INTERACTION OF *LABEO ROHITA* LECTIN (LRP) WITH MYCOBACTERIAL SURFACE OLIGOSACCHARIDE

S. Mitra and H.R. Das*

Proteomics and Structural Biology Unit, Centre for Biochemical Technology, Mall Road, Delhi University Campus, Delhi-110007, India

ABSTRACT

A C-type mannose specific lectin (LRP) isolated from plasma of fresh water fish *Labeo rohita* showed 500 times higher specificity towards cell surface oligosaccharide (LAM) of *Mycobacterium tuberculosis* (H37Rv). Using biotinylated LRP, binding between lectin and LAM was demonstrated by ELISA and it was observed that even 3ng of oligosaccharides might be detected using only 1 μ g of biotinylated lectin.

KEY WORDS: *Labeo rohita*, lipoarabinomannan (LAM), plasma lectin.

INTRODUCTION

The carbohydrate binding properties of lectins have been found in many diverse applications in biomedical research. Use of lectin-affinity electrophoresis (1), lectin based ELISA (2,3,4) are well known techniques for quantification and characterization of glycoforms. Recently a novel mannose-binding lectin is being purified from the plasma of fresh water fish *Labeo rohita* in our laboratory (5). On the surface of *Mycobacteria tuberculosis* lipoarabinomannan (LAM) is known to be present (6,7). To develop a rapid procedure for detection of *M. tuberculosis* a series of interaction studies between this lectin (LRP) and LAM isolated from *M. tuberculosis* (H37Rv) were conducted. The results of these interaction studies are presented in this report.

MATERIALS AND METHODS

Bovine serum albumin, orcinol, glucose, N-acetylglucosamine, galactose, N-acetyl-galactosamine, methyl- α -D mannopyranoside, neuraminidase, biotinylating reagents including biotin (long arm) hydrazide, 1-(3-dimethylaminopropyl) -3-

ethylcarbodiimide EDC and hydroxysuccinimide, streptavidin peroxidase, ortho-phenylenediamine dihydrochloride (OPD), polyoxyethylenesorbitan monolaurate (Tween-20) were all purchased from Sigma, USA; gelatin from Qualigens, nitrocellulose paper from Millipore and microelisa plates from Nunck. All other reagents were of local analytical grade.

The mannose-binding lectin has been isolated from fish plasma (*L. rohita*) as described elsewhere (5). The protein concentration was measured by Lowry *et al.* (8). Lipoarabinomannan (LAM) has been isolated from *M. tuberculosis* strain H37Rv following the method of Khoo *et al.* (9). The total sugar content of LAM was determined by orcinol-sulphuric acid method (10).

Sugar inhibition assay of the lectin was performed using serially diluted LAM (150 μ g/ml to 1 μ g/ml concentration) and 4 HA unit of lectin (eg.20 μ g). The mixture was incubated for 20 min at room temperature. Then neuraminidase treated 4% rabbit erythrocytes, washed with Tris buffered saline (TBS: 10mM Tris-HCl, pH 7.2 containing 150mM NaCl, 1mM CaCl_2), was added and incubated further for 30 min and inhibition of agglutination was measured as described earlier (11). To quantify the binding of lectin to LAM, the lectin was tagged with biotin by carbodiimide coupling method (Sigma's manufacturer protocol). The biotinylation of lectin was confirmed by dot blot and ELISA assays were performed in microtiter

Author for correspondence:

Dr. Hasi R. Das, at above address

E-mail : hdas@cbt.res.in, hasidas@yahoo.com

Fax: 091-011-766-7471

polystyrene plates as reported earlier (12). The ratio of LAM and lectin was optimized for high absorbance. The LAM was serially diluted with a starting concentration of 100 ng in 100 μ l of 0.1M carbonate buffer (pH 9.5) and fixed to the well for overnight at 37°C. The plates were washed four times with phosphate buffered saline (PBS) containing 0.02% Tween-20 (PBS-T). Non-specific binding sites were blocked by 4% gelatin (periodate treated) by incubating the plate at 37°C for 3 hours. After washing with PBS-T, the plate was incubated with biotinylated lectin (1 μ g) in 100 μ l of PBS for 3 h at 37°C. Washed the wells with PBS-T thrice. Peroxidase labeled streptavidin at a concentration of 0.1 mg/ml in 1:700 times dilution was added and incubated for 45 min at 37°C. The plate was finally washed with PBS-T and the color was developed by addition of 100 μ l of ortho-phenylenediamine and H₂O₂ in 100mM citrate buffer (pH 5.6). The reaction was terminated by adding 50 μ l of 2N H₂SO₄ and the samples were read at 492 nm in an ELISA reader (Anthos II). All the assays were performed in triplicates for 3-4 times. The reading of the well without lectin was treated as negative control.

