ISSN 1007-7626
CN 11-3870/Q
中国生物化学与分子生物学报
Chinese Journal of Biochemistry and Molecular Biology
2004 年 8 月
20(4) :434～439

Purification and Characterization of a Novel α-Amylase Inhibitor from Wild Amaranth(Amaranthus paniculatus) Weeds

WANG Lin , ZHOU Tie-yang , TANG Xing ,WANG Xian-chun * , LIANG Song-ping *
(*College of Life Sciences , Hunan Normal University , Changsha* 410081 , *China*)

Abstract A novel proteinaceous inhibitor of α-amylase was purified from the wild amaranth (*Amaranthus paniculatus*) seeds. The inhibitor , named WAI-1 ,has a molecular weight of 986.5 determined by MALDI-TOF mass spectrometry. It is the smallest proteinaceous inhibitor of α-amylase found so far. Preliminary compositional and structural analysis indicated that WAI-1 is a nonapeptide with N-terminal pyroglutamate. Purified directly by reversed-phase HPLC, WAI-1 potently inhibited the α-amylase activity of the insect (*Periplaneta Americana*) digestive duct in a noncompetitive manner and did not inhibit the human salivary α-amylase. WAI-1 inhibited α-amylase activity of *Periplaneta Americana* digestive duct evidently under mild acid conditions , with optimal inhibitory pH 6.0. WAI-1 exhibited the highest inhibitory activity after preincubation with the enzyme at 37 ℃for about 30 min. When a fixed amount of α-amylase used , along with the increase of the inhibitory/enzyme ratio the inhibition percentages of the α-amylase activity were linearly increased up to about 50 % , and then increased slowly up to a maximum of about 65 %.

Key words proteinaceous inhibitor ,α-amylase , amaranth

野生苋属植物籽实中新型α淀粉酶抑制剂的分离纯化及其性质研究

王 琳 , 周铁杨 , 唐 兴 , 王贤纯 * , 梁宋平 *
(湖南师范大学生命科学学院 ,长沙 410081)

摘要 从野生苋属植物(*Amaranthus paniculatus*)籽实中分离纯化出α淀粉酶的一种新型蛋白质类抑制剂. 该抑制剂被命名为 WAI-1. MALDI-TOF 质谱测得其分子量为 986.5 ,是目前报道的α-淀粉酶的蛋白质类抑制剂中分子量最小的. 初步的组成和结构分析结果表明 ,WAI-1 由 9 个氨基酸残基组成 ,其 N 端为焦谷氨酸. 直接用 RP-HPLC 纯化后 , WAI-1 能在弱酸性条件下 , 以非竞争性抑制作用方式有效抑制美洲蜚蠊消化道α淀粉酶的活性 , 最适抑制 pH 6.0 , 但对人唾液淀粉酶活性无影响. WAI-1 在 37 ℃下与酶预温浴约 30 min 后显示最大抑制活性. 当α淀粉酶用量一定时 , α淀粉酶活性的抑制率在约 50 %的范围内随抑制剂/酶比例的增大而呈线性增加 ,超过 50 %后 , 抑制率随抑制剂/酶比例的增大而缓慢上升 ,最终达到最大值(约 65 %) .

关键词 蛋白质类抑制剂 ,α淀粉酶 , 苋属植物
中图分类号 Q5 ,Q51 ,Q55

Received :November 3 ,2003 ;Accepted ;January 30 ,2004
Supported by National Natural Science Foundation of China (No. 30270285)
* Corresponding authors. Tel : 0731-8872556 ; Fax :0731-8861304 ; E-mail :liangsp @public. cs. hn. cn ; Wang . xianchun @263. net
收稿日期 :2003-11-03 ,**接受日期** :2004-01-03
国家自然科学基金 (No. 30270285) **资助课题**
* **联系人** Tel :0731-8872556 ; Fax :0731-8861304 ; E-mail :liangsp @public. cs. hn. cn ; Wang . xianchun @263. net ;
王琳 ,**女** ,1978 **年** 2 **月生** ,**硕士研究生**

