ULTRASTRUCTURAL DEMONSTRATION OF HELIX POMATIA LECTIN-BINDING SITES IN GOBLET CELLS OF HUMAN CONJUNCTIVA

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SUMMARY: The Lectin-binding sites in goblet cells of normal human conjunctiva were investigated at the ultrastructural level by means of post-embedding staining of resin-embedded thin sections, using Helix pomatia lectin-gold complex (HPL-GC). The satisfactory labeling and good fine structure preservation were obtained by mild aldehyde fixation, Agar resin 100 embedding and post-embedding staining protocol. The Helix pomatia lectin-binding sites were intensely observed over mucus droplets of goblet cells. This finding demonstrates that N-acetyl-D-galactosamine is present in the glycoconjugates of goblet cells of human conjunctiva.

Key Words: Lectin, glycoconjugate, goblet cell, conjunctiva.

INTRODUCTION

The ocular mucus, especially the glycoprotein part (mucin glycoproteins) is considered essential for stability of the tear film and maintenance of the wettability of the ocular surface epithelium (5,14-16, 22-25, 38), and defense of the ocular surface against bacteria and other infectious agents (8).

Most of mucus material originates from the conjunctival goblet cells (mucus-secreting cells); other proposed sources are tear gland and the conjunctival epithelial cells (5,16).

The mucin glycoproteins contain many short 0-linked glycans. Many proteins carry covalently attached oligosaccharide or polysaccharide chains. The 0-linked glycans are usually attached by a glycosidic bond between N-acetyl galactosamine and hydroxyl group of threonin or serine residues (27). Lectins are non-immunological proteins/glycoproteins that interact with specific, non-reducing, terminal glycosol groups of complex oligosaccharides and glycoproteins (9).

Lectins are the valuable tools for light and electron microscopic localization of sugar sequences in oligosaccharide unites of glycoconjugate (28, 31). Since they are not electron-opaque, lectins can not be directly visualized at the electron microscope level, and consequently have to be conjugated to an electron-dense marker (17). Recently, colloidal gold has been introduced in the field of cytochemistry as an alternative particulate marker (12,18). Because gold particles are electron-dense, uniform in size, and are reproducibly and easily prepared, they represent actually the most attractive markers for both transmission and scanning electron microscopy (4).

In contrast to the extensive use of lectin-gold complex in the cell surface studies (19), their application for the

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detection in the intracellular receptors is relatively new (32).

Lectin application for detection of intracellular binding sites by electron microscopy is limited because of their poor penetration into cells and tissues. Different approaches exist to circumvent this problem and they include the labeling of cellular fractions (7,13, 30, 37), the incubation of thick frozen sections in a pre-embedding technique (3,11, 34, 40), or the staining of ultra thin sections from resin-embedded specimens in a post-embedding technique (10, 32, 35, 36).

In this study we investigated the binding-sites of Helix pomatia lectin, specific for terminal N-acetyl-D-galactosamine residues (2,12, 21) in goblet cells of human conjunctiva, using Agar resin 100 in a post-embedding technique. The labeling of thin sections was performed as one step (direct) lectin-gold technique.

MATERIALS AND METHODS

The tissue pieces were taken from the conjunctiva of normal human. For thin section post-embedding labeling, small fragments of the tissue were fixed at room temperature for 2 hours in 0.5% glutaraldehyde diluted in phosphate buffered saline (PBS; pH 7.4). After fixation, the tissue fragments were rinsed in PBS, and free aldehyde groups were blocked by incubation in 0.5M NH_4CL in PBS for 1 hour at room temperature. After further rinses in PBS, the tissue fragments were dehydrated in a graded series of ethanol at room temperature and embedded in Agar resin 100. Thin sections were cut by glass knives and picked up on 200 mesh, parlodion-carbon coated nickel grids.

