Preparation And Application of A - Amylase Inhibitors

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Abstract. Alpha amylase inhibitors are a class of natural substances that inhibit the breakdown and absorption of alpha starches. By inhibiting the activity of Alpha amylase in the gut, they prevented the hydrolysis and digestion of starch in food, thus reducing the absorption of sugar by human body and lowering the sugar content in human blood. This article mainly introduced the plant-derived alpha-amylase inhibition and action mechanism. And the preparation process of plant-derived alpha-amylase inhibitors was introduced in detail. The alpha-amylase inhibitors that have appeared in the market so far were mostly protein inhibitors extracted from plants such as white kidney beans, while other non-protein inhibitors, such as polyphenolic flavonoids, alkaloids and other compounds, are in the research stage and have not been applied in natural foods and drugs. Alpha amylase inhibitors were not only able to reduce blood sugar and lose weight in humans, but also beneficial to insect prevention and control, which had a huge potential for development.

Keywords: α-AI, Adhibition, Protein, Polyphenol.

1. Introduction

Carbohydrate is the main source of energy for human life activities, which plays an important role in maintaining metabolism and other life activities. People can supplement by ingesting food. For example, starch enters the body and undergoes hydrolysis in several stages. Part of the starch is catalysed through saliva and amylase to produce maltose. The rest of the starch is hydrolyzed by amylase in the small intestine to produce maltose. Maltose is catalysed by maltose enzymes in the intestinal fluid to hydrolyse into glucose which may be absorbed by the human body. The glucose that the body eventually gets is passed around the body through your bloodstream to help metabolize energy.

A long-term diet with high carbohydrates as the staple food will lead to a lot of energy accumulation in the body, long-term energy accumulation can lead to obesity or lead to serious diseases such as diabetes. The major enzyme that hydrolyzed starch was alpha-amylase (EC.3.2.1.1), which catalyzed the hydrolysis of starch into a variety of oligosaccharides such as glucose, maltose, maltotriose and dextrin.

Obstructing one or more of the steps in a series of starch hydrolysis processes can lead to a decrease in starch hydrolyzed products, thus reducing the accumulation of sugars in the body. A class of substances found in microorganisms, beans, grains, etc., can inhibit the hydrolysis of starch, thus reducing the accumulation of sugars; making it into drugs can effectively reduce the hydrolysis of starch in the body, so as to reduce blood sugar and lose weight.

The so-called class of substances is alpha-amylase inhibitors, which belong to a class of glycoside hydrolase inhibitors [1]. Its function is to inhibit the degradation of starch compounds, such as starch, into oligosaccharides, and prevent the breakdown of carbohydrates, such as starch, into glucose, in order to reach the goal of hypoglycemia. α-amylase inhibitors are special organic compounds, belonging to non-competitive inhibitors, which do not compete with amylase in the process of blocking starch hydrolysis, but form enzyme-inhibitor complex with amylase, so as to change the conformation of amylase to inactivate it, thus preventing the hydrolysis of starch. It is known that α-amylase inhibitors from different sources are generally divided into two groups in terms of chemical structure, namely protein α-amylase inhibitors and non-protein α-amylase inhibitors.

At present, people have made a lot of research progress on α-amylase inhibitors. For example, α-amylase inhibitors extracted from white kidney beans can be made into drugs by controlling the key enzyme activity of sugar metabolism in the digestive tract, which has the effect of reducing blood

sugar and losing weight. Some of them have been produced. Other compounds, such as polyphenols, flavonoids and alkaloids, are under laboratory study and not yet available in drugs or natural foods. Therefore, the development and utilization of α-amylase inhibitors have broad application prospects in the fields of hypoglycemia and lipid reduction.

Alpha-amylase inhibitors also play a role in insect control. In the protective mechanism of higher plants, α-amylase inhibitors which naturally exist in higher plants can inhibit the activity of alpha-amylase in digestive tract and reduce the digestibility of starch and other carbohydrates by insects, thus reducing the main energy source of insects. At the same time, alpha-amylase inhibitors form complexes with amylase that cause anorexia in insects, thus achieving anti-insect effects. The discovery and development of insect amylase inhibitors will provide a new way to increase the yield of agricultural crops.

