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Table 1. Sulphotransferase activity in cell extracts

<table>
<thead>
<tr>
<th>Cell line</th>
<th>N-Sulphotransferase (c.p.m. min⁻¹ μg⁻¹ of protein)</th>
<th>O-Sulphotransferase (c.p.m. min⁻¹ μg⁻¹ of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>210C</td>
<td>53.7</td>
<td>8.3</td>
</tr>
<tr>
<td>215CSC</td>
<td>39.4</td>
<td>6.2</td>
</tr>
<tr>
<td>219CT</td>
<td>36.2</td>
<td>5.9</td>
</tr>
</tbody>
</table>

The factors controlling distribution of sulphate groups along the chain are poorly understood. Structural and some biosynthetic evidence shows that O-sulphation and epimerization reactions occur mainly at regions containing N-sulphate groups (Feingold et al., 1981). It is currently believed that N-sulphation in turn is controlled by activity of the de-N-acetylase. It is probable that the enzymes involved in biosynthesis of heparan sulphate are arranged in a multi-enzyme complex, and therefore the organization of this complex might control the final structure of the product. If such were the case, the alteration in tumour cell heparan sulphate might be due to disruption of the normal organization of this complex in the Golgi apparatus.

Proteoglycans in cellular differentiation and neoplasia

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The extracellular matrix is a dynamic, highly organized, mechanically cohesive system that maintains the overall structure and form of organs and tissues and also profoundly influences the behavioural properties of the cells associated with it. Proteoglycans are suitably located to mediate the cell regulatory functions of the external domain since they are constituents of both the stromal matrix itself and the cell-surface membrane. The questions then arise of how supramolecular aggregates elicit specific cellular responses.

Molecular affiliations of proteoglycans

Proteoglycans are a special class of glycoproteins in which long, linear, glycosaminoglycan chains are linked covalently to protein (Lindahl & Hook, 1978). Several types of proteoglycan are recognized on the basis of these polysaccharide substituents. The chondroitin sulphate proteoglycans of cartilage represent the largest molecular size class of proteoglycan and they bind specifically with hyaluronic acid and link proteins to produce large complexes which retain water and expand the collagen fibre network (Hcasall & Heinegard, 1974; Hardingham, 1979). Dermatan sulphate proteoglycans and heparan sulphate proteoglycans contain fewer glycosaminoglycan chains than chondroitin sulphate derivatives but the sugar units are more variable in structure and contain, in addition to glucuronic acid found in chondroitin sulphate, sulphated iduronic acid, and, in the case of heparan sulphate, N-sulphated glucosamine residues. These copolymeric chains play an important role in the interaction of the proteoglycans with other extracellular matrix glycoproteins such as collagen, laminin and fibronectin (Obrink, 1972; Timpl et al., 1985; Yamada, 1983). Matrix glycoproteins have distinct structural regions for binding proteoglycans and the ensuing patterns of association yield a molecular skeleton at the cell periphery. For example, at the surface of cultured cells, heparan sulphate and fibronectin co-distribute to form an ordered fibrillar network (Hayman et al., 1982). Interactions between heparan sulphate and laminin have been proposed as essential features of a functional basement membrane (Timpl et al., 1983) and an abnormally high rate of heparan sulphate degradation may explain structural imperfections in the basal laminae produced by breast tumour cells in culture (David & Bernfield, 1982). Heparan sulphate and dermatan sulphate glycosaminoglycans of cog-
nate structure self-associate under experimental conditions and these properties may be relevant in vivo in the context of cell-cell and cell-matrix recognition (Fransson et al., 1981). In detailed electron microscopic analyses of the association of collagen and proteoglycan in rat tail tendon, Scott and coworkers reinforced the concept of an orderly extracellular matrix by visualizing the molecular 'morphology' of the binding sites (Scott et al., 1981); dermalat sulphate proteoglycans form orthogonal arrays along the collagen fibre axis with a specific recognition site for the transverse elements at the 'd' band in the gap zone (Scott & Orford, 1981). Human skin fibroblasts also produce a collagen-binding dermalat sulphate of high iduronic acid content (Gallagher et al., 1983a), which may be similar to that found in tendon. Recent fine structural analyses of various dermalat sulphate species has shown that collagen-binding properties are associated with repeat sequences of greater than 25 iduronic acid residues and some collagen-binding chains contain up to 80 iduronic acid repeats representing virtually the entire hexuronic acid content of the polymer (Hampson & Gallagher, 1983).

