Available online at www.sciencedirect.com
ScienceDirect
Phytomedicine 16 (2009) 594–600
Phytomedicine
www.elsevier.de/phymed

# *Musa acuminata* (Del Monte banana) lectin is a fructose-binding lectin with cytokine-inducing activity

Allen H.K. Cheung, Jack H. Wong, T.B. Ng*

*Department of Biochemistry, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China*

## Abstract

A homodimeric, fructose-binding lectin was isolated from Del Monte bananas by using a protocol that involved ion-exchange chromatography on DEAE-cellulose and SP-Sepharose, and gel filtration by fast protein liquid chromatography on Superdex 75. Not only fructose, but also glucose, mannose, rhamnose and glucosamine could inhibit the lectin. The N-terminal amino acid sequence of its identical 15-kDa subunits was similar to lectins from other *Musa* species except for the deletion of the N-terminal glycine residue in Del Monte banana lectin. The hemagglutinating activity was stable up to 80 °C and also stable in the range pH 1–13. However, the hemagglutinating activity dwindled to an undetectable level at 90 °C. The lectin was capable of eliciting a mitogenic response in murine splenocytes and inducing the expression of the cytokines interferon-gamma, tumor necrosis factor-alpha, and interleukin-2 in splenocytes. The lectin also inhibited proliferation of leukemia (L1210) cells and hepatoma (HepG2) cells and the activity of HIV-1 reverse transcriptase. The additional information obtained in the present study includes demonstration of fructose-binding activity and cytokine-inducing activity of Del Monte banana lectin. Fructose binding is an unusual characteristic of plant lectins. It is possible that the banana lectin can be developed into a useful anti-HIV, immunopotentiating and antitumor agent in view of its trypsin stability and thermostability.


***Keywords:*** Fructose-binding lectin; *Musa acuminata*; Banana; Isolation; Cytokine-inducing activity

## Introduction

Lectins are carbohydrate-binding proteins possessing at least one non-catalytic domain, which binds reversibly to a specific saccharide (Peumans and Van Damme 1995). The binding of a lectin to a sugar residue is as specific as antigenic recognition by antibodies or enzyme–substrate specificity. Lectins have been found in diverse types of organisms. They display a variety of interesting biological activities such as blood group specificity (Khan et al. 2002), antitumor (Abdullaev and de Mejia 1997), immunomodulatory (Rubinstein et al. 2004), antifungal (Herre et al. 2004), and anti-insect (Macedo et al. 2003) activities.

Glycosylation is the most common post-translational modification of proteins. It changes immature proteins into biologically active proteins. Currently, there are some technologies available for glycan analysis, such as mass spectrometry, Western blotting, and chromatography, but they are time consuming and require expertise making it difficult for the average researcher to access and master. Nowadays, a new technology, lectin microarray, has become a rapid and a simple tool of protein glycosylation. So lectins also have immense value in glycomics study (Pilobello et al. 2005).

Del Monte banana is a consumable fruit available in most supermarkets in the western countries. Banana

*Corresponding author. Tel.: +852 26098031; fax: 852 26035123.
*E-mail address:* b021770@mailserv.cuhk.edu.hk (T.B. Ng).

0944-7113/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2008.12.016

lectins have been reported by several research groups, *Musa paradisiac* lectin by (Koshte et al. 1990), *Musa acuminata* lectin by (Peumans et al. 2000), and *Musa basjoo lectin* by our group (Wong and Ng 2006). The objective of the present investigation was to isolate the lectin from *Musa acuminata* and further characterize it and compare it with the lectins isolated earlier.