© 1994-2006 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

Many plants contain proteinaceous substances which are specifically inhibitory to hydrolytic enzymes. α-Amylase (α-1, 4-glucan-4-glucanohydrolases, EC 3.2.1.1) inhibitors, which have been found for over half a century[1,2], are a family of such substances. So far, α-amylase inhibitory activity has been detected in wheat *Triticum aestivum*[3~5], barley *Hordeum vulgareum*[6,7], sorghum *Sorghum bicolor*[8], rye *Secale cereale*[9,10], rice *Oryza sativa*[11], pigeonpea *Cajanus cajan*[12], cowpea *Vigna unguiculata*[13], bean *P. vulgaris*[14~17] and many other plants. These inhibitors show remarkable structure variety leading to different modes of inhibition and different specificity profiles against diverse α-amylases. Such inhibition specificity of these inhibitors has aroused interest in their inhibition mechanisms and potential applications. Some inhibitors may play a vital role in controlling endogenous α-amylase activity and therefore regulating starch metabolism in plants[18]. A knowledge of these inhibitors could improve our understanding of α-amylases and the related metabolism. On the other hand, the inhibitors which specifically inhibit insect and microbial α-amylases may be used to play a role in preventing insect and microbial[19~21] for crop. The selective inhibition of human α-amylases is of high potential pharmaceutical value as regulative agents in reducing digestive-starch degradation in patients suffering from diabetes and obesity[22,23]. In addition, the unique property of some inhibitors to specifically inhibit endogenous cereal α-amylases synthesized *in vivo* during germination[18] may be of importance to the baking industry. Endogenous α-amylase inhibitors have been used as natural additives in doughs prepared from sprouted wheat flour[24]. It is worthwhile to note that flour processed from sprout-damaged wheat is not suitable for bread making because of increased levels of α-amylase.

We have been attempting to identify naturally occurring proteinaceous inhibitors that have strong activity against insect digestive enzymes but little or none against mammalian enzymes. Our ultimate wish is to transfer genes that encode inhibitors selective for insect digestive enzymes into crops by genetic engineering, with the goal of creating new insect-resistant crop varieties. In the process of surveying more than fifteen wild plants for potential inhibitor of insect α-amylase, a novel proteinaceous inhibitor was found in wild amaranth weeds. The purification and preliminary study of the inhibitor is reported in this paper.

1 Materials and Methods

1.1 Extraction and ultrafiltration

Weeds of wild Amaranth (*A. paniculatus*) were collected from the open country. The weeds (5 g) were soaked in distilled water (20 ml) for 1 hour at room temperature, then disintegrated and homogenized in a mortar. Crude extract was obtained by centrifugation (10 000 g for 10 min). The extraction was repeated three times. Crude extract was pooled and then heated at 70 ℃ for 15 min to inactivate β-amylase[6]. The resulting turbid solution was centrifuged (15 000 g for 10 min). The clear supernatant was ultrafiltered (Elite, 0.22 μm) and subsequently used for RP-HPLC.

1.2 Reversed-phase high performance liquid chromatography (RP-HPLC)

RP-HPLC was conducted firstly using a Waters 2010 HPLC station with a preparative column (Elite 10 ×250 mm, Hypersil 10 μm C8, 30 nm of pore size), and then using a Waters Alliance2690 HPLC station with an analytical column (Waters 4.6 mm ×250 mm, Vydac 5 μm C18, 30 nm of pore size). Solvent A was 0.1 % TFA in water, and solvent B was 0.1 % TFA in acetonitrile. All the solvens were of HPLC or analytical grade. Water was redistilled.

1.3 Preparation of insect α-amylases[25]

The digestive ducts of adult cockroach (*Periplaneta Americana*) were homogenized with PBS containing 0.02 mol/L NaCl and 0.1 mmol/L $CaCl_2$ (pH 6.7), and centrifuged at 15 000 g for 10 min. The supernatant was used as an insect α-amylase preparation. The specific activity is about 1200.

