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Fractionation of *Canavalia lineata* Bean Lectin by Gel-Filtration

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SUMMARY

Canavalia lineata lectin was fractionated into three fractions by Bio-Gel A-1.5 m column (2 x 85 cm) chromatography using 0.01 M Tris-HCl buffer pH 9.0 as eluant. After rechromatography of these three fractions, five fractions were obtained (Frs. A1, A2, B1, B2, and C). Fraction A1, which was excluded by the Bio-Gel column, had high hemagglutination activity, and its hemagglutination titer was equal to that of the original lectin. The result of SDS-polyacrylamide gel electrophoresis revealed that fraction A1 was composed of the intact subunit (mol. wt. 29,000).

Key words: Canavalia lineata lectin, Gel-filtration, Hemagglutination, Intact subunit.

The lectin purified from the bean meal of *Canavalia lineata* was not electrophorectically homogeneous. Existence of this lectin in multiple molecular species, especially at pH 9, had also been demonstrated by gel-filtration[1]. The intact subunit of this lectin had been purified by gel-filtration in the presence of 1% sodium dodecyl sulfate (SDS), but its hemagglutination activity was 15-fold less than that of the original lectin[2]. Effects of *C. lineata* lectin on the proliferation of S-180 sarcoma cells in mice had been studied in our laboratory[3]. The results indicated that the lectin-bound tumor "vaccine" could prolong the life span of tumor-bearing animals, however, the cure rate was low. We are going to study the anti-tumor effect of the components of *C. lineata* lectin, and search for the component which had better anti-tumor activity. Since the lectin exists in multiple molecular species at pH 9, we had obtained five fractions from the lectin by using this property and technique of gel-filtration. One of the fractions obtained (Fr. A1) was composed of the intact subunit only, and had high hemagglutination activity. The method of fractionation is described in this report.

MATERIALS AND METHODS

C. lineata lectin was purified by adsorption on a Sephadex G-50 column

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and eluted with maltose solution[1]. Concanavalin A (*Canavalia ensiformis* bean lectin) purified by the method of Olson and Liener[4] was purchased from Sigma company. Sephadex G-50 was bought from Pharmacia Fine Chemicals. Bio-Gel A-1.5 m was obtained from Bio-Rad Laboratories. Dialysis membrane tubing (Spectrapor mol. wt. cutoff 3,500) was the product of Spectrum Medical Industries, INC. Hemagglutination titers toward rat erythrocytes were determined in a series of test tubes as previously described[1]. SDS-polyacrylamide gel electrophoresis without mercaptoethanol was carried out in tube gels according to the method described by Weber and Osborn[5]. 0.1 mg lectins or fractions were used. Percentages of total acrylamide and cross-linker of the gels (0.5 x 11 cm) were 10.4 and 3.9. The gels were stained in 7% acetic acid containing 1% amido black 10B, and destained in 7% acetic acid. Molecular weights were determined by plotting the relative mobility against logarithm of molecular weight. Low molecular weight calibration kit containing phosphorylase b, albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, and alphalactalbumin was obtained from Pharmacia Fine Chemicals.

RESULTS

Bio-Gel A-1.5 m column chromatography of *C. lineata* lectin:

One hundred mg *C. lineata* lectin was dissolved in 10 ml 0.01 M Tris-HCl buffer pH 9.0 and filtered through a Whatman No. 1 filter paper. The filtrate was applied on to a Bio-Gel A-1.5 m column (2 x 85 cm) and eluted with 0.01 M Tris-HCl buffer pH 9.0 at a flow rate of 4 ml/tube/20 min. The elution profile was shown in Fig. 1. The substance which was excluded by the column (first peak, tubes 20 to 31) was collected as fraction 1. The last peak which appeared in tubes 55 to 62 was collected as fraction 3. The components which were eluted between fractions 1 and 3 (tubes 36 to 54) were pooled as fraction 2.

Rechromatography of fractions 1, 2, and 3:

Fraction 1 obtained from 200 mg *C. lineata* lectin was lyophylized and dissolved in 15 ml 0.01 M Tris-HCl buffer pH 9.0. This solution was reapplied on to the Bio-Gel A-1.5 m column and eluted with the Tris-HCl buffer. As shown in Fig. 2, in addition to the substance which was excluded by the column (fraction A1, tubes 19 to 31), a second peak appeared in tubes 54 to 60 and was collected as fraction A2.

Fraction 2 obtained from 300 mg *C. lineata* lectin was lyophylized and dissolved in 15 ml 0.01 M Tris-HCl buffer pH 9.0. This solution was reapplied on to the Bio-Gel A-1.5 m column and eluted with the Tris-HCl buffer. The elution profile shown in Fig. 3 indicated that the major peak unexpectedly appeared after tube 50. Tubes 31 to 49 were pooled as fraction B1, and the major peak (tubes 53 to 64) was collected as fraction B2.

