SUMMARY AND CONCLUSION

Chapter I

Some kinds of leguminous seeds harvested mainly in the tropical and subtropical areas were analyzed for their nutrient components and antinutritional factors. The seeds contained a high level of protein (20% or more). Protein was extracted sequentially with 2% NaCl, 30% isopropyl alcohol, 4% lactic acid and 0.5% KOH from the seeds. The NaCl extract had the highest range of protein concentration (50% or more). The saline extract was separated into two fractions based on solubility in water. The water-soluble fraction (WS) was made up of albumins and the water-insoluble (WI) of globulins. In all cases, the content of WI was higher than that of WS. The amino acid compositions of the flour from the seeds and of WI and WS were determined. High contents of lysine, aspartic acid, glutamic acid and leucine were obtained, whereas the contents of the sulfur containing amino acids were low. Trypsin and chymotrypsin inhibitory activities were generally observed in the leguminous seeds. Hemagglutinating activity was also found in the legumes. The seeds had high phosphorus contents including phytate-phosphorus.

Among the legumes, winged bean (Psophocarpus tetragonolobus) seeds had the highest range of protein (27.8-36.6%) and fat (14.8-17.9%). The values were similar to those of soybean. However, winged bean milk did not form solid curd like soy curd (tofu) under the same condition as manufacturing tofu. Winged bean contained tannin (1.35-6.75 mg/g of bean) and phytic acid (7.77-12.03 mg/g of bean) as non-protein antinutritional factors. Phytate-phosporus represented a considerable percentage of the toatal phosphorus (44.3-54.8%). Chymotrypsin inhibitory activity was about twofold higher than trypsin inhibitory activity. α-Amylase inhibitory activity could not be detected in winged bean. Hemagglutinating activity was observed with respect to all types of human erythrocytes (A, B and O). However this activity disappeared completely after heating in a boiling water bath for 10 min.

Chapter II

The α-amylase inhibitory activities were found in various kidney bean (<u>Phaseolus vulgaris</u>) seeds but not in other kinds of leguminous seeds. When kidney bean (var. Cranberry) seeds were soaked in distilled water for 15 h at room temperature, the inhibitory activity decreased, and after boiling treatment for 10 min it completely disappeared. However, dry heat treatment at 110℃ for 30 min caused no loss of the activity. The activity level of α-amylase inhibitor in cranberry bean reached a maximum 4 days germination. A similar change was observed for the trypsin inhibitory activity.

α-Amylase inhibitors were purified from two cultivars of kidney beans (<u>Phaseolus vulgaris</u>), cranberry bean and kintoki bean, respectively. Both inhibitors were glycoprotein. Their molecular weights and carbohydrate contents were 45,000 and 14% for the inhibitor from cranberry bean, and 45,000 and 15% for that from kintoki bean, respectively.

The cranberry bean α-amylase inhibitor (CBAI) exhibited inhibitory effects on pancreatic α-amylases from the following mammals: pig, dog, cat, horse, sheep, cow, rabbit, guinea pig, rat and mouse. CBAI showed a maximal inhibition at pH 5.5 against porcine pancreatic α-amylase (PPA). It was confirmed by gel filtration that a complex was formed in the 1:1 ratio between CBAI and PPA when they were incubated at 37℃ for 30 min at pH 5.5. A similar inhibition pattern was also observed at pH 6.9 that is optimal for the amylase reaction, but much higher concentrations of CBAI were required to give 50% inhibition at pH 6.9 than at pH 5.5.

CBAI was composed of three different subunits not linked by disulfide bridges and only one of them contained carbohydrate. Although the inhibitor was stable at pH 3 to 7, it was heat labile at pH 3 and 5. Chemical modification of the amino groups and the guanido groups in CBAI molecule resulted in rapid loss of the inhibitory activity, respectively. Oxidation of the tryptophan residues also led to loss of the activity. On the other hand, reductive methylation of the amino groups scarcely affected the activity. CBAI was quite resistant to the proteolytic digestions by pepsin and trypsin, while it was seemingly susceptible to the action of chymotrypsin.

