viability during the culture process was measured to determine the effect of polypeptides on the cell viability. Pinto bean polypeptide was found to inhibit amylase at a low dose, but with little effect on cell viability at this dose.  

Some substances have been found to affect the secretion of α-amylase by regulating the signaling pathway. To determine the effect of α-amylase on pancreatic acinar cells, the morphology of zymogen granules and the expression of the biological signals associated with the secretion of amylase in pancreatic acinar cells were studied. The results showed that leucine promotes the phosphorylation of PI3k, Akt, mTOR, and S6K1 by modulating the PI3K-mTOR signaling pathway, thereby increasing the expression of α-amylase in fetal bovine pancreatic acinar cells. Furthermore, the MIST-1 signal has been reported to enhance the expression of salivary amylase 1 in vitro.

**Vivo experiments**

The vivo activity of the α-amylase inhibitor was determined by the activity of α-amylase in the pancreatic tissue homogenate of rats treated with the inhibitor and the expression of α-amylase m-RNA. The presence of α-amylase inhibitors in the digestive tract can delay the digestion and absorption of starch, which is important for the relief of postprandial blood glucose spikes. The vivo activity of α-amylase inhibitors can also be characterized by glucose levels in the gut and in the blood. In one study, intestinal cavity eversion was used to measure the absorption of glucose in the small intestine. Glucose and inhibitors were added to the intestinal cavity after the fat and mesentery fragments were removed for in vitro culture, and glucose levels were measured at different sampling times. The results showed that protein hydrolyzate with a molecular weight below 1 kDa inhibited α-amylase activity and reduced glucose uptake in the small intestine.

At present, measurements of α-amylase inhibitory activity in vitro are quite mature, and based mainly on the quantification of amylase activity, so that the activity of the inhibitors can be obtained indirectly. However, amylase activity is affected by many factors that are more complex in vivo than those in vitro, so the development of a more targeted in vivo assay method is essential. With advancements in computer technologies, the combination of inhibitors and amylase by software simulation has become an increasingly significant method with which to screen inhibitors.

**Study of the structure and mechanism of proteinaceous α-amylase inhibitors**

Because of its conserved nature in evolution, α-amylase share similar catalytic residues with amylase from different sources. Proteinaceous amylase inhibitors are classified into seven types, as mentioned above, according to their origin and structure, namely the microbial type, Knottin-like type, γ-thionin-like type, cereal type, Kunitz type, thaumatin-like type, and lectin-like type. Data on each of these is presented in Table 2, below. According to their structural characteristics, different α-amylase inhibitors inhibit specific amylases. The active sites of amylases usually comprise three amino acids, namely Asp, Glu and Asp. The α-amylase binding site could hold at least six monosaccharide structures and be cleaved between the third and fourth pyranose residues by a double displacement mechanism. For example, the triple catalytic site Glu233 in pig pancreatic α-amylase and Arg19 in tandemistat (streptomyces inhibitor) forms a salt bridge, and the Arg7 in the amaranth α-amylase inhibitor (AAI) amino acid identity forms a salt bridge with Asp287.

**Microbial type**

A wide variety of proteinaceous α-amylase inhibitors originate from α-amylase genes in the genus streptomyces, such as tandemistat from *S.tendae*, Z-2685 (parvulustat) from *S.parvulus*, Haim from *S.griseoporeus*, Paim from *S.olivaceoviridis*, AI-409 from *S.chartreusis*, AI-3688 from *S.aureofaciens*, T-76 from *S.nitroporeus*, and MA-4680 from *S.avermitilis*. Streptomyces α-amylase inhibitors are typically composed of 75 amino acids that have been separated from different
streptomyces. They have approximately 30% sequence similarity, with a structure that exhibits two β-barrel folds and comprises a pair of twisted sheets. Each twisted sheet is composed of three anti-parallel β sheets and connects through disulfide bridges. In one study of tandemstat, once the protein had been broken down to obtain the different peptides, the interaction of amylase between cycle and line peptide was examined and the importance of the orbital was discovered. The combination of tandemstat and porcine pancreatic amylase (PPA) is shown in Figure 2(a). Parvulustat has a similar structure to that of tandemstat, however it is more stable because it has a longer and more flexible C-terminal. When the inhibition mechanism parvulustat combines with amylase, it will induce changes in the amylase structure to inhibit its activity. α-Amylase inhibitors are produced by transferring amylase inhibitory genes into engineering bacteria. The residues contain the conserved amino acid including Try-Arg-Tyr (WRY), which is where the inhibitor interacts with mammalian amylase and it also has inhibitory effects on insect amylase, insect growth and total soluble protein production.

**γ-Thionin type**

γ-Thionin is a kind of 5 kDa cysteine-rich peptide with a certain proportion of hydrophobic amino acid. Its secondary structure consists of a three-chain antiparallel β-sheet connected by three disulfide bonds to α-helix, and its configuration is a cysteine-stabilized αβ (CSαβ) motif. The α-core structure is a loop comprising the first β chain and the helix, while the γ-core structure is an area comprising the hairpin loop and it connects the second and third β-chains.