RESULTS

A novel mannose-binding lectin (LRP) has been purified from the plasma of Indian major carp, *Labeo rohita*. The lectin was found to agglutinate neuraminidase treated rabbit erythrocytes (ntRBC) the most (5). This lectin showed strong affinity towards methyl- α -D mannopyranoside as determined by hemagglutination inhibition assay using the ntRBC (11). The cell wall polysaccharides of *M. tuberculosis* consist of lipoarabinomannan (LAM) and are extensively capped with mannose in the case of pathogenic strains and are termed as ManLAM (13). Preliminary experiments showed that LAM isolated from H37Rv strain could inhibit the hemagglutinating activity of this lectin. To find out the exact concentration of LAM required to inhibit 4HA unit of lectin, an experiment was designed by diluting the LAM serially for the inhibition assay. The inhibition experiment giving 50% inhibition of 4HA unit of lectin was observed at 0.146 μ g/ml of LAM as shown in Figure 1. Comparative inhibition potency was measured between LAM and various monosaccharides using 4HA unit of lectin. It was observed that LAM at the

Fig. 1. LRP binding to LAM of *M. tuberculosis* (H37Rv) as measured in terms of % of hemagglutination inhibition.

concentration of 0.146 $\mu\text{g/ml}$ is 500 times more potent than the potent monosaccharide inhibitor mannose (Table 1). Lectin-based ELISA is one of the most sensitive techniques in determining the lectin binding to various glycoconjugates. Using biotinylated LRP, binding of lectin

Table 1. Sugar Inhibition of hemagglutinating activity of purified *Labeo rohita* plasma lectin (LRP)

Sugar inhibitor	Concentration ^a mM	Relative inhibitory potency ^b
Mannose	0.39	1.0
Methyl- α -D-Mannopyranoside	0.20	2.0
Galactose	100*	-
N-Acetyl-D-Glucosamine	1.56	0.25
N-Acetyl-D-Galactosamine	100*	-
LAM ^c (<i>M. tuberculosis</i>)	0.14 ($\mu\text{g/ml}$)	500

^a Minimal concentration required for 50% inhibition of 4HA units of purified plasma lectin.

^b The inhibitory potency of Mannose is arbitrarily set as 1.0 for purified lectin.

^c The LAM concentration is in terms of their carbohydrate content as described in 'methods'.

*Non-inhibitory at the indicated final concentration.

Fig. 2. Binding of LAM from *M. tuberculosis* H37 Rv to biotinylated LRP as detected by ELISA. Each assay was done in triplicate and the values represent the averages and standard deviations from three experiments.

towards LAM was determined quantitatively at much lower concentration. The ratio of lectin and LAM was optimized and found that 3ng of LAM gives a detectable O.D. using only 1 μg of biotinylated lectin. Upto 100 ng concentration of LAM, the absorbance at 492nm increases logarithmically and above 100ng, the O.D. reaches its saturation (Fig. 2).

DISCUSSION

Lipoarabinomannan (LAM) is considered as a major antigen in mycobacterial cell wall envelope (6,7). Mannose capping is considered to be a common feature of LAM from all strains of *M. tuberculosis* Erdman, H37Ra and H37Rv (14). Comparative analysis of the antigens of H37Rv strain with other mycobacterial antigens (15) have indicated that this strain shares the antigens mostly polysaccharide in nature with other mycobacterial strains, although there are few protein antigens also found to be shared. Interaction studies between LRP and cell surface oligosaccharide of H37Rv strain may offer an important approach to identify this polysaccharide antigen in virulent strains of mycobacteria. Results of these studies shows strong affinity of this lectin towards cell surface macromolecule of this organism. It is known that the attachment of *Mycobacterium* spp. to macrophages is the preliminary step prior to invasion of the macrophages by the bacteria. Human mannose-binding protein (MBP) was also reported to interact with *Mycobacterium* (16) suggesting a possible host defense against this organism. This interaction study between LRP and mycobacterial LAM may offer an important approach towards prevention of infection by blocking the receptor (LAM) by this lectin. However, the results of this interaction study may help in developing a method for identification of virulent strains of *Mycobacterium*. Presently a rapid and economical method for detection of mycobacterial infection using this lectin is in progress in this laboratory.

