REVERSIBLE DISRUPTION OF CULTURED ENDOTHELIAL MONOLAYERS BY SULPHATED FUCANS

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SUMMARY
We have examined the effects of a variety of polysaccharides and glycoconjugates on the organization and morphology of cultured A14CL-1 endothelial monolayers. The sulphated fucose-containing polysaccharides, fucoidin and sea-urchin egg jelly fucan, induce a dramatic disruption of the organization of the monolayers, characterized by the retraction of adjacent borders of cells exposing areas of the subendothelial matrix. This effect, which occurs at a fucoidin concentration of 10 μg/ml, is rapidly reversible after the fucoidin-containing medium is removed. Within 1 h after replacement with fresh medium many cell contacts are re-established; within 20 h the fucoidin-treated monolayers closely resemble the untreated controls. The effect of the sulphated fucose-containing polysaccharides is specific. Of a wide variety of sulphated polysaccharides and glycoconjugates tested, only fucoidin and the egg jelly fucan produce a detectable alteration in the morphology of cultured endothelial monolayers. The endothelial monolayer has specific binding sites for fucoidin. The binding of fucoidin is saturable and a maximum of $4.5 \times 10^5$ molecules of fucoidin are bound per cell with an apparent affinity of $2.3 \times 10^{-7} \text{M}$. A significant proportion (26%) of the total monolayer-associated fucoidin is apparently internalized by the endothelial cells after incubation with fucoidin for 1 h at 37 °C. The morphological response to fucoidin is probably not due to its internalization, since the effect is observed at 7 °C where little uptake (3-5%) occurs. Fucoidin appears to bind at two distinct sites on endothelial monolayers. One site is inhibitable by heparin, while the other site seems to be specific for fucoidin. The observation that fucoidin still induces the retraction of the endothelial cells in the presence of a 100-fold excess of heparin, suggests that binding at the fucoidin-specific site is responsible for the morphological effect of fucoidin. In addition, fucoidin has no detectable effect on monolayers of 3T3 and BHK fibroblast-like cells at 1 mg/ml, 100-fold higher than the concentration required to produce an effect on endothelial cells. Among the possible interpretations of these results is that sulphated fucose-containing glycoconjugates may play a role in the adhesive interactions of endothelial cells.

INTRODUCTION
Endothelial cells form the lining of the vascular system and play important roles in the regulation of blood-vessel permeability and the maintenance of a continuous non-thrombogenic surface (Gimbrone, 1976; Simionescu, Simionescu & Palade, 1976; Anderson, 1981). The disruption of the integrity of the vascular endothelium may contribute to pathogenic states such as inflammatory reactions and atherosclerosis. In

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atherosclerosis local disruption of the endothelium is thought to be the initial event leading to plaque formation (Thorgeirsson & Robertson, 1978). Thus the factors that participate in the maintenance of endothelial integrity are essential for the normal function of the vascular system. These factors might include the cell surface components that participate in intercellular adhesion between adjacent endothelial cells and factors that mediate the attachment of endothelial cells to the subendothelial matrix. Fibronectin has been implicated as a mediator of the attachment of endothelial cells to the extracellular matrix (Birdwell, Gospodarowicz & Nicolson, 1978), but the molecular nature of the components that participate in the adhesion of the lateral surfaces of endothelial cells remains unknown.

Cell surface carbohydrate-binding components or lectins are believed to play an important role in intercellular adhesion in a wide variety of systems (Frazier & Glaser, 1979; Barondes, 1981). The purpose of this study was to investigate the possible existence of specific cell surface lectins on cultured bovine aortic endothelial monolayers. In this paper, we report that fucoidin, a sulphated polysaccharide containing predominantly L-fucose, disrupts the morphological integrity of cultured endothelial monolayers in a manner consistent with the disruption of the lateral adhesive contacts and retraction of the borders between adjacent endothelial cells. This effect of fucoidin is specific: many other sulphated polysaccharides have no effect on monolayers. In addition, two cell lines of fibroblast-like cells, 3T3 and BHK, show no detectable change in morphology upon treatment with fucoidin at a concentration 100-fold higher than that required to produce an effect in endothelial cells. We also demonstrate that cultured endothelial cells possess a specific binding site for fucoidin, the properties of which suggest that the morphological response to fucoidin is due to the binding of fucoidin to the endothelial monolayer.

