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Biochemical Properties of Lectin Isolated from Raw and Boiled Peanut

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ABSTRACT

We have studied biochemical properties of peanut (*Arachis hypogaea* L.) seed lectin through amounts of protein and carbohydrate, hemagglutination activity, molecular weights, optimal temperature, thermal stability, and pH. Lectin was isolated from raw and boiled peanut. Because the lectin was not detected in boiled peanut, lectin isolated from raw peanut was used in this study. The molecular weights of raw peanut lectin determined by SDS-PAGE were 59 and 24 kDa. The isolated lectin did not agglutinate trypsin-treated as well as non-treated human ABO bloods, but agglutinated trypsin-treated rabbit blood. The optimal temperature of isolated peanut seed lectin was 30°C, and thermal stability was 40°C. The maximal pH of this lectin was pH 3.2.

Key words : Biochemical property, Hemagglutination activity, Lectin, Peanut

Introduction

Peanut (*Arachis hypogaea* L.) belonging to *Leguminosae* family is an annual grass, which blooms, fertilizes, withers, and then its lower part of ovary grows into the soil and forms pods where seeds grow [1]. While a plant generally bears its fruit on its above ground part, peanut bears its fruit in the soil, of which seeds grow underground and have hard shells [2].

It has been known that 100 g of peanut contains 7.6% of moisture, 25.6% of protein, 46.6% of lipid, 16.4% of carbohydrate, 1.7% of fiber, and 2.0% of ashes in general. For minerals, calcium content is relatively higher and for vitamin content, niacin as well as vitamin B1, B2, and E is higher also, so peanut is cooked for unique staple or side dishes and used also as favorite food [3]. Peanut has been used as fried peanut for snack or boiled green peanut, but self-sufficiency, cultivating area, and production of domestic peanuts continue to be reduced from increase of imported foreign cheap peanuts and nuts after entry to WTO system [4].

A lectin is a material existing in almost all organisms, which has been known for functions such as nutrient delivery and storage, antibody, and anticancer factor as well as cell agglutinating ability as a protein or glycoprotein to bind carbohydrates, so is being studied continuously and is currently used as an anticancer agent [5].

The first lectin study was performed by Sillmark in 1988, where ricin, a protein agglutinating red blood cells from castor bean, was found [6] hand then about 300 kinds of lectin isolated from various origins such as microorganisms and animals as well as plants have been reported currently. Like this, hemagglutination assay has been often used for identification and isolation of lectin existing widely in the nature system.

It was reported that lectin had 2-6 sugar binding sites, consisted of 2 or 4 subunits, and bound to specific sugar receptors on the cell surface with high affinity [7,8]. It has been studied and developed continuously, so a number of lectins are commercialized and used. For example Concanavalin A, Wheat germ agglutinin, peanut agglutinin, *Phaseolus vulgaris* agglutinin, ricin, and abrin are often used in immunological study [9].

In this study, lectin was isolated from raw peanuts and boiled peanuts to study their biochemical properties by measuring pro-

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tein and carbohydrate content, hemagglutination, molecular weight, optimal temperature, thermal stability, and pH stability.

Materials and Methods

1. Materials and chemicals

Peanut seeds grown in Youngduck Gyeongsangbukdo were purchased from a local market. In our study, raw and boiled peanuts were used as the materials for biochemical properties determination of lectin. Refrigerator centrifuge (Kontron T-324), fraction collector (Bio-Rad 2110), UV-VIS spectrophotometer (Gene Quant 100), and ELISA microplate reader (Bio-Rad 680) were used in this study. All chemicals were obtained from Sigma-Aldrich (St. Louis, MO USA) unless noted otherwise.

2. Isolation of lectin

The lectin was isolated from peanut seeds by the method of Kilpatrick [10]. Peanut seeds extracts in 0.15 M NaCl/0.1 M sodium phosphate buffer (pH 7.0) were centrifuged at 1,000 × g for 10 mins. Solid $(NH_4)_2SO_4$ was slowly added to 50% saturation to supernatant. The precipitate was collected after 24 hrs by centrifugation at 40,000 × g for 1 hr, and then resuspended in 0.9% neutral saline. Resuspended solution was dialysed against 4 liters of neutral saline for 48 hrs, with a change of saline after 24 hrs.