Fraction 3 obtained from 300 mg *C. lineata* lectin was lyophylized and dissolved in 15 ml 0.01 M Tris-HCl buffer

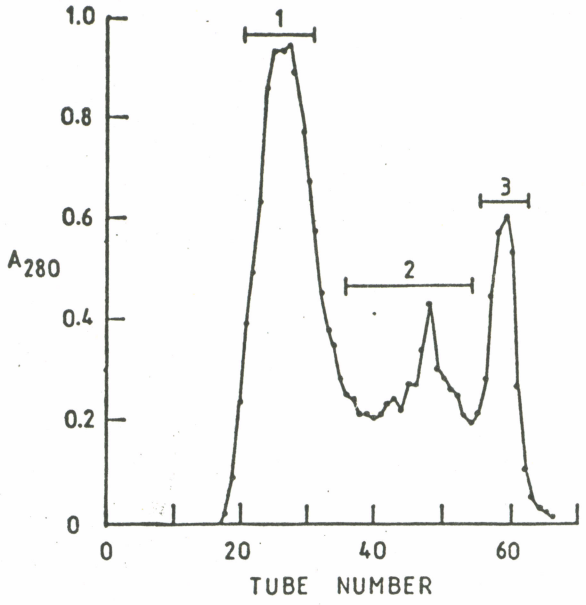
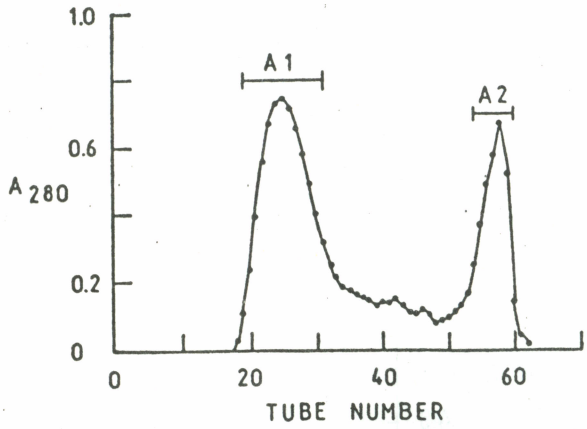


Fig. 1.
Bio-Gel A-1.5 m column (2 x 85cm) chromatography of *C. lineata* lectin. 100mg lectin in 10ml 0.01M Tris-HCl pH 9.0 was applied on to the column and eluted with the same buffer at a flow rate of 4ml/tube/20min. Tubes 20 to 31 were pooled as fraction 1, tubes 36 to 54 were pooled as fraction 2, and tubes 55 to 62 were pooled as fraction 3.

Fig. 2.
Rechromatography of fraction 1. Fr. 1 obtained from 200mg *C. lineata* lectin was lyophilized, and dissolved in 15ml 0.01M Tris-HCl pH 9.0. The solution was reapplied on to a Bio-Gel A-1.5m column (2 x 85cm) and eluted with the Tris-HCl buffer at a flow rate of 4ml/tube/20min. Tubes 19 to 31 were pooled as fraction A1, and tubes 54 to 60 were pooled as fraction A2.



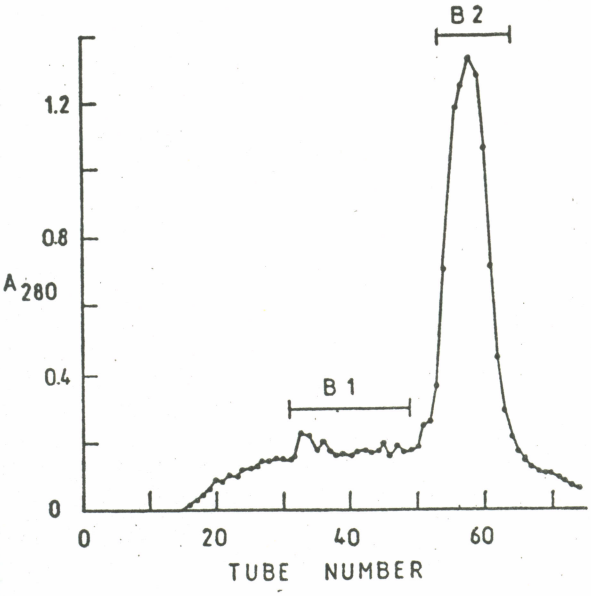


Fig. 3.
Rechromatography of fraction 2. Fr. 2 obtained from 300mg *C. lineata* lectin was lyophylized, and dissolved in 15ml 0.01M Tris-HCl pH 9.0. The solution was reapplied on to a Bio-Gel A-1.5m column (2 x 85cm) and eluted with the Tris-HCl buffer at a flow rate of 4ml/tube/20min. Tubes 31 to 49 were pooled as fraction B1, and tubes 53 to 64 were pooled as fraction B2.

