Cytochemical Characterization of Sialoglyconjugates on Rat Photoreceptor Cell Surface

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Terminal saccharide sequences in rat photoreceptor cell surface glycoconjugates were characterized. Lectin cytochemistry and electron microscopy were used for preembedding cytochemical localization of surface carbohydrates. Neuraminidase digestion was employed for the exposure of penultimate saccharides in sialoglycoconjugates. Isolated rat retinas were incubated with ferritin-labeled wheat germ agglutinin (WGA), peanut agglutinin (PNA), and soybean agglutinin (SBA) prior to and after neuraminidase digestion. PNA and SBA did not label untreated photoreceptors. WGA densely labeled the photoreceptor surface and interphotoreceptor matrix (IPM) components. Following neuraminidase treatment, PNA, but not SBA, labeled the photoreceptor surface and the IPM. WGA labeling of the IPM was abolished, and the labeling of the photoreceptor surface was reduced. Based on the lectin specificity, it was concluded that photoreceptor surface glycoconjugates in the rat retina contain a terminal trisaccharide: sialic acid-D-galactose-(β1→3)-N-acetyl-D-galactosamine. Invest Ophthalmol Vis Sci 28:640-645, 1987

Sialic acid residues are common components on the surface of various cells and secretory products. In several cellular systems, sialic acids have been implicated in intercellular interactions, both by affecting cell surface negative charge, and as a masking agent of recognition determinants. Removal of sialic acids from sialoglycoconjugates is associated with intercellular phenomena such as polyagglutination of red blood cells, which is mediated by the exposed T antigen, and the uptake of asialoglycoproteins by liver cells.

Recently, Cohen and Nir demonstrated the presence and distribution of sialic acid moieties on the surface of rat photoreceptor and pigment epithelium cells by means of colloidal iron (pH 1.8) cytochemistry. In view of the possible role of penultimate saccharides in sialoglycoconjugates in recognition events, it was of interest to determine the nature of penultimate saccharides in the photoreceptor sialoglycoconjugates. These saccharides, if exposed, might be involved in the photoreceptor-pigment epithelium interaction, for which a role for the surface saccharides in a recognition process has been suggested.

The most common penultimate sugars in sialoglycoconjugates are galactose and N-acetylgalactosamine. The current study used lectin cytochemistry for the localization of penultimate saccharides in sialoglycoconjugates in the rat photoreceptor. Peanut agglutinin (PNA) was used for the visualization of terminal galactose residues, and soybean agglutinin (SBA) for the localization of terminal N-acetylgalactosamine. Penultimate sugars in sialoglycoconjugates were exposed by means of neuraminidase digestion of the retina. Wheat germ agglutinin (WGA) labeling coupled with neuraminidase digestion was used for localization of sialic acid residues.

Materials and Methods

Tissue Preparation

Normal pigmented RCS rats were maintained on a 12-h light/12-h dark cycle. Animal care and treatment in this investigation were in compliance with the ARVO Resolution on the Use of Animals in Research. Eyes were enucleated from 15-20-day-old dark-adapted rats, 30 min before the onset of light. Retinas were separated from the pigment epithelium which remained attached to the posterior eyecup and were rinsed in cold phosphate-buffered saline (PBS). The tissues were then fixed in 1% glutaraldehyde in 0.15 M phosphate buffer (pH 7.0) at room temperature. Following fixation, the tissues were cut into small (2 mm × 1 mm) strips, and rinsed in PBS for 10 min, and then in 0.05 M glycine in PBS for 20 min.

Lectin Cytochemistry

Ferritin-labeled lectins, PNA, SBA, and WGA, were obtained from E-Y Laboratories, San Mateo, CA. Fixed retinal tissue was incubated with 500 μg/ml (PBS) of...
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lectin for 90 min at room temperature. The tissue was then rinsed for 60 min in PBS, osmicated with 1% OsO₄ in 0.15 M phosphate buffer for 30 min, dehydrated in ethyl alcohol, and embedded in Epon.

Neuraminidase Digestion

Vibrio cholerae neuraminidase, free of protease activity, was obtained from Behringwerke AG, Marburg, West Germany. Unfixed retinal tissue was incubated in a medium containing 0.2 U/ml neuraminidase in an incubation mixture of 0.145 M NaCl, 0.003 M CaCl₂, and 0.004 M NaHCO₃ (pH 6.5). Incubation was carried out for 60 min at room temperature. The retinal tissues were then rinsed in saline bicarbonate buffer and labeled with the lectins.