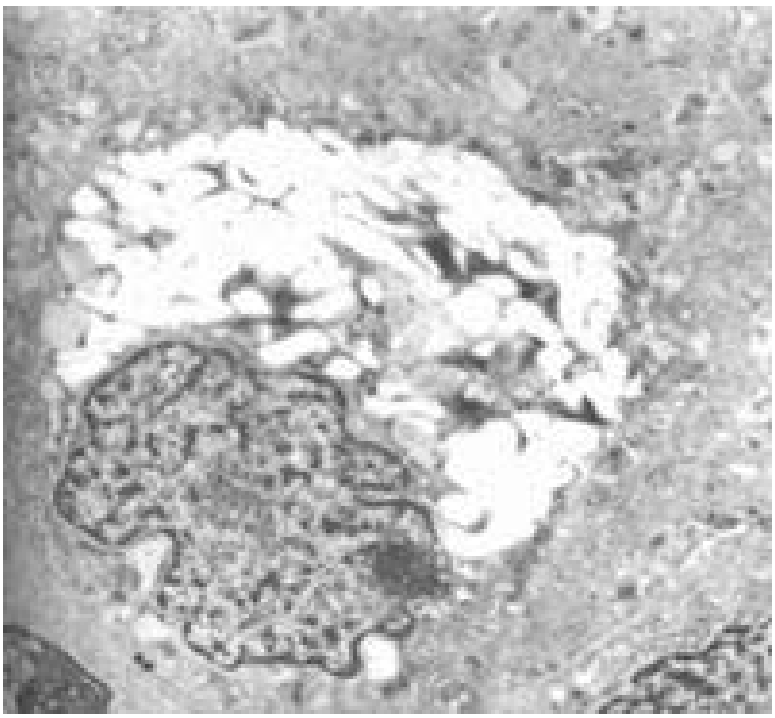
Staining protocol

Incubations were always performed at room temperature. Initially all of the grids with the attached sections were placed on a drop of PBS for 5 min.

One-step method (direct labeling)

Thin sections were incubated for 60 min with 14 nm Helix pomatia lectin gold-complex (HPL-GC) diluted with 0.02% polyethylene glycol (PEG) in PBS (3:1). HPL-GC was kindly provided

Figure 1: An electron micrograph of goblet cell of normal human conjunctiva fixed in glutaraldehyde, embedded in Agar resin 100, and incubated with Helix pomatia lectin-gold complex (HPLC-GC). Black gold particles representing Helix pomatia lectin-binding sites are seen over mucus droplets. X 13500.



by Dr. Frederic W.K. Kan (Department of Anatomy, Faculty of Medicine, University of Montreal). Afterwards sections were washed with PBS (2 times for 2-3 min each) and bi-distilled water and counterstained with 3% aqueous uranyl acetate (7 min) and lead citrate (5-7 min). All sections were examined under the electron microscope, Carl Zeiss EM 9S-2.

Control for specificity

Specificity of lectin-binding was tested by incubation of sections with excess of native lectin (1 mg/ml; 30 min) before the applications of the corresponding Helix pomatia lectin -gold complex to which unlabeled lectin was also added.

RESULTS

Control of specificity

Almost complete abolition of staining was observed by pre-incubation of thin sections with the no labeled lectin.

Thin sections of Agar resin 100-embedded conjunctival tissue incubated with HPL-GC revealed rather intense labeling over mucus droplets of the conjunctival goblet cells (Figures 1, 2 and 3).