In one study, it was found that cowpea's plant defense protein VuD1 binds to the active site of amylase mainly through an N-terminal structure. Lys1 is obtained from VuD1, while Asp240 is obtained from *Z. subfasciatus* α-amylase (ZSA). The interaction between Asp240 and ZSA at the catalytic site and this interaction is the main way in which digestive enzymes are inhibited. There are salt bridge and hydrogen bond interactions between VuD1 and ZSA. Moreover, the model revealed a positively charged region at the site where the enzyme interacted with VuD1 and a core region (white) of nonpolar residues surrounded by positively and negatively charged residues. None of the hydrophobic residues seemed to be in contact with the enzyme, suggesting that the enzyme inhibition may be mainly mediated by ionic and hydrogen bonds. The Asn40 of the third ring of VuD1 is an uncharged amino acid. It is important for mammalian α-amylase inhibitors to have positively charged amino acids at this site. The fact that VuD1 has no charged residues in this region may explain why it does not inhibit PPA and has an inhibitory effect on insect amylase. In addition, it has been pointed out that the longer loop of a PPA may hinder VuD1 bonding.
Knottin type

The AAI is the smallest $\alpha$-amylase protein inhibitor ever found, with only 32 residues and 3 disulfide bonds. The structure of the protein is determined by nuclear magnetic resonance, including a desmin fold, three antiparallel chains, and a characteristic disulfide topology. AAI has a specific inhibitory effect on insect $\alpha$-amylase only. It binds to the gap between the A and B domains of the catalytic site of yellow meal worm $\alpha$-amylase (TMA), one of which is the Asp residue, and forms a salt bridge with the AAI Arg, while other catalytic residues are connected to the AAI via a complex network of water-mediated hydrogen bonds. Their combined structure is shown in Figure 2(d). The AAI-TMA complex is characterized by a high degree of complementarity between the interacting surfaces, and the specificity of AAI to insect amylase makes it an excellent candidate for insect resistant genetically modified crops.

Cereal type

Cereal $\alpha$-amylase inhibitors are composed of 120–160 amino acid residues, forming five disulfide bonds. Among the most studied are exogenous wheat $\alpha$-amylase inhibitor 0.19 and Elesusine coracana Gaertn bi-function $\alpha$-amylase/trypsin inhibitor (RBI). Wheat $\alpha$-amylase inhibitor 0.19 has 124 amino acids, named after its gel electrophoretic mobility for bromophenol blue, that inhibit $\alpha$-amylase in mammals and insects. When X-ray crystallography was used to study the $\alpha$-amylase inhibitor 0.19 and determine its structure, it was found to have a secondary structure consisting of five spirals up and
down. Furthermore, the formation of disulfide bonds is dependent on L-cysteine residues. \cite{53} Their combined structure is shown in Figure 2(b).

The bi-functional \(\alpha\)-amylase trypsin inhibitor is another prototype of the grain inhibitor family. The inhibitor is a stable monomer composed of 122 amino acids. It has five disulfide bonds and is resistant to urea, guanidine hydrochloride and thermal denaturation. The bi-functional inhibitor exhibits a three-dimensional structure very similar to that of the 0.19 inhibitor, with four helical spherical folds, a simple “up and down” topology and a small antiparallel sheet. These inhibitors may competitively inhibit a variety of amylase. By binding to the active site of the enzyme, the inhibitor prevents contact between the substrate and the enzyme, and also reduces the affinity of the enzyme to the substrate. \cite{24}

**Kunitz type**

Kunitz-like \(\alpha\)-amylase inhibitors contain about 180 amino acids and four cystine and are found in grains such as barley, wheat and rice. \cite{73,74} The results of a study using circular dichroism showed that the ratio of the \(\beta\)-fold was 39% in the secondary structure of the Kunitz inhibitor, and that the thermal stability of the protein was high. The disulfide bond between Cys44 and Cys91 was found to be conserved. \cite{56} The most widely studied Kunitz family is the barley \(\alpha\)-amylase/subtilisin inhibitor (BASI), the structure of which has two disulfide bonds and a \(\beta\) trefoil topology. The mechanism by which BASI inhibits amylase is different from that of several other inhibitors, which do not come into direct contact with the binding sites of amylase, instead forming 12 hydrogen bonds, two salt bridges and multiple electrostatic forces that strongly interact with the active regions A and B of the catalytic site, thus preventing the substrate from entering. The cavity at the enzyme-inhibitor interface contains calcium ion, which can enhance the network of water molecules at the interface of the complex and improve the stability of the complex, \cite{53,58} as shown in Figure 2(c). These amylase inhibitors mechanism also summarized in Table 3.