Control Experiments

PNA binding specificity was studied by including 0.2 M galactose in the rinsing and incubation media. WGA binding specificity was evaluated by the addition of 0.1 M chitotriose to the reaction mixtures. The specificity of the enzymatic digestion was evaluated by denaturing the neuraminidase at 65°C for 30 min.

Results

PNA

The surface of untreated photoreceptor cells in the rat retina was not labeled with PNA (Fig. 1). Following neuraminidase digestion, substantial binding to the surface of the rod outer segments (ROS) was noted (Fig. 2). The neuraminidase treatment also exposed numerous PNA-binding sites in the interphotoreceptor matrix (IPM) (Fig. 2). Labeled IPM components were seen in close proximity to the photoreceptor surface. However, due to the high resolution of the ultra-cytochemical procedure, a clear distinction could be made between PNA binding to the photoreceptor surface (Fig. 2) and to the adjacent IPM. In control experiments, the addition of galactose to the PNA incubation medium completely abolished PNA binding to neuraminidase-treated retinas. The inhibition of PNA binding was observed both to the photoreceptor cell surface and in the IPM (Fig. 3). Incubation of the retina with heat-inactivated neuraminidase did not expose PNA binding sites (data not shown).

WGA

Since WGA binds both to sialic acid and N-acetylglucosamine, the contribution of sialic acids to the binding pattern could be determined only by comparison of the lectin binding to the photoreceptors prior to (Fig. 4) and after (Fig. 5) neuraminidase digestion. In untreated cells, dense WGA binding to the ROS plasma membrane was seen (Fig. 4). Substantial binding to the IPM was also observed. Following neuraminidase digestion, labeling of the photoreceptor plasma membrane was still observed. However, labeling density was lower in comparison with untreated retinas. While untreated photoreceptor plasma membrane was covered by a continuous array of ferritin particles (Fig. 4), in neuraminidase-treated cells, an apparent reduction in labeling density was noted (Fig. 5). Binding of WGA to the IPM was completely abolished by the neuraminidase digestion (Fig. 5). Thus, while binding of WGA to the ROS plasma membrane was apparently mediated by sialyl and N-acetylglucosamine residues, binding to IPM components was mediated entirely by sialic acid residues. In the presence of a competing hapten saccharide, chitotriose, binding to both the IPM and the photoreceptor surface was prevented (data not shown).
Fig. 2. PNA binding to retina treated with neuraminidase prior to incubation with the lectin. The enzyme digestion exposed numerous PNA-binding sites on the photoreceptor surface (arrowheads). PNA-binding sites (arrows) were exposed also in the IPM (X75,000).

SBA

This lectin did not bind to the ROS plasma membrane, either prior to or after neuraminidase digestion (Fig. 6). Thus, the SBA-specific saccharide, N-acetyl-galactosamine, could not be detected in the rat retina, either as a terminal saccharide in glycoconjugates or in the penultimate position in sialoglycoconjugates.

A summary of the labeling patterns of the various lectins in the rat retina is presented in Table I.

Discussion

In the current study, we observed the absence of PNA binding to rod photoreceptor surfaces in the rat retina. These results agree with various reports concerning the labeling of retinas from different species with PNA. Preferential staining of cone photoreceptors with fluorescence-labeled PNA has been reported in monkey, human, rabbit, chick, fish, and mouse retinas.13-15 Although PNA might react with terminal nonreducing galactose residues,9 the absence of PNA binding does not indicate the absence of galactose residues from the terminal position in photoreceptor surface glycoconjugates. Recessus communis agglutinin, which is specific to terminal galactose residues,16 does label ROS in the frog,17 bovine,18 and rat retinas (D. Cohen and I. Nir, unpublished observations).