Obesity and its health hazards, including type 2 diabetes, cardiovascular disease and metabolic syndrome, are global sub-health problems [2]. The global prevalence of type 2 diabetes has been on the rise over the past few decades due to the increasing prevalence of obesity [3]. People with type 2 diabetes experience a rise in blood sugar after a meal because sugar in food is digested into glucose and passed into the bloodstream. However, alpha-amylase inhibitors can inhibit the activity of alpha-amylase and reduce the breakdown of starches and other foods into glucose, thus lowering blood sugar. So, the research and development of α-amylase inhibitors is of great significance.

In this paper, protein α-amylase inhibitors extracted from plants are studied, and plant α-amylase inhibitors and non-protein α-amylase inhibitors are introduced in detail. The preparation method of α-amylase inhibitor from plants is introduced, including the method of protein extraction and the selection of materials. The application of α-amylase inhibitors of different classes is also introduced.

2. Protein alpha-amylase inhibitors

2.1. Preparation Method

2.1.1 Principle

At present, most of the widely used protein alpha-amylase inhibitors are polypeptides. Because proteins consist of different amino acids and different spatial structures, the degree of digestion and absorption in the human body varies. Therefore, hydrolysis of proteins into polypeptides can improve the absorption rate of the human body and make it play a better role. So far, protein-like α-amylase inhibitors have been isolated from wheat, barley, sorghum, peanut, cereal, soybean, taro root and other plants. Most alpha-amylase inhibitors have molecular weights ranging from 1.0 to 6.0 million, but there are also polypeptides with molecular weights in the low thousands, consisting of one or more subunits. Molecular weight varies from source to source (See Table 1) [4].

Table 1 The chemical and physical activity of α-amylase inhibition in plant [4]

Source	Molecular Weight (Da)	Subunit	Carbohydrate	pI
Wheat	51,000	4		7.3
Barley	20000			
Millet	9333	1		
Sorghum	21000	2		
Black beans Ⅰ	49000	4	8.6-13.0	
Black beans Ⅱ	60000			4.86
Grain	29000	1		
Peanut	25000			
Cabbage beans	38000	2	9-10	
Potatoes	12000			4.6
Soybean	14000			4.

To get an alpha-amylase inhibitor, you have to get the protein from the raw material. Firstly, the alkali-soluble acid precipitation method, solvent extraction method, reverse micellar extraction technology, membrane separation method and enzyme method were used for protein extraction. Alkali-soluble acid precipitation method is a method of separation according to the different acid and alkaline properties of each substance in the mixed liquid [5]. Specific operation: First, raw materials (such as white kidney beans, wheat, quinoa, etc.) are crushed and dissolved in organic solvents to remove the fats. Second, place the raw material for lipid removal in an alkaline solution for extraction. Next, adjust the pH of the solution so that the protein can be precipitated under acidic conditions. Finally, the experimental materials were obtained after freeze-drying and stored. The main value is that proteins extracted from different raw materials have different extraction requirements, so it is necessary to search data or use orthogonal tests to get the best conditions. And in the process of the experiment, attention must be paid to the strength of alkalinity and acidity, heating temperature and time, to avoid changes in the structure of some compounds under the condition of too acid or too base, resulting in the loss of protein activity.

Solvent extraction is widely used in the separation and extraction of natural products because of its simple operation and low cost. It is a method to extract the target extract from the raw material by using the different solubility of different substances in the solvent, choosing the solvent with greater solubility to the target extract and smaller solubility to other substances. Different kinds of proteins can be extracted, but this method has the disadvantages of high solvent consumption and low product purity. The reverse micelle extraction technique micelles are nanoscale aggregates dispersed in continuous organic phases and stabilized by surfactants. In the process of extraction, the extraction rate of bioactive substances can be greatly improved by selecting different reverse micellar systems or using auxiliary ultrasonic technology and supercritical technology. Reverse micellar extraction technology has been widely used in the separation and extraction of protein, and good results have been obtained in the extraction of soybean protein, peanut protein, tomato seed protein and sesame residue protein. But at present, this method is still in the experimental research stage, and this kind of technology needs to be further explored.