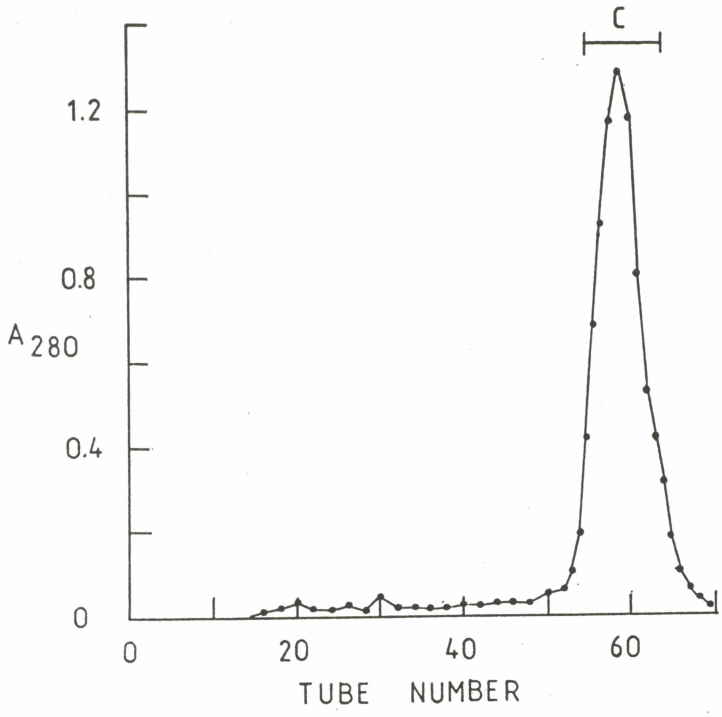


Fig. 4. Rechromatography of fraction 3. Fr. 3 obtained from 300mg *C. lineata* lectin was lyophylized, and dissolved in 15ml 0.01M Tris-HCl pH 9.0. The solution was reapplied on to a Bio-Gel A-1.5m column (2 x 85cm) and eluted with the Tris-HCl buffer at a flow rate of 4 ml/tube/20min. Tubes 55 to 64 were pooled as fraction C.

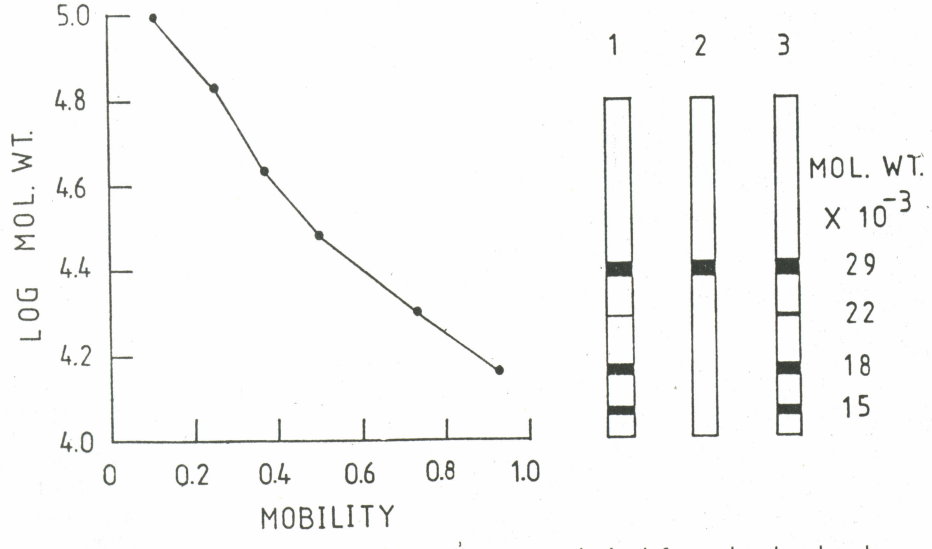


Fig. 5. Left: Molecular weight calibration curve obtained from phosphorylase b (94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and alpha-lactalbumin (14,400).
Right: SDS-polyacrylamide gel electrophoretic patterns of 1. *C. lineata* lectin, 2. fraction A1, 3. concanavalin A and molecular weights of the subunits.

pH 9.0. This solution was reapplied on to the Bio-Gel A-1.5 m column and eluted with the Tris-HCl buffer. The elution profile was shown in Fig. 4, and tubes 55 to 64 were pooled as fraction C.

