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Comparison of Biochemical Characterization of Korean and Chinese Mung Bean Lectin

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The lectins were separated from Korean and Chinese mung bean seeds finally via chromatography using Sephadex G-100 and their biochemical features were studied and compared. They showed no hemagglutination with human red blood cells regardless of trypsin treatment and showed hemagglutination with only trypsin treated rabbit red blood cells. The molecular weights of two lectins were identified as 54 kDa and 28 kDa by SDS-PAGE. It was found that while the optimal reaction temperature of the lectin from Korean mung bean was 60℃, that of the lectin from Chinese mung bean seeds was 50℃. It was found also that the most thermal stable temperature of the seed lectin from Korean mung bean seeds was 50℃ and the lectin from Chinese mung bean was 40-50℃. The lectin from Korean mung bean seeds showed the highest activity at pH 3.2 and the lectin from Chinese mung bean showed the highest activity at pH 6.2. It was identified that when treating a denaturant, thiourea and guanidine-HCl resulted in no hemagglutination, so they induced denaturalization. It was identified also that there was no hemagglutination with urea, so it did not induced denaturalization. They showed no septicity to 6 types of carbohydrates including D-glucose. In addition, the lectins from the two mung bean seed had specificity to metal ions.

Key words : Biochemical characterization, Korean and Chinese mung bean seed, lectin

Introduction

Mung bean (*Phaseolus radiatus* L. var.) is an annual plant which belongs to *Leguminosae* and is originated from India and cultivated also in Korea. Length of its stem is 60~80 cm and brown pili are spread on the whole of plant. Its pod is 5~6 cm in length, has slender and long shape with rough pili and projections, and contains 10-15 fruits. As it is highly nutritious, containing about 60% of starch and 21% of protein as main ingredients, it serves an important nutrition source to animals as well as humans and is also used as a major protein source due to its higher fiber content and lower fat content [28].

Lectin is one of natural proteins cohering reversibly to specific monosaccharide and polysaccharide and has specific binding affinity to carbohydrates [30], so involves in interaction between proteins and sugars [14]. Lectin is featured by various biochemical functions such as anticancer [8], anti-insect [36], anti-mold [41], antibacteria [15], and anti-HIV [2].

It is widely distributed across plants, animals and microorganisms in nature [26]. Plant lectin is mainly isolated from dried seeds, but exists also in leaves, stems, roots, and tubers [39]. Particularly it is rich in seeds of leguminous plants among them [5, 27].

The lectin is synthesized in ribosome of plant cells, transferred to secretion system in form of glycoprotein, accumulated highly in vacuoles and cell walls [6], and then used in growth of young plants. In additions it serves various physiological roles, including recognition of nitrogen fixation bacteria on the surface of roots, growth inhibition of plant pathogenic sources, and delivery of sugars, hormones, and glycoproteins [13].

Along with recent rise of price of mung bean produced in Korea, the amount of mung bean seeds imported from China increases. However it has been reported that the Chinese mung bean (CMB) seeds are inferior to the Korean mung bean (KMB) seeds in the flavor and the quality and most of all, there are distinctive differences in properties, in comparing the KMB and the CMB seeds.

Thus to identify the effect of place of origin on the lectin, this study was intended to identify existence of lectin in the KMB and the CMB seeds and compare biochemical proper-

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ties of the lectin between the KMB and the CMB seeds by estimating protein and carbohydrate content, molecular weight, hemagglutination, optimal reaction temp., thermal and pH stability, and effects of denaturant, metal ion and carbohydrate.

Materials and Methods

Plant materials and chemicals

KMB and CMB seeds used as materials were purchased from a local market of Yechon Gyunsangbukdo, Korea and Henan province, China, respectively. All chemicals were obtained from Sigma-Aldrich (St. Louis, MO) unless noted otherwise.