A rabbit was immunized with CBAI and the specific antiserum for it was obtained. The inhibitor distribution in legumes was re-examined by the use of the antiserum. As the result, the inhibitor identical in antigenicity was proved to be exclusively occurring in cultivars of the same kind (Phaseolus vulgaris) but not in those of other kinds. Additionally its in vitro digestibility was investigated from both immunochemical and amylase-inhibitory viewpoints. The inhibitor was gradually inactivated by chymotrypsin digestion, although being resistant to pepsin and trypsin digestions, respectively. The antigenicity did not correspond with the inhibitor activity with regard to their loss in process of digestion; the former was left alive by half after the latter had disappeared.

CBAI was treated with $[^{14}C]HCHO$ and $NaBH_4$ to modify the lysyl ε-amino group. $[^{14}C]$Labeled inhibitor thus obtained was used as a radioactive probe. The reductive methylation of CBAI caused neither a significant change in its susceptibility to digestive enzymes nor in its immuno-reactivity with rabbit antiserum. Consequently, gastric emptying and intestinal transit of the inhibitor was represented by radioactivity measurement and simultaneously complemented by quantification due to single radial immunodiffusion. As the result, the bulk of the inhibitor was found not to undergo digestion and absorption but to pass through the small intestine without a considerable loss of antigenicity and radioactivity.

No considerable increase was observed in the levels of intraluminal α-amylase activity, blood sugar and plasma insulin in the

animals given CBAI at a dose of 10 mg each. These results suggest that the purified preparation from cranberry bean serves in fact as a potent inhibitor of rat pancreatic α-amylase.

The results of radioactivity and antigenicity measurements implied that CBAI rid of digestion and absorption was transferred to the ileocaecum to some extent. Even so it is doubtful whether the inhibitor activity of CBAI remains intact there. However, it is highly probable that CBAI escapes intraluminal digestion beyond expectations, when the use of too much protease in the in vitro model experiment is taken into consideration. Actually, intraluminal α-amylase activity was significantly low in duodenojejunum relative to the reference without CBAI loading. Therefore, CBAI would be useful as a non-poisonous pharmaceutics for the improvement in hyperglycemia and corpulency.

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Chapter I.

Section 1.

1. Kanamori, M., Ikeuchi, T., Ibuki, F., Kotaru, M. and Kan, K.K.: Amino acid composition of protein fraction extracted from Phaseolus beans and the field bean (Vicia faba L.).
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Section 2.

2. Ibuki, F., Kotaru, M., Yoshikawa, H.,Ikeuchi, T. and Kanamori, M.: Protein content of leguminous seeds imported for food and their antinutritional factors. (in Japanese)
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3. Kotaru, M., Yoshikawa, H., Ikeuchi, T. Ibuki, F. and Kanamori, M.: Mineral and phytic acid contents of leguminous seeds imported for food. (in Japanese)
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学 位 論 文 要 旨

Studies on Antinutritional Factors in Leguminous Seeds

（豆種子の反栄養因子に関する研究）

小 垂　眞

Ⅰ. はじめに

豆科植物は根に共生する根粒菌の作用によって固定された大気中の窒素を利用することができ、その種子や葉はタンパク質含量が高く、とくに完熟種子は保存性に優れる。熱帯・亜熱帯産の豆類には限られた地方でしか栽培されていないものや未利用のものが多く、その生産性向上も二期作、三期作によって可能であることから、熱帯・亜熱帯地域での豆類栽培は、牧畜や酪農の適さない当該地域での貴重なタンパク質資源として有望視される。一方、豆科植物には消化酵素に対する阻害物質(インヒビター)やレクチンなどの反栄養因子が存在することが知られており、豆種子を有効に利用するためには、消化吸収に悪影響を及ぼすおそれのあるこれら反栄養因子の性質や作用について明らかにしておく必要がある。なかでも、タンパク質性の消化酵素阻害物質についてはトリプシンやキモトリプシンに対する“プロテアーゼ・インヒビター”を中心によく研究されているが、 α-アミラーゼ・インヒビターに関しての知見は少ない。

本論文は、豆種子の有効利用に際して問題となるタンパク質やその他の成分について栄養学的見地から分析を行うと共に cranberry bean (Phaseolus vulgaris)から単離・精製したα-アミラーゼ・インヒビターのタンパク質的性状並びに動物消化管における挙動と作用発現の有無について検討を加えたものである。