1.4 Assay for α-amylase and inhibitor activities

The activity of the crude insect α-amylase was measured using a modified Bernfeld method[26]. Namely, a suitable amount of α-amylase preparation was incubated with 100 μl of 10 g/L soluble starch solution in 0.2 mol/L sodium phosphate buffer (pH 6.0) at 37 ℃. After 5 min the reaction was stopped by the addition of 500 μl of 3,5-dinitrosalicylic acid and heated in boiling water for 10 min. After standing for 15 min at room temperature, the absorbance of the solution was read at 546 nm. The amount of α-amylase used in the assay was properly adjusted so that the A_{546} values were in the range of 0.4—0.7. The α-amylase activity was expressed in A_{546} values directly. Inhibitor activity was expressed as a percentage of inhibited enzyme activity out of the total enzyme activity used in the assay.

2 Results

2.1 Purification and molecular weight of the inhibitor

The result of RP-HPLC using the preparative C8 column is shown in Fig. 1. Using the method described above, the peak labeled with an asterisk was identified to be active toward the insect α-amylase used (Table 1). The active peak fraction was further purified by the Vydac analytical C18 column (Fig. 2 A). Active peaks from the second HPLC separation were collected and lyophilized. Fig. 2 B is the chromatogram of the inhibitor further purified. Only one sharp and symmetric peak appeared, indicating high purity of the inhibitor. MALDI-TOF mass

© 1994-2006 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

spectrometric analysis indicated that the obtained inhibitor, named WAI-1 by us, has a molecular weight of 986.5 (spectrum not shown).

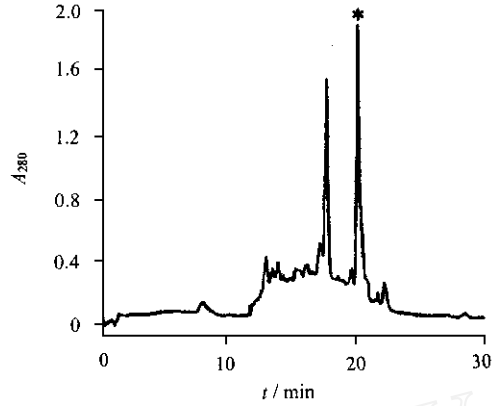

Fig. 1 Chromatogram of RP-HPLC of the extract with a preparative column

Table 1 Identification of active peak

	Control	Active peak
A_{546}	0.409	0.186

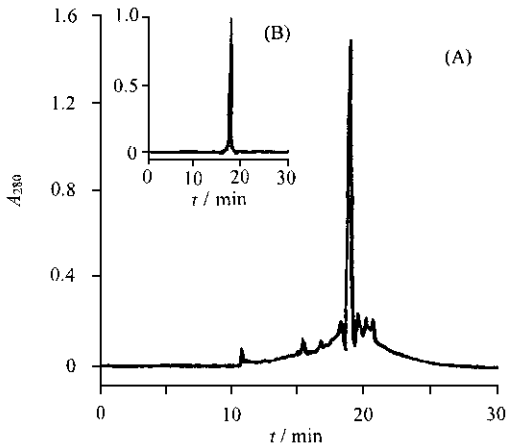

Fig. 2 Chromatogram of RP-HPLC of the active peak with an analytical column

2.2 Effect of pH on inhibitory activity

The extent of inhibition of cockroach α-amylase was measured at different pH values by preincubation of WAI-1 (2.6 μg) and enzyme in PBS buffer. The α-amylase activity remaining after preincubation at 37 °C for 20 min was determined. Controls were included to correct for the loss of enzyme activity at various pH during preincubation. The results (Fig. 3) show that WAI-1 inhibits cockroach α-amylase activity significantly under mild acid conditions, with optimal inhibitory pH 6.0.

2.3 Effect of preincubation duration on inhibitory activity

For a given amount of α-amylase and WAI-1 (2.5 μg), the percentage inhibition of α-amylase activity was determined at different durations of preincubation of the enzyme and inhibitor and at 37 °C pH 6.0. Results (Fig. 4) showed that the inhibitory activity of WAI-1 was related to the preincubation duration. In the early preincubation, the inhibitory activity of WAI-1 increased as the preincubation duration extended. However, the inhibitory activity reached a maximum after a certain length of time. Under the experimental conditions used, the maximum extent of inhibition (%) was observed at preincubation duration of about 30 min.

