Effect of benzyl β -D-xyloside on the biosynthesis of chondroitin sulphate proteoglycan in cultured human monocytes

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Monocytes isolated from human blood differentiate into macrophage-like cells when maintained *in vitro* for 3–5 days on plastic or glass culture dishes. In the process the cells display characteristic morphological changes, and in addition, a transition in glycosaminoglycan biosynthesis, from the production of chondroitin 4-sulphate to the formation of a polysaccharide containing 20% 4,6-disulphated disaccharide units [Kolset, Kjellén, Seljelid & Lindahl (1983) *Biochem. J.* **210**, 661–667]. Cells were incubated with inorganic [35 S]sulphate on day 1 or day 6 in culture, in the presence or absence of benzyl β -D-xyloside, and labelled polysaccharide was isolated from the culture medium. In the presence of xyloside, the secretion of proteoglycans (90% galactosaminoglycan) was inhibited in a dose-dependent fashion and replaced by release of single polysaccharide chains, the size of which decreased with increasing dose of xyloside. The single polysaccharide chains produced on day 6 in the presence of 0.5 mM-xyloside showed the same proportion of disulphated disaccharide units as did the corresponding control material. Day-1 polysaccharide contained negligible amounts of this component, irrespective of the presence or absence of xyloside. It is concluded that the regulatory mechanism that induces 'oversulphation' during the differentiation process operates independently of any association between the polysaccharide chains and the core protein. Moreover, cells maintained in the presence of 0.5 mM-xyloside throughout a 6-day culture period showed the same morphological change, indicative of differentiation into macrophage-like cells, as did untreated control cells. The xyloside did not significantly affect the cytotoxicity of the monocytes, or of the differentiated macrophage-like cells, toward tumour cells.

INTRODUCTION

The biological functions (or biological effects) of glycosaminoglycans generally depend on the polyelectrolyte properties of these molecules, and often involve ionic binding of other macromolecules (Lindahl & Höök, 1978). Such interactions may vary in specificity, from relatively nonspecific co-operative electrostatic binding, to highly specific 'lock and key' binding. In the latter case, binding involves a strictly defined region in the polysaccharide molecule, and will depend on the structural integrity of this region to the extent that even minor modifications may abolish the interaction. One example of such binding is the interaction between heparin and antithrombin, which requires the presence of at least four sulphate groups in certain positions within a pentasaccharide sequence (Lindahl *et al.*, 1983).

In glycosaminoglycan (proteoglycan) biosynthesis the formation of polysaccharide chains is initiated by xylosylation of preformed (or nascent) polypeptide core (Rodén, 1980). Sulphate groups are incorporated after formation of the polysaccharide backbone, sometimes in concert with other polymer-modification reactions. The regulation of these reactions, as required to form polysaccharide sequences of specific structure, is enigmatic; in particular, the role of the core protein has attracted considerable attention. Exogenous β -D-xylosides will act as artificial initiators of polysaccharide chain elongation, in competition with the endogenous, xylosyl-

ated core protein (Okayama *et al.*, 1973; Schwartz *et al.*, 1974; Robinson *et al.*, 1975). Since biosynthesis induced by these compounds will by-pass the formation of core protein, the process should be insensitive to any regulatory mechanisms that depend on the integrity of the proteoglycan structure. In the present study a xyloside has been used according to this concept to probe the regulation of chondroitin sulphate biosynthesis during the differentiation *in vitro* of human monocytes into macrophage-like cells. In a previous study (Kolset *et al.*, 1983) we found that this cellular transition, which occurred over a period of about 4 days, was accompanied by a change in polysaccharide biosynthesis, such that the chondroitin 4-sulphate produced during the first day in culture was replaced by a more highly sulphated species that contained an appreciable proportion of 4,6-disulphated *N*-acetylgalactosamine residues. We now report that this distinct change in sulphation pattern will occur irrespective of whether the polysaccharide chains during biosynthesis are bound to the endogenous core protein or to an exogenous xyloside.

