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Immunoreactive Lectins in Leaf Gel from *Aloe barbadensis* Miller

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Lectins isolated from the gel portion of leaves of *Aloe barbadensis* Miller by differential centrifugations and gel filtration were found to have strong haemagglutination and mitogenic activities. Haemagglutination titres induced by these lectins were markedly higher than those induced by lectins isolated similarly from *Aloe saponaria* Haw and *Aloe chinesis*. The levels of lymphocyte blastogenesis using the *Aloe barbadensis* Miller lectins were high, but markedly less than those detected using positive control lectins concanavalin A and phytohaemagglutinin. Results of Western blot transfers followed by immunoblots using specific receptor antisera suggested that the *Aloe barbadensis* Miller lectins were active at alpha D-glucose and mannose sites and not at *n*-acetyl glucosamine sites. The haemagglutination activities of these *Aloe* substances were also observed to decrease as a function of time frozen at liquid nitrogen temperatures for up to 9 years.

Keywords: Aloe; lectins; mitogens; sugar receptors; liquid nitrogen stability.

INTRODUCTION

Substances with lectin-like properties have been isolated and purified from a wide variety of plant sources. Many of these plant lectins have been extensively studied and have been found to be of considerable biomedical and commercial value (Bog-Hansen and Freed, 1988). Lectins are included within a wide range of multimeric glycoproteins which bind to carbohydrate groups displayed on the surfaces of cells. Accordingly, two major biological responses which can occur as a consequence of lectin binding to such cell surface glycoproteins are mitogenesis and agglutination of cells. Mitogens activate cells and induce them to divide, while agglutinins, by binding one cell to another, induce cell aggregation and clump formations. Some lectins are able to agglutinate cells, but are not mitogenic, for example wheat germ agglutinin (WGA). Moreover, many, but not all, lectins are both agglutinins and mitogens, such as concanavalin A (Con A) and phytohaemagglutinin (PHA) (reviewed by Ashman, 1984). Substances extracted and purified from the leaves of *Aloe arborescens* Miller (Fugita *et al.*, 1978; Suzuki *et al.*, 1979; Yoshimoto *et al.*, 1987) and from the gel portion of leaves of *Aloe barbadensis* Miller (Winters *et al.*, 1981; Winters, 1990, 1991) and *Aloe saponaria* Haw (Winters *et al.*, 1981; Winters, 1990) have been shown to have biological activities consistent with the lectin-like activities of agglutination, mitogenesis or both.

In the present studies the aims were to more completely identify and characterize agglutinin and mitogenic activities of substances prepared from *Aloe barbadensis* Miller, an *Aloe* species most commonly cultivated and utilized as a phytotherapeutic agent in the United States and Mexico. Accordingly, in these studies partially purified substances from *A. barbadensis* Miller with agglutinin activities were compared with substances similarly prepared from *Aloe saponaria* Haw and *Aloe chinesis*, two other species also frequently grown in these areas, and with well recognized reference plant derived lectins such as Con A. Furthermore, the substances from *A. barbadensis* Miller were tested by standard quantitative methods for mitogenic (lymphocyte blastogenesis) activities and site specific glycoprotein binding and for haemagglutinin stability after long term freezing.

MATERIALS AND METHODS

Aloe substances were freshly extracted and prepared from the gel portion of leaves of *Aloe barbadensis* Miller, *Aloe saponaria* Haw and *Aloe chinesis* using methods of serial differential centrifugations and gel fractionation chromatography as previously described (Winters *et al.*, 1981; Winters, 1990; Suzuki *et al.*, 1979). Haemagglutination assays as described by Suzuki *et al.* (1979) were performed as modified by Winters *et al.* (1981) for red blood cells of canine and human origin. Lymphocyte blastogenesis assays using human cells and serially diluted concentrations of each of the *Aloe* fractions and reference mitogens were performed as described by Lesourd *et al.* (1986). Briefly, peripheral blood leukocytes were collected from 22 normal healthy volunteers (age range 18–50 y old; 11 females and 11 males). Mononuclear cells were isolated by Ficoll-Triosil (Pharmacia Chemicals, Uppsala, Sweden) methods and prepared for blastogenesis assays in RPMI 1640 medium containing 10% heat inactivated human serum, 1% glutamine and 1% penicillin–streptomycin solution. Cells were dispensed into 96-well plates at 100 000 cells per well and in triplicates, no antigen control, positive antigen control and *Aloe* fractions in dilutions were added. After incubation and addition of ^{3}H-thymidine (1 μC/well) the cells were harvested using a MASH II device and blastogenesis was measured by the amount of radioisotope incorporated into cells as assessed by liquid scintil-

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Table 1. Haemagglutination (HA) titres of *Aloe* substances.