Isolation of lectin

Lectins were isolated from KMB and CMB seeds according to the method of Kilpatrick [18]. Seeds were ground to a fine power in liquid nitrogen and stirred overnight in 0.1 M sodium phosphate buffer (pH 7.2) containing 0.15 M NaCl. Powder of $(NH_4)_2SO_4$ for 50% final concentration added to supernatant collected by centrifuged at 1,000× *g* for 10 mins. After 24 hr, $(NH_4)_2SO_4$ fractions were collected by centrifugation at 40,000× *g* for 1 hr. Pellets resuspended in neutral saline (0.9% NaCl adjusted to pH 7.0 with Na_2HPO_4) were dialyzed against 5 l of neutral saline for 48 hr at 4℃, with a change of saline after 24 hr. After centrifugation at 40,000× *g* for 30 min, the supernatant solution was added to trypsin-treated human erythrocytes, and the mixture was shaken gently at room temperature for 15 min. The lectin-agglutinated erythrocytes were then harvested at 1,000× *g* for 5 min at room temperature. The cells were then washed three times with 5 volumes of neutral saline. The lectin was recovered from the washed erythrocytes by resuspending the cells in a mixture of *N*-acetylglucosamine oligomers. After being shaken for 5 mins at room temperature, the cells were harvested at 1000× *g* for 5 min and the supernatant retained. A further the preparation of *N*-acetylglucosamine oligomers was added to the cell pellet and the procedure was repeated. The combined supernatants were then dialysed against 5 l of neutral saline for 5 days at 4℃ with changes of saline every 24 hr. The dialysed solution was concentrated by ultrafiltration in an Amicon cell fitted with a PM-30 membrane filter, then loaded on a Sephadex G-100 column (1.5×20 cm) equilibrated with neutral saline. The column washed with 0.9% neutral saline until the A_{280} fell below zero, and then eluted with 0.9% neutral saline at a flow rate of 0.3 ml/min; 3 ml fractions were collected using fraction collector (Bio-Rad 2110). The fractions containing greatest lectin activity were pooled to provide the isolated lectin preparation for hemagglutination activity. The fractions kept at 0℃ for further assay.

All purification processes were done at 4℃ except as indicated.

Blood activation and hemagglutination activity determination

ABO human and rabbit blood were activated using trypsin suspension (25%, v/v) in neutral saline containing 0.25% trypsin. The blood cells were centrifuged at 8,000 rpm for 5 min after incubation at 37℃ for 5 min, and then harvested at room temperature. The cells were subsequently washed four times in neutral saline, and then hemagglutination activity was determined.

Hemagglutination activity was determined by a 2-fold serial dilution using the method of Takatsy [38]. Bloods were prepared by a 2% cell suspension in 0.9% neutral saline, respectively. Each sample was serially diluted in neutral saline, and a 2% suspension of blood was added to each well of a microplate, and agglutination was determined after incubation at 37℃ for 1 hr. The degree of agglutination was assessed by eyes. The reciprocal of the highest dilution of the lectin showing complete agglutination was taken as the hemagglutination titer.

Measurement of protein and carbohydrate contents

Protein contents were measured at 595 nm according to Bradford method [3] using microplate reader (Bio-Rad 680) with bovine serum albumin as a standard. Carbohydrate contents were measured at 490 nm by the phenol/H_2SO_4 method of Dubiso et al. [11] with glucose as a standard.

SDS-PAGE and molecular weight determination

12% SDS-PAGE was performed at room temperature by the method of Laemmli [21]. The lectin isolated by affinity chromatography on Sephadex G-100 was denatured in a boiling H_2O for 10 min before loading on the gel. The gels were run at 30 mA for 1 hr. The bands were stained with Coomassie Brilliant Blue R-250, and then destained by 7.5% acetic acid. The molecular weight was determined by the method of Weber and Osborn [40]. The molecular weight markers were rabbit muscle phosphorylase b (97 kDa), bo-

vine serum albumin (66 kDa), chicken egg white ovalbumin (45 kDa), bovine erythrocyte carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa).

Determination of temperature effect and thermal stability

The effect of temperature on activity of lectin was measured at the range from 10℃ to 90℃. The dialysates containing isolated lectin was incubated for 10 min at 10~90℃, respectively, and then hemagglutination activity was determined by a serial 2-fold dilution method using trypsin treated rabbit blood. The isolated lectin was incubated in the range from 20℃ to 90℃ for 10 min. After cooling immediately in ice water bath, the hemagglutination activity was determined by a serial 2-fold dilution method using trypsin treated rabbit blood for thermal stability.