EXPERIMENTAL

Materials

Monocytes were isolated from human blood and cultured in 16 mm Costar wells (1×10^6 cells/well) as described previously (Kolset *et al.*, 1983). HeLa cells

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derived from human cervical carcinoma cells maintained in RPMI containing 10% fetal calf serum (Gibco Bio-Cult, Paisley, Renfrewshire, Scotland, U.K.). Human melanoma (MDA) cells were obtained from Dr. Ø. Fodstad of the Norwegian Radium Hospital, Oslo, Norway, and were cultured in MEM (minimum essential medium) supplemented with 10% fetal calf serum.

The glycosaminoglycan and oligosaccharide preparations used have been described previously (Kolset *et al.*, 1983). In addition, chondroitin 4-sulphate reference standards of known M_r (Wasteson, 1971) were given by Dr. Å. Wasteson, University of Uppsala, Uppsala, Sweden. Benzyl β -D-xyloside was a gift from Dr. H. C. Robinson, Monash University, Melbourne, Australia. Sepharose CL-6B, Sephadex G-50 and DEAE-Sephacel were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden; bacterial chondroitinases AC (chondroitin AC lyase, EC 4.2.2.5) and ABC (chondroitin ABC lyase, EC 4.2.2.4) from Seikagaku Fine Chemicals, Tokyo, Japan, and testicular hyaluronidase (EC 3.2.1.35) from AB Leo, Helsingborg, Sweden. Inorganic [35 S]sulphate (carrier-free) and [3 H]thymidine were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

Methods

Scanning electron microscopy of monocytes was performed as described previously (Kolset *et al.*, 1984), except that the cells were kept on glass cover slips.

In cytotoxicity assays HeLa and MDA tumour cells were used as target cells, after labelling with [14 C]thymidine (0.5 μ Ci/ml) for 24 h. The cells were washed, detached by trypsinization [0.05% trypsin in phosphate-buffered saline (0.14 M-NaCl/2 mM-KCl/8 mM- Na_2HPO_4 /1.5 mM- KH_2PO_4 , pH 7.4) containing 1 mM-EDTA], washed again (by centrifugation), and finally suspended in the same medium (2×10^5 cells/ml) used to culture the monocytes. After adding prelabelled, viable target cells (2×10^4 cells/well) to monocyte/macrophage cultures, medium samples of 50 μ l were collected and analysed for radioactivity (indicating cell lysis; Kaplan, 1983). The values given in Figs. 6 and 7 correspond to single wells of monocyte/tumour cell co-cultures, and are corrected for the (almost negligible) release of radioactivity from target cells cultured in the absence of monocytes.

For biosynthetic labelling of glycosaminoglycans, cells were incubated for 24 h in sulphate-depleted medium supplemented with inorganic [35 S]sulphate (50 μ Ci in 1 ml of medium/well), in the absence or presence of benzyl β -D-xyloside. Labelled polysaccharide was isolated from the cell fractions or from culture media by proteolytic digestion followed by gel chromatography on Sephadex G-50 (Kolset *et al.*, 1983). Alternatively, samples were treated with 1% SDS at 100 °C for 3 min and analysed by gel chromatography on Sepharose CL-6B.

Digestions of glycosaminoglycans with chondroitinase AC or ABC were performed as described previously (Kolset *et al.*, 1983). For digestion with testicular hyaluronidase the labelled polysaccharide, mixed with 0.5 mg of chondroitin 4-sulphate internal standard, was incubated with 50 units of enzyme in 0.5 ml of 0.15 M-sodium acetate/0.2 M-NaCl, pH 5.5, at 37 °C for 20 h.

Cleavage of proteoglycans by alkaline β -elimination,

to yield single polysaccharide chains, was achieved by treatment with 0.5 M-NaOH at room temperature for 15 h.

Hexuronic acid was determined by the carbazole method of Bitter & Muir (1962). Radioactivity was determined in a Packard model 2405 liquid-scintillation spectrometer. Additional analytical methods are described in the legends to Figures.

RESULTS

Effects of benzyl β -D-xyloside on proteoglycan biosynthesis

The incorporation of [35 S]sulphate into polysaccharide increased with increasing concentration of benzyl β -D-xyloside in the culture medium. In the presence of 0.5 mM-xyloside the overall formation of labelled polysaccharide in cultures maintained for 1 day (monocytes) and for 6 days (macrophage-like cells) was stimulated about 2-fold and 5-fold, respectively. Selective degradation of the products by deamination with nitrous acid or digestion with chondroitinase ABC (Kolset *et al.*, 1983) indicated > 90% galactosaminoglycan in xyloside-treated as well as in control cultures.

