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A study of oligosaccharide determinants expressed by prostatic glandular epithelium of the normal adult rat*

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Summary. Normal prostates from Copenhagen/Fischer F1 hybrid rats were removed at 14 months of age. After routine formalin fixation and paraffin embedding, the expression of seven oligosaccharide structures by prostatic epithelial cells was assessed by an examination of lectin binding sites before and after neuraminidase digestion. Con-A bound to plasma membranes as well as the cytoplasm of all cells, thus confirming the presence of complex-type glycoconjugates. However, only two other oligosaccharides, apart from Con-A, were freely expressed on epithelial luminal plasma membranes. These were the Type I structure (Galβ1→3GalNAc–) identified by PNA-binding and $(GlcNAc\beta1\rightarrow4GlcNAc\beta1\rightarrow4-)_n$ identified by WGA. PNA, WGA, UEA-1 and SBA bound to the cytoplasm of almost all epithelial cells, although their intracellular distribution was not identical. DBF binding was not identified. ECG bound to only a very few cells and then only after digestion with neuraminidase when it was localised to the cytoplasm. Following removal of sialic acid groups by neuraminidase digestion, PNA-binding became more prominent, SBA-binding appeared localized to paranuclear intracellular vesicles and WGA binding sites were abolished. This study has now characterized the major oligosaccharide determinants expressed by rat normal prostatic epithelial cells and provides a baseline against which alterations occurring during ontogenesis and oncogenesis may be compared.

Key words: Lectins – Glycoconjugates – Rat prostate

Oligosaccharides of cellular glycoconjugates alter during tissue ontogenesis [18]. Although the precise functions of many cell-surface glycoconjugates have yet to be elucidated, some are recognized to be growth-factor receptors eg insulin receptor [7] while others are involved in binding matrix proteins eg fibronectin [23]. In each of these situations, glycosylation of individual proteins is highly regulated and results in construction of specific oligosaccharide sequences at defined sites on the protein [21]. Alterations in patterns of glycosylation which occur as a result of neoplastic transformation also appear to be consistent and result in a significantly different intracellular distribution of many glycoproteins [13]. Such changes observed during neoplastic transformation have been associated with tumour-cell behaviour [8]. In particular, sialylation of cell-surface oligosaccharide determinants is a frequent finding in malignant epithelial tumours where it has been associated with increased metastatic potential [10].

Herein we assess the expression of glycoconjugates by non-neoplastic prostatic epithelial cells of the normal adult rat. We employ lectin histochemistry together with neuraminidase digestion to elucidate the major oligosaccharide structures synthesized and expressed by these cells. Consequent upon this study we will be able to identify and to interpret the altered expression of prostatic epithelial-cell glycoconjugates which are observed during development and following malignant transformation.

Materials and methods

Rats

Male Copenhagan/Fischer rats were donated by Dr. Altman (Miami, USA). Animals were maintained 6 per cage, and fed standard rat chow. At 14 months of age, 12 animals were sacrificed by carbon dioxide asphyxiation and prostates removed intact. Tissues representative of the whole prostate were fixed in buffered neutral formalin and routinely processed to paraffin wax.

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Abbreviations: Con-A = Canavalia ensiformis; SBA = Glycine max; DBF = Dolichos biflorus; PNA = Arachis hypogaea; ECG = Erythrina cristagalli; WGA = Triticum vulgaris; UEA-1 = Ulex europaeus-1

Table 1. Binding-specificities of lectins employed in this study

Lectin	Abbr.	Structure recognized	Reference
Concanavalin A (Jack bean)	ConA	Manα1→3[Manα1→6]Manβ1→4GlcNAcβ1→4	[5]
Glycine max (Soya bean)	SBA	α/βGalNAc–	[9]
Dolichos biflorus (Horse Gram)	DBF	GalNAcα1→3GalNAc–	[12]
Arachis hypogaea (Peanut)	PNA	Galβ1→3GalNAc–	[16]
Erythrina cristagalli (Coral tree)	ECG	Galβ1→4GlcNAc–	[15]
Triticum vulgaris (Wheat germ)	WGA	$(GlcNAc\beta1\rightarrow4GlcNAc\beta1-)_n$	[20]
Ulex europaeus I (Gorse)	UEA-I	Fucα1→2Gal(GalNAc–) (Blood Group H(O))	[17]