Determination of pH effect

The effect of pH on activity of lectin was investigated by measurement in buffer of various pH values (0.025 M glycine-HCl buffer, pH 2.2; 0.2 M acetate buffer, pH 3.2, 4.2; 0.01 M phosphate buffer, pH 6.2, 7.2; 0.2 M tris-HCl buffer, pH 8.0, 9.1; 0.2 M sodium carbonate-bicarbonate buffer, pH 10.0). The isolated lectin was preincubated in buffers with different pH for 4 hr at 4℃, and the hemagglutination activity was determined by a serial 2-fold dilution method using trypsin treated rabbit blood.

Determination of carbohydrate specificity

Hemagglutination activity by carbohydrates was determined by the method of Allen et al. [1]. 200 mM D-fructose, 200 mM D-galactose, 200 mM D-maltose, 200 mM D-sucrose, 200 mM D-mannose, 200 mM D-glucose, and 200 mM N-acetyl-D-glucosamine were used in this study. Hemagglutination activity was determined by a serial 2-fold dilution method using trypsin treated rabbit blood.

Determination of metal ion effect

To determination of metal ions effect on lectin, the isolated lectin solution was pre-incubated with 20 mM metal ions such as $CaCl_2$, $CoCl_2$, $CuSO_4$, $FeSO_4$, $MgSO_4$, and $MnSO_4$ for 15 min at 20℃. Hemagglutination activity was determined by a serial 2-fold dilution method using trypsin treated rabbit blood.

Determination of denaturants effect

3 M urea, 3.5 M thiourea, and 3 M guanidine-HCl were used as denaturants. Hemagglutination activity was determined by a serial 2-fold dilution method using trypsin treated rabbit blood.

Results

Isolation of lectin

The lectin was isolated finally using neutral saline solution on Sephadex G-100. While the KMB seeds showed activity in the 2^{nd} -6^{th} fraction, of which the 3^{rd} fraction has the highest protein content and activity (Fig. 1), the CMB seeds showed activity in 2^{nd}~8^{th} fraction, of which the 3^{rd} fraction has the highest protein content and activity (Fig. 2). Therefore, the fraction with the highest activity was used to identify molecular weight, optimal reaction temperature, thermal and pH stability, modulator, and effect of metal ions and carbohydrates.

Contents of protein and carbohydrate

By measuring content of protein and carbohydrate of lectin from KMB seeds, it was found that the protein and carbohydrate content were 0.896 mg/ml and 0.545 mg/ml, respectively. Content of protein and carbohydrate of lectin from CMB seeds were 0.793 mg/ml and 1,044 mg/ml, respectively (Table 1).

Hemagglutination activity and specificity

Human ABO blood and rabbit blood were divided into

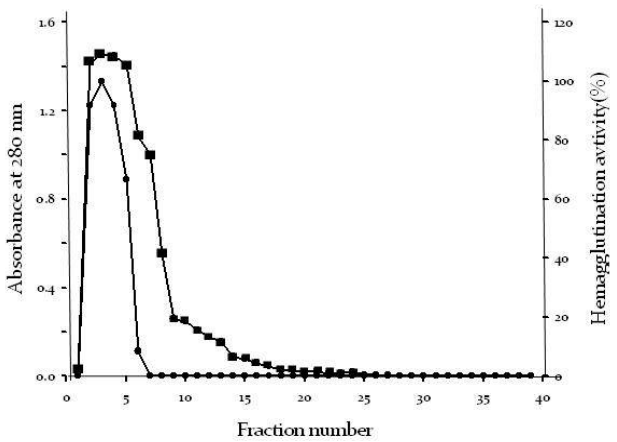

Fig. 1. Fractionation profile for lectin isolated from Korean mung bean on Sephadex G-100 finally. The bound lectin was eluted with neutral saline. Hemagglutination activity was determined using rabbit blood. ■–■: optical density, ●–●: hemagglutination activity.

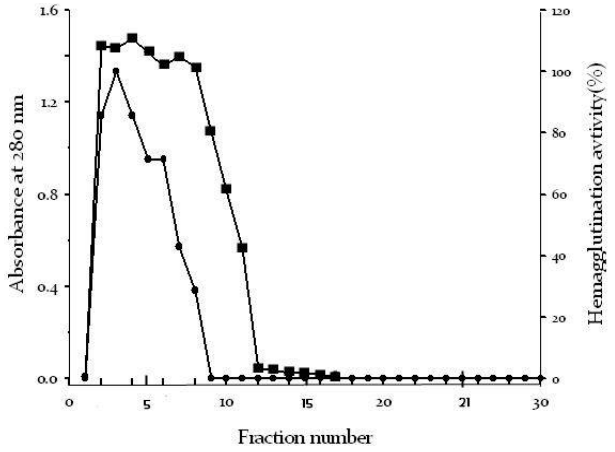

Fig. 2. Fractionation profile for lectin isolated from Chinese mung bean on Sephadex G-100 finally. The bound lectin was eluted with neutral saline. Hemagglutination activity was determined using rabbit blood. ■–■: optical density, ●–●: hemagglutination activity.