Macromolecular properties of [35 S]galactosaminoglycans. Previous studies showed that the 35 S-labelled polysaccharide released by the macrophage-like cells (or monocytes) into the culture medium occurred almost exclusively in proteoglycan form (Kolset & Kjellén, 1986). Gel chromatography of such material on Sepharose CL-6B accordingly showed an essentially single peak of labelled material (proteoglycan), which was quantitatively replaced by a more retarded component (free polysaccharide chains) after treatment with alkali, under conditions known to cleave the polysaccharide-protein linkage (Fig. 1a). In the presence of increasing concentrations of xyloside, polysaccharide synthesis was progressively shifted from the assembly of proteoglycan molecules to the generation of single polysaccharide chains (Figs. 1b–1d). Already at 0.01 mM-xyloside concentration the latter components accounted for about two-thirds of the total polysaccharide formed, and 0.5 mM-xyloside practically eliminated proteoglycan formation. The results illustrated in Fig. 1 pertain to macrophage-like cells, labelled on day 6 in culture. Similar results were obtained with monocytes, labelled on day 1 (results not shown), except that proteoglycan formation was less susceptible to inhibition by xyloside, and accounted for 30% of total polysaccharide biosynthesis even at 0.5 mM-xyloside concentration.

Fig. 1 also illustrates a striking effect of the xyloside on the molecular size of the newly-synthesized polysaccharide. At low xyloside concentration (0.01 mM), the polysaccharide chains formed on the artificial initiator apparently attained the same size (Fig. 1b) as the constituent chains of the proteoglycans in the untreated control cultures (Fig. 1a). However, at higher xyloside concentrations the resulting single polysaccharide chains were of progressively smaller size (Figs. 1c and 1d). Molecular sizes calculated after calibration of the column with well-characterized chondroitin 4-sulphate standards (Wasteson, 1971) showed a > 4-fold decrease in M_r , from 23600 for the polysaccharide chains of proteoglycans synthesized in control cultures to 5200 for the chains

Fig. 1. Gel chromatography of ^{35}S -labelled macromolecules synthesized by macrophage-like cells (day 6) in the presence or absence of benzyl β -D-xyloside

Cultures were incubated on day 6 with inorganic ^{35}S sulphate for 24 h (see the Methods section) in the absence (a) of benzyl β -D-xyloside, or in the presence of (b) 0.01 mM-, (c) 0.05 mM- or (d) 0.5 mM-xyloside. Samples (0.1 ml) of the culture media were mixed with SDS (final concentration, 1%), boiled for 3 min, and applied to a column (1 cm $\times$ 90 cm) of Sepharose CL-6B, which was then eluted with 0.05 M-Tris/HCl/0.15 M-NaCl/0.1% SDS, pH 8.0, at a rate of 4.5 ml/h. Fractions (approx. 1.5 ml) were collected and analysed for radioactivity. The three peaks shown in each panel correspond, in the order of elution, to proteoglycans, single polysaccharide chains, and inorganic sulphate, respectively. The filled symbols in (a) correspond to alkali-treated medium (see the Methods section).

Table 1. Approximate molecular sizes of ^{35}S galactosaminoglycan chains produced by cultured monocytes

Cultures were incubated on day 1 (monocytes) or day 6 (macrophage-like cells) with inorganic ^{35}S sulphate in the absence or presence of benzyl β -D-xyloside, as indicated. Samples of the culture media were treated with 1% SDS and subjected to gel chromatography on Sepharose CL-6B (Fig. 1). The K_{av} values given correspond to the peak elution positions of the single polysaccharide chains formed in the presence of xyloside (Figs. 1b–1d), or generated by alkali treatment of control proteoglycan (Fig. 1a). M_r values were calculated from a standard curve of $\log(M_r)$ versus K_{av} , constructed by use of well-characterized chondroitin 4-sulphate fractions (Wasteson, 1971).