Haemagglutinin source	HA erythrocyte sources	
	Human RBCs	Canine RBCs
Aloe barbadensis Miller		
Fraction I pool[a]	7144±2048[b]	1536±512[b]
Fraction II pool[a]	1536±512	768±256
Aloe saponaria Haw		
Fraction I pool	64±32	16±8
Fraction II pool	96±32	192±64
Aloe chinesis		
Fraction I pool	96±32	24±8
Fraction II pool	24±8	12±4
Reference lectin		
Concanavalin A	12294±4098	7144±2048

[a] Fractions I and II pools from each aloe species were prepared and tested at approximately the same protein concentration.
[b] HA titres reflect the mean±SE of three samples tested for each *Aloe* pool. Each pool was composed of substances extracted from at least six individual leaves.

lation counting. Polyacrylamide gel electrophoresis (PAGE) followed by Western blot transfers and immunoblot assays with specific antibodies to lectins and to glycoprotein components were performed as previously described (Parker *et al.*, 1988; Winters, 1991). Protein concentrations were determined using BioRad protein standard assay techniques. Reference lectins were prepared in the same solutions used for the *Aloe* substances. Stability tests for haemagglutinins and growth factors were performed using *Aloe* stock and reference samples collected over several years which had been frozen in liquid nitrogen immediately after the completion of bioassays showing positive responses for both of these properties. These deep frozen samples were evaluated at the time of bioassays after rapid thawing and dilution with the appropriate solutions or media. Statistical analysis of the study data was performed using the Stat-View 512+ statistical program in a Macintosh 30SE computer drive system.

RESULTS AND DISCUSSION

In the studies of haemagglutination activities of *Aloe* lectins, human red blood cells (RBCs) were observed to be more sensitive to haemagglutinins (HA) from the three species of *Aloe* than canine RBCs, while both human and canine RBCs were equally sensitive to Con A. HA from *A. barbadensis* Miller was markedly stronger reacting than the HA from *A. saponaria*, which in turn was stronger than the HA from *A. chinesis*. HA titres with Con A were markedly higher than HA titres from any of the three *Aloe* species when tested under the same assay conditions (Table 1).

Lectins prepared from the gel portions of leaves of *A. barbadensis* Miller, *A. saponaria* Haw and *A. chinesis* in these studies showed similar levels of haemagglutination of human and canine RBCs as lectins with haemagglutinating activities previously detected in extracts of gel portions of *A. barbadensis* Miller and *A. saponaria* Haw (Winters *et al.*, 1981; Winters, 1990; Winters, 1991). HA titres measured in the fractions prepared from the gel portion of *A. barbadensis* Miller (Tables 1 and 2) were markedly higher than those reported by Suzuki and co-workers (1979). The reasons for these differences may be related to the types of processing steps, the source of the starting materials and the species of *Aloe*. For example, in the present studies, *A. barbadensis* Miller and not *A. arborescens* Miller was used, the gel portion of the leaf and not whole leaves were sources of starting materials and the gel extracts were not repurified by repeated chromatographic steps (Suzuki *et al.*, 1979).

In the studies of mitogenic activities of *Aloe* lectins, substances prepared by serial differential centrifugations from *A. barbadensis* Miller were further fractionated by gel filtration on Sephadex G100 as described by Suzuki *et al.* (1979) and Winters (1991). The fractions which contained *Aloe* substances with highest titres of HA by haemagglutination assays were then further tested for mitogenic activities. Approximately equal concentrations of all of the *Aloe* fractions tested were found to induce increased levels of lymphocyte blastogenesis, albeit one of the fractions, Fraction 9, induced 2–3 times less than the other four fractions (Table 2). Under similar conditions of blastogenesis assays, but using 10 fold less concentrations, the positive control mitogenic lectins, Con A and PHA induced similar levels of stimulation of lymphocyte blastogenesis as the *Aloe* substances. Other reference lectins, poke weed mitogen (PWM) and wheat germ agglutinin (WGA), respectively reacted with partial and little mitogenic activities (Table 2).