The membrane separation method is a general name for the method of selective penetration of some components in solution by using films of different materials. Commonly used membrane separation methods include dialysis, electrodialysis, reverse osmosis, ultrafiltration, microfiltration, and so on. Ultrafiltration and reverse osmosis are the most widely used. The method has the advantages of strong selectivity, low energy consumption, and can be carried out at room temperature, but it also has some disadvantages, such as low membrane utilization, membrane pollution, and inability to separate isomers. Before performing the experiment, consult the data to find out which way, environmental conditions, etc. can extract the protein from the raw material with maximum benefit.

2.1.2 Extraction

Secondly, the extracted protein is decomposed under the action of protease, so as to obtain polypeptide. There are many ways of protein hydrolysis, such as acid hydrolysis, alkali hydrolysis and enzyme hydrolysis, among which enzyme hydrolysis can obtain more functions than the original protein to prepare bioactive peptide, but also can maintain the natural properties of peptide, so choose enzyme hydrolysis. Of course, before enzymolysis, appropriate proteases should be selected for hydrolysis, such as trypsin, flavor protease, alkaline protease, papain, neutral protease and complex protease. In addition, under the optimum pH value and temperature of different proteases, the same amount of enzyme addition, enzymatic hydrolysis time, polypeptide content and inhibition rate are used as evaluation indexes to comprehensively screen out proteases with reducing ability and a relatively high degree of hydrolysis (See Table 2). If necessary, the check of protease should be carried out.

Table 2. Optimal enzymatic hydrolysis conditions of single and combined enzymes [6]

The kinds of enzyme	Enzymatic	
	Temperature/℃	pH
Trypsin	50	8.0
Flavor Protease	50	6.0
The alkaline Protease	50	8.0
Papain	50	6.5
Neutral Protease	50	7.0
Compound Protease	50	7.5
Trypsin +flavor Protease	50	7.0
Trypsin +alkaline Protease	50	8.0
Flavor Protease +alkaline Protease	50	7.5

2.1.3 Preparation

After selecting the appropriate protease, the amylase inhibition rate was used as the index to determine the final preparation environment of α-amylase inhibitor polypeptide under the conditions of different pH, temperature, times and enzyme dosage by response surface method. By direct interaction of α-amylase with substrate starch, it can be screened in vitro and the inhibition rate can be obtained. Dinitrosalicylic acid (DNS) was used to determine the amount of reducing sugar to determine the activity of α-amylase, so as to obtain the inhibition rate of α-amylase inhibitors.

3. Non-protein inhibitors

3.1. Polyphenol compounds

Polyphenols generally exert their inhibitory activity by binding to alpha-amylase molecules. The interactions between the two include hydrogen bonding (between the hydroxy group and the enzyme-catalyzed active seat) and hydrophobicity (between the aromatic ring of the polyphenol and the tryptophan residue of alpha-amylase). Therefore, the inhibitory effect of polyphenols on alpha-amylase is strongly linked to its molecular structure. Polyphenols are phenolic acids, such as hydroxytyrosol HPLC grade, tannic acid, and gallic acid, which contain a C6 aromatic ring. The delocalization of electrons between C=C (or C=O) in polyphenols and the aromatic cycle strengthens the hydrophobic (π-π) interaction with alpha-amylase. Thus, aromatic rings containing one or more hydroxyl groups in polyphenols play an important role in inhibiting α-amylase. Examples include a C6 aromatic ring, stilbenes containing a C6-C2-C6 structure, flavonoids containing a C6-C3-C6 structure, and lignans containing a C6-C4-C6 structure. C=C (or C=O) in polyphenols and electronic delocalization between aromatic rings enhance the hydrophobic (π-π) interaction between polyphenols and alpha-amylase, thus affecting their ability to hydrolyze starch [7].

Gomes et al. studied α-amylase and hypoglycemic effects of a leaf extract from Terminalia phaeocarpa, an endemic tree species in Brazil [8]. The ethanol extract, ethyl acetate extract and hydrogenated methanol extract from T. phaeocarpa leaf had significant inhibition on α-amylase. The experimental study showed that there were 38 phenolic compounds in the three extracts, mainly phenolic acids, ellagitannins and flavonoids. Therefore, T. phaeocarpa leaf extract is a potentially effective medication to treat type 2 diabetes. These results indicate that plant-derived polyphenolic compounds extract can inhibit α-amylase activity and can be used as a potential source of alpha-amylase inhibitors. However, at present, it is in the experimental research stage, and there are not many in vivo and in vitro experiments to verify its effect and safety.