PNA preferentially binds to the terminal disaccharide sequence D-galactose-(β1—3)-N-acetyl-D-galactosamine.9 In the retinal cones, as in a variety of other cell types which are labeled with PNA,19-21 this disaccharide apparently occupies a terminal position in glycoconjugates. Retinal rods can be included in another group of cell types, in which only neuraminidase pretreatment imparts PNA reactivity by removing sialic acids from terminal positions in glycoconjugates.22 In these cells PNA binding depicts the localization of a terminal trisaccharide: sialic acid-(α-2—>3,6)-D-galactose-(β1—3)-N-acetyl-D-galactosamine.22

The nature of the sialoglycoconjugates on the photoreceptor surface is not known. It might be either glycolipids or glycoproteins. However, in view of the molecular organization of known sialoglycolipids (gangliosides),23 desialization is not expected to expose PNA binding sites.

In glycoproteins, the carbohydrate side chains are linked to the protein either O-glycosidically or N-glycosidically.24 The core structure of O-glycosidically linked chains generally consists of the disaccharide D-galactose-(β1—3)-N-acetyl-D-galactosamine (toward which PNA has the highest affinity) linked to the hydroxyl of serine or threonine in the peptide. Glycoproteins with O-glycosidically linked side chains are associated mainly with secretion of epithelial cells, but also with plasma membranes or their glycoalyx.25

In the central nervous system, O-glycosidically linked
side chains have been demonstrated in the brain tissue of rats. In the retina, O-glycosidically linked side chains have been observed in the bovine IPM. Presently, the labeling of desialated rat photoreceptors with PNA indicates that sialoglycoproteins with O-glycosidically linked side chains are a constituent of the rod membrane.

The extent of desialization by neuraminidase was followed by WGA labeling. Binding of WGA to the IPM was completely inhibited by the neuraminidase digestion, thus indicating extensive desialization in this domain. Binding of WGA to the photoreceptor surface was only moderately reduced by the neuraminidase. The remaining WGA labeling of the photoreceptor surface was probably due to N-acetylglucosamine residues, toward which WGA is specific, in addition to sialic acids. The photoreceptor outer segment plasma membrane is rich in opsin molecules. The opsin oligosaccharides, which contain N-acetylglucosamine residues, might provide the WGA with binding sites in desialated outer segments. Since opsin oligosaccharide is devoid of PNA-specific sugars, it is apparent that following desialization, PNA and WGA label two different glycoconjugates in the ROS plasma membrane.

Neuraminidase digestion of the retina exposed PNA binding sites in the IPM also. The possibility that IPM components might adhere to the photoreceptors, thus affecting their labeling pattern, cannot be excluded. To resolve this question, positive identification of intrinsic plasma membrane sialoglycoconjugates is required. Although such information is not yet available, the following preliminary observations should be considered. In some experiments, the photoreceptors have been isolated, digested with neuraminidase, and rinsed extensively to maximize the removal of IPM, which is highly water soluble. Following this treatment, the photoreceptor surface has been clearly labeled with PNA. In addition, initial analysis of desialated rat ROS revealed PNA binding to isolated membrane proteins (A. Polans, personal communication).

Terminal sialic acid can be bound to N-acetylgalactosamine residues such as in various mucins. The
Table 1. Comparative binding of ferritin-labeled lectins to the surface of rod outer segments and interphotoreceptor matrix

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<th>Lectin</th>
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<th>Interphotoreceptor matrix</th>
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The indicated labeling density is based on a subjectively-estimated scale: (-) nonreactive, (+) moderate density, (++ high density.

The absence of SBA labeling in the rat retina, which was subjected to neuraminidase digestion, indicates that sialoglycoconjugates with penultimate N-acetylgalactosamine cannot be detected in the rat photoreceptor surface or in the IPM.

Fig. 5. WGA binding to retina treated with neuraminidase prior to incubation with the lectin. The enzyme digestion removed all binding sites from the IPM. Labeling of the photoreceptor surface, although reduced, is not completely abolished (×75,000).

Fig. 6. SBA binding to retina treated with neuraminidase prior to incubation with the lectin. The enzyme digestion did not expose lectin-binding sites (×72,500).

The results of the current study are consistent with the presence of rat photoreceptor surface glycoconjugates, which contain a terminal trisaccharide: sialic acid-D-galactose-(β1–3)-N-acetyl-D-galactosamine. A terminal disaccharide, galactose-(β1–3)-N-acetyl-D-galactosamine, could not be detected. N-Acetylgalactosamine residues were not detected in sialoconjugates, either in the terminal position or as a penultimate saccharide.

Key words: rat photoreceptors, sialoglycoconjugates, lectins, electron microscopy

References