## Materials and methods

Ripe Del Monte bananas (*Musa acuminata*) were purchased from a local supermarket. The pulps of the bananas were blended in distilled water using a Waring blender. To the supernatant obtained after centrifugation (15 000*g*, 30 min) of the homogenate, $(NH_4)_2SO_4$ was added to achieve 10% saturation. The mixture was centrifuged again (15 000*g*, 30 min) and $(NH_4)_2SO_4$ was added to attain 80% saturation. The supernatant obtained after centrifugation was then dialyzed against distilled water at 4 °C to get rid of $(NH_4)_2SO_4$. Tris–HCl buffer (pH 7.8) was added to the dialyzed supernatant until the concentration of tris attained 10 mM. The supernatant was then loaded on a $3 \times 10$ cm column of DEAE-cellulose (Sigma) in 10 mM Tris–HCl buffer (pH 7.8). After removal of the unadsorbed fraction (D1), adsorbed fractions (D2 and D3) were eluted with starting buffer containing 0.2 and 1 M NaCl, respectively. After examination of the hemagglutinating effect of fractions D1–3, the lectin-enriched fraction D2 was dialyzed against 10 mM $NH_4OAc$ buffter (pH 4.5) at 4 °C, and then chromatographed on a $1.5 \times 10\,cm^2$ column of SP-Sepharose (Amersham Biosciences), which had previously been equilibrated with and was then eluted with 10 mM $NH_4OAc$ buffer (pH 4.5). Following elution of unadsorbed materials (fraction S1), adsorbed proteins were desorbed with two-stepwise NaCl concentrations (0.4 and 1 M) in 10 mM $NH_4OAc$ buffer (pH 4.5). The adsorbed fraction (S2), which was eluted by 0.4 M NaCl, was the lectin-enriched fraction. It was dialyzed, lyophilized, and subjected to gel filtration on a Superdex 75 HR 10/30 column (Amersham Biosciences) using an AKTA Purifier (Amersham Biosciences) in 10 mM $NH_4HCO_3$ buffer (pH 9.4). The lectin was adsorbed on Superdex 75, and was eluted by $NH_4HCO_3$ buffer (pH 9.4) containing 200 mM glucose. The eluted fraction consists of purified lectin.

### Molecular-mass determination by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and by FPLC-gel filtration

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in accordance with the procedure of Nielsen and Reynolds (1978), using a 12% resolving gel and a 5% stacking gel. At the end of electrophoresis, the gel was stained with Commassie brilliant blue. FPLC-gel filtration was carried out using a Superdex 200 HR 10/30 column that had been calibrated with molecular-mass standards (Amersham Biosciences).

### Analysis of N-terminal amino acid sequence

Amino acid sequence analysis was carried out using an HP G1000A Edman degradation unit and an HP 1000 HPLC system (Lam et al. 1998).

### Assay of hemagglutinating activity

In the assay for lectin (hemagglutinating) activity, a serial two-fold dilution of the lectin solution in microtiter U-plates (50 μL) was mixed with 50 μL of a 2% suspension of rabbit red blood cells in phosphate-buffered saline (pH 7.2) at 20 °C. The results were recorded after about 1 h when the blank containing only red cells had fully sedimented and appeared as a dot at the bottom of the well. The hemagglutination titer, defined as the reciprocal of the highest dilution exhibiting hemagglutination, is defined as one hemagglutination unit. Specific activity is the number of hemagglutination units per mg protein (Wong and Ng 2003a).

### Inhibition of lectin-induced hemagglutination by carbohydrates

The hemagglutinating inhibition tests to investigate inhibition of lectin-induced hemagglutination by various carbohydrates for 30 min at room temperature were performed in a manner analogous to the hemagglutination test (Wong and Ng 2003a).

### Effect of temperature and pH on lectin-induced hemagglutination

The effects of temperature and pH on hemagglutinating activity of the lectin were examined as previously described (Wong and Ng 2003a).

### Assay of mitogenic activity

Incubation of splenocytes from BALB/C mice (20–25 g) was carried out at 37 °C in a humidified atmosphere of 5% $CO_2$ in the presence or absence of the banana lectin for 24 h in a 96-well culture plate, 10 μL (methyl $^3H$)-thymidine (0.25 μCi, Amersham Biosciences) was added before the splenocytes were incubated for another 6 h under the same conditions. The splenocytes were then harvested onto a glass fiber filter,