MATERIALS AND METHODS

Fucoidin was purchased from ICN K&K (Plainview, N.Y.). Heparan sulphate was a kind gift from Dr A. Linker, VA Hospital, Salt Lake City, Utah. Polysaccharide stock solutions were routinely boiled for 10 min at pH 7.2 before use. All other reagents were from Sigma (St Louis, Mo). Eagle's minimal essential medium (MEM) was obtained from the cell culture facility at the University of California, San Francisco. 'Hyclone' calf serum was obtained from Sterile Systems (Logan, Utah). Carrier-free 125I (NEZ033) was from New England Nuclear (Boston, MA).

Cell culture

Primary cultures of foetal bovine aortic endothelial cells (A1CL-1) were a kind gift from Dr Peter Jones, Department of Pediatrics, Childrens Hospital of Los Angeles (Jones, 1979). A1CL-1 cells were cultured in MEM containing 10% heat-inactivated (30 min at 56°C) calf serum, 2% tryptose phosphate broth and penicillin/streptomycin on tissue-culture dishes treated with 0.1% gelatin. The original culture was passed at a ratio of 1:5 twice, and the resulting cultures were stored frozen in ampules. These subcultures were passed a maximum of six times at 1:8 before being discarded. A1CL-1 cells are positive for bovine Factor VIII antigen by immunofluorescence (Jones, 1979). BHK and 3T3 cells were cultured as described (Rosen, Singer, Glabe & Grabel, 1981) and passaged onto 0.1% gelatin-coated dishes. The bovine aortic endothelial cell line, BAE, originally isolated by D. Gospodarowicz, was obtained from Dr R. Kramer, University of California, San Francisco, and grown on gelatin-treated plates in Dulbecco's modified Eagle's medium containing 1 g of glucose per litre, 10% calf serum, penicillin/streptomycin.
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$^{125}$I-labelled polysaccharide binding

Fluorescein derivatives of fucoidin and heparin were prepared by reacting fluoresceinamine with cyanogen bromide-activated polysaccharides as will be described in detail (Glabe, Harty & Rosen, 1983). Briefly, 20 mg of polysaccharide in 2 ml of water was activated with 20 mg CNBr (Sigma Chemicals) by titrating to pH 11 for 8 min. The solution was desalted rapidly by gel filtration on a 1 cm x 20 cm column of Sephadex G-50 (Pharmacia, Piscataway, N.J.) in 0.2 M-sodium borate, pH 8.0. The polysaccharide-containing fractions were immediately pooled and reacted with 2 mg of fluoresceinamine (Sigma Chemicals) for 24 h, after which the derivatized fucoidin was separated from the unincorporated fluoresceinamine by gel filtration on Sephadex G-50 in Ca,Mg-free Dulbecco's phosphate-buffered saline (CMF). The resulting fluorescein derivatives are stable and contain between 1 and 3 mol of fluorescein/1000 mol of saccharide residues (Glabe et al. 1983). $^{125}$I-labelled polysaccharides were prepared by reacting 150 μg of the fluorescein derivatives of polysaccharides and 0.2 mCi of $^{125}$I (New England Nuclear, Boston, MA) in 0.2 ml sodium borate buffer (pH 8.0) with a 4 μg film of Iodogen (Pierce, Rockford, IL) as described (Fraker & Speck, 1978). Specific activities ranging from $4 \times 10^5$ to $10^6$ c.p.m./μg of polysaccharide were obtained. Confluent monolayers of cells (5 x 10⁵ cells) grown in 35 mm dishes were incubated with various amounts of $^{125}$I-labelled polysaccharide in complete medium. After incubation, the medium was removed and the monolayers washed three times with 2 ml of medium. The monolayer was solubilized in 0.4 ml of 0.1 % sodium dodecyl sulphate (SDS) and the dish rinsed with two additional 0.4 ml portions of 0.1 % SDS. The amount of $^{125}$I was quantified in a gamma counter (Nuclear Chicago, Chicago, IL). Less than 1 % of the total cell-associated radiation remained with the dish after washing with 0.1 % SDS. The internalization of $^{125}$I fucoidin by endothelial cells was investigated by harvesting cells with trypsin. The cell-associated $^{125}$I fucoidin was determined by the centrifugation assay previously described (Stahl et al. 1980).

