edge of structure, and can be successfully applied to mix-
tures. Sensitivity improvements of two orders of magnitude
or more could be achieved if proposed new instrumentation
incorporating post-acceleration array detection becomes a
reality (H. R. Morris, R. A. McDowell & A. Dell, unpub-
lished work).

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Proteoglycan–protein interaction in arterial tissue

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Arterial smooth muscle cells synthesize both in vivo and in
vitro three types of sulphated proteoglycans: proteochond-
rin sulphate proteoglycan, proteodermatan sulphate and proteo-
heparan sulphate, and distribute them preferably to the
extracellular and cell-surface associated compartment. The
proteoglycan–protein interaction of the dermatan-sulphate-
rich proteoglycan (DS-PG) and heparan sulphate proteo-
glycan (HS–PG) has been studied in more detail.

The DS-PG synthesized by arterial smooth muscle cells
belongs to the group of small proteoglycans. It has a molecu-
lar mass of about 180 000 and six to seven dermatan-sulphate-rich side-chains. The [35S]methionine-
labelled protein core has a molecular mass of about 48 000
(Schmidt & Buddecke, 1985, 1987). About 70% of DS-PG
synthesized by the cells is released into the culture medium.
In bovine and human arterial tissue the extracellular DS–PG
is associated with collagen fibrils. After specific staining of
DS–PG with Cuprolinic Blue, the DS–PG can be visualized
in electron micrographs as proteoglycan/Cuprolinic Blue
precipitate linked to adjacent collagen fibrils at the periodi-
cally spaced d-band regions of collagen. The protein core of
DS–PG is probably responsible for the binding to collagen
fibrils. If the glycosaminoglycan side-chains, which are highly
negatively charged, were responsible one would expect an
electrostatic binding, but the d-band where the DS–PG is
located, is not positively charged. Therefore, electrostatic
binding of DS–PG to the collagen fibril is unlikely.

In the course of the catabolic pathway the DS–PG is
released from collagen and internalized from the extracel-
ular compartment by endocytosis. Endocytosis was found to
be a specific and saturable process involving specific inter-
action of DS–PG to cell surface receptors (Schmidt &
Buddecke, 1985). Uptake of DS–PG shows saturable kinet-
tics. At saturation one cell may endocytose up to 1.5 x 10⁶
DS–PG molecules/h. Endocytosis of DS–PG may be inhib-
ited competitively by non-labelled DS–PG isolated from cell
cultures or from bovine aorta. A Kᵣ value of 18.3 mmol/ml
was calculated in the presence of 85 mmol of non-labelled
DS–PG/ml. No inhibition of endocytosis of DS–PG was
observed in the presence of a 5-fold excess of non-labelled
chondroitin-sulphate-rich proteoglycan isolated from cell
cultures or bovine aorta. The latter is also internalized by
arterial smooth muscle cells, but at a 10-fold lower rate. The
slow uptake of [35S-labelled chondroitin-sulphate-rich prote-
glycan was not influenced by non-labelled DS–PG thus indi-
cating that DS–PG and the chondroitin-sulphate-rich PG
do not compete for the same binding site. Free chondroitin
sulphate and dermatan sulphate chains, up to a concentra-
tion of 1.6 mmol/ml, did not inhibit endocytosis nor were
these polysaccharides endocytosed when presented to the
cells as [35S]glycosaminoglycans. From these data it is con-
cluded that the protein core of DS–PG interacts with a
DS–PG-specific cell surface receptor as the initial step for
endocytosis.

Using proteoglycan–gold conjugates, which appear in
electron micrographs as pearl-string-like gold strands of
about 170 nm length, binding, endocytosis and intracellular
translocation of DS–PG can be visualized (Völker et al.,
1984). The proteoglycan–gold conjugates bind to coated
as well as to non-coated cell surface membrane areas at 4°C.
Post-incubation at 37°C leads to a time-dependent uptake of
DS–PG via non-coated and coated vesicles, which after
fusion are translocated to multi-vesicle bodies and to large-
-sized vesicles within 1 h. After conversion of these vesicles to
lysosomal compartments, the gold particles are uncoupled
from the proteoglycan and are concentrated within residual
vacuoles.