Cell cultures	[Benzyl β -D-xyloside] (mM)	K_{av}	$10^{-3} \times M_r$
Day 1	– (control)	0.47	23.0
	0.01	0.48	22.6
	0.05	0.49	22.1
	0.5	0.60	12.7
Day 6	– (control)	0.46	23.6
	0.01	0.46	23.6
	0.05	0.59	13.3
	0.5	0.69	5.2

formed in the presence of 0.5 mM-benzyl β -D-xyloside (Table 1). A similar, but less pronounced, effect was noted with monocyte cultures labelled on day 1 (Table 1). Moreover, a concomitant decrease in molecular size was noted for the residual proteoglycans (Fig. 1). Repeated gel chromatography following alkali treatment of the isolated proteoglycan fractions yielded elution patterns (not shown) which closely coincided with those of the corresponding xyloside-attached single polysaccharide chains (Fig. 1). It is concluded, then, that the polysaccharide chains attached to the endogenous core protein, and to the exogenous xyloside, were modulated in a parallel fashion with regard to molecular size. In addition, the smaller size of the proteoglycans in the xyloside-treated cultures may be due to a decrease in the number of polysaccharide chains attached to the protein core (Lohmander *et al.*, 1979).

In the absence of exogenous xyloside, the labelled polysaccharide released into the culture medium during a 24 h incubation with inorganic ^{35}S sulphate corresponded to about 70% of the total glycosaminoglycan synthesized during this period. For cultures (day 1 or day 6) treated with 0.5 mM-benzyl β -D-xyloside this value increased to > 90%. Gel chromatography of the intracellular material, solubilized by treatment with 1% SDS, demonstrated the same general increase in the ratio of single polysaccharide chains/proteoglycan with increasing xyloside concentration as found for the extracellular material (results not shown).

Polysaccharide structure. Previous studies (Kolset *et al.*, 1983, 1984) showed that the differentiation *in vitro* of monocytes into macrophage-like cells is coupled to the formation of a galactosaminoglycan containing 4,6-disulphated *N*-acetylgalactosamine residues. The resulting 'oversulphated' species is readily detected by its retarded elution position on ion-exchange chromatography [com-

Fig. 2. Ion-exchange chromatography of [^{35}S]galactosaminoglycans labelled on day 1 or on day 6 in the absence or presence of benzyl β -D-xyloside

Cell cultures were incubated on day 1 (*a,c*; monocytes) or day 6 (*b,d*; macrophage-like cells) with inorganic [^{35}S]sulphate (see the Methods section), in the absence (*a,b*) or presence (*c,d*) of 0.5 mM-benzyl β -D-xyloside. Labelled polysaccharide was isolated from the culture media (by papain digestion followed by gel chromatography), and samples (approx. 5000 c.p.m.) were applied to a column (1 cm $\times$ 6 cm) of DEAE-Sephacel, along with internal standards of chondroitin 4-sulphate (1 mg) and heparin (2 mg). The column was eluted at a rate of about 9 ml/h with a gradient extending from 0.05 M- to 1.5 M-LiCl in 0.05 M-acetate buffer, pH 4.0. Fractions (3 ml) were collected and analysed for radioactivity ($\circ$) and for hexuronic acid ($\bullet$). The two hexuronic acid peaks in each chromatogram correspond to the chondroitin sulphate (less retarded) and heparin internal standards.

pare the elution pattern of polysaccharide labelled on day 6 in culture (Fig. 2*b*) with that of material labelled on day 1 (Fig. 2*a*). The question was raised whether the perturbation of the biosynthetic process, caused by the presence of exogenous xyloside, would interfere with the change in sulphation pattern induced during the differentiation of cultured monocytes. To settle this matter, medium polysaccharide was isolated (by papain digestion and gel chromatography) from cells labelled with [^{35}S]sulphate on day 1 or day 6, in the presence or absence of benzyl β -D-xyloside, and subjected to ion-exchange chromatography on DEAE-Sephacel. Polysaccharides produced in the presence of xyloside (Figs. 2*c* and 2*d*) generally displayed less polyanionic character than the corresponding controls (Figs. 2*a* and 2*b*); this effect was dose-dependent and was more pronounced for day-6 (Figs. 2*b* and 2*d*) than for day-1 (Figs. 2*a* and 2*c*) material. A preliminary interpretation of these findings implied a decreased degree of sulphation for the polysaccharide produced by the xyloside-treated cells. However, the results of ion-exchange chromatography must be evaluated with caution, since the elution properties of polyelectrolytes such as glycosaminoglycans