Proteoglycans in cell development

In embryogenesis proteoglycans have reciprocal functions with hyaluronic acid. Stroma rich in hyaluronate are associated with the extracellular matrix. In vitro, the presence of proteoglycans in the pericellular matrix of progenitor cells to their final sites of maturation (Hay, 1981). Migration arrest and subsequent cell development is accompanied by degradation of hyaluronic acid and a concurrent rise in the concentration of proteoglycan (Toole, 1976). However, the above generalizations may not apply to the highly disorganized tissues of the vascular and nervous systems where specific classes of proteoglycan may provide a guide path along which cell movement and development may occur (Collins & Garrett, 1980; Ausprunk et al., 1981). Hyaluronate inhibits vascularization but hyaluronic oligosaccharides produced by hyaluronidase digestion stimulate blood- vessel formation (D. West, S. Anes & S. Kamur, personal communication), suggesting a mechanism for ensuring a blood supply to forming tissues once the immature cells have migrated to their developmental sites.

In the nervous system, the development of cellular interactions and the expression of neuronal cell functions are closely correlated with the metabolism and distribution of heparan sulphate. The synthesis of heparan sulphate increases selectively during post-natal development of the brain (no changes occur in other glycosaminoglycans) and the timing of the increase is associated with, and may be associated with, the acquisition of a functional neural transmitter system (Margolis et al., 1975). Heparan sulphate proteoglycan promotes neurite outgrowth in culture and it is very significant that the concentration of neuronal cell surface heparan sulphate increases considerably during growth of ganglionic neurones and dendritic expansions (Grief & Reichardt, 1982). Other evidence implicating heparan sulphate in nervous tissue physiology stems from studies on mucopolysaccharides in which defects of heparan sulphate degradation (Hunter & Hurler syndromes) cause severe neurological dysfunction, whereas lesions in chondroitin or dermatan sulphate metabolism lead primarily to skeletal abnormalities (Dorfman & Matalon, 1972).

The pericellular matrix of tumour cells

In cultured cells transformed by oncogenic viruses, immunological and biochemical studies have demonstrated the loss of laminin, fibronectin, collagen and heparan sulphate from the pericellular region (Alitalo et al., 1982; Hayman et al., 1982). During matrix biogenesis, the order in which the different constituents are laid down is unknown but, by virtue of its capacity to associate specifically with fibronectin and laminin, heparan sulphate could be a key molecule in the formation of lateral interactions. Possibly the reduced 'O'-sulphate content of transformed-cell-derived heparan sulphate (Winterbourne & Mora, 1981) impairs its ability to 'organize' other matrix constituents. In view of the proposed role of the extracellular matrix in the regulation of cell growth and differentiation, the absence of an ordered micro-environment around tumour cells is essential for the full expression of the malignant phenotype.

Heparan sulphate seems to be a key target in the disruption of the subendothelial basil laminae by metastatic tumour cells which apparently secrete a glycosidase with specificity for this glycosaminoglycan (Kramer et al., 1982). The release of such an enzyme might be causally related to the loss of an identifiable matrix from transformed cells in culture.

β-D-Xylosides as regulators of proteoglycan synthesis and cell development

Although the evidence implicating the extracellular matrix in regulating cell behaviour is very strong, the mechanistic aspects of the controlling events are unknown. Use of β-D-xylosides has emphasized the importance of proteoglycans in such regulatory processes. In most native proteoglycans xylose is linked to serine residues in the protein core of the proteoglycan biosynthesis these xylose substituted serine residues are the endogenous sites for the assembly of glycosaminoglycan chains. β-D-Xylosides act as competitive acceptors for the initiation and polymerization of glycosaminoglycans (Robinson et al., 1975). In so doing, β-D-xylosides stimulate the synthesis of free glycosaminoglycan chains (which are chondroitin sulphate) and inhibit proteoglycan synthesis. However, their inhibitory effects are not uniform. Proteoglycans distributed to the culture medium by human skin fibroblasts and mouse bone marrow cells are almost completely inhibited by β-D-xylosides, whilst proteoglycans associated with the cell surface membrane are little affected (Gallagher et al., 1983b). The collagen-binding dermalat sulphate proteoglycans produced by human skin fibroblasts grown on collagen gels are only partially inhibited by β-D-xylosides, whereas corresponding collagen-binding proteoglycans produced by identical cells grown on plastic (and released into the culture medium in the absence of the collagenous substrate) are strongly inhibited. These studies show that separate compartments for proteoglycan biosynthesis exist which are responsive to the micro-environment and which represent precursor pools destined for different topographical locations on and around the cell.

