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STUDIES ON *ALOE VERA* L. Burm. f. LEAF GEL LECTIN

ALOE VERA L. Burm. f. (sarısabır) YAPRAKLARININ JEL KISMINDAKİ LEKTİN ÜZERİNE ÇALIŞMALAR

SERPİL MALKOÇ, NURİYE AKEV

Department of Biochemistry, Faculty of Pharmacy, University of Istanbul 34452, Istanbul, Turkey

A single lectin was isolated from the gel portion of the leaves of Aloe vera L cultivated in Turkey, by extraction, ammonium sulfate precipitation, hydroxylapatite column chromatography and some of its biochemical properties were examined.

The 350 fold purified lectin was of glycoprotein structure and did not agglutinate any of the blood groups of human erythrocytes. Besides, it was observed that rat erythrocytes agglutinating activity of the lectin was weaker than that of rabbit erythrocytes and agglutination occured within a longer period.

Türkiye'de kültür bitkisi olarak yetiştirilen Aloe vera L. (sarısabır) bitkisinin yaprak jelinden, ekstraksiyon, amonyum sülfatla çöktürme ve hidroksilapatit sütun kromatografisi yöntemleri kullanılarak elde edilen tek bir lektin, kısmen saflaştırıldı ve bazı biokimyasal özellikleri incelendi.

350 kez saflaştırılan lektinin glikoprotein yapısında olduğu, hiçbir gruptaki insan eritrositlerini aglutine etmediği ve sıçan eritrositlerini tavşan eritrositlerine, oranla daha düşük aktivitede ve daha uzun sürede aglutine ettiği gözlemlendi.

Keywords: *Aloe vera L.; Leaf gel lectin*

Anahtar kelimeler: *Aloe vera L(Sarısabır); Yaprak jeli lektini*

Introduction

Substances derived from the leaves of *Aloe* plants have worldwide importance in the field of phytotherapy. Although substances of a protein nature constitute only a small percentage of the total solids present in the extracts of leaves, they have been reported to have several biological activities. Among them, lectins which are cell-agglutinating, sugar-specific proteins, have been isolated from the leaves of some *Aloe* species (1-7).

In 1981, based on Goldstein's (8) proposal, The International Nomenclature Commitee of the Union of Biochemistry has defined the lectins as molecules consisting of protein or glycoprotein, not derivated from the immunological system, having more than one sugar binding sites, capable to aggregate and precipitate the cells and carbonhydrate compounds (9).

Recently, much has been written about the lectins present in *Aloe* species. In 1979, two lectins have been purified for the first time, from the leaves of *Aloe arborescens* Mill. (1). Later, Winters (4) examined *Aloe barbadensis* Mill. (=*Aloe vera L.*), *Aloe saponaria* Haw., *Aloe chinesis* and by fractional precipitation of the leaves gel, has partially isolated some substances and investigated them for hemagglutinating and mitogenic activity.

In 1996, Bouthet et al.(6) obtained substance that showed hemagglutinating activity from the leaf extract of *Aloe barbadensis* Mill. In their work, the effect of treatment with proteases, the influence of pH and heat on hemagglutinating activity have been investigated, but no further purification has been carried out.

The aim of this study was to isolate and purify the lectin/s from the gel portion of *Aloe vera L.* leaves and to investigate some of its/their properties.

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Materials and Methods

Plant material

Specimens of *Aloe vera* (L.) Burm.fil.* were collected from Kale (Demre) in Antalya, and cultivated in the greenhouse of our Faculty. In this study the fresh leaves of this cultivated plant were used.

Chemicals

Hydroxylapatite was prepared in our laboratory according to Tiselius et al. (10). All the other chemicals used were analytical reagent grade.

Assay methods

Hemagglutinating tests were performed by making serial dilutions of samples in PBS (Phosphate Buffered Saline, pH 7.4) using either microplates (in 50 µl) or 10 mm x 75 mm test tubes (in 100 µl). After the serial dilutions, to each well or tube 4% erythrocyte suspension prepared from rabbit blood was added. Hemagglutination was determined visually after keeping at room temperature overnight (11,12) Hemogglutination titer was expressed as the reciprocal of the highest dilution showing detectable agglutination and the activity (HU/mg) was calculated (11).

Protein contents of the samples obtained during the purification processes were determined by the method of Lowry (13) using bovine serum albumine as a standart.

Carbohydrate contents were determined by the phenol-sulfuric acid method of Dubois et al. (14), using D-mannose as a standart.

Specificity towards human and rat erythrocytes:

Peripheral blood from healthy donors of A Rh(+), B Rh (+), 0 Rh (+) and 0 Rh (-) blood groups were collected on sodium oxalate. 4% erythrocyte suspensions were prepared for each blood group. Besides, 4% eryythrocyte suspension was prepared with blood taken by heart puncture from a Wistar rat.