Table 1. Amounts of protein and carbohydrate in isolated lectin

	Amounts (mg/ml)	
	Korean mung bean	Chinese mung bean
Protein	0.896	0.793
Carbohydrate	0.545	1.044

trypsin treated- and untreated group and hemagglutination of each group was estimated. For the trypsin treated group, both lectin from KMB and CMB seeds showed agglutination only in rabbit blood and entirely no agglutination in human ABO blood. In addition, for the trypsin-untreated blood, no agglutination was shown in both bloods (Fig. 3). Thus the trypsin treated rabbit blood was used for biochemical characterization study of the lectin.

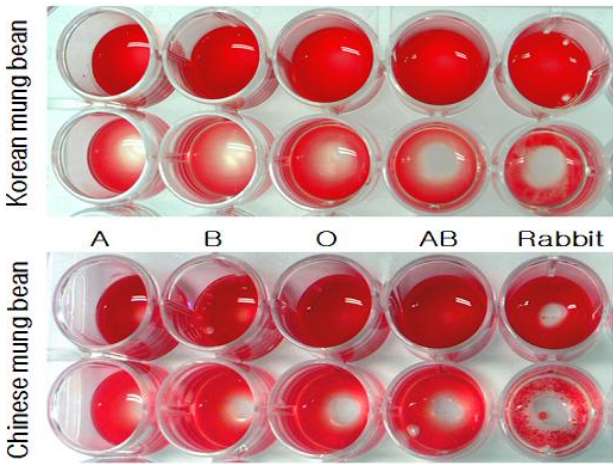

Fig. 3. Hemagglutination effects of Korean and Chinese mung bean lectin on human ABO and rabbit blood. Upper on each plate: no treated with trypsin. Lower on each plate: treated with trypsin.

SDS-PAGE and molecular weight

Two bands were found in both lectins from KMB and CMB seeds by SDS-PAGE. Molecular weight of these bands were measured by comparing relative mobility to reference proteins, and it was identified that the bands separated from the lectin had 54 kDa and 28 kDa of molecular weight, respectively (Fig. 4).

Effect of temperature and thermal stability on lectin activity

The lectin from KMB seeds showed the highest activity of 100% at 60℃ and higher activity of 90% also at 70℃. However, it showed lower activity as less than 60% below 50℃ and lost its activity below 10℃ and over 80℃. The lectin from CMB seeds showed the highest activity of 100% at 50℃ and at least 60% of activity at 20-40℃. However, its activity was reduced significantly at 10℃ and 60℃ and disappeared completely over 70℃ (Fig. 5).

It was found that the most stable reaction temperature

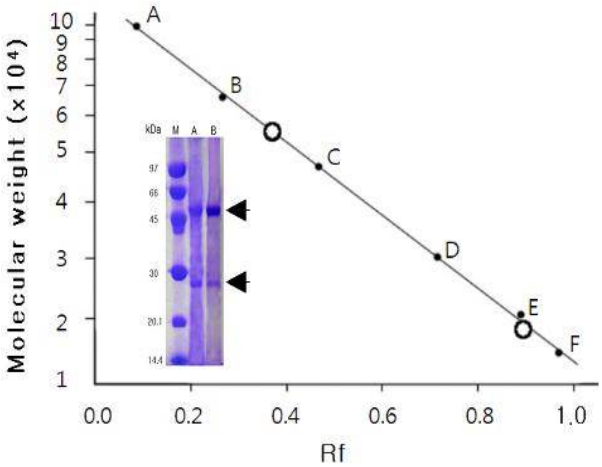

Fig. 4. 12% SDS-PAGE pattern and determination of molecular weight of lectin isolated from Korean and Chinese mung bean. The gels was run at 30 mA for 1 hr and stained with Coomassie brilliant blue R-250. Arrows indicate lectin isolated by affinity chromatography on Sephadex G-100. Lanes: M, molecular weight marker; A, isolated lectin from Korean mung bean; B, isolated lectin from Chines mung bean. Open circles (○) indicate isolated lectin. The molecular weight markers (●) were rabbit muscle phosphorylase b (97 kDa), bovine serum albumin (66 kDa), chicken egg white ovalbumin (45 kDa), bovine erythrocyte carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa).