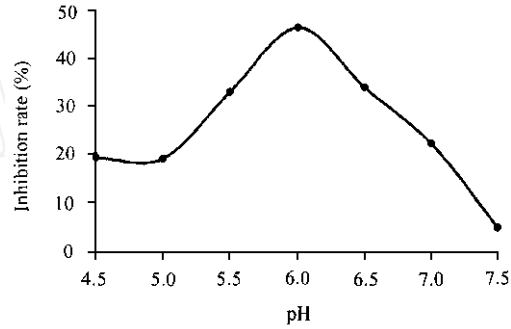

Fig. 3 pH dependence of inhibitory activity of WAI-1

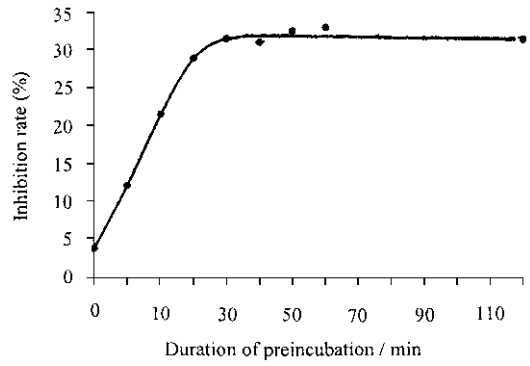

Fig. 4 Effect of preincubation duration on inhibitory activity

2.4 Effect of WAI-1 concentration on α-amylase activity

When a fixed amount of α-amylase was used, the enzyme and WAI-1 of different concentrations were preincubated at pH 6.0 and 37 °C for 20 min prior to the addition of starch solution. The results (Fig. 5) showed that the level of inhibition was dependent on the inhibitor/enzyme ratio. The percentage inhibition of α-amylase activity increased linearly up to about 50% with increasing WAI-1 concentration, then deviated from linearity at inhibition (%) higher than 50%, and attained finally a plateau at about 65%.

2.5 Nature of α-amylase inhibition by WAI-1

To ascertain whether inhibition of cockroach α-amylase by WAI-1 was competitive or not, Lineweaver-Burk plots were drawn for the uninhibited and partially inhibited enzyme. The rate of α-amylase action was

© 1994-2006 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

determined at different starch concentrations in digests (1.0 ml) containing soluble starch (0.2, 0.4, 0.5 and 1.0 mg/ml) in the absence and presence of a fixed amount of inhibitor WAI-1 at 37 ℃ and pH 6.0, respectively. Reaction velocity (v) was expressed in μmol maltose liberated per minute. Lineweaver-Burk double reciprocal plot for the uninhibited and partially inhibited enzyme intersected on the abscissa (Fig. 6), indicating that WAI-1 is a noncompetitive inhibitor of cockroach α-amylase.

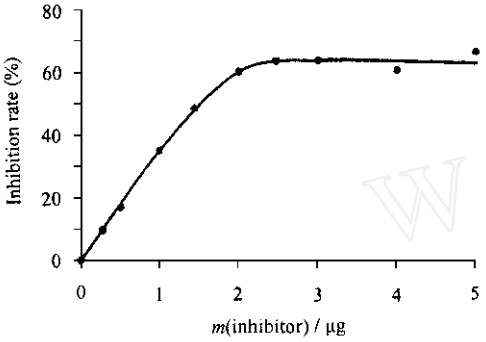

Fig. 5 Effect of WAI-1 concentration on α-amylase activity

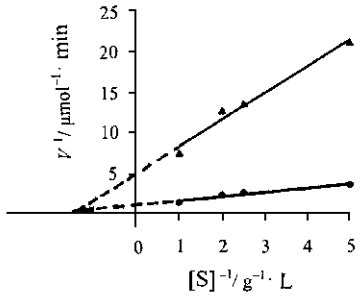

Fig. 6 Lineweaver-Burk double reciprocal plot for the uninhibited (·) and partially inhibited (▲) enzymes

2.6 Specificity of WAI-1

The purified WAI-1 was tested for its ability to inhibit α-amylases from *Periplaneta Americana* digestive ducts and human saliva from laboratory postgraduates simultaneously. When tested at similar concentrations, WAI-1 was found to potently inhibit α-amylases from the insect digestive ducts, having no inhibitory effect on human salivary α-amylase (Table 2).