Dialyzed solution was applied to a column (1.5 × 20 cm) of Sephadex G-100 equilibrated with 0.9% neutral saline before loading the sample. The column was washed with the same buffer until the absorbance at 280 nm read zero. The column was eluted with neutral saline at a flow rate of 0.3 mL/min and 3 mL fractions were collected. The fractions containing greatest protein concentration and lectin activity were pooled to determined the hemagglutination activity.

All purification process were done at 4˚C except as indicated.

3. Determination of hemagglutination activity

ABO human and rabbit blood were activated using trypsin by method of Kilpatrick [10]. Bloods were trypsin-treated by suspension (25%, v/v) in neutral saline containing 0.25% trypsin, respectively. After incubation at 37˚C for 5 mins, the cells were centrifuged at 8,000 rpm for 5 mins, and then harvested at room temperature. The cells were subsequently washed four times in neutral saline before determination of hemagglutination activity.

Hemagglutination activity of lectin was determined by a 2-fold serial dilution method of Takatsy [11]. Bloods were prepared by a 2% cell suspension in 0.9% neutral saline. 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˚C for 1 hr. The degree of agglutination was assessed by eyes and microscope. The reciprocal of the highest dilution of the lectin showing complete agglutination was taken as the hemagglutination titer.

4. Determination of protein and carbohydrate contents

Protein determinations were made at 595 nm by the method of Bradford [12] using bovine serum albumin as the standard. Carbohydrate was assayed at 490 nm by the phenol/H_2SO_4 method of Dubois et al. [13] using glucose as standard.

5. SDS-PAGE and molecular weight determination

SDS-PAGE was performed on a 12% polyacrylamide gel by the method of Laemmli [14] using molecular weight markers [rabbit muscle phosphorylase B (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and lysozyme (14.3 kDa)]. The isolated lectin was denatured in a boiling H_2O for 10 mins before being loaded on the gel, and then gels were run at 30 mA for 1 hr 30 min. For visual protein bands gel were stained with Coomassie Brilliant Blue R-250, and then destained by 7.5% acetic acid. The molecular weights of bands were determined by the method of Weber and Osborn [15]. The molecular weight markers used were same as SDS-PAGE.

6. Determination of optimal temperature

For measurement of optimal temperature, the isolated lectin was incubated at temperatures of the range of 10-90˚C for 10 mins, and then the determination of hemagglutination activity was carried out using trypsin treated rabbit blood by a serial 2-fold dilution method.

7. Thermal stability

For thermal stability, the isolated lectin was incubated in the range of 20-90˚C for 10 mins. The hemagglutination activity was determined by a serial 2-fold dilution method using trypsin

treated rabbit blood.

8. Stability of pH

The effect of pH on hemagglutination activity of the isolated lectin was measured in buffer of various pH values at 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), and 0.2 M sodium carbonate-bicarbonate buffer (pH 10.0). For pH stability, residual activity was measured after 4 hrs of incubation at 4°C in buffers of the same pH.

Results

1. Isolation of lectin

Precipitates obtained by adding 50% $(NH_4)_2SO_4$ solution were dissolved in neutral saline and dialyzed to isolate lectin through Sephadex G-100 with neutral saline solution. The lectin isolated from raw peanuts showed its activity in 1-7th fraction, among which 2nd-3rd fraction showed the highest lectin content and activity (Fig. 1). However, the lectin obtained from boiled peanuts showed no activity in any fraction (Fig. 2). Therefore, the 3rd fraction from raw peanuts was used to study molecular weight, optimal reaction temperature, heat stability, and pH stability.

2. Protein and carbohydrate content

As results of measuring protein and carbohydrate content of the lectin from raw peanuts, it was found that the protein content was 0.946 mg/mL and the carbohydrate content was 0.682 mg/mL (Table 1).