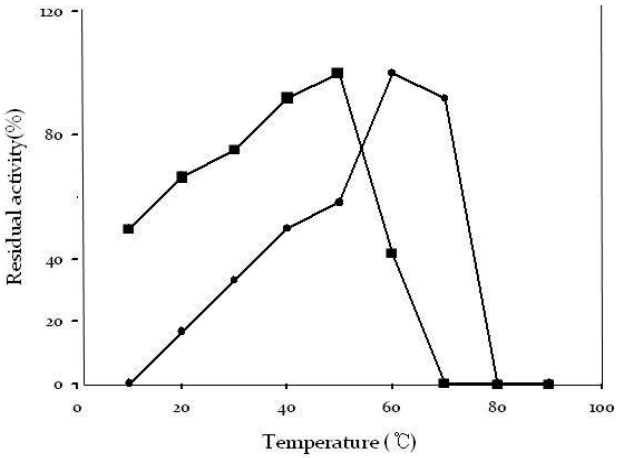

Fig. 5. Effect of temperature on hemagglutination activity of lectin isolated from mung bean. The lectin activity was tested by incubation at 10-90℃, respectively. ●-●: lectin from Korean mung bean, ■-■: lectin from Chinese mung bean.

of the lectin from KMB seeds was 50℃, where it showed the highest activity as 100% and that its activity was stable also at 40℃ as 60%. However, its activity was reduced significantly to very low level at 60℃ and lost over 70℃. For the lectin from CMB seeds, it was found that it most stable reaction temperature was 40-50℃, where it showed the highest activity as 100%. It was shown also that its activity was very stable also at 20-30℃, but decreased dramatically at 70 ℃ and lost completely over 80℃ (Fig. 6).

Effect of pH on lectin activity

The lectin isolated from the KMB seeds showed the highest activity as 100% at pH 3.2. In addition, it showed at least 60% of activity also at pH 2.2, pH 4.2 and pH 6.2. However, its activity was decreased dramatically over pH 7.2 and lost completely at pH 10.0. The lectin isolated from the CMB seeds showed the highest activity as 100% at pH 6.2. In addition, it showed at least 60% of activity also at pH 3.2, pH 4.2 and pH 7.0. However, its activity was decreased dramatically over pH 7.2 and lost completely at pH 10.0 (Fig. 7).

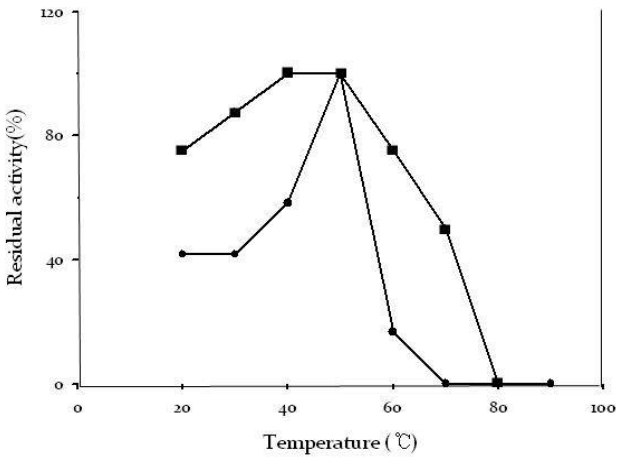

Fig. 6. Thermal stability of lectin isolated from mung bean. The lectin was preheated for 10 min at 20-90℃, respectively. ●-●: lectin from Korean mung bean, ■-■: lectin from Chinese mung bean.