Table 2 Results of specificity of WAI-1

	Control	Insect amylase	Human amylase
A_{546}	0.564	0.242	0.559

2.7 Amino acid composition and N-terminal of WAI-1

In order to investigate the composition of WAI-1, the inhibitor was hydrolyzed in 6 mol/L HCl at 110 ℃ for 24 hours. Analysis of the hydrolysate indicated that WAI-1 was composed of nine amino acids. However, Edman degradation of WAI-1 did not give any signal of phenylthiohydantoin amino acid (Fig. 7A), suggesting that its N-terminal was blocked. After WAI-1 was treated with 1 mol/L HCl at 60 ℃ for 4 hours according to the method described by Hashimoto T *et al*.[27], its molecular weight increased 18 determined by MALDI-TOF mass spectrometry (mass spectrum not shown) and Edman degradation gave an obvious signal of Glu (Fig. 7B), indicating that the N-terminal residue of WAI-1 is pyroglutamate.

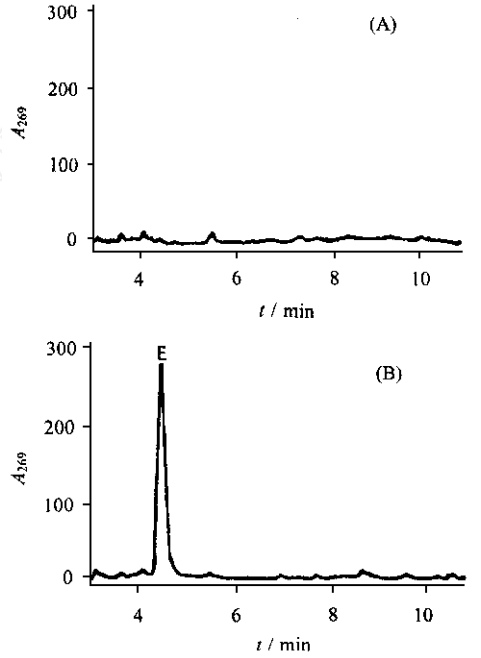

Fig. 7 Identification of WAI-1 N-terminal before (A) and after (B) hydrolysis in 1 mol/L HCl

3 Discussion

Internet search indicates that WAI-1 is the smallest known proteinaceous inhibitor of α-amylase. Such a small proteinaceous inhibitor can be purified from natural materials readily by RP-HPLC widely used in the purification of peptides[28~31]. Chagolla-Lopez *et al*. purified an α-amylase inhibitor (AAI) from the seeds of *Amaranthus hypocondriacus*, a variety of the Mexican crop plant amaranth[32]. AAI with a molecular weight of 3 586.1 was reputed as the major α-amylase inhibitor present in the amaranth seeds and the shortest α-amylase inhibitor described. Interestingly, when WAI-1 from wild amaranth seeds in southern China was purified, AAI could hardly be found, suggesting that the α-amylase inhibitor distribution profile of Chinese wild amaranthus plants is different from that of Mexican crop plant amaranth.

Like most other α-amylase inhibitors, the inhibitory activity of WAI-1 is dependent on the preincubation time, pH and temperature, etc. The inhibition takes place much more rapidly at 37 ℃ than at 0 ℃ and 25 ℃ (data

© 1994-2006 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

not shown). Inhibition studies carried out between pH 4.5 and 7.5 showed that WAI-1 exhibited the highest inhibitory activity at pH 6.0. Therefore, the detailed investigation of the effects of WAI-1 on insect α-amylases was made at 37 ℃ and pH 6.0. When fixed amounts of WAI-1 and α-amylase were preincubated for different time periods, about 4 % inhibition was obtained with a 0-min preincubation time and the maximum inhibition required a preincubation period of about 30 min, indicating the equilibrium nature of the inhibition reaction.