Acknowledgement

Suchareeta Mitra is thankful to the University Grants Commission for providing senior research fellowship.

REFERENCES

1. Inoue, T., Yamauchi, M. and Ohkawa, K. (1999) Structural studies on sugar chains of carbohydrate-deficient transferrin from patients with alcoholic liver disease using lectin affinity electrophoresis. *Electrophoresis*. 20, 452-457.
2. Ray, S. and Chatterjee, B.P. (1992) Lectin binding reactions monitored by enzyme immunoassay exemplified by carbohydrate determination in ant egg glycoprotein. *Biochemical Archives*. 8, 295-300.
3. Van der Schall, I.A.M., Logman, T.J.J., Diaz, C.L. and Kune, J.W. (1984) An enzyme-linked lectin binding assay for quantitative determination of lectin receptors. *Anal. Biochem.* 140, 48-55.
4. Müller, W.E.G., Matthes, E., Bachmann, M., Kljajic, Z., Gasic, M. and Forrest, J.S.M. (1993) Novel, lectin-based enzyme-linked immunoassay for the quantitation of envelope glycoprotein 120 of human immunodeficiency virus. In : *Lectins, Biology, Biochemistry, Clinical Biochemistry*. Eds. E. Van Driessche, H. Franz, S. Beeckmans, U. Pfüller, A. Kallikorm., T.C. Bog- Hansen., Textop, Hellerup, Denmark Vol 8, p 341-347.
5. Mitra, S. and Das, H.R. (2001) communicated to *Fish Physiology and Biochemistry*.
6. Chatterjee, D. and Khoo, K.H. (1998) Mycobacterial lipoarabinomannan: an extraordinary lipoheteroglycan with profound physiological effects. *Glycobiology*. 8, 113-120.
7. Vercellone, A., Nigou, J. and Puzo, G. (1998) Relationships between the structure and the roles of lipoarabinomannans and related glycoconjugates in tuberculosis pathogenesis. *Front. Biosci.* 3, 149-163.
8. Lowry, O.M., Rosenbrough, N.J., Fars, A.L. and Randall, R.J. (1951) Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193, 265-275.
9. Khoo, K.H., Dell, A., Morris, H.R., Brennan, P.J. and Chatterjee, D. (1995) Structural definition of acylated phosphatidylinositol mannosides from *Mycobacterium tuberculosis*: definition of a common anchor for lipomannan and lipoarabinomannan. *Glycobiology*. 5, 117-127.
10. White, C. A. and Kennedy, J.F. (1986) Oligosaccharides In: *Carbohydrate analysis, a practical approaches*. Eds. M.F. Chaplin and J.F. Kennedy IRL Press, Oxford, U.K. p. 37-54.
11. Manihar, S.R., Das, R.H. and Das, H. R. (1991) A T-antigen-binding lectin from *Channa leucopunctatus* (Murrel) plasma. *Carbohydr. Res.* 213, 251-261.
12. Jayaraman, V. and Das, H.R. (1998) Interaction of peanut root lectin (PRA II) with rhizobial lipopolysaccharides. *Biochim. Biophys. Acta.* 1381, 7-11.
13. Chatterjee, D., Khoo, K.H., McNeil, M.R. and Brennan, P.J. (1993) Structural definition of the non-reducing termini of mannose-capped LAM from *Mycobacterium tuberculosis* through selective enzymatic degradation and fast atom bombardment-mass spectrometry. *Glycobiology*. 5, 497-506.
14. Khoo, K.H., Dell, A., Morris, H.R., Brennan, P.J. and Chatterjee, D. (1995) Inositol phosphate capping of the nonreducing termini of lipoarabinomannan from rapidly growing strains of *Mycobacterium* *J. Biol. Chem.* 270, 12380-12389.
15. Navakar, R.G. (1977) Comparative analysis of the antigens of *M. tuberculosis* H37Rv with various mycobacterial antigens. *Zentralbl. Bakteriell. [Orig A]*. 237, 330-336.
16. Polotsky, V.Y., Belisle, J.T., Mikusova, K. Ezekowitz, R.A. and Joiner, K.A. (1997) Interaction of human mannose-binding protein with *Mycobacterium avium*. 175, 1159-1168.