yielded liver metastases (median values of 40, 10 and 1, respectively) 4 weeks after intrasplenic inoculation [6, 7].

Cells in stationary growth phase were incubated with \(^3H\)-labelled amino acid mixture (10 µCi/ml, Amersham, U.K.) for 24 h. Fractions enriched in cell membranes, in nuclei (nuclear sediment) and in the cytoplasm were separated as described [8]. The proteins associated with the membranes and with the nuclear sediments were solubilized in phosphate-buffered saline, pH 7.4, supplemented with 0.5% (w/v) Triton X-100 and proteinase inhibitors. The heparin-binding proteins were isolated by affinity chromatography on Heparin-Ultrogel eluted with 0.5% (w/v) heparin (Laboratoires Choay, France) in phosphate-buffered saline, pH 7.4. Some residual radioactivity (about 10% of that eluted by the heparin) was recovered by 1 M-NaCl solution from the affinity column. The heparin-binding protein label in the membrane and in the nuclear sediment material decreased with the increase of the metastatic potential of the cells (Table 1). The most marked differences in the decrease and in the distribution of the labelled heparin-binding proteins were observed between the tumorigenic HT 18 cells and the HT 168 and M1 metastatic variants. H.p.l.c. of heparin-binding proteins on a TSK SW 300 column in 4 M-guanidinium chloride, 0.05 M-Tris/HCl, pH 7.5 yielded a large fraction in the molecular mass range of 100–200 kDa and a low molecular mass component of about 20 kDa. The 20 kDa protein accounted for about 30% and 50% of the heparin-binding proteins in the membranes and in the nuclear fraction, respectively. The 20 kDa protein was eluted on a Mono Q (Pharmacia) ion-exchange column with a 0–1 M-NaCl gradient in 0.05 M-Tris/HCl, pH 7.4, as a single peak at 1 M-NaCl concentration.

The cells were assayed for their interactions with exogenous heparan sulphate from bovine kidney (Sigma). The uptake of added heparan sulphate by the low metastatic cells at 4°C was dose dependent and saturable. A non-specific binding was shown in the highly metastatic (M1) variant.

Considering these data and the secretion of heparan sulphate in melanoma cells. These results, taken together with earlier findings [3, 4], suggest that the decrease in the amount of the biosynthetically labelled heparan-binding proteins in the malignant cells can be related to the expression of the metastatic phenotype.

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**Table 1. Distribution of \(^3H\)-labelled total cell proteins (T) and \(^3H\)-labelled heparin-binding proteins (HB) in the membranes, cytoplasm and in the nuclear sediment from human melanoma cell variants**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Membrane</th>
<th>Cytoplasm</th>
<th>Nuclear sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>HB</td>
<td>T</td>
</tr>
<tr>
<td>HT 118</td>
<td>11.3</td>
<td>8</td>
<td>71.4</td>
</tr>
<tr>
<td>HT 168</td>
<td>2.7</td>
<td>0.22</td>
<td>92.1</td>
</tr>
<tr>
<td>M1</td>
<td>1.2</td>
<td>0.08</td>
<td>95.4</td>
</tr>
</tbody>
</table>

**Interaction of ram and boar proacrosin with \(^{125}I\)-fucoidan**

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Proacrosin (the zymogen form of the acrosomal proteinase acrosin, EC 3.4.21.10) has been found to be a major receptor for probes made from zona pellucida glycoproteins and other neoglycoproteins which have been used to mimic the initial binding between sperm proteins and zona components at fertilization [1]. In recent experiments, fucoidan, a highly sulphated 100 000 M, polysaccharide, was found to be a potent inhibitor of binding of zona or neoglycoprotein probes to proacrosin. Therefore, it was concluded that the proacrosin was interacting with fucoidan in a way comparable with its interaction with the zona pellucida. Further investigation of this molecular interaction should provide more information about the nature and site of binding of proacrosin to the zona pellucida.

Experiments described here used proacrosin purified by electroelution from SDS/PAGE separations of pH 3 extracts from ram and boar sperm. The protein was immobilized by dot-blotting on to nitrocellulose and a series of competition assays carried out to investigate the nature of binding of \(^{125}I\)-fucoidan.

Crude fucoidan (from Sigma) was purified by \(\beta\)-elimination and pronase digestion [2], followed by conjugation with fluoresceinamine [3] and iodination using iodogen [4]. A variety of mono- and poly-saccharides were tested for their ability to block the interaction between the sperm protein and the polysaccharide probe. Blots were blocked with 5% (w/v) bovine serum albumin for 2 h at 23°C and then exposed to various dilutions of the saccharide blocking solution, pH 7.2, for 1 h at 23°C. Subsequently, the blocking agent was removed and the iodinated probe (~400 000 c.p.m./ml) added for 1 h. After washing, the radioactivity retained on the nitrocellulose membrane was counted, background values were subtracted, and the number of counts relative to control samples was calculated.

These results were plotted against the log of the saccharide concentration and the concentration required to

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1990
give 50% inhibition of binding (IC50) was derived from these graphs. The values for ram proacrosin were as follows: unlabelled fucoidan (19.1 nM), dextran sulphate 500 000 Mw (31.6 nM), dextran sulphate 5000 Mw (251 nM), polyvinyl-
sulphate (326 nM), xylan (299 μM) and mannan (1000 μM). The following solutions were tested, but did not reach 50% inhibition at the concentrations given: dextran 506 000 Mw (160 μM), d + f-fucose (0.8 M), d + f-galactose (0.8 M), d + l-mannose (0.8 M), lactose (0.4 M), glucoseamine (0.5 M), galactosamine (0.5 M), chondroitin sulphates A and C (0.4 mM). Comparable results were obtained using boar proacrosin. 