3.2. Flavonoids compounds

Flavonoids are a kind of rich natural phenolic compounds. According to the change of carbon ring and the different connections between benzopyranol and phenyl, they are divided into six categories:

flavonoids, flavonols, flavanols, isoflavones, flavan-3-alcohols and anthocyanins. Their activity was directly related to their structure. Methylation and methoxylation of flavonoids reduced the number of hydrogen-bound receptors/donors and significantly weakened the inhibition of alpha-amylase in vitro. The hydrogenation of C2 = C3 double binding and flavonoid glycosylation considerably weakened the inhibition of α-amylase in vitro. Hydroxylation of flavonoids improves the inhibition of alpha-amylase in vitro [9]. Its principle of action is the formation of hydrogen bonds between the phenolic -OH groups and the amino acid side chains of the active α-amylase sites (like Asp197 and Glu233) [10]. For this reason, the hydroxyl (-OH) group of flavonoids plays a critical role in inhibiting the activity of α-amylase.

3.3. Phenolic acid

Phenolics are aromatic phenols, secondary metabolites of plants with functional groups of carboxylic acid, which are widely distributed throughout the plant kingdom. Natural plant phenolics contain predominantly hydroxycinnamic acid and hydroxybenzoic acid. The basic molecular structure of phenolic acids is relatively stable, but the substituents (hydroxyl and methoxy) in the aromatic ring change the structural properties of the molecule, such as polarity. In the molecular structure of hydroxycinnamic acid in phenolic acids, the C=C double bond can be conjugated with the carbonyl group, resulting in electron transfer between the acrylic acid and the benzene ring portion. So, hydroxycinnamic acids can form highly conjugated systems. When it binds to the active site of α-amylase, it can form a stable chemical structure, resulting in α-amylase fire, and achieve the purpose of inhibition. Therefore, the main principle of inhibiting α-amylase by hydroxycinnamic acid in phenolic acid is that it forms a conjugate structure with α-amylase and has multiple hydroxyl groups. [10]

4. Comparison of protein and non-protein inhibitors of alpha-amylase

Both alpha-amylase protein inhibitors and non-protein inhibitors come from different plants and have different modes of action. In general, alpha-amylase protein inhibitors are mostly derived from the seeds of crops, while non-protein inhibitors are mostly derived from leaves, seed-coats, seeds, etc. There is a preliminary sound system for protein inhibitors, and some have even produced drugs for use. In contrast to non-protein inhibitors, most of them are under in vitro study or laboratory stage, such as determining that these substances have an inhibitory effect on alpha-amylase and have potential medicinal value, which can be used as future research objects. However, most of the non-protein inhibitors cannot be used in natural foods and drugs, so further exploration and experiments are needed.

5. Conclusion

It was found that polyphenols and proteins had strong inhibitory activity on α-amylase. Now there have been many studies on alpha-amylase inhibitors, but most of them are still in the laboratory stage. The current availability-amylase inhibitor was derived from white kidney beans and was shown to improve blood sugar after meals. However, other polyphenols, flavonoids and alkaloids with α-amylase inhibition effect have not been used in drugs and natural foods. Alpha-amylase inhibitors come from a wide range of sources and have been isolated from plants, animals and microorganisms, and are classified into protein and non-protein alpha-amylase inhibitors. It forms a complex with alpha-amylase and inactivates it for hypoglycemic purposes. Therefore, α-amylase inhibitors can be used in medicine to control the rise of blood glucose in patients with type 2 diabetes after taking meals. It also reduces the conversion of sugar to fat and increases the amount of fat consumed. Therefore, it is also used as a diet drug to reduce fat to achieve the purpose of losing weight. Is currently on the market for the treatment of type 2 diabetes and weight loss is relatively safe a class of substances. In agriculture, it can be used as an anti-insect gene, as a drug to make bugs

malnourished or anorexic, or as a biological preservative to avoid pests. Whether in the field of medicine or agriculture, α-amylase inhibitors have a lot of development space, which is worth exploring.

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