Table 2. See text

Tissue structure	Before neuraminidase digestion							After neuraminidase digestion			
	Con-A	SBA	DBF	PNA	ECG	WGA	UEA-1	SBA	PNA	ECG	WGA
Epithelial luminal membranes	+++	–	–	++	–	++	–	–	+++	–	–
Epithelial cytoplasm	+++	+++	–	++	–	+++	++	+++	+++	+	–

Lectin histochemistry

All seven lectins employed in this study were obtained native from Sigma (Poole, UK) and are summarised in Table 1. Each was diluted to 10 μg/ml in Tris buffered saline (TBS; pH 7.6) containing 1 mmol each of calcium chloride, magnesium chloride and manganese chloride. TBS was also used for washing. Sections were cut at 3μ, dewaxed in xylene and brought to water. Sites of endogenous peroxidase activity were blocked using methanol (400 ml) containing hydrogen peroxide (3 ml of 100 vol) for 15 min. Sections were then washed in tap water. Lectins were incubated on sections for 60 min at room temperature which were then washed in TBS. Rabbit anti-sera to the lectins (Dako, High Wycombe, UK) were diluted 1:100 in TBS, incubated on sections for a further 60 min at room temperature and washed in TBS. Swine anti-rabbit IgG (Dako) was diluted 1:100 in TBS and incubated on sections for 30 min. After further washing in TBS, rabbit PAP complexes (Dako), diluted 1:200 with TBS, were added for 30 min. Peroxidase activity was localized by addition of 3,3-diaminobenzidine tetrahydrochloride at 250 μg/ml in TBS containing 500 μl of 100 vol (0.4%) hydrogen peroxide per ml for 10 min. Sections were counterstained in Harris' haematoxylin, dehydrated, cleared and mounted. Lectin binding was assessed and graded, according to light microscopic appearances, from negative (–) to strongly positive (+++). Localization of staining to subcellular organelles was defined.

Neuraminidase digestion

In addition, binding of SBA, ECG, WGA and PNA was examined after digestion with neuraminidase (ex Vibrio cholerae; obtained from BDH Laboratories, Dagenham, UK) used at a dilution of 1:10 in 200 mM sodium acetate buffer (pH 5.5) at room temperature for 60 min. Before lectin histochemistry was performed, according to the protocol already described, sections were washed three times in tap water. Binding of the other lectins (Con-A, DBF and UEA-1)was not studied after neuraminidase because these determinants are not masked by sialic acid in normal cells [4].

Normal controls

Paraffin sections of normal human kidney were used as positive controls [14]. For negative controls, the native lectin step was omitted.

Results

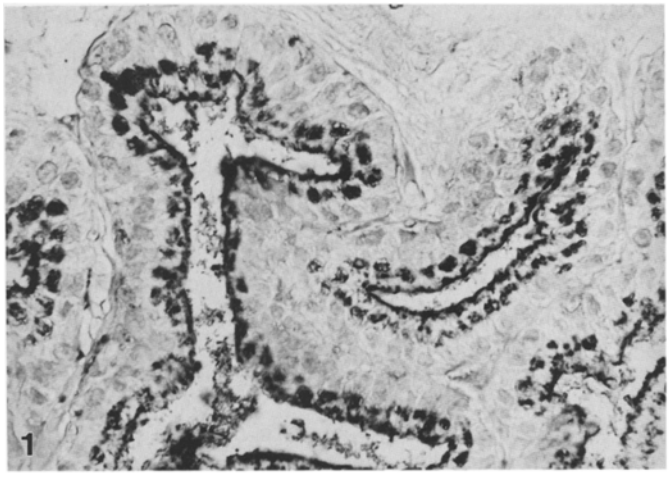
The patterns of lectin-binding to rat normal prostatic epithelial cells, and the oligosaccharide structures identified within these tissues are presented in Table 2. All prostatic epithelial cells bound Con-A and hence expressed complex-type branched mannose core oligosaccharide structures: Manα1→3[Man 1→6]Manβ1→4GlcNAcβ1→4.