depend not only on the charge density of the molecules but also on their molecular size (Hallén, 1972). Indeed, paper electrophoresis of disaccharides obtained by digesting the samples with chondroitinase ABC clearly showed the same high proportion of disulphated disaccharides ($\sim 20\%$ of total disaccharide units) for the day-6 polysaccharide, produced in the presence of 0.5 mM-xyloside (Fig. 3*c*), as for the control material formed in the absence of exogenous xyloside (Fig. 3*b*). In contrast, no significant amounts of disulphated disaccharide were obtained from samples labelled on day 1 in culture (Fig. 3*a*), irrespective of xyloside addition (cf. Kolset *et al.*, 1983). The biosynthetic mechanisms that induce the formation of disulphated disaccharide units thus may act on (artificially induced) single polysaccharide chains as well as on intact proteoglycan molecules.

Previous studies showed that the galactosaminoglycans produced before and after the conversion *in vitro* of monocytes into macrophage-like cells differed not only in sulphate content but also with regard to susceptibility towards enzymic degradation (Kolset *et al.*, 1983). Whereas day-0 to day-2 material was extensively degraded to disaccharides on incubation with the

Fig. 3. Paper electrophoresis of disaccharides formed by digestion of [^{35}S]galactosaminoglycans with chondroitinase ABC

Cell cultures were incubated on day 1 (*a*; monocytes) or day 6 (*b,c*; macrophage-like cells) with inorganic [^{35}S]sulphate (see the Methods section), in the absence (*a,b*) or presence (*c*) of 0.5 mM-benzyl β -D-xyloside. Labelled polysaccharide was isolated from the culture media and samples (approx. 5000 c.p.m.) were digested with chondroitinase ABC (0.01 unit) in 50 μl of 0.05 M-Tris/HCl, pH 8.0, containing 0.05 M-sodium acetate and 0.05 mg of bovine serum albumin/ml. After incubation for 8 h at 37 $^{\circ}\text{C}$ the samples were applied directly to Whatman 3MM paper and separated by high-voltage electrophoresis in 1.6 M-formic acid, pH 1.7, at 40 V/cm for 80 min. The standards shown below the tracings are: (I) monosulphated and (II) disulphated hexuronosyl-2,5-anhydro[1- ^3H]mannitol disaccharides.

bacterial eliminase chondroitinase AC, a significant portion, about 30%, of the day-5 to day-7 material resisted degradation under the same conditions. The resistant portion was readily depolymerized by treatment with chondroitinase ABC, and was therefore tentatively considered to represent dermatan sulphate. However, Yanagishita *et al.* (1979) noted a particular galactosaminoglycan sequence that resisted chondroitinase AC digestion in spite of a very low iduronic acid content, and ascribed this finding to the predominant exoenzyme nature of the enzyme preparation used. It thus appears

Fig. 4. Gel chromatography of products obtained on digesting oversulphated [^{35}S]galactosaminoglycan with chondroitinase AC or with testicular hyaluronidase

Labelled polysaccharide was isolated from the medium of cultures labelled on day 6 with inorganic [^{35}S]sulphate. Samples (approx. 5000 c.p.m.) were mixed with 0.5 mg of chondroitin 4-sulphate internal standard and incubated with chondroitinase AC (*a*) or with testicular hyaluronidase (*b*), as described in the Methods section. The products were applied to a column (1 cm $\times$ 90 cm) of Sephadex G-50, and eluted with 0.2 M- NH_4HCO_3 at a rate of 6 ml/h. Fractions (approx. 2 ml) were collected and analysed for radioactivity ($\circ$) and for hexuronic acid ($\bullet$).