Fig. 1 shows the data for four blocking agents binding to ram proacrosin. The significance of molecular mass was demonstrated by the more potent effect of 500 000 Mw over 5000 Mw dextran sulphate. The importance of sulphation is shown by the lack of inhibition with dextran (Mw 506 000), versus dextran sulphate. However, absence of an inhibitory effect with chondroitin sulphates A and C (Mw 40 000) demonstrates that the stereochemical arrangement of sulphate groups on the polysaccharide structure must also be significant in order to block the fucoidan-binding sites on proacrosin.

As proacrosin is a serine proteinase, other members of this family (chymotrypsinogen, trypsinogen, thrombin, elastase, plasminogen, pepson, Streptomyces griseus protease) have been screened for fucoidan binding activity. One chymotrypsinogen and trypsinogen retained significant amounts of the probe with Kd values of 1.4 x 10^-6 M and 3.0 x 10^-5 M respectively [5]. The affinity of fucoidan for proacrosin was Kd 4.9 x 10^-6 M. This unusual property of carbohydrate-binding activity suggests a second function for this family (chymotrypsinogen, trypsinogen, thrombin, elastase, plasminogen, pepson, Streptomyces griseus protease) have been screened for fucoidan binding activity. One chymotrypsinogen and trypsinogen retained significant amounts of the probe with Kd values of 1.4 x 10^-6 M and 3.0 x 10^-5 M respectively [5]. The affinity of fucoidan for proacrosin was Kd 4.9 x 10^-6 M. This unusual property of carbohydrate-binding activity suggests a second function for these three proteinases. This activity was not blocked by proteinase inhibitors, indicating that the fucoidan-binding site is different from the substrate-binding site. However, the tertiary structure of the molecule is important, as reduction with 2-mercaptoethanol caused a 50% decrease in binding of the polysaccharide probe.

These fucoidan-binding properties of proacrosin are comparable with those reported for bindin, the sea urchin sperm adhesion molecule [6]. Using group-specific chemical modification of basic amino acids, DeAngelis & Glabe [6] showed that arginine, lysine and histidine residues were important in fucoidan binding. To determine the location of basic residues in chymotrypsinogen, trypsinogen and proacrosin, X-ray crystallographic data was used to construct three-dimensional molecular models using Evans and Sutherland computer programs. As there is ~40% linear sequence similarity between boar proacrosin and trypsinogen, a three-dimensional model of proacrosin was predicted based on comparisons with similar regions of trypsinogen. The models show that in each of the three proteins basic residues are located on the surface and not on internal β-sheets. In proacrosin there are many surface arginine residues which could provide fucoidan-binding sites.


Characterization of receptors for sulphated polysaccharides on guinea-pig spermatozoa

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Fertilization in mammals involves complementary recognition and fusion between two morphologically disparate gametes, the sperm and egg. Receptors for spermatozoa on the zona pellucida (the extracellular matrix that surrounds mammalian eggs) have been shown to reside in the oligosaccharide moieties of one or several constituent glyco-proteins [1]. However, less is known about the nature of the ligand molecule or molecules on spermatozoa and their mechanism of interaction with the carbohydrate residues on zona glycoproteins. It would seem that in most species spermatozoa attach to the surface of the zona with an intact plasma membrane, overriding the acrosomal matrix. However, a different situation is found in the guinea-pig. In this species acrosome-reacted, rather than acrosome-intact, spermatozoa bind to the zona in vitro [5] suggesting that receptor molecules must be located within the acrosomal matrix or bound to acrosomal membranes. The aim of the present study was to identify these receptors using fucoidan as a probe. Fucoidan (a sulphated polysaccharide from Fucus vesiculosus) is a potent inhibitor of fertilization in a wide variety of species and has been shown to mimic binding of zona glycoproteins to spermatozoa [6, 7].

Guinea-pig spermatozoa were washed once with 10 vol. of phosphate-buffered saline (PBS) and proteins extracted at pH 3 as described [7] or solubilized with 1% (v/v) Triton X-100/1% (w/v) sodium deoxycholate/1 mM-p-aminobenzamidine/10 μg of aprotinin/ml/10 μg of leupeptin/ml, described in this region, e.g. galactosyltransferase [2], rabbit sperm autoantigens glycoproteins [3] and tyrosine kinase [4]. However, a different situation is found in the guinea-pig. In this species acrosome-reacted, rather than acrosome-intact, spermatozoa bind to the zona in vitro [5] suggesting that receptor molecules must be located within the acrosomal matrix or bound to acrosomal membranes. The aim of the present study was to identify these receptors using fucoidan as a probe. Fucoidan (a sulphated polysaccharide from Fucus vesiculosus) is a potent inhibitor of fertilization in a wide variety of species and has been shown to mimic binding of zona glycoproteins to spermatozoa [6, 7].

Guinea-pig spermatozoa were washed once with 10 vol. of phosphate-buffered saline (PBS) and proteins extracted at pH 3 as described [7] or solubilized with 1% (v/v) Triton X-100/1% (w/v) sodium deoxycholate/1 mM-p-aminobenzamidine/10 μg of aprotinin/ml/10 μg of leupeptin/ml.

Vol. 18