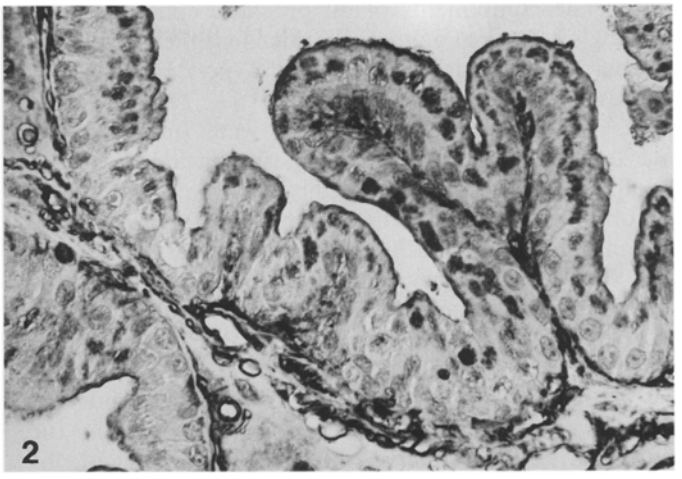
Prior to neuraminidase digestion

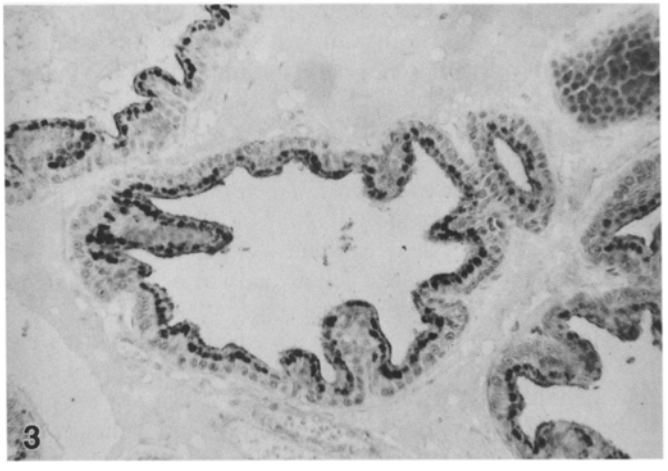
Con-A bound strongly to plasma membranes and to cytoplasmic structures of all epithelial cells. PNA readily identified that the Type I structure: Galβ1→3GalNAc was expressed simultaneously as a luminal plasma membrane determinant as well as a component of intracytoplasmic vesicles, probably Golgi apparatus (Fig. 1). WGA-binding ($(GlcNAc\beta1\rightarrow4GlcNAc\beta1\rightarrow4-)_n$) appeared identical to that of PNA (Fig. 2). Two other oligosaccharide structures: α/βGalNAc-, recognized by SBA binding (Fig. 3) and Fucα1→2Gal (GalNAc), recognized by UEA-1 binding were also expressed by the epithelial cells. However, these latter sequences were only identified within supranuclear cytoplasmic compartments, probably regions within Golgi apparatus and were not identified as luminal membrane determinants. The structure: GalNAcαl→3GalNAc, recognized by DBF and the Type II terminal oligosaccharide sequence: Galβ1→4GlcNAc– recognized by ECG were not demonstrated to occur in rat normal prostatic epithelium under these conditions.