Hemagglutination was tested in 10 mm x 75 mm test tubes with 0.2 ml from each erythrocyte suspension added to 0.5 ml of the lectin solution. The occurence of hemagglutination was estimated visually after standing one night at room temperature.

Purification steps

1.*Preparation of the crude extract*

Leaf gel was separated from 1250 g fresh *Aloe vera* L. leaves by scratching with a spoon. The 658 g gel was homogenized with PBS by means of Moulinex Masterchief. The extract was filtered through cloth, the filtrate centrifuged at 20 000 rpm for 30 minutes at +2°C in a refrigerated centrifuge (Cryofuge 20-3 Heraeus-Christ) and the clear yellow supernatant (Crude leaf gel extract) was collected.

2.*Ammonium sulfate fractionation*

The crude extract was first precipitated by ammonium sulfate at 20% saturation and left overnight in the refrigerator. The precipitate, which did not show any hemagglutination activity, was separated by means of centrifugation at 20 000 rpm (-10°C) for 30 minutes and discarded. The supernatant was brought up to 40% saturation with ammonium sulfate and left again overnight at 4°C. The precipitate which showed hemagglutinating activity was separated by centrifugation at 20 000 rpm (-10°C) for 30 minutes, suspended and then dissolved in PBS by magnetic stirring for two hours and dialysed against the same buffer. This clear solution filled in a dialysis bag was plonged in crystallized sugar and left overnight in the refrigerator. The protein solution thus concentrated was dialysed against PBS (+4°C) and used for further purification (20%-40% $(NH_4)_2SO_4$ cut).

3.*Hydroxylapatite column chromatography*

The clear dialysate (20%-40 $(NH_4)_2SO_4$ cut) was applied to a hydroxylapatite column equilibrated with 1 mM sodium-potassium phosphate buffer (pH 6.8). The elution was performed by washing the column stepwise with 5 mM, 50 mM, 100 mM and 200 mM phosphate buffer (pH 6.8). The fractions corresponding to the peak showing hemagglutination activity were pooled separately, concentrated and subsequently dialysed against PBS.

Results

One protein peak showing hemagglutinating activity was eluted with 50 mM phosphate buffer and named as "leaf gel lectin" (Fig.1).

Protein 85 mg in 15 ml 1 mM phosphate buffer was applied to a column (2 cm x 18 cm) equilibrated with the same

*Identified by Prof.Dr. Nurhayat Sütlüpınar
Department of Pharmacognosy, Faculty of Pharmacy, University of Istanbul

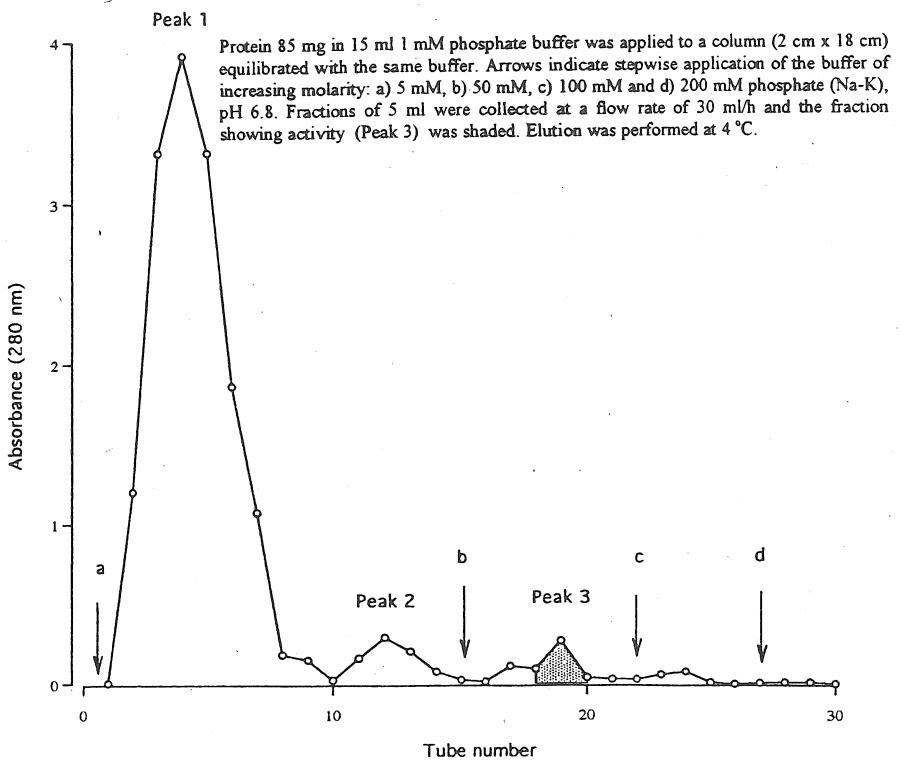

Fig.1. Hydroxylapatite column chromatography elution profile of the fraction obatined by 20%-40% ammonium sulfate saturation of the crude leaf gel extract

buffer. Arrows indicate stepwise application of the buffer of increasing molarity: a)5 mM, b)50 mM, c)100 mM and d)200 mM phosphate (Na-K), pH 6.8. Fractions of 5 ml were collected at a flow rate of 30 ml/h and the fraction showing activity (Peak 3) was shaded. Elution was performed at 4°C.