**Lectin type**

Plant lectin-like amylase inhibitors, termed \(\alpha\)-AI1 and \(\alpha\)-AI2, are purified from a variety of kidney bean seeds. These two kinds of allelic variation protein inhibitors exert different inhibitory properties, mainly on different kinds of amylase. \(\alpha\)-AI1 has been found to inhibit both mammalian and two kinds of insects amylases come from \(C\).maculatus and \(C\).chinensis. \(\alpha\)-AI2 can only inhibit the insects amylase from \(Z\).subfasciatus. In addition, the comparison of the amino acid sequences of these inhibitors with other plant defense proteins indicates that the translation of precursors to form active tetramers requires Arg residues near the processing site. \cite{75}

A heterotetramer of \(\alpha\)-AI1 binds to two PPA molecules and inhibits their activity. Structural determination of the complex of \(\alpha\)-AI1 with PPA and TMA reveals that two hairpin rings in \(\alpha\)-AI1 bind to the TMA reaction, the substrate-enzyme binding is blocked and the hydrogen bond network is established with the residues in the substrate-binding region. The \(\alpha\)-amylase inhibitor in white kidney bean consists of two glycosyl polypeptides, which inhibit mammalian \(\alpha\)-amylase and form a complex

| Inhibitor | \(\alpha\)-amylase | Binding site | Substrate similar |
|-----------|------------------|--------------|-------------------|
| Tendamistat | PPA \cite{54} | Arg19 forms salt-bridge to Glu233 | NO |
| RBI | TMA \cite{65} | RATI Ser1 N, O, Oy hydrogen bond with the catalytic proton donor, nucleophile, and the third catalytic acid | NO |
| BASI | AMY2 \cite{53} | Through Ca\textsuperscript{2+} and H\textsubscript{2}O bonding to the AMY2 | NO |
| AAI | TMA \cite{77} | AAI Arg7 and Tyr28 connect with the proton donor and the catalytic nucleophile in TMA | YES |
of amylase and inhibitor in a ratio of 2:1. Previous studies have shown that the amylase inhibitor has two binding sites to amylase and can bind to amylase independently. [76]

**Thaumatin-like type**

Thaumatin-like α-amylase inhibitors are proteins with a molecular weight of approximately 22 kDa. They are so named because of the significant sequence similarities between the pathogenesis-related group and a sweet protein called thaumatin. The most studied of these inhibitors is Zeamatin from maize, which has a significant inhibitory effect on insect alpha-amylase, but not on mammals. [58]

**Other types**

A novel type of α-amylase inhibitor, made up of two subunit chains (α + β) each with a cupin structure, had led to the discovery that this cupin structure is the main α-amylase site. [77] The peptides produced by enzymatic hydrolysis usually do not have high-level structures, and it is the composition and sequence of amino acids in a peptide that determines its biological activity. Studies have shown that watermelon polypeptide fragments hydrolyzed by alkaline protease have stronger α-amylase inhibition, and their hydrolyzates exhibit noncompetitive inhibition effects on α-amylase. [29,60,78,79]

Furthermore, a set of plant lipid transfer proteins, with a molecular weight of about 22 kDa, is a class of plant defensins and has been found to have a significant inhibitory effect on insect α-amylase. The structure of the α-amylase-LTP from Vigna unguiculata was studied using three-dimensional modeling and polypeptide synthesis in vitro. The inhibitor consists of 91 amino acids with an approximate molecular size of 9 kDa. There were 4-helix and 3/10 helix in its secondary structure. The inhibitor’s polypeptide ring can be exposed to a solvent and positively charged amino acids bind to the active site of the α-amylase and do not require a complete protein structure in the protein, as specific regions of the molecule alone act as α-amylase inhibitors. [18,80,81]

In summary, extensive research has long been conducted into the inhibitors of proteinaceous amylase activity, with the structure and mechanism of various inhibitors being studied in more depth. In recent years, with the increasing research in small molecule proteins, it has become necessary to build a database of inhibitors and their genes to manage the discovery of novel protein amylase. The properties of the various inhibitors need to be more clearly defined in order to distinguish between the same class of protease inhibitor enzymes, and to identify the clear differences between different kinds of proteases.

**Conclusion**

Due to the impact of insect pests, α-amylase inhibitors have developed as important physiological functions in plants, and plant derived α-amylase inhibitors have broad prospects for research. In recent years, α-amylase has played a huge role in medicine, lowering blood sugar, losing weight, as well as in the general pursuit of good health. In view of the remarkable structural and functional diversity of α-amylase inhibitors in nature, the crystal structures of increasing numbers of amylase inhibitors have been studied. Moreover, with the development of sequence analysis and computer modeling, the specificity of amylase observed in experiments has been solved.

The different kinds of amylase inhibitors vary greatly in structure and molecular weight, directly affecting their ability to inhibit specific kinds of amylase. Different inhibitors, even of the same amylase, have different inhibition mechanisms. The inhibitors of small molecules, for example, can block the binding of the substrate to the active site through steric hindrance, however, the inhibitors with higher molecular weight can inhibit amylase activity through the interaction of specific amino acid residues. This is of great significance for the artificial screening and synthesis of amylase inhibitors.
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Conflicts of Interest

The authors declare no conflict of interest.

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