that resistance of galactosaminoglycans towards chondroitinase AC may be due to structural features other than consecutive iduronic acid-containing disaccharide units. In order to investigate this point further, polysaccharide labelled at day 6 was digested with testicular hyaluronidase, which cleaves the β 1-4 linkage between hexosamine and D-glucuronic, but not L-iduronic, acid residues. Gel chromatography of the resulting products on Sephadex G-50 showed a broad peak of oligosaccharides, all retarded in relation to the excluded volume (V_0) of the column (Fig. 4*b*). In contrast, after digestion of the same polysaccharide preparation with chondroitinase AC, part of the product was excluded from the gel (Fig. 4*a*). This material was apparently susceptible to hyaluronidase and would thus seem to contain D-glucuronic rather than L-iduronic acid units. The reason for the partial resistance to chondroitinase AC remains unclear. However, taken together, the results of the enzymic depolymerization suggest that the highly sulphated galactosaminoglycan produced by the macrophage-like cells is largely, or exclusively, chondroitin sulphate.

Fig. 5. Scanning electron microscopy of human monocytes

The micrographs show monocytes cultured *in vitro* on glass cover slips for 1 day (a,b) or for 7 days (c,d), in the absence (a,c) or presence (b,d) of 0.5 mM-benzyl β -D-xyloside. The bars represent 10 μ m.

Effects of benzyl β -D-xyloside on cell morphology and function

Monocytes cultured for extended periods of time on plastic or glass substrates showed the characteristic morphological change observed previously (Kolset *et al.*, 1983, 1984). After about 4 days the cells thus had flattened out and assumed a rounded appearance with a 3–4-fold increase in diameter and a centrally located nucleus (Figs. 5a and 5c). The process expressed by this change in morphology has been related to the differentiation *in vivo* of monocytes into tissue macrophages, but also bears some resemblance to the development of epithelioid cells seen in certain granulomatous diseases, such as tuberculosis or leprosy (Johnson *et al.*, 1977; Kaplan & Gaudernack, 1982; Kaplan, 1983). Judging from morphological criteria alone, the differentiation process appeared unaffected by the presence of benzyl β -D-xyloside in the culture medium. Even at the highest concentration tested (0.5 mM), cells maintained in the presence of xyloside throughout a 6-day culture period were morphologically indistinguishable from untreated control cells (Figs. 5a–5d). Furthermore, the ability of monocytes/macrophage-like cells to lyse human tumour cells was investigated, in two separate sets of experiments. Firstly, HeLa cells were added to day-1 cultures of

monocytes, and the cells were co-cultured for a period of 5 days, in the presence or absence of 0.5 mM-benzyl β -D-xyloside. The xyloside-treated monocytes did not differ significantly from control cells in their ability to lyse target cells (Fig. 6). In a second experiment, monocytes were maintained in culture for 1 week, in the presence or absence of xyloside, and were then exposed to MDA target cells. The cytotoxic capacity of the resulting macrophage-like cells was apparently the same regardless of whether xyloside was present or not (Fig. 7). Within the limitations of these experiments thus neither the differentiation of monocytes into macrophage-like cells, nor the cytotoxic action of these cells, were appreciably affected by the xyloside.

DISCUSSION

The fine structure of sulphated glycosaminoglycan chains results from the interaction between nonsulphated precursor polysaccharides and the enzymes that catalyse the polymer-modification reactions involved in the biosynthetic process (Rodén, 1980). Such interaction is presumably rapid and transient, yet strictly regulated as evidenced by the occurrence of specific structural sequences in polysaccharide molecules. Little is known of

Fig. 6. Cytotoxic activity of monocytes against HeLa cells

Monocytes were cultured *in vitro* in the absence or presence of 0.5 mM-benzyl β -D-xyloside. After 1 day, 2×10^4 prelabelled HeLa cells were added to each well and co-cultures were maintained for 5 days in the absence (open bars) or presence (hatched bars) of xyloside. After the indicated periods of time, medium samples of 50 μ l were collected and analysed for radioactivity. The results shown represent one of three experiments with similar results. For additional information see the Methods section.