In the present study, the curves relating WAI-1 concentrations with the residual activity of insect α-amylase showed that the inhibition increased almost linearly up to the point where about 50 % reduction in enzyme activity was effected. Similar curves have previously been reported for other α-amylase-inhibitor systems[33,34]. The enzyme-inhibitor complex has been reported to have some amylolytic activity[35,36], which probably is the cause of the plateau at higher WAI-1 concentrations.

The binding site of inhibitor to the α-amylase molecule is different from that of the enzyme to starch[33]. An α-amylase inhibitor, therefore, is effective only against certain α-amylases which probably have suitable groups capable of binding with it. Since WAI-1 can selectively inhibit insect α-amylases, it is apparent that insect α-amylases have sites suitable for binding with WAI-1. Substrate can not dissociate the enzyme-inhibitor complex, and the inhibition is noncompetitive in nature.

Preliminary composition and structural analysis indicated that WAI-1 is a nonapeptide with N-terminal pyroglutamate. Some of the amino acids are modified or uncommon amino acids. The primary structure of WAI-1 is partially cyclic. Further studies are in progress to determine the detailed molecular structure and inhibition mechanism of WAI-1.

Acknowledgements We wish to thank teachers Xie Jinyun, Cao Menglin, Hu Weijun, Chen Ping, Zeng Xiongzhi for their supports and encouragements during the course of this work.

References

1 Keen E, Sandstedt R M. An amylase inhibitor for certain cereals. *J Am Chem Soc*, 1943, **65**: 1247

2 Keen E, Sandstedt R M. Distribution and general properties of an amylase inhibitor in cereals. *Arch Biochem*, 1946, **9**: 235～249

3 Franco O L, Rigden D J, Melo F R, Bloch Jr C, Sliva C P, Grossi de Sá M F. Activity of wheat α-amylase inhibitors towards bruchid α-amylases and structural explanation of observed specificities. Eur J Biochem, 2000, **267**(8): 1466～1473

4 Petrucci T, Rab A, Tomasi M, Silano V. Further characterization studies of the alpha-amylase protein inhibitor of gel electrophoretic mobility 0.19 from the wheat kenel. *Biochim Biophys Acta*, 1976, **420**: 288～297

5 Feng G H, Richardson M, Chen M S, Kramer K J, Morgan T D, Reek G R. α-amylase inhibitor from wheat: a sequences and patterns of inhibition of insect and human α-amylases. *Insect Biochem Mol Biol*, 1996, **26**: 419～426

6 Randall J W, Alexander W M, Robert D H, Harry W D. Purification and characteristics of an endogenous α-amylase inhibitor from barley kernels. *Plant Physiol*, 1983, **73**: 1008～1012

7 Abe J I, Sidenius U, Svensson B. Arginine is essential for the α-amylase inhibitory activity of the α-amylase/subtilisin inhibitor (BASI) from barley seeds. *Biochem J*, 1993, **293**: 151～155

8 Bloch Jr C, Richardson M. A new family of small (5 kD) protein inhibitors of insect α-amylase from seeds of sorghum (*sorghum bicolor* (L.) Moench) have sequence homologies with wheat δ-purothionins. *FEBS Lett*, 1991, **279**: 101～104

9 Iulek J, Franco O L, Silva M, Slivinski C T, Bloch Jr C, Rigden D J, Grossi de Sá M F. Purification, biochemical characterization and partial primary structure of a new α-amylase inhibitor from Secale cereale (Rye). *Int J Biochem Cell Physiol*, 2000, **32**: 1195～1204

10 Garcia-Casado G L, Sanchez-Monge R, Lopez-Otin C, Salcedo G. Rye inhibitors of animal α-amylases shown different specificities, aggregative properties and IgE-binding capacities than their homologues from wheat and barley. *Eur J Biochem*, 1994, **224**: 525～531

11 Yamagata H K, Kunimatsu K, Kamasaka H, Kuramoto T, Iwasaki T. Rice bifunctional α-amylase/subtilisin inhibitor: characterization, localization, and changes in developing and germinating seeds. *Biosc Biotechnol Biochem*, 1998, 62: 978～985