Light microscopy

Samples for light microscopy were fixed with 2.5 % glutaraldehyde in CMF containing 50 mM-cacodylate (pH 7.4) for 1 h at 20°C. Fixed samples were dehydrated with a graded series of ethanol to 100 % ethanol and air-dried. Samples were photographed on Kodak Pantonic-X with an Olympus BH microscope equipped with 20x Hoffman modulation contrast objectives.

Other procedures

Fucoidin was desulphated by solvolysis (Usov et al. 1971). The resulting fucoidin was 95 % desulphated (Grabel, Rosen & Martin, 1979) and was not substantially degraded, as judged by the fact that it continues to void on a Sephadex G-50 column. The sulphated fucan from sea-urchin egg jelly was prepared as described (SeGall & Lennarz, 1979).

RESULTS

Effect of fucoidin on endothelial monolayers

We initially investigated the effect of fucoidin on A14CL-1 monolayers by incubating confluent monolayers in medium containing 10 μg/ml fucoidin at 37°C for 1 h. The control endothelial cells adopt a characteristic 'cobblestone' morphology (Fig. 1). The borders between individual endothelial cells in the confluent monolayer are difficult to resolve. After treatment with 10 μg/ml fucoidin, the endothelial cells have separated from each other exposing the subendothelial matrix (Fig. 1b). The borders between adjacent endothelial cells are now distinct. The effect of fucoidin is specific: heparin, heparan sulphate, chondroitin sulphate and dextran sulphate have no effect on the morphology of endothelial monolayers at concentrations of 100 μg/ml (Fig. 2).
A variety of other saccharides and glycoconjugates listed in the legend to Fig. 2, are also inactive. Another sulphated fucose polysaccharide, isolated from sea-urchin egg jelly, also induces the separation of endothelial cells at 100 µg/ml (Fig. 2E). Sulphation of fucoidin appears to be necessary for its activity since desulphated fucoidin (95% desulphated) is inactive at 100 µg/ml (Fig. 2F).
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Fig. 2. Specificity of the morphological response of endothelial monolayers to treatment with sulphated glycoconjugates. A1C1-1 monolayers were treated with 100 µg/ml of polysaccharide for 2 h at 37 °C. A. Control monolayers; B, chondroitin sulphate; C, dextran sulphate; D, heparin; E, egg jelly fucan; F, desulphated fucoidin. The following glycoconjugates were also inactive at 100 µg/ml: dermatan sulphate; desulphated fucoidin; dextran; heparan sulphate; hyaluronic acid; yeast mannan; xylan. The following saccharides were inactive at 0.2 M: D-mannose, D-galactose, D-glucose, L-fucose, N-acetyl-D-glucosamine, sialic acid. ×265.

The effect of fucoidin is reversible (Fig. 3). After treatment of the endothelial cells with fucoidin for 2 h, followed by removal of the fucoidin, washing of the monolayer and replacement with fresh media, many of the intercellular contacts are apparently re-established within 1 h (Fig. 3c). After 20 h following removal of fucoidin, the endothelial monolayers closely resemble the control monolayers not exposed to fucoidin (Fig. 3b).

Binding of fucoidin to endothelial monolayers

The binding of fucoidin to endothelial cell monolayers was investigated using an
Fig. 3. Reversibility of the effect of fucoidin on endothelial monolayers. a. Control A14CL-1 monolayers incubated with fresh medium for 2 h. b. Monolayers treated with medium containing 50 μg/ml fucoidin for 2 h. c. Fucoidin-treated monolayers 1 h after removal of fucoidin and replacement with fresh medium. d. Fucoidin-treated monolayers 20 h after removal of fucoidin and replacement with fresh medium. ×265.