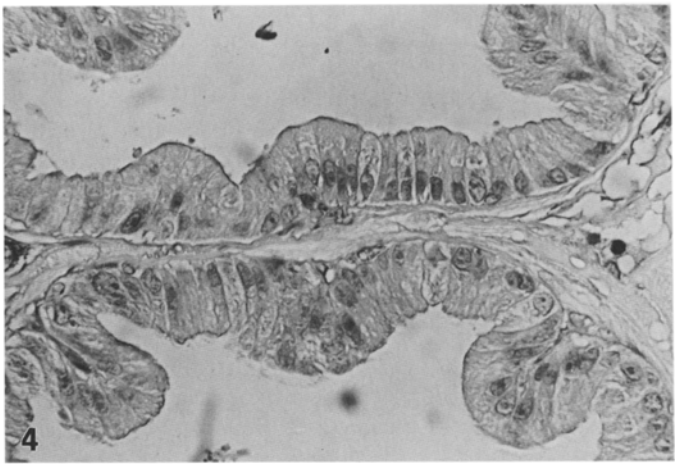
Following neuraminidase digestion

Expression of Galβ1→3GalNAc– (PNA-binding) particularly along luminal plasma membranes, became en-









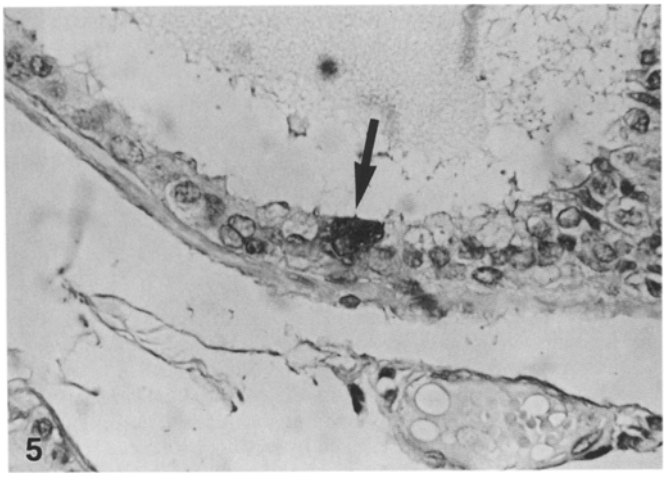


Fig. 1. PNA staining luminal plasma membranes and intracytoplasmic vesicles of all prostatic epithelial cells. The appearances with and without neuraminidase digestion were identical (×1,500)

Fig. 2. WGA staining (without neuraminidase digestion) of luminal plasma membranes and intracytoplasmic vesicles of all epithelial cells.These appearances are similar to those observed for PNA, but were abolished after neuraminidase digestion (×1,500)

Fig. 3. SBA staining (without neuraminidase digestion) of intracytoplasmic vesicles within all the epithelial cells. Membrane staining and stromal staining are absent (×500)

Fig. 4. WGA staining (after neuraminidase digestion) was absent (×1,500)

Fig. 5. ECG staining (after neuraminidase digestion) distributed throughout the cytoplasm of individual cells (*arrow*) (×1,750)

hanced. However, all WGA-binding was abolished by this procedure (Fig. 4). The lectin ECG was now observed to bind to a very few epithelial cells within each of the prostatic glands (Fig. 5) thus indicating the presence of structure: Galβ1→4GlcNAc- in all prostates examined. The pattern of ECG-binding was diffuse throughout the cytoplasm of these few cells and was localized neither to plasma membranes nor to intracytoplasmic vesicles. Without lectin-binding, epithelial cells binding the lectin ECG were morphologically indistinguishable from adjacent negative cells. The distribution of SBA-binding-sites (α/βGalNAc-) was not altered by neuraminidase digestion.

Discussion

The studies described herein reveal adult rat normal prostatic epithelium to synthesize two distinct groups of cellular glycoconjugates: plasma membrane-associated and cytoplasmic. Concanavalin A recognizes the core structure [Manα1→3]Manβ1→4GlcNAcβ1→4) of complex oligosaccharides and bound to cytoplasmic and

membrane components of all prostatic epithelial cells. The major terminal oligosaccharide identified on luminal plasma membranes was the Type I structure: (Galβ1→3GalNAc–) recognized by PNA. These determinants were freely expressed and were only partially masked by sialic acid. Type II oligosaccharide structures: (Galβ1→4GlcNAc–) recognized by ECG were not detected on luminal plasma membranes but were detected within the cytoplasm of a very small number of cells and only then following neuraminidase digestion. The apparent absence of Type II oligosaccharide structures from luminal plasma membranes is supported by the observation that UEA-1, known to recognize Fucα1→2Gal(GalNAc)- structures on Type II but not Type I backbones [22] was present only within the cytoplasm of the epithelial cells. The significance of this restricted oligosaccharide expression on luminal plasma membranes, as defined by ECG, is not presently apparent, but may indicate a sub-population of prostatic epithelial cells of novel and specific phenotype. Although WGA-binding (GlcNAcβ1→4GlcNAcβ1→4–)$_n$ was also present on luminal plasma membranes, this ligand does not discriminate between Type I and Type II structures.