12 Giri A P, Kachole M V. Amylase inhibitors of pigeonpea (*Cajanus cajan*) seeds. *Phytochemistry*, 1998, **47**: 197～202

13 Melo F R, Sales M P, Silva L S, Franco O L, Bloch C Jr, Ary M B. α-Amylase inhibitors from cowpea seeds. *Prot Pept Lett*, 1999, **6**: 387～392

14 Grossi de Sá M F, Mirkov T E, Ishimoto M, Colucci G, Bateman K S, Chrispeels M J. Molecular characterization of a bean α-Amylase inhibitor that inhibits the α-Amylase of the Mexican bean weevil Zabrotes subfasciatus. *Planta*, 1997, **203**: 295～303

15 Young N M, Thibault P, Watson D C, Chrispeels M J. post-translational processing of two α-Amylase inhibitors and an arcelin from the common bean, *Phaseolus vulgaris*. *FEBS Lett*, 1999, **446**: 203～206

16 王贤纯, 梁宋平. 豆科植物籽实中昆虫消化道α-淀粉酶抑制剂的研究(Ⅰ). 生物技术 (Wang Xian-chun, Liang Song-ping. The study on the insect α-amylase inhibitors from Leguminus seeds (Ⅰ). *Biotechnology*), 1994, **5**: 18～21

17 王贤纯, 梁宋平. 豆科植物籽实中昆虫消化道α-淀粉酶抑制剂的研究(Ⅱ). 生物技术 (Wang Xian-chun, Liang Song-ping. The study on the insect α-amylase inhibitors from Leguminus seeds (Ⅱ). *Biotechnology*), 1995, **2**: 29～31

18 Munck L, Mundy L, Vaag P. Characterization of enzyme inhibitors in barley and their tentative role in malting and brewing. *Amer Soc Brew Chem J*, 1985, **43**: 35～38

19 Franco O L, Rigden D J, Melo F R, Grossi-de-Sá. Plant α-amylase inhibitors and their interaction with insect α-amylases: Structure, function and potential for crop protection. *Eur J Biochem*, 2002, **269**: 397～412

20 Ishimoto M, Sato T, Chrispeels M J, Kitamura K. Bruchid resistance of transgenic azuki bean expressing seed α-amylase inhibitor of common bean. *Entomol Exp Appl*, 1996, **79**: 309～315

21 Morton R L, Schroeder H E, Bateman K S, Chrispeels M J, Armstrong E, Higgins T J V. Bean alpha-amylase inhibitor 1 in transgenic peas (*Pisum sativum*) provides complete protection from pea weevil (*Bruchus pisorum*) under field conditions. *Proc Natl Acad Sci USA*, 2000, **8**: 3820～3825

22 Powers J R, Whitaker J R. Purification and some physical and chemical properties of red kidney beans (*Phaseolus vulgaris*) α-amylase inhibitor. *J Food Biochem*, 1977, **1**: 217～228

23 Lankisch M, Layer P, Rizza R A, DiMagno E P. Acute postprandial gastrointestinal and metabolic effects of wheat amylase inhibitor (WAI) in normal, obese, and diabetic humans. *Pancreas*, 1998, **2**: 176～181

24 Zawistowska U. Langstaff J, Bushuk W. Improving effect of a natural α-amylase inhibitor on the baking quality of wheat flour containing malted barley flour. *J Cereal Sci*, 1988, **8**: 207～209

25 Ishimoto M, Kitamura K. Growth inhibitory effects of an α-amylase inhibitor from the kidney bean, *phaseolus vulgaris* (L.) on three species of bruchids (Coleoptera: bruchidae). *Appl Ent Zool*, 1989, **24**(3): 281～286