Protein contents and hemagglutinating activities of the samples were determined at each purification step and the results were presented (Table 1).

Carbohydrate content: The leaf gel lectin contained 3.5 % neutral sugar. This result suggests that leaf gel lectin is of glycoprotein structure.

Specificity towards human and rat erythrocytes: Agglutinating activity of leaf gel lectin was tested on human erythrocytes of blood group types A Rh(+), B Rh(+), 0 Rh(+) and 0 Rh(-) as well as rat erythrocytes, in comparison with rabbit erythrocytes.

Table 1. Partial purification of *Aloe vera* L. leaf gel lectin (Starting with 507 g leaf gel obtained from 1250 g fresh leaves)

Purification step	Total protein (mg)	Purification fold	Specific activity (HU/mg)
Crude extract	3299	1	6.5
20%-40% $(NH_4)_2SO_4$ cut	170	200	11.3
Hydroxylapatite	85	200	11.3
Applicate	0.28	350	2285
Gel lectin (Peak 3)			

The leaf gel lectin did not agglutinate any of the blood groups of human erythrocytes. Besides, it was observed that rat erythrocytes agglutinating activity of the lectin was weaker than that of rabbit erythrocytes and agglutination occured within a longer period.

Discussion

Recently, two lectins named Aloctin I and Aloctin II were separated from the leaf pulp of *Aloe vera* L. in our laboratory (15). It can be considered that the leaf gel lectin purified in the present study might be equivalent to Aloctin II, as they were both eluted with 50 mM phosphate buffer through hydroxylapatite column. Further characterizations of the lectins are needed to prove this consideration.

It has been determined that the lectins obtained from *Aloe arborescens* Mill. contain more than 18% neutral carbohydrates (1). Yagi at al. (7) have isolated a mitogenic glycoprotein from the leaf gel of *Aloe vera* L., but hemagglutination activity was not mentioned. The lectin obtained from the leaf gel in the present study was also a glycoprotein containing 3.5% neutral sugar conforming to the literature.

It was reported that the two lectins purified from *Aloe arborescens* Mill. agglutinated both human and rabbit erythrocytes (1) and that *Aloe vera* extract agglutinated human erythrocytes on blood type (6). But in this study, the lectin of *Aloe vera* L. leaf gel only agglutinated rabbit erythroctes, known to be the most sensitive to agglutination by other lectins (16,17).

In this study, the separation and 350 fold purification of one lectin from the leaf gel of *Aloe vera* L. was achieved. Further purification of the leaf gel and pulp lectins and comparison of their biochemical properties is our aim for the future continuation of this research.

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References

1. Suzuki, I., Saito, H., Inoue, S., et al. : J. Biochem. 85, 163 (1979)
2. Winters, W.D., Benavides, R., Clouse, W.J.: Economic Botany 35, 89 (1981)
3. Saito, H.: Phytotherapy Research 7, S14 (1993)
4. Winters, W.D.:Ibid, 7, S23 (1993)
5. Koike, T., Beppu, H., Kuzuya, H. et. al. : J. Biochem. (Tokyo) 118, 1205 (1995); (Ref.) Chem. Abst. 124. 168770d (1996)
6. Bouthet, C.F., Virgil, R.S., Winters, W.D.: Phytotherapy Research 10, 54 (1996)
7. Yagi, A., Egusa, T., Arase, M., et al.: Planta Medica 63, 18 (1997)
8. Goldstein, I.J., Hughes, R.C., Monsigny, M., et al.: Nature 285, 66 (1980)
9. Dixon, H.B.F.: Nature 292, 192 (1981)
10. Tiselius, A., Hjérten, S., Levin, Ö.: Arch. Biochem. Biophys. 65, 132 (1956)
11. Liener, I.E., Hill, E.G.: J. Nutrition 49, 609 (1953)
12. Lis, H., Sharon, N.: Methods Enzymol. 28 Part B 366 (1972)
13. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: J. Biol. Chem. 193, 265 (1951)
14. Dubois, M., Gilles, K.A., Hamilton, J.K. et al.: Anal. Chem. 28, 350 (1956)
15. Akev, N.: XIII. Ulusal Biyokimya Kongresi (Uluslararası katılımlı) Antalya, Özet Kitabı/Abstract Book C-205 (1996)
16. Liener, I.E., Pallansch, M.J.: J. Biol. Chem. 197, 29 (1952)
17. Park, J., Kim, H.: Korean Biochem. J. 20, 208 (1987)

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