Ⅱ. 豆種子タンパク質及び反栄養因子の分析

熱帯・亜熱帯産豆種子の栄養成分と反栄養因子について分析した。これらの豆種子は、概ね20%程度のタンパク質を含み、それらの大半は 2% NaClで抽出された。アミノ酸分析の結果、含硫アミノ酸含量は低かったが、リジン含量は高く、穀物との混食によるアミノ酸スコアの一層の改善が示唆された。熱帯産の四角豆 (Psophocarpus tetragonolobus)のタンパク質含量 (27.8-36.6%)と脂肪含量 (14.8-

17.9%)は大豆のそれにほぼ匹敵した。今回の実験で試料とした豆種子中には、反栄養因子として一般的なタンニン、フィチン酸、レクチン、トリプシン・インヒビターおよびキモトリプシン・インヒビターが含まれていることを確認した。

Ⅲ. 豆種子α-アミラーゼ・インヒビターに関する研究

(1) α-アミラーゼ・インヒビターの分布と発芽中の消長

各種の豆種子について α-アミラーゼ・インヒビター活性を検討したところ、Phaseolus vulgarisに属する栽培種にのみインヒビター活性が見出された。その一種（阻害活性の最も高い） cranberry bean の水浸漬を室温で 15時間続けると α-アミラーゼ・インヒビター活性は80%に減少し、さらに沸騰水中で10分間加熱するとインヒビター活性は消失した。しかし、豆種子を直接 110℃で30分間乾熱処理しても α-アミラーゼ・インヒビター活性に変化はみられなかった。 Cranberry bean 発芽種子中の α-アミラーゼ・インヒビター活性は、発芽開始後四日目で最大となり、その後次第に減少する傾向を示した。

(2) Cranberry bean α-アミラーゼ・インヒビターの単離と性質

粉砕した cranberry beanより水抽出、熱処理、アルコール沈殿、DEAE-cellulose クロマトグラフィー及びSephacryl S-200ゲル濾過により電気泳動的に均一な標品を得（以下、CBAIと略記）、その性質を調べた。CBAIは分子量約45,000の糖タンパク質（糖含量14%）で、ジスルフィド結合によって結合していない 3種の異なるサブユニットから構成され、そのうちの 1種のみが糖を含んでいた。CBAIの阻害作用は哺乳動物膵α-アミラーゼに特異的で、 微生物や植物のアミラーゼには作用しなかった。CBAI とブタ膵α-アミラーゼを pH 5.5、37℃、 30分間以上放置しておくと、両者はモル比 1：1 の EI complexを形成した。CBAI構成アミノ酸中の アルギニン残基を 1,2-cyclohexanedioneにより、トリプトファン残基を N-bromosuccinimideにより、また末端アミノ基を 2,4,6-trinitrobenzenesulfonate によって修飾すると阻害活性の急速な低下がみられた。これに反し、リジン残基のε-アミノ基を formaldehydeで還元メチル化しても阻害活性に影響しなかった。また、人工消化試験の結果によると、キモトリプシン消化に対してはやや不安定と思われたが、ペプシンとトリプシン消化に対しては極めて安定であった。

(3) In vivo 胃腸管でのCBAIの消化抵抗性と生理作用

[^{14}C]メチル標識CBAIと 抗CBAIウサギ抗血清を作成し、これを用いてCBAIのラット消化管における挙動を検討した。胃腸管各部位での放射活性及び免疫活性（抗原量）測定の結果、投与されたCBAIの多くが消化吸収を受けずにそのままの形態で胃腸管を通過していく様子が観察された。この知見をもとに、CBAIを他の食餌成分と共にmeal-feedingの条件下にあるラットに経口投与し、食後の血中グルコース濃度及び血中インシュリン濃度の変化を追跡した。その結果CBAI投与ラットの腸腔内プロテアーゼ活性に変化はないが α-アミラーゼ活性は明らかな低値を示し、食後しばらく血糖値及びインシュリン濃度も低く抑えられることが判明した。

Ⅳ. 結びにかえて

Cranberry bean からα-アミラーゼインヒビターを均一に精製しその in vivoにおける消化抵抗性と投与効果について調べたところCBAIは腸腔内において膵から分泌されたプロテアーゼの作用をほとんど受けずに α-アミラーゼを阻害しデンプン消化を妨げた。このことは、CBAIが高血糖症や肥満などの治療目的に応用可能であることを示唆する。