3. Hemagglutination and specificity of blood

In order to check blood specificity of the lectin from raw and boiled peanuts, human ABO blood and rabbit blood were divided into trypsin treated and untreated group to measure each hemagglutination. From the results, it was shown that the lectin from raw peanuts showed agglutination only with trypsin treated rabbit blood and showed no agglutination with other blood. For the lectin from boiled peanuts, it was found that there was no agglutination to any blood (Fig. 3). Therefore in this study, the lectin from raw peanuts was used for studying its biochemical properties and the trypsin treated rabbit blood was used for agglutination reaction.

4. SDS-PAGE and molecular weight determination

The fraction with the highest activity isolated from raw peanuts was separated through 12% SDS-PAGE. It was found that the lectin from raw peanuts had 2 bands. By comparing and measuring molecular weight of the above bands with relative mob-

Table 1. Amounts of protein and carbohydrate in isolated lectin

	Amounts (mg/mL)
	Raw peanut
Protein	0.946
Carbohydrate	0.682

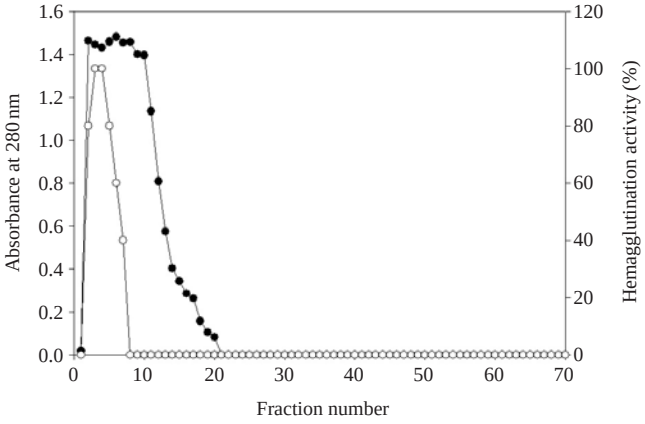

Fig. 1. Fractionation profile for lectin isolated from raw peanut on Sephadex G-100 finally. The bound lectin was eluted with neutral saline. Hemagglutination activity was determined using rabbit blood. ●-●: optical density, ○-○: hemagglutination activity.

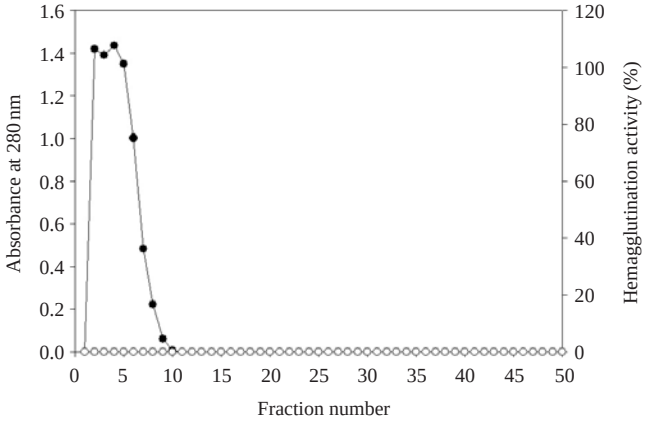

Fig. 2. Fractionation profile for lectin isolated from boiled peanut on Sephadex G-100 finally. The bound lectin was eluted with neutral saline. Hemagglutination activity was determined using rabbit blood. ●-●: optical density, ○-○: hemagglutination activity.

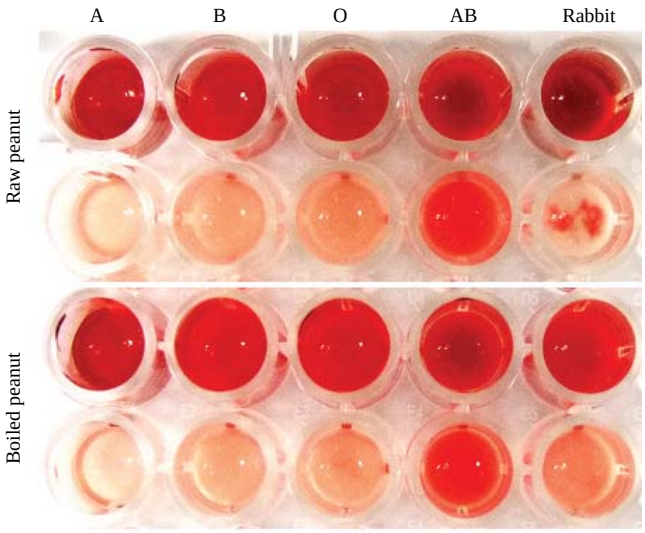

Fig. 3. Hemagglutination effects of raw peanut lectin on human ABO and rabbit blood. Upper on each plate: no treated with trypsin. Lower on each plate: treated with trypsin.