Fractions A1, A2, B1, B2, and C obtained as described above were dialyzed in Spectrapor membrane tubing against distilled water and lyophylized. The yields of the fractions were given in Table 1.

Hemagglutination titers of the fractions, *C. lineata* lectin, and concanavalin A:

The results were shown in Table 1. The lectins isolated from the two species of *Canavalia* had equal agglutination activity toward rat erythrocytes. Among the fractions obtained from *C. lineata* lectin, only fraction A1 exhibited high hemagglutination activity, and the titer was equal to that of the original lectin. Although the elution volumes of fractions A2, B2, and C in Bio-Gel A-1.5 m column chromatography were very closed, the hemagglutination titers of these fractions were different. The results suggested that fractions A2, B2, and C might be the different components of *C. lineata* lectin.

SDS-polyacrylamide gel electrophoresis:

The molecular weight calibration curve obtained from the standard proteins and electrophoretic patterns were shown in Fig. 5. The results indicated that *C. lineata* lectin and concanavalin A had same electrophoretic pattern. The molecular weight of the intact subunit of these two lectins was estimated to be 29,000. In addition to the two major fragmented subunits (mol. wt. 18,000 and 15,000), a minor fragmented subunit (mol. wt. 22,000) could also be detected. Electrophoretic pattern of fraction A1 revealed that this fraction had intact subunit only. The subunit structures of fractions A2, B1, B2, and C were unknown, since no protein band was obtained from these fractions.

DISCUSSION

The lectins purified from *C. lineata* and *C. ensiformis* (jack bean) had been compared in this work. The results indicate that these lectins have same hemagglutina-

Table 1. Hemagglutination Titers and Yields of the Fractions Obtained from *Canavalia lineata* Lectin

Fraction or Lectin	Hemagglutination titer (ml/mg)	Yield (mg/100mg lectin)
Fr. A1	256	13
Fr. A2	<1	37
Fr. B1	8	8
Fr. B2	8	25
Fr. C	2	10
C lineata lectin	256	
Concanavalin A	256	

tion activity toward rat erythrocytes, and same subunit structure. The infrared absorption spectra of these two lectins are different (data not shown). Therefore, we can conclude that the structures of these lectins are very similar but not identical.

The intact subunit of *C. lineata* lectin had been purified by gel-filtration in the presence of 1% SDS[2]. However, the purified intact subunit can not bind with dextran, and its hemagglutination activity is 15-fold less than that of the original lectin. Therefore, the intact subunit purified in the presence of detergent is not suitable for the study of its biological properties. We have found in this work that the intact subunit of this lectin will aggregate in weak alkaline solution. By using this property, we have obtained a fraction (Fr. A1) containing intact subunit only and having high hemagglutination activity.

In addition to fraction A1, we also obtained four fractions from *C. lineata* lectin and expected that these fractions might contain different subunit. But the subunit structures of these fractions are unknown, since no protein band is obtained from these fractions in SDS-polyacrylamide gel electrophoresis by the method of Weber and Osborn[5]. SDS-polyacrylamide gel electrophoresis of the fractions had also been carried out in a discontinuous buffer system described by Neville[6]. However, the same results were obtained.

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利用膠濾法分離濱刀豆細胞凝集素的成分

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濱刀豆細胞凝集素溶解於弱鹼溶液後，會呈現多種分子量不同的成分。本研究乃利用此種特性，應用膠濾法來分離此種細胞凝集素的成分。濱刀豆細胞凝集素溶解於 0.01 M Tris-HCl pH 9.0溶液，過濾後通入Bio-Gel A-1.5m管柱並以0.01 M Tris-HCl pH 9.0溶液通洗，結果可得到Fr. 1, Fr. 2 和Fr.3 三個部分。Fr. 1, Fr. 2 和Fr. 3 經冷凍乾燥後，分別再依前述方法處理一次，最後得到Fr. A1, Fr. A2, Fr. B1, Fr. B2 和Fr. C 五個部分。Fr. A1 對老鼠紅血球有相當強的凝集作用，其凝集價和濱刀豆細胞凝集素相同。利用 SDS 聚丙烯醯胺膠電泳法分析次單元結構，得知濱刀豆細胞凝集素含有一種分子量 29,000 的完整次單元，並含有三種由完整次單元斷裂而成的片斷次單元，其分子量分別爲 22,000 、 18,000 和 15,000 。經電泳分析 Fr. A1 ，結果顯示此部分只含有完整次單元。本研究同時做刀豆細胞凝集素和濱刀豆細胞凝集素的比較，結果發現此二種細胞凝集素，有相同的紅血球凝集價和次單元結構。

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民國七十五年三月十日受理