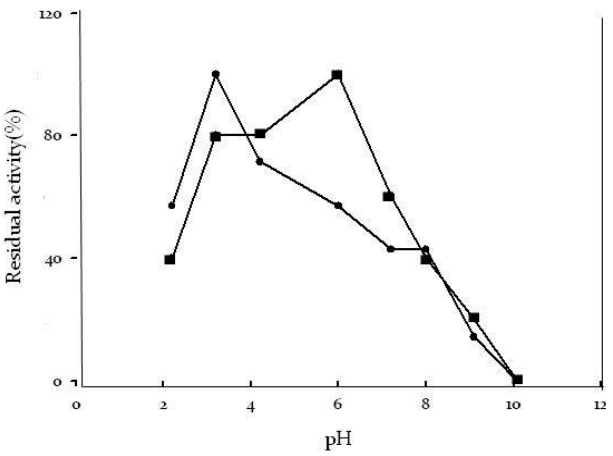

Fig. 7. Effect of pH on hemagglutination activity of lectin isolated from mung bean. The lectin was incubated different pH for 4 hr at 4℃. ●-●: lectin from Korean mung bean, ■-■: lectin from Chinese mung bean.

Effect of denaturants on lectin activity

While the lectin from the KMB seeds showed 100% agglutination in treating urea, no agglutination in treating thiourea and guanidine-HCl. The lectin isolated from the CMB seeds showed same results to the lectin from the KMB seeds (Table 2). Thus, it was suggested that for thiourea and guanidine-HCl, the lectin was denaturalized by the denaturants, but for urea, the lectin was not denaturalized, so showed 100% of agglutination identical to the control group.

Carbohydrate specificity

By studying minimum activity inhibitory concentration of the lectin by carbohydrate, it was found that the lectin from

Table 2. Effect of denaturants on lectin activity

	Relative activity (%)	
	Korean mung bean	Chinese mung bean
Control	100	100
Urea	100	100
Thiourea	0	0
Guanidine-HCL	0	0

Table 3. Effect of carbohydrates on lectin activity

Carbohydrates	Minimal Inhibitory conc. (mM)	
	Korean mung bean	Chinese mung bean
D-glucose	>6.25	>6.25
D-galactose	>6.25	>6.25
D-maltose	>6.25	>6.25
D-mannose	>6.25	>6.25
D-fructose	>6.25	>6.25
N-acetyl-	>6.25	>6.25
D-glucosamine	>6.25	>6.25
D-sucrose	>6.25	>6.25

the KMB showed hemagglutination to all carbohydrate levels in this study, from 6.25 mM to 200 mM. It was found also that the lectin from the CMB seeds showed hemagglutination to all carbohydrate levels, from 6.25 mM to 200 mM (Table 3). Therefore, it was suggested that the lectin from the KMB and the CMB seeds had no specificity to the carbohydrate used in this study.

Effect of metal ion on lectin activity

As results of measuring minimum inhibitory concentration of the lectins by effects of metal ion, it was found that the lectin from KMB seeds showed agglutination with all metal ion levels from 1.25 mM to 20 mM. The lectin from the CMB seeds also showed agglutination with metal ions within the range identical to that of the lectin from the KMB bean (Table 4). Therefore, it was suggested that the lectin from the KMB and the CMB seeds had no specificity to the metal ions used in this study.

Discussion

The characterization of lectin has been widely studied in biochemical and medical section [31] and its major feature is to bind sugars on cell surfaces and make sugar composite, which is referred as the origin of biological feature of the lectin [13]. Using these characterizations of lectin, the lectin can be used as a useful tool for immunological study, screening and separation of sugar composites and their characterization [34]. This lectin exists often in leguminous plants [22] and in this study, mung bean seeds, one of the leguminous plants, was used to compare and analyze biochemical characterizations of lectin.

Table 4. Effect of metal ions on lectin activity

Metal Ions	Minimal Inhibitory conc. (mM)	
	Korean mung bean	Chinese mung bean
$CaCl_2$	> 1.25	> 1.25
$CoCl_2$	> 1.25	> 1.25
$CuSO_4$	> 1.25	> 1.25
$FeSO_4$	> 1.25	> 1.25
$MgSO_4$	> 1.25	> 1.25
$MnSO_4$	> 1.25	> 1.25

As there was about 0.1 mg/ml of difference between protein contents in the lectin from the KMB and the CMB seeds, it was suggested that the KMB seeds contained more protein. When comparing this result with protein content of lectins from brown soybean [24] and yak-kong [37], it was higher as much as 1.13 and 1.18 times than the result of this study, respectively. The carbohydrate content of the lectin from CMB seeds was higher as much as two times than that from KMB seeds. When comparing this result with carbohydrate content of lectin in this study, carbohydrate content of peanut lectin was higher as much as 1.25 times than that of KMB seeds and lower than that of CMB seeds [29]. By identifying the protein and carbohydrate content in the lectin from KMB and CMB seeds, it was suggested that the lectin was a glycoprotein.