Fig. 7. Cytotoxic activity of monocytes (macrophage-like cells) against MDA cells

Monocytes were cultured *in vitro* in the absence or presence of 0.5 mM-benzyl β -D-xyloside. After 7 days, 2×10^4 prelabelled MDA cells were added to each well and co-cultures were maintained for 11 days in the absence (open bars) or presence (hatched bars) of xyloside. After the indicated periods of time, medium samples of 50 μ l were collected and analysed for radioactivity. The results shown represent one of three experiments with similar results. For additional information see the Methods section.

the regulation of polymer modification, except that it depends on the substrate specificity of the enzymes involved. Since both the enzymes and their polysaccharide substrate are membrane-bound, factors such as the spatial distribution of the enzymes and the mode of transport of the polysaccharide between these enzymes should be of importance. The role of the core protein is

particularly intriguing, since this component may anchor the proteoglycan to the membrane surface during the process of biosynthesis. The present investigation is concerned with a specific regulatory event which induces a subtle but distinct transition in the sulphation pattern of chondroitin sulphate produced by cultured human monocytes. As a result of this transition, a limited number of 6-sulphate groups are introduced into otherwise 4-sulphated polysaccharide. This structural change was found to be similarly expressed by polysaccharide chains initiated on exogenous xyloside or on endogenous core protein. It should therefore be ascribed to a modulation of the interactions between polysaccharide chains and sulphotransferases that occurs independently of any association between these chains and a core protein. Interestingly, the change in sulphation pattern was expressed regardless of the appreciable decrease in chain length displayed by the polysaccharide synthesized in the presence of xyloside. Similar effects of xylosides on the molecular size of newly-synthesized chondroitin sulphate have been found in other systems (Lohmander *et al.*, 1979; Mitchell & Hardingham, 1982); the mechanism behind this phenomenon has not been established with certainty.

Proteoglycans of different structure, in different cell types, and in different subcellular compartments, are not uniformly affected by xylosides. The monocytes presently studied were only minimally perturbed by xyloside, and thus retained the same ability as control cells to respond to environmental changes by producing a slightly modified chondroitin sulphate. In other cell types the addition of xylosides may result in dramatically changed patterns of glycosaminoglycan synthesis. For instance, heparin-producing mast cells turned to chondroitin sulphate synthesis under the influence of xyloside (Robinson & Lindahl, 1981; Stevens & Austen, 1982); in fact, rat serosal mast cells produced an oversulphated chondroitin sulphate similar to the day-6 material synthesized by the monocytes in the present study (Stevens *et al.*, 1983). Apparently, xylosides may unmask a biosynthetic potential that is normally not expressed by the cells.

The ability of exogenous xylosides to modulate the biosynthesis of glycosaminoglycans has been exploited extensively to probe the role of these macromolecules in cell development and differentiation (Gallagher & Hampson, 1984). In view of the varied effects of xylosides on glycosaminoglycan biosynthesis in different cells it is not surprising that their cellular effects have been found highly diversified (see, e.g., Kinoshita & Saiga, 1979; Lohmander *et al.*, 1979; Thompson & Spooner, 1983; Spooner *et al.*, 1983). The present study shows that the cytotoxic capacity of human mononuclear phagocytes is unimpaired by the presence of 0.5 mM-benzyl β -D-xyloside. Moreover, the differentiation *in vitro* of human blood monocytes into macrophage-like cells is unaffected by the xyloside, as judged by morphological criteria. Yet under the same conditions the xyloside blocks the synthesis of a proteoglycan which normally displays a specific structural change (occurrence of di-O-sulphated N-acetylgalactosamine units) during the differentiation process; in contrast, it promotes the formation of the corresponding free polysaccharide chains. While the biological function of the polysaccharide is unknown, a functional role in relation to the differentiation process appears plausible, since both

the characteristic change in cellular morphology and the change in polysaccharide sulphation could be prevented by subtle modifications of the culture conditions (Kolset *et al.*, 1984). It cannot be excluded that the single polysaccharide chains retain the ability to perform a specific function that is normally exerted by the proteoglycan.

This work has been supported by grants from the Norwegian Council for Science and the Humanities, the Norwegian Cancer Society, the Swedish Medical Research Council (no. 2309, 6525), the Swedish Council for Forestry and Agricultural Research (no. A5861) and the Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences. L.K. is the recipient of a research fellowship from the Juvenile Diabetes Foundation. The skilful technical assistance of Ms. Helena Grundberg is gratefully acknowledged.

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Received 31 January 1986; accepted 24 April 1986