The recognition site of DS–PG required for interaction
with the cell-surface receptor depends on the presence of a
recognition marker which resides in the protein core of
DS–PG and involves lysine residues (Glössl et al., 1983), but
the chemical features of the proteoglycan-specific cell sur-
face receptor are unknown. Binding experiments of
3H[35S]DS–PG to cultured smooth muscle cells at 4°C gave
evidence for high-affinity and low-affinity binding sites, but
the variation of the values, probably due to the polyanionic

Abbreviations used: DS–PG, dermatan-sulphate-rich proteo-
glycan; HS–PG, heparan sulphate proteoglycan.
nature of DS-PG and its self-aggregation tendency, did not allow the calculation of the number of high-affinity binding sites. However, when the DS chains of DS-PG are degraded by chondroitin ABC lyase, the isolated and $^{35}$S-labelled protein core binds to cultured smooth muscle cells at 4°C in a saturable manner. From Scatchard plots 4.2 x 10$^3$ binding sites per cell were calculated.

To characterize a DS-PG core protein-binding cell-surface protein, cultured arterial smooth muscle cells were labelled with $^{35}$S-methionine. The cell membranes were then isolated by standard methods, solubilized and submitted to affinity chromatography using a Sepharose-bound core protein of DS-PG. The fraction eluted from the affinity column gave radioactivity bands at $M_r$ 61,000 and 44,000 (double band) on SDS electrophoresis (Fig. 1).

In independent experiments the interaction of HS-PG of arterial wall and arterial smooth muscle cell proteins was studied. HS-PG was isolated from bovine arterial tissue and cultured arterial smooth muscle cells within 48 h resides in the cell layer. It was characterized as a proteoglycan with an apparent $M_r$ of 200,000 consisting of a protein core ($M_r$ 91,000) to which three to four heparan sulphate side-chains are covalently linked. Pulse-chase experiments revealed that about 40% of the cell-layer-associated HS-PG is released into the medium and that the majority is internalized and converted to smaller species.

In native arterial tissue the extracellular HS-PG is shown by electron microscopy to be associated mainly with the external surface of elastic fibres (Volker et al., 1986) and is identified by its susceptibility to heparan-sulphate-degrading enzymes. Since under conditions in vivo the majority of HS-PG is located extracellularly, it was isolated from bovine arterial tissue by sequential collagenase and elastase digestion in the presence of proteinase inhibitors. The HS-PG of arterial tissue has an $M_r$ 175,000 and possesses four heparan sulphate side-chains ($M_r$ 32,000) covalently bound to the protein core via a galactose- or xylose-containing polysaccharide protein-binding region. The protein core of the arterial tissue HS-PG was found to be significantly smaller ($M_r$ 38,000) than that of the cell-membrane-integrated HS-PG of cultured arterial smooth muscle cells.

Extracellular HS-PG is endocytosed by arterial smooth muscle cells via saturable kinetics, but in contrast to DS-PG, the heparan sulphate side-chains (and not the protein core) bind to a specific cell-surface protein (Krüger & Kresse, 1986).

A special interest in the HS-PG of arterial smooth muscle cells has been stimulated by the report of Fritze et al. (1985) that post-confluent arterial smooth muscle cells produce a cell-surface heparan-sulphate species with antiproliferative activity. We have confirmed this observation and found that the cell-surface heparan sulphate, but not the cell-surface chondroitin sulphate, and dermatan sulphate have an antiproliferative activity significantly higher than that of equivalent amounts of heparin. Moreover, in contrast to heparin, the antiproliferative heparan sulphate preparation had no antithrombogenic effect, which was tested by the ability of heparin and heparan sulphate to prevent the conversion of prothrombin to thrombin. From these data it is concluded that there are different structural requirements for the antiproliferative and antithrombogenic function of heparan sulphate and heparin, respectively. The antiproliferative activity of the heparan sulphate side-chains of HS-PG suggests that bovine arterial smooth muscle cells are capable of controlling their own growth and raises the question of the structural determinants of the antiproliferative domain of heparan sulphate.


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