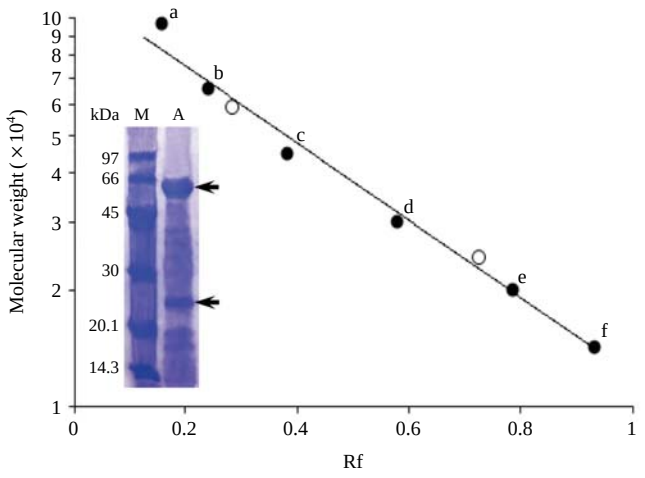

Fig. 4. 12% SDS-PAGE pattern and molecular weight of lectin isolated from raw peanut. The gels were run at 30 mA for 1 hr 30 min and stained with Coomassie brilliant blue R-250. Arrow indicate lectin isolated by affinity chromatography on Sephadex G-100. Lanes: M, molecular weight marker; A, purified lectin from raw peanut. White circles (○) indicate lectin isolated on Sephadex G-100. The molecular weight markers (●) were phosphorylase B (a, 97 kDa), bovine serum albumin (b, 66 kDa), ovalbumin (c, 45 kDa), carbonic anhydrase (d, 30 kDa), soybean trypsin inhibitor (e, 20.1 kDa), and lysozyme (f, 14.3 kDa).

ility against standard molecular weight, it was calculated that the bands of lectin had 59 kDa and 24 kDa, respectively (Fig. 4).

5. Optimum temperature of lectin

In order to measure optimal reaction temperature of the lectin from raw peanuts, its activity depending on temperature change of 10-90˚C was studied. It was found that the activity was the highest as 100% at 30˚C. In addition, it showed over 80% of activity even at 10-20˚C. However, the activity was reduced below 50% from 40˚C and lost at 70˚C (Fig. 5).

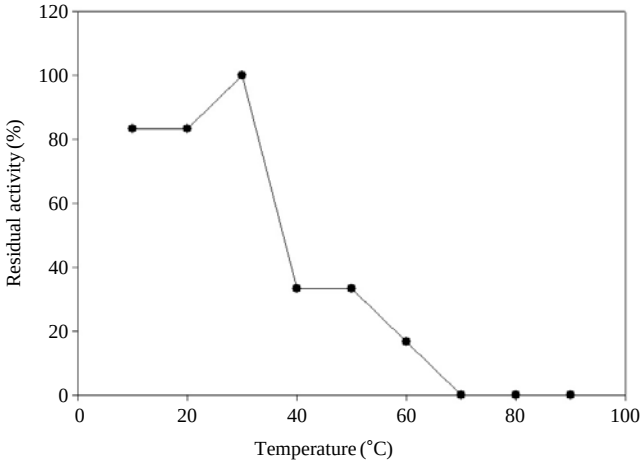

Fig. 5. Effect of temperature on hemagglutination activity of lectin isolated from raw peanut. The lectin activity was tested by incubation at 10-90˚C, respectively.