© 1994-2006 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

26 Bernfeld P. Amylases, α- and β-. In: *Methods in Enzymology*, Vol1 (S. P Colowick and N. O. Kaplan, eds). New York: Academic Press, 1955: 149～158
27 Hashimoto T, Ohki K, Sakura N. Hydrolytic cleavage of pyroglutamyl-peptide bond. I. The susceptibility of pyroglutamyl-peptide bond to dilute hydrochloric acid. *Chem Pharm Bull* (*Tokyo*), 1995, **43** (12): 2068～2074
28 陈平,谢锦云,梁宋平. 虎纹捕鸟蛛毒素-I 中组氨酸残基的修饰与活性的关系. 中国生物化学与分子生物学报 (Chen Ping, Xie Jin-yun, Liang Song-ping. The relationship between chemical modification of histidine of HWTX-I and bioactivity. *Chin J Biochem Mol Biol*), 2001, **17** (5): 642～646
29 Wang X C, Chen P, Liang S P. Liquid chromatography-tandem mass spectrometry analysis of a synthetic nonapeptide and its main by-products. *Chin J Biochem Mol Biol*, 2003, **19** (5): 576～581
30 王蔚,胡玮,高培基. 密粘褶菌 Gloeophyllum trabeum 胞外低分子量多肽的分离纯化及其对纤维素生物降解作用. 中国生物化学与分子生物学报 (Wang Wei, Hu Wei, Gao Pei-ji. The purification and role in fungal oxidative cellulose degradation of a low molecular weight peptide from brown rot fungi Gloeophyllum trabeum. *Chin J Biochem Mol Biol*), 2002, **18** (2): 202～208
31 戴捷,梁宋平. 两种虎纹捕鸟蛛昆虫毒素的分离纯化及生物学活性鉴定. 中国生物化学与分子生物学报 (Dai Jie, Liang Song-ping. Purification and characterization of Huwentoxin-VII and Huwentoxin-VIII, two novel insecticidal neurotoxins from the Chinese bird spider *Selenocosmia huwena*. *Chin J Biochem Mol Biol*), 2003, **19** (4): 488～492
32 Chagolla-Lopez A, Blanco-Labra A, Patthy A, Sanchez R, Pongor S. A novel alpha-amylase inhibitor from amaranth (*Amaranthus hypocondriacus*) seeds. *J Biol Chem*, 1994, **38**: 23675～23680
33 Saunder R M. Alpha-amylase inhibitors in wheat and cereals. *Cereal Food Word*, 1975, **20**: 282～285
34 Buonocore V, Poerio E, Pace W, Petrucci T, Silano V, Tomasi M. Interaction of *Tenebrio molitor* L. alpha-amylase with a wheat flour protein inhibitor. *FEBS Lett*, 1976, **67**: 202～206
35 Buonocore V, Petrucci T, Silano V. Wheat protein inhibitors of alpha-amylase-review. *Phytochemmistry*, 1977, **16**: 811～820
36 O'Connor C M, McGeeney K F. Isolation and characterization of four inhibitors from wheat flour which display different inhibition specificities for human salivary and human pancreatic α-amylase. *Biochim Biophys Acta*, 1981, **658**: 387～396

2004 年国际生物芯片技术论坛 10 月在北京召开

2004 年 10 月 21～24 日,北京中关村生命科学园

会议由清华大学、生物芯片北京国家工程研究中心、科技部、教育部、国家自然科学基金委等单位共同主办,由生物芯片北京国家工程研究中心具体筹办.

会议议题主要包括以下内容:

1. DNA、蛋白质、细胞及组织微阵列芯片技术;
2. 微流体芯片及缩微芯片实验室技术;
3. 芯片药物筛选技术;
4. 生物信息学技术.

会议已邀请到 30 余位国际上最具权威性的生物学专家作大会特邀报告,其中既有从事生物芯片前沿性探索研究的科研院校的著名教授,也有从事生物芯片研发的知名商业公司的总裁或部门经理,是国内学者和投资机构进行交流和学习的良好机会.

会议网站:http://www.capitalbio.com/IFBT2004/
会议联系人:任琛
联系电话:010-62772239, 13651155414
传真电话:010-62773059
E-mail:chren@capitalbio.com
地址:北京市海淀区 清华大学生物科学与技术系 301 室
邮编:100084

© 1994-2006 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net