Fig. 4. Kinetics of the binding of fucoidin to A14CL-1 monolayers. 125I-labelled fucoidin was incubated with endothelial monolayers at a concentration of 10 μg/ml. After incubation for various lengths of time, the amount of bound fucoidin was determined (see Materials and Methods). (●—●) incubated at 37°C; (○---○) incubated at 7°C.
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$^{125}$I-labelled fluorescein derivative of fucoidin previously described (Glabe, Grabel, Vacquier & Rosen, 1982). The kinetics of association of $^{125}$I-fucoidin with A14CL-1 monolayers at 37°C and 7°C is shown in Fig. 4. At 37°C, the association of fucoidin is linear over the 2 h period of incubation. At 7°C, the binding of fucoidin approaches a maximum by approximately 1 h. The binding of fucoidin is rapidly reversible in the presence of excess unlabelled fucoidin ($t_{1/2} = 5$ min). The fact that the association of $^{125}$I-fucoidin is linear at 37°C and approaches a plateau at 7°C suggests that fucoidin may be internalized at 37°C. We have examined this possibility by determining the amount of internalized fucoidin at both 37°C and 7°C, as defined as the amount of cell-associated fucoidin that is not released from the cells by trypsin treatment (Table 1). After incubation of the endothelial cells with 10 μg/ml $^{125}$I-fucoidin for 1 h at 37°C, 26% of the total cell-associated fucoidin is resistant to release by trypsin, suggesting that it is internalized. The same fraction of fucoidin (26%) is also resistant to displacement by a 100-fold excess of unlabelled fucoidin (1 mg/ml). In contrast, after incubation at 7°C, only 3-5% of the total cell-associated fucoidin is retained by cells indicating that there is greatly reduced uptake at 7°C. The uptake of fucoidin by endothelial cells appears to depend on cell surface binding rather than fluid-phase pinocytosis since the internalization is blocked by addition of excess unlabelled fucoidin (1 mg/ml), which competes for the binding of $^{125}$I-fucoidin without decreasing the concentration of $^{125}$I-fucoidin in the medium. If the effect of fucoidin on the morphology of endothelial monolayers depends on its uptake, then the effect should be greatly reduced at 7°C. Endothelial monolayers treated with fucoidin at 7°C are shown in Fig. 5. Fucoidin has the same effect on the monolayers at 7°C as it does at 37°C, consistent with the idea that the effect of fucoidin is not due to internalization.

We have examined the concentration dependence of fucoidin binding at 7°C using a 120 min incubation period. The binding of $^{125}$I-fucoidin is saturable (Fig. 6). The endothelial monolayer ($5 \times 10^5$ cells) binds a maximum of 3.75 pmol of fucoidin. This gives an estimate of $4.5 \times 10^5$ molecules of fucoidin bound per cell (assuming a molecular weight of 100 000 for fucoidin). The Scatchard plot of the binding data (Fig. 6, inset) is approximately linear ($r^2 = 0.990$). The average apparent affinity constant $K_d$ is $2.3(\pm 0.9) \times 10^{-7}$ M, calculated on the basis of four separate determinations.

Table 1. Temperature dependence of the uptake of $^{125}$I-fucoidin by endothelial monolayers

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>37</th>
<th>7</th>
</tr>
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<tbody>
<tr>
<td>Total specific cell-associated (c.p.m.)</td>
<td>3098</td>
<td>3084</td>
</tr>
<tr>
<td>Released by trypsin (c.p.m.)</td>
<td>2290</td>
<td>2974</td>
</tr>
<tr>
<td>Cell-associated after trypsin (c.p.m.)</td>
<td>808</td>
<td>110</td>
</tr>
<tr>
<td>Percentage of total internalized (%)</td>
<td>26</td>
<td>3-5</td>
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Monolayers were incubated with fucoidin for 1 h and processed as described in Materials and Methods. The values shown are means of duplicate determinations.
Fig. 5. Effect of incubation at 7°C on the morphological effect of fucoidin on ALCL-1 monolayers. A. Control monolayers incubated at 7°C. B. Endothelial monolayers incubated with 10 μg/ml for 2 h at 7°C. The endothelial cells still retract when exposed to fucoidin at 7°C.

Fig. 6. Concentration dependence of the binding of [125I]fucoidin (10^6 c.p.m./μg) to endothelial monolayers at 7°C. The data shown have been corrected for non-specific binding as defined as the amount of [125I] associated with the cell monolayer in the presence of a 50-fold excess of unlabelled fucoidin. The non-specific binding was less than 15% of the total specific binding over the linear part of the concentration curve. Inset: Scatchard analysis of binding data.
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concentration dependence of fucoidin binding is very similar to the concentration dependence of the morphological response. The binding of fucoidin is half-maximal at 23 μg/ml, while the morphological effect is qualitatively maximal in the range of 10–20 μg/ml.