Of particular interest in the present study were the partially purified lectin substances in all the *A. barbadensis* Miller fractions tested which showed both haemagglutination and mitogenic activities, similar to those of Con A (Table 2). In previous studies, Suzuki and co-workers (1979) reported lectin substances highly purified from *A. arborescens* Miller were of two types: one (S-1) with strong HA activities and the other (P-2) with both HA and mitogenic activities. Another highly purified lectin from *A. arborescens* Miller, ATF1011

Table 2. Lymphocyte blastogenesis responses to *Aloe* substances and reference mitogens

Mitogen source[a]		Concentration[b] (µg/mL)	Blastogenesis[c] (% stimulation)	HA Titre[c]
Aloe barbadensis Miller				
Fraction	7	100	1500±150	2040±32
	8	100	1100±100	1024±32
	9	100	400±50	1024±64
	15	200	1400±100	512±32
	16	100	900±50	1024±16
Con A		10	1300±50	4096±8
PHA		15	720±50	2048±8
PWM		10	200±20	NT
WGA		15	>10	NT

[a] Con A, concanavalin A; PHA, phytohaemagglutinin; PWM, poke weed mitogen; WGA, wheat germ agglutinin; NT, not tested.
[b] Concentrations of *Aloe* and reference mitogen test substances were based on values obtained using BioRad standard protein assays.
[c] Percent (%) stimulation of blastogenesis and HA titres reflect mean±SE values for three repeat assays of each mitogen source. *Aloe* fractions 7, 8, 9, 15 and 16 from two sources were tested.

(Yoshimoto *et al.*, 1987) was reported to have little agglutinating ability for erythrocytes of various species and no mitogenic effects.

In studies of receptor antibody responses to *Aloe* lectins, additional similarities between the activities of lectins from *A. barbadensis* Miller and Con A were revealed when PAGE-Western blot transfers followed by immunoblot assays using specific antisera showed that the agglutinin-mitogens in the *A. barbadensis* Miller fractions demonstrated specific binding with Con A receptor, but not WGA receptor antibodies. These results suggested that these *Aloe* lectin substances were active at alpha D-glucose and mannose sites and not at *n*-acetyl glucosamine receptor sites (data not shown). Comparisons of the *A. arborescens* Miller lectin, ATF 1011, with Con A in assays of binding and effects on growth of tumour cells (Yoshimoto *et al.*, 1987) have shown that affinity constants and ratios of binding sites for the murine normal and tumour cells were very similar, as were concentration dependent effects on the growth of specific murine tumour cell types.

Studies of long term freezing effects on *Aloe* lectins, with particular emphasis on our studies to determine the stability of the haemagglutination activities of the lectins from *A. barbadensis* Miller, indicated that HA levels decreased with time in liquid nitrogen storage (Table 3). Effects of repeated freeze–thaw cycles on degradation of lectins such as PHA and Con A are well recognized. Indeed, it is generally recommended by commercial lectin sources that immediately after the initial preparation of such a lectin into a stock solution, the solution should be prepared in small portion aliquots and these should be then deep frozen until used individually. Any remnants of aliquots used during experiments and testing should be discarded. Our results generally confirm these recommendations, since Set 1 samples, which were used 1–2 times, and thus were freeze–thawed 1–2 times, showed greater loss of HA activities than the samples in Set 2 which had not been used after their initial freeze (Table 3).

Table 3. Effects of liquid nitrogen freeze storage on haemagglutination titres of *Aloe barbadensis* Miller substances

Time of preparation	HA titre[a]
Set 1	
July 1981	64±4 (1024±16)
April 1985	128±8 (2048±32)
March 1988	256±8 (1024±32)
April 1989	128±4 (2048±32)
Set 2	
October 1981	128±4 (2048±8)
September 1988	256±8 (1024±16)
August 1989	512±8 (1024±16)
September 1990	51±4 (1024±32)

[a] HA titres reflect mean±SE of three tests of two *Aloe* samples tested per time interval. Values in parenthesis indicate titres observed prior to initial freeze in liquid nitrogen.

The present results suggest that there may be a future potential for the use of purified *Aloe* lectins in immunobiological studies.

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