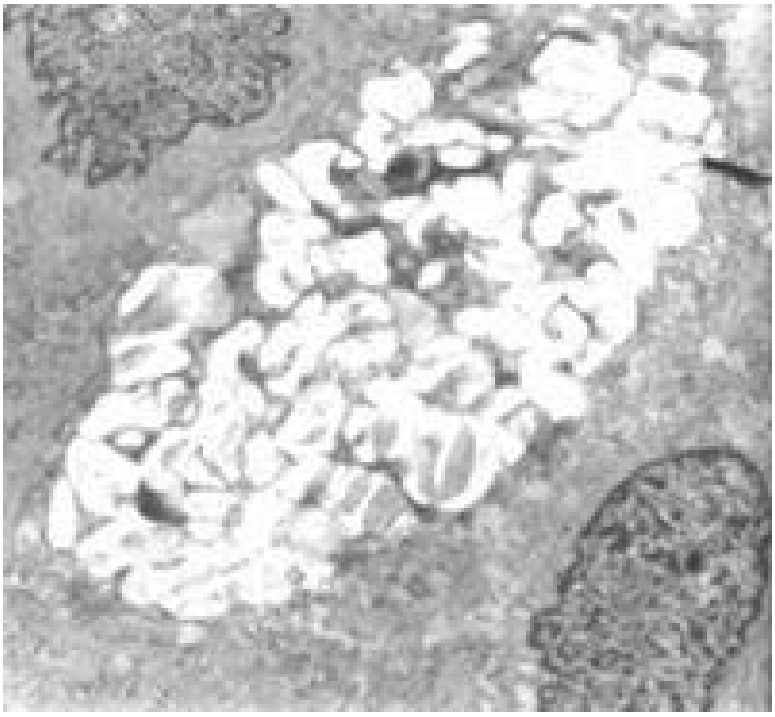
DISCUSSION

Numerous studies, dealing with the localization of glycoconjugates in various tissues, have utilized the remarkable capacity of lectins for binding specifically to certain sugar residues (1, 6, 24, 26, 32, 33, 36, 39). Various fixatives and embedding materials have been tested in these studies. According to the results, embedding of aldehyde fixed animal tissue in the acrylic LR white and lowicryl K4M have provided high preservation of both cellular fine structure and lectin-binding sites.

The present work demonstrates that the Helix pomatia lectin-binding sites and the fine structure of goblet cells of human conjunctiva are also well preserved after using mild aldehyde fixation and Agar resin 100 embedding material.

It has been shown that the mucus droplets of goblet cells of normal human conjunctiva, labeled positively with Helix pomatia lectin (HPL), Peanut agglutinin (PNA), wheat germ agglutinin (WGA), Succinylated wheat germ agglu-

Figure 2: The mucus droplets of goblet cell show positive labeling with Helix pomatia lectin-gold complex. X 13500.



tinin (S-WGA), Soybean agglutinin (SBA), Dolichos biflorus agglutinin (DBA), Ricinus communis agglutinin I (RCA I) and Griffonia simplicifolia (GS-I) at the light and electron microscope levels (20, 23, 29, 36, 39). However, there are four N-acetyl-D-galactosamine 'specific' lectins: Helix pomatia A hemagglutinin (HPA), Soybean agglutinin (SBA), Lima bean lectin (LBL), and Dolichos biflorus lectin (DBL) (2,12).

In our study the intense labeling of goblet cell with HPL-GC, indicates that they are rich in oligosaccharides containing various linkages of terminal N-acetyl-D-galactosamine on the mucin gylcoproteins of mucus droplets.

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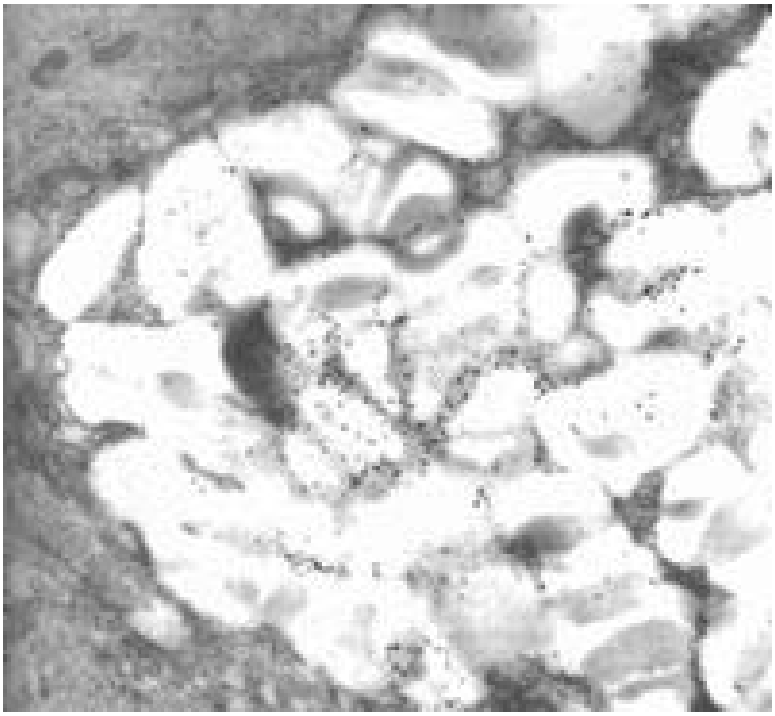
Figure 3: An electron micrography of higher magnification of one of the cytoplasmic part of goblet cell in Figure 2. The colloidal gold particulars are intensely observed over mucus droplets. X 28500.



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