Using a property of lectin to agglutinate specifically with mammalian red blood cells [12], the activity of lectin was determined [35]. The lectin from KMB and CMB seeds showed hemagglutination only with trypsin treated rabbit blood and no hemagglutination with other bloods. Therefore, it was identified that the lectin from KMB and CMB seeds reacted specifically to only trypsin activated rabbit blood. On the contrary, the lectin isolated from *Erythrina speciosa* showed hemagglutination with all of human ABO type blood, wherein it showed stronger hemagglutination with A, B, AB type blood, but relatively weaker hemagglutination with O type blood. In addition, it showed hemagglutination also with rabbit, mouse, and sheep blood, but no reaction with horse blood [20].

Although the lectin isolated from wild sunflower showed no hemagglutination with all of human ABO type blood, it developed hemagglutination with all of rabbit blood regardless of trypsin treatment, which was different from the results of this study. Moreover, the lectin from *Fusarium solani* had blood specificity with neuraminidase or pronase treated human ABO type blood and no blood specificity with trypsin treated or untreated human blood [17]. From these results, it was suggested that lectin had different specificity to other enzymes according to red blood cell features, type of enzyme and its treatment.

Most lectin existing in nature has 26-400 kDa of molecular weight and consists of 2-18 homogeneous or heterogeneous subunits [4]. In this study, it was identified that both the lectin from KMB and CMB seeds had two bands, which had 54 and 28 kDa of molecular weight respectively, and they had same molecular weight. As the results of this study were different from that of the mung bean lectin, 34 kDa [16], it was suggested that molecular weight of the lectin may be varied even in same plant species. It was different also from the lectin of *Dolichos lablab* [23], which consisted of two subunits, 31 kDa and 29 kDa, and had 120±5 kDa of molecular weight and showed some difference also from the lectin of *Erythrina speciosa* seeds consisting of two subunits with 27.6 kDa [20].

The most optimal reaction temperature of the lectin from KMB seeds was 60℃ and that of the lectin from CMB seeds was 50℃. While the lectin from KMB seeds showed higher activity as 90% even at 70℃, the lectin from CMB lost its activity over 70℃. The lectin from CMB seeds had higher activity below 50℃, and the lectin from KMB seeds had higher activity over 50℃. The optimal reaction temperature of the lectin separated from horse bean shoots was 40℃ and decreased over 60℃ [33]. Different from the above two lectins from mung bean seeds, although the lectin separated from *Arisaema tortuosum* had optimal activity up to 55℃, its activity was reduced to the half and lost over 85℃ [9]. From these results, it was cleared that the lectins of the above two mung bean seeds had similar optimal reaction temperature to those of other leguminous plants. It seems that the lectin had species specificity to optimal temperature and different optimal temperature according to its origins even within same species.

It was found that the lectins from KMB and CMB seeds were the most stable at 50℃ and the lectin from CMB seeds showed activity even at 70℃, so it was more thermal stable than that from KMB seeds. The lectin from *Dolichos lablab* seeds showed stable activity up to 40℃, but lost its activity at 50-90℃ [9]. Although the results of this study were varied in comparing the above result, they showed generally higher stability at 40-60℃ and lost their activity with rise of temperature.

As the lectin from KMB seeds showed the most stable reaction at strong acid solution and the lectin from CMB seeds showed the most stable reaction at weak acid solution near neutral, it was identified that although the lectins from the above two mung bean seeds had some difference in optimal stability to pH, they had similar features of lectin as same species but their characteristics were different depending on their origins. In addition, it was found that as they were stable in the range of pH 4-7 and showed no agglutination over pH 8.0, their features were similar to the lectin from *Flammulina velutipes* [19] and different from the lectin from *Erythrina speciosa* seeds [20]. Therefore, it was suggested that the lectins by seeds had specific tendency to pH.