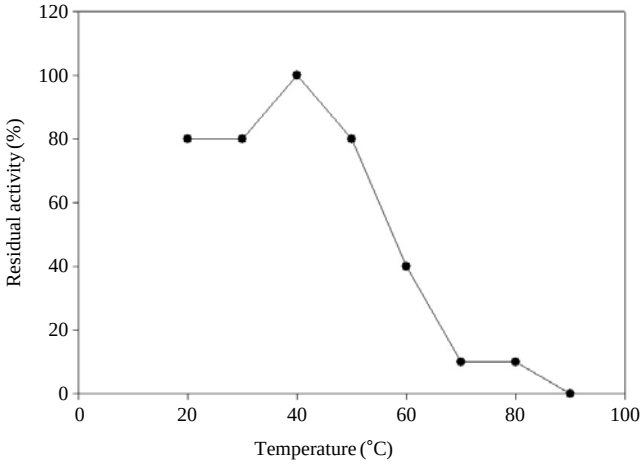

Fig. 6. Thermal stability of lectin isolated from raw peanut. The lectin was preheated for 10 min at 20-90˚C, respectively.

6. Thermal stability of lectin

In order to measure heat stability of the lectin from raw peanuts, its activity was studied through hemagglutination in the range between 20 and 90˚C. The results showed the highest activity, 100%, at 40˚C and showed relatively stable activity even at 20-30˚C and 50˚C. However, the activity was dramatically reduced to below 50% over 60˚C, very low over 70˚C,

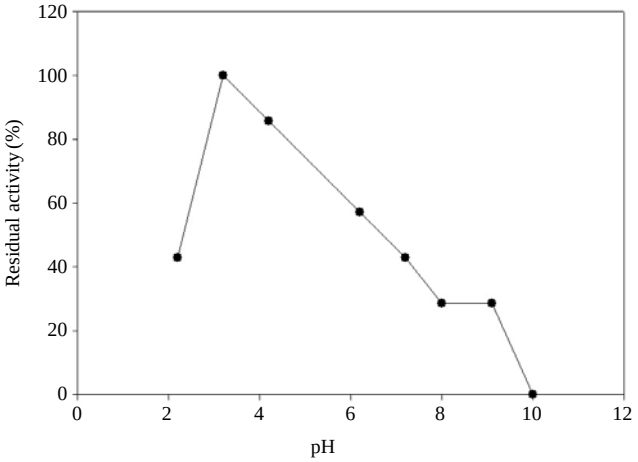

Fig. 7. Effect of pH on hemagglutination activity of lectin isolated from raw peanut. The lectin was incubated different pH for 4 hr at 4˚C.

and lost completely at 90˚C (Fig. 6).

7. pH stability of lectin

In order to study pH stability of the lectin from raw peanuts, its activity was measured through hemagglutination between pH 2.2 and 10.0. As the results, it was shown that the activity was the highest, 100%, at pH 3.2 with acetate buffer and still high, near 90%, at pH 4.2. The activity was reduced to about 50% at pH 2.2 and gradually over pH 6.2 and lost completely at pH 10.0 (Fig. 7).

Discussion

In this study, domestic peanuts was selected to isolate lectin separately from raw and boiled peanuts by extracting them in sodium phosphate buffer containing NaCl and passing them through Sephadex G-100. As results of identifying lectin in raw and boiled peanuts with hemagglutination, it was found that the lectin was identified in raw peanuts, but not identified in boiled peanuts. It is considered that in the boiled peanuts after heat treatment, the protein denatured by heat. Tertiary structure of the protein is destroyed and denatured by heat.

There was no hemagglutination in the boiled peanuts, so the lectin isolated from raw peanuts was used to test protein and carbohydrate content, molecular weight, optimal reaction temperature, heat stability and pH stability.

By measuring protein and carbohydrate content of the lectin from raw peanuts, it was found that it contained 0.946 mg/mL of protein and 0.682 mg/mL of carbohydrate. For Yak-kong lectin, it was shown that it contained 0.774 mg/mL of protein and 0.849 mg/m of carbohydrate content [16].

As shown in the above, it was identified that the lectin extracted from raw peanuts consisted of glycoprotein by confirming the protein and carbohydrate content in it, even though the carbohydrate content was lower than the protein content.

