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

ALOE VERA L. Burm. f. (sarisabir) YAPRAKLARININ JEL KISMINDAKI LEKTİN ÜZERİNE ÇALIŞMALAR

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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 occurred within a longer period.

Keywords: Aloe vera L.; Leaf gel lectin

Anahtar kelimeler: Aloe vera L(Sarasabir); Yaprap jelli 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 carbohydrate 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) Hemagglutination 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% erythrocyte 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 occurrence of hemagglutination was estimated visually after standing one night at room temperature.

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*Identified by Prof.Dr. Nurhayat Sütünpınar  
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128*
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.

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

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

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein (mg)</th>
<th>Purification fold</th>
<th>Specific activity (HU/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>3299</td>
<td>1</td>
<td>6.5</td>
</tr>
<tr>
<td>20%-40% (NH₄)₂SO₄ cut</td>
<td>170</td>
<td>200</td>
<td>11.3</td>
</tr>
<tr>
<td>Hydroxylapatite Applicate</td>
<td>85</td>
<td>200</td>
<td>11.3</td>
</tr>
<tr>
<td>Gel lectin (Peak 3)</td>
<td>0.28</td>
<td>350</td>
<td>2285</td>
</tr>
</tbody>
</table>

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 occurred within a longer period.
Discussion

Recently, two lectins named Alocitin I and Alocitin 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 Alocitin 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 et 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 erythrocytes, 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

16. Liener, I.E., Pallansch, M.J.: J. Biol. Chem. 197, 29 (1952)

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