Heparan sulphate alterations in tumour cells

DAVID J. WINTERBOURNE and ZEENA KHAN
Department of Biochemistry, St. George’s Hospital Medical School, Cranmer Terrace, London SW17 0RE, U.K.

Heparan sulphate proteoglycan is widely distributed, being found inserted into plasma membranes of most cells (Kjellen et al., 1981; Norling et al., 1981) and as a significant component of basement membranes (Kanwar et al., 1980; Vlodavsky et al., 1980) and regulation of enzyme activity [e.g. lipoprotein lipase (Shimada et al., 1981; Cheng et al., 1981) and serine proteases (Colburn & Buonassissi, 1982)]. These functions could underlie the role that heparan sulphate appears to play in growth control.

Heparan sulphate obtained from cells transformed by viruses has consistently been found to have a lower degree of sulphation than the glycosaminoglycan obtained from control cells (Underhill & Keller, 1975; Winterbourne & Mora, 1980; Frolinson et al., 1981). A similar defect has been observed when heparan sulphate from both rat and human hepatocytes has been compared with normal hepatocytes (Nakamura et al., 1978; Nakamura & Kojima, 1981).

In our studies we have used established mouse cell lines which have overcome the normal restrictions on indefinite growth in vitro. Such immortalized cells have progressed along the multi-step route to carcinogenesis to a stage where growth in vitro. Such immortalized cells have progressed into the medium and material isolated from trypsinized cells. These results show that reduced sulphation of heparan sulphate is increased because these reactions do not go to completion. Sulphate and iduronic acid residues are not distributed regularly along the chain, but occur in highly substituted stretches separated by varying lengths of unmodified polysaccharide chain.

Structural analysis of the heparan sulphate from the medium and material isolated from trypticized cells. These results show that reduced sulphation of heparan sulphate is closely associated with the progression to highly tumourigenic cells.

At least five enzymes are involved in the maturation of the glycosaminoglycan side chains of heparan sulphate. The concerted action of these enzymes result in the introduction of N- and O-sulphate groups on glucosamine residues and 2-O-sulphate groups on iduronic residues (which arise by epimerization of glucuronic acid residues within the polymer) (Feingold et al., 1981). The structural complexity of heparan sulphate is increased because these reactions do not go to completion. Sulphate and iduronic acid residues are not distributed regularly along the chain, but occur in highly substituted stretches separated by varying lengths of unmodified polysaccharide chain.

Structural analysis of the heparan sulphate from the cell surface of control and tumour cells demonstrated that the reduced affinity for DEAE-cellulose of tumour cell heparan sulphate arose from a reduction in the degree of O-sulphation with unchanged or even increased N-sulphation (Winterbourne & Mora, 1981). The reduction occurred in 6-O-sulphate groups located in regions of alternating N-sulphated and N-acetylated glucosamine residues. Excision of these regions by nitrous acid, which cleaves the polysaccharide at N-sulphated glucosamine residues, yields a variety of tetrasaccharides. It is within the group of monosulphated tetrasaccharides that the defect was located (Winterbourne & Mora, 1981). These results were confirmed in the analysis of the heparan sulphate from the eight recently isolated cell lines (not shown).

Such subtle changes in the structure of heparan sulphate have a marked affect on the properties of the glycosaminoglycan as judged by the inability of these molecules to self-associate (Fransson et al., 1981) and their reduced affinity for cell-surface receptors (Winterbourne, 1982). These alterations might be involved in defective cell-cell or extracellular matrix interactions, possibly resulting in the reduced capacity of transformed cells to organize an extracellular matrix at their surface (Norling et al., 1981; Alitalo et al., 1982).

Where altered sulphation of proteoglycans has been reported previously, the increased (Conrad & Woo, 1980) or decreased (Sugahara & Schwartz, 1979) sulphation has been assigned to alterations in levels of enzymes involved in sulphate activation when assayed in vitro, suggesting that defective sulphation in tumour cells occurs by a different mechanism from that previously reported. In agreement with this, the specific activities of both N- and O-sulphotransferases in the tumour- (210CT) and virus-transformed (215SCSC) cells were reduced by 30% compared with control cells (210C, Table 1).