Although it has been reported that denaturants such as urea, thiourea, and guanidine-HCl digest hydrogen bond in polypeptide chain or inhibit activity of lectin by interfering hydrophobic interaction [9], treatment with thiourea and guanidine-HCl brought no hemagglutination and urea treatment resulted in guanidine-HCl. Different from this study, it was found that the lectin from sea cucumber had 50% reduced activity in 4 M urea [10] and the lectin from *Arisaema tortuosum* showed 50% reduced activity in 3 M urea and thiourea and 50% reduced activity in 3.5 M of guanidin-HCl [9]. Therefore, it was identified that although urea gave no effect to the lectins from both mung bean seeds, thiourea and guanidine-HCl affected them. Additionally, it was identified that the lectins by origins had different inhibitory reaction depending on concentration of urea.

The lectins from KMB and CMB seeds showed hemagglutination with all the carbohydrate samples used in this study under 200 mM. The results of this study that hemagglutination of the above lectins were not inhibited by carbohydrate means that the mung bean lectin had no specificity to carbohydrate. However, the lectin from *Schizophyllum commune* showed significantly high sugar specificity to lactose and N-acetyl-D-galactosamine [7]. In addition, the lectin from *Dolichos* lectin showed sugar binding specificity to galactose, *N*-acetylgalactosamine and MeβGal and played a role as a blocker over 100 mM of glucose, mannose, and *N*-acetylglucosamine [23]. From these, it was suggested that sugar specificity are various according to lectins. The lectins show its activity by reacting metal ions or plays role as a blocker from reaction of binding site between metal ion and lectin [19]. Moreover as the lectin may need Ca^{2+} and Mn^{2+} in binding with sugars and these metal ions are located close to sugar binding site of the lectin, the metal ions contributes to maintaining stability of lectin unit and helps arrangement of amino acid residues from sugar binding [25].

Considering the lectins from the two mung bean seeds, it was found that they had no specificity to these bivalent metal ions below 20 mM of concentration. Although for the

lectin of egg plants, no specificity to bivalent metal ions was found identically to the results of this study [32], the lectin from *Erythrina* seeds must need Ca^{2+} and Mn^{2+} for its activity [20]. Therefore, it was identified that the lectins from two mung bean seeds had no specificity to metal ions in the binding site of lectin.

In conclusion, existence of lectin in KMB and CMB seeds was identified through hemagglutination and SDS-PAGE. While they showed identical results in blood specificity, molecular weight, optimal thermal stability, and effects of regulator, metal ions, and carbohydrate, they showed different results in protein and carbohydrate content, optimal reaction temperature, and optimal pH. It means that even same mung bean seeds may have difference of biochemical features by origins. It is considered that these results may be used as biochemical index discriminable between KMB and CMB seeds.

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초록 : 한국산 녹두와 중국산 녹두에 있어서 Lectin의 생화학적 특성 비교

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한국산과 중국산 녹두 종자에서 0.15 M NaCl/0.1 M sodium phosphate buffer (pH7.0)에 의한 추출, $(NH_4)_2SO_4$ 침전, 최종적으로 Sephadex G-100을 이용한 affinity chromatography에 의해 lectin을 분리한 다음, 이들의 생화학적 특성을 조사, 비교하였다. 사람의 적혈구는 trypsin의 처리 유무와 상관없이 응집반응이 일어나지 않으며, 토끼의 적혈구에서는 trypsin을 처리한 경우에만 응집반응이 일어났다. 두 녹두 종자 lectin의 분자량은 SDS-PAGE를 통해 54 kDa와 28 kDa로 확인되었다. 한국산 녹두 종자 lectin의 최적 반응 온도는 60℃이며, 중국산 녹두 종자 lectin의 경우는 50℃로 나타났다. 종자 lectin이 열에 가장 안정한 온도는 한국산의 경우는 50℃이며, 중국산의 경우 40-50℃로 밝혀졌다. 한국산 녹두 종자 lectin은 pH 3.2에서 가장 높은 활성을 보였으며, 중국산 녹두 종자 lectin은 pH 6.2에서 가장 높은 활성을 보였다. 변성제를 처리했을 때, thiourea와 guanidine-HCl과는 혈액 응집이 일어나지 않아 변성작용이 일어남을 알 수 있었고, urea와는 혈액 응집이 일어나지 않아 변성작용이 일어나지 않음을 알 수 있었다. D-glucose외 6가지의 탄수화물에 대한 특이성이 나타나지 않았다. 또한 두 녹두 종자의 lectin은 Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+} 및 Mn^{2+} 등의 금속이온에 대한 특이성이 없었다.