Bearing in mind the selective nature of the inhibitory effects of β-D-xylosides on proteoglycan synthesis, it is intriguing to note that these low molecular weight glycosides have a powerful influence on cell development. They inhibit specifically the maturation of sea urchin embryos at the blastula stage by blocking the synthesis of a proteoglycan which binds to nuclear chromatin in a process essential for progression to the gastrula (Kinoshita & Saiga, 1979). Although chondrogenesis of chick limb bud mesenchyme proceeds normally in the presence of β-D-xylosides the matrix is depleted in chondroitin sulphate proteoglycans and the tissue reduced in size (Subramander et al., 1979). β-D-Xylosides also inhibit branching morphogenesis of mouse salivary gland cultures without affecting cell proliferation (Thompson & Spooner, 1983). In contrast to the foregoing results, all observed in embryonic tissues, β-D-xylosides have the surprising effect of stimulating the haemopoietic activity of mouse bone marrow cultures derived from adult animals (Scoopncer et al., 1983). In this system immature and mature haematopoietic cells were equally stimulated and the available data suggested that the primary target for xylsode
action was the pluripotential haemopoietic stem cell (CFU-S). Whenever they were tested, free glycosaminoglycan chains were unable to reproduce β-D-xylodine influences on cell behaviour. On the basis of the cited biochemical evidence it seems likely that the xylodine effects are mediated through alterations in surface membrane proteoglycans. Most probably the xylodine-induced changes follow from a reduction in proteoglycan concentration in the extracellular matrix and/or the associated tissue fluids. It should also be remembered that, in contrast to the humble sea urchin, xylodine influences on the formation of nuclear proteoglycans in mammalian systems have not been investigated and represent an important area for future studies. Nevertheless, the use of β-D-xylodines has emphasized the great significance of proteoglycans in normal cellular proliferation and morphogenesis and these sugar derivatives will remain powerful tools for further experiments at the molecular and cellular levels.


Carbohydrate differentiation and cancer-associated antigens detected by monoclonal antibodies

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During differentiation and oncogenic transformation the structures of complex carbohydrates on the cell surface change as a result of a modulation of the levels of glycosyltransferases. Many monoclonal antibodies which detect differentiation or transformation antigens are directed against these carbohydrates. Of 325 monoclonal antibodies that we have obtained from different laboratories, 97 bind carbohydrates (Table 1).

Cell-surface carbohydrates can exist in either glycoproteins or glycolipids and in many cases identical oligosaccharides are found on both types of molecules. As glycolipids have a relatively simple structure and contain one hapten oligosaccharide, they are ideal for the chemical characterization of cell-surface-carbohydrate antigens.

A gastrointestinal cancer-associated antigen detected by monoclonal antibody 19-9 is a glucose-containing sialylated lacto-N-fucopentaose II in colorectal carcinoma cell line SW 1116

Monoclonal antibody 19-9, produced by hybridomas obtained from mice immunized with a human colon adenocarcinoma cell line, has an apparent specificity for human intestinal tumours (Koprowski et al., 1979). Binding of this antibody to tumour cells is inhibited by serum from most patients with pancreatic and gastrointestinal cancer, but not by the serum of normal individuals, patients with inflammatory bowel diseases, or most patients with other malignancies (Herlyn et al., 1982). The antigen for this antibody in the cell line used for immunization is a monosialo-ganglioside (Magnani et al., 1982) and was purified with the aid of a method we developed that allows the visualization of glycolipid antigens by direct binding of radioactive antibodies to thin-layer chromatograms followed by autoradiography (Magnani et al., 1981, 1982). About 30 μg of ganglioside antigen is obtained from 1 g wet weight of tissue culture cells.

The structure of the carbohydrate was determined by methylation analysis, and combined gas chromatography and mass spectroscopy of the triluroacetylated derivative (Magnani et al., 1982), to be:

\[
\text{NeuNac} 2\rightarrow3\text{Gal} 4\underset{\text{Fuc}}{\overset{\text{Fucz}}{\text{Fuc}}} 3\text{Glc} \]

This is a sialylated derivative of the normal Le\(^+\) blood group-active pentasaccharide, lacto-N-fucopentaose II. Both fucose and sialic acid are immunodominant sugars as neither lacto-N-fucopentaose II nor LS-tetrasaccharide a (the defucosylated stalyoligosaccharide) bind the antibody.