Analysis of the heparan sulphate produced by two such variants, isolated from separate clones, demonstrated that in both cases the glycosaminoglycan was reduced in sulphation (Winterbourne & Mora, 1981). More recently we have repeated these studies with eight tumour cell lines isolated separately from one of the clones. In each case the heparan sulphate bound less strongly to DEAE-cellulose than the heparan sulphate from the parent clone. A typical result for cell surface glycoconjugates is shown in Fig. 1. The same alteration was observed in heparan sulphate secreted into the medium and material isolated from trypticized cells. These results show that reduced sulphation of heparan sulphate is closely associated with the progression to highly tumourigenic cells.

At least five enzymes are involved in the maturation of the glycosaminoglycan side chains of heparan sulphate. The concerted action of these enzymes result in the introduction of N- and O-sulphate groups on glucosamine residues and 2-O-sulphate groups on iduronic residues (which arise by epimerization of glucuronic acid residues within the polymer) (Feingold et al., 1981). The structural complexity of heparan sulphate is increased because these reactions do not go to completion. Sulphate and iduronic acid residues are not distributed regularly along the chain, but occur in highly substituted stretches separated by varying lengths of unmodified polysaccharide chain.

Structural analysis of the heparan sulphate from the cell surface of control and tumour cells demonstrated that the reduced affinity for DEAE-cellulose of tumour cell heparan sulphate arose from a reduction in the degree of O-sulphation with unchanged or even increased N-sulphation (Winterbourne & Mora, 1981). The reduction occurred in 6-O-sulphate groups located in regions of alternating N-sulphated and N-acetylated glucosamine residues. Excision of these regions by nitrous acid, which cleaves the polysaccharide at N-sulphated glucosamine residues, yields a variety of tetrasaccharides. It is within the group of monosulphated tetrasaccharides that the defect was located (Winterbourne & Mora, 1981). These results were confirmed in the analysis of the heparan sulphate from the eight recently isolated cell lines (not shown).

Such subtle changes in the structure of heparan sulphate have a marked affect on the properties of the glycosaminoglycan as judged by the inability of these molecules to self-associate (Fransson et al., 1981) and their reduced affinity for cell-surface receptors (Winterbourne, 1982). These alterations might be involved in defective cell-cell or extracellular matrix interactions, possibly resulting in the reduced capacity of transformed cells to organize an extracellular matrix at their surface (Norling et al., 1981; Alitalo et al., 1982).

Where altered sulphation of proteoglycans has been reported previously, the increased (Conrad & Woo, 1980) or decreased (Sugahara & Schwartz, 1979) sulphation has been assigned to alterations in levels of enzymes involved in sulphate activation when assayed in vitro, suggesting that defective sulphation in tumour cells occurs by a different mechanism from that previously reported. In agreement with this, the specific activities of both N- and O-sulphotransferases in the tumour- (210CT) and virus-transformed (215SCSC) cells were reduced by 30% compared with control cells (210C, Table 1).

![Fig. 1. Trypsin-released glycoconjugates from control cells and a highly tumourigenic variant](image-url)

The control clone (210C) (-----) was labelled with 5 µCi/ml [3H]glucosamine and one of the tumourigenic variants (230CT) (-----) with 1 µCi/ml [14C]glucosamine for 24h. Material released from the washed cell sheet by trypsin was applied directly to a 1 cm x 4 cm column of DEAE-cellulose, washed with 0.2M-NaCl in 10mM-Tris/HCl, pH 8.4, until fraction 16, and then eluted with a linear gradient of 0.2-0.6M-NaCl in Tris buffer. Aliquots of 1 ml were counted from each 4ml fraction. Under these conditions most of the heparan sulphate elutes between fractions 33 and 43.
**Proteoglycans in cellular differentiation and neoplasia**

JOHN T. GALLAGHER* and IAN N. HAMPSON†

*CRC Department of Medical Oncology and †Clinical Research, Christie, Hospital, Wilmslow Road, Manchester M20 9BX, U.K.

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 recognizably expressed on the basis of these poly-saccharide substitents. 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 (Hascall & 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 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., 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-

Table 1. Sulphotransferase activity in cell extracts

<table>
<thead>
<tr>
<th>Cell line</th>
<th>N-Sulphotransferase (c.p.m. min⁻¹ µg⁻¹)</th>
<th>O-Sulphotransferase (c.p.m. min⁻¹ µg⁻¹)</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-acetylas. 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.