In addition to Con-A and PNA, the group of cytoplasmic glycoconjugates comprised: α/βGalNAc– recognized by SBA (GlcNAcβ1→4GlcNAcβ1→4–)$_n$ recognized by WGA and Fucα1→2Gal(GalNAc) recognized by UEA-1 binding. Since only Type I oligosaccharides have been identified within the vast majority of non-neoplastic prostatic epithelial cells, it is most likely that these latter three groups represent specific modifications to this basic backbone structure.

Oligosaccharide structures such as those recognized by SBA and UEA-1, (α/βGalNAc– and Fucα1→2Gal(GalNAc)–) respectively, are known to function as terminators to glycoconjugate oligosaccharide sequences [11, 24]. Therefore, identification within Golgi apparatus, but not on plasma membranes of the same cells, suggests that these two sequences might be involved in modulating the intracellular trafficking of cell-surface glycoconjugates within prostatic epithelium. For example, cleavage of these oligosaccharides in the trans-Golgi, or within post-Golgi vesicles, could allow completion of glycoprotein transit from the site of synthesis of the protein moieties at the rough endoplasmic reticulum through glycosylation in the Golgi apparatus to insertion into plasma membranes. A similar regulatory role for the sugar identified by WGA is less certain because neither the natural biological structure identified by the lectin, nor its relationship to sialic acid, have yet been confirmed precisely. In particular, it is now accepted that sialic acid groups do not comprise an integral part of the determinant identified by WGA [19] although it is recognized that there is a requirement for sialic acid residues to be in close proximity to the dominant oligosaccharide structure: (GlcNAcβ1→4GlcNAcβ1→4–)$_n$ in order that WGA binding might occur. However, partial sialylation of the structure identified by PNA (Galβ1→3GalNAc–) at the plasma membrane suggests a further control of expression and hence modulation of the function of the associated intact glycoconjugates.

Current work in this laboratory suggests lectin binding sites differ between species. We have previously reported the distribution of lectin binding sites in formalin-fixed and paraffin-wax embedded sections of adult human prostate [2]. Con A, WGA and PNA bound to over 90% and DBA to less than 10% of human prostate epithelial cells, a result similar to the present work. In contrast, UEA-1 and SBA binding sites were detected in less than 10% of human prostate cells. Other investigators [6] have shown PNA binding to prostatic epithelium of mature humans was high. However, before puberty and in old age PNA-binding was low, suggesting the possibility of an association with levels of circulating testosterone.

A further refinement to our present technique would be the concurrent study of frozen material [1]. Tissues processed through formalin-fixation and subsequent routine paraffin-wax embedding undergo extraction with lipid solvents. Whilst glycoproteins are, almost certainly, unaffected by these processes, glycolipid components may be variably extracted [25]. It is a possibility that DBF binding sites (GalNAcα1→3GalNAc–) are components only of glycolipids – which would explain why they were undetected in the present work. An additional factor is the possibility that expression of some oligosaccharide determinants are cell-cycle dependent – either being expressed only during the S-phase of cell division or not at all during this period.

Alterations in cell surface glycoconjugates are now recognized to occur during cellular ontogenesis and oncogenesis [24]. Strong evidence indicates these changes to be associated with the control of tissue morphogenesis as well as influencing the biological behaviour of tumour cells [3]. The results of the work reported herein provide a firm basis for further studies to define the altered expression of cell-surface oligosaccharide structures during ontogenesis and oncogenesis in the rat prostate.

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