Lectin has been known as a protein or a glycoprotein to agglutinate cells including blood cells, lymphocytes, fibroblasts, spermatozoids, bacteria, and fungi and bind to carbohydrate [8,17]. Therefore in this study, the lectin isolated from raw and boiled peanuts was used to measure hemagglutination to human ABO blood and rabbit blood divided into trypsin treated and untreated group in order to test blood specificity and activity of the lectin. In our study, it was shown that the lectin from raw peanuts showed agglutination only with trypsin treated rabbit blood and showed no agglutination with other blood. For the lectin from boiled peanuts, it was found that there was no agglutination to any blood. When comparing these results with the results of hemagglutination with lectin from peanut seeds produced in China, where it showed no hemagglutination with neuraminidase untreated human ABO blood and strong hemagglutination with neuraminidase treated human blood [18], it was suggested that the result might be different according to treatment of red blood cell and enzyme types.

Contrary to the above results, it was found that raw kidney beans showed hemagglutination with human red blood cells and heat treated kidney beans showed almost no hemagglutination because of inactive lectin. From these, it was suggested that even though the lectin was extracted from same leguminous plants, its specificity might be different depending on pretreatment of experimental materials, how to measure hemagglutination, and types and treatments of enzyme.

In this study, it was shown that there were 2 bands in the lectin from raw peanuts and they had 59 kDa and 24 kDa of molecular weight respectively, comparing and measuring their relative mobility against standard molecular weight, which is different from 33 kDa of peanut root lectin from India [19] and 29 kDa of peanut seed lectin from China. In general, the molecular weight of legume lectin is known as 30 KDa, which consists of about 250 amino acids [20].

By measuring activity depending on temperature change in 10-90˚C in order to test optimal reaction temperature of raw peanut lectin, it was found that it showed the highest activity, 100%, at 30˚C. In addition, it showed over 80% of activity also even at 10-20˚C.

However, the activity was reduced below 50% from 40˚C and

lost at 70˚C. From these, it was suggested that optimal reaction temperature of the peanut lectin was 30˚C. Although there was some difference from a result of peanut seed produced in China that its activity was reduced over 55˚C [18], it was identified that the activity of peanut lectin would be lost at high temperature. From these results, it seems that each lectin has specific optimal temperature even in same species and lectin produced in different origins has different optimal temperature even in a species.

Usually, most lectin shows the highest stability at 30-60˚C and loses its activity by protein denaturation over 80˚C. In order to test heat stability of lectin isolated from raw peanuts, its activity was measured through hemagglutination at 20-90˚C. As the results, it was found that it showed the highest activity, 100%, at 40˚C and stable activity also at 20-30˚C and 50˚C. However, the activity was dramatically reduced to below 50% over 60˚C, very low over 70˚C, and lost completely at 90˚C. From the above results for optimal reaction temperature and heat stability of lectin from raw peanuts, it was shown that the lectin extracted from peanuts boiled at 100˚C for 20 min lost its activity at high temperature, so showed no hemagglutination. It was also suggested that although the lectin had relatively high heat stability at 40-50˚C, it lost its activity at higher temperature. On the contrary, when comparing it with a result that a lectin of horse bean grown in a tropical climate maintains at least 80% of its activity at 50-70˚C [21], it was identified that even same species might have different heat stability.

In order to study pH stability of the lectin from raw peanuts, its activity was measured through hemagglutination between pH 2.2 and 10.0. The activity was reduced to about 50% at pH 2.2 and gradually over pH 6.2 and lost completely at pH 10.0. The lectin from raw peanuts showed the highest activity, 100%, at pH 3.2, which suggests that it was somewhat unstable at highly acidic and basic condition and showed the most stable activity in strong acid. These results showed some differences from a result of pH stability that activity of a lectin extracted from peanut seeds produced in China decreased below pH 5 or over pH 11 [18]. When comparing it with other leguminous plants, it showed some differences from Lima bean [22] having same optimal pH of lectin to horse bean seeds, pH 7.0 and peanut root lectin [23] and was also different from winged bean lectin [24] which showed high activity at pH 1.8-10.0. Thus it was suggested that even same species might have different pH specificity and the pH stability range might be different according to habitats and processing methods.

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