Specificity of fucoidin binding

The specificity of the binding of fucoidin to endothelial cells was investigated by examining the effectiveness of a variety of glycoconjugates as competitors of the binding of [125I]fucoidin (Fig. 7). Unlabelled fucoidin competes effectively for the binding of [125I]fucoidin. The Kᵦ for unlabelled fucoidin is approximately 10⁻⁷ M, which compares quite favourably with the Kᵦ determined for [125I]fucoidin, indicating that the binding properties of labelled and unlabelled fucoidin are very similar. Heparin competes for a fraction of the fucoidin binding sites, but a significant amount of the bound fucoidin is resistant to displacement by heparin. An average of 55 % of the total bound fucoidin is resistant to heparin displacement, based on three separate determinations. Dextran sulphate also competes for a fraction of the total bound fucoidin, displacing a maximum of 30 % of the total bound fucoidin. A wide variety of glycoconjugates and saccharides have no effect on the binding of fucoidin to endothelial monolayers (Fig. 7). Sulphation of fucoidin appears to be necessary for its binding activity, since desulphated fucoidin is inactive as an inhibitor of fucoidin.

Fig. 7. Specificity of the binding of [125I]fucoidin to endothelial monolayers; 10 μg/ml of [125I]fucoidin was incubated with endothelial monolayers in the presence of varying amounts of unlabelled polysaccharides. The amount of specific binding in the presence of unlabelled polysaccharides as competitive inhibitors is expressed as the percentage of the maximum specific binding in the absence of any unlabelled polysaccharide. A, Fucoidin (○ — ○); B, heparin (○ — ○); C, dextran sulphate (▲ — ▲); D, dermatan sulphate (△ — △); E, inactive polysaccharides: chondroitin sulphate, heparan sulphate, hyaluronic acid, yeast mannan, desulphated fucoidin (■ — ■; □ — □).
binding. However, the specificity of the binding of fucoidin cannot be explained on
the basis of ionic interactions due to the density of the charged sulphate groups. The
charge density of fucoidin is less than 1 negative charge per saccharide residue (Mian
& Percival, 1973; Medcalf & Larsen, 1977), whereas the charge density of heparin is
between 1 and 2 (Rodén, 1980) and for chemically sulphated dextran sulphate, the
value is 2.4.

The results of the investigations of $[^{125}\text{I}]$fucoidin binding are consistent with the
existence of two different classes of fucoidin binding sites on endothelial monolayers.
One class of sites may be related to the heparin binding site described for human
umbilical-cord endothelial cells (Glimelius, Busch & Höök, 1978). We have directly
investigated this possibility using $[^{125}\text{I}]$heparin. Heparin binds to Al4CL-1
monolayers but does not saturate within the concentration range in which fucoidin
saturates (Fig. 8). The inhibition of $[^{125}\text{I}]$heparin binding by unlabelled heparin is
directly proportional to the reduction of the specific activity by dilution with un-
labelled heparin, indicating that the $[^{125}\text{I}]$labelled heparin has the same binding
properties as the unlabelled heparin (Fig. 9). Unlabelled fucoidin competes for
$[^{125}\text{I}]$heparin binding to the same extent as the unlabelled heparin. Thus it appears
that heparin and fucoidin share at least one class of binding sites while another class
of binding sites is specific for fucoidin.

Is the binding of fucoidin to the heparin site responsible for the morphological
effect on endothelial cells? Since the binding of fucoidin to this site can be blocked
with an excess of heparin, under these conditions we would expect no effect of

![Fig. 8. Concentration dependence of the binding of $[^{125}\text{I}]$heparin (4 $\times$ 10$^4$ c.p.m./µg) to
Al4CL-1 endothelial monolayers at 7°C. The binding assay was performed as described in
Materials and Methods. The data shown are from two separate determinations and have
been corrected for non-specific binding.](image-url)
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Fig. 9. Specificity of the binding of $^{125}$I-labelled heparin to endothelial monolayers; 10 μg/ml of $[^{125}$I]heparin was incubated with endothelial monolayers in the presence of varying amounts of unlabelled heparin or fucoidin. Heparin (○—○); fucoidin (●—●).

Fig. 10. Effect of fucoidin on A1,CL-1 monolayers in the presence of excess heparin. A. 10 μg/ml fucoidin in the presence of 1 mg/ml heparin; B. control monolayers – no addition.

fucoidin if binding to the heparin site is critical. In the presence of a 100-fold excess of heparin, 10 μg/ml fucoidin still induces the retraction of the lateral contacts between adjacent cells (Fig. 10), indicating that the heparin site is not involved in the morphological response to fucoidin.
Fig. 11. Effect of fucoidin on BAE endothelial monolayers. A. Control monolayers, incubated in the absence of fucoidin; B, monolayers treated with 10 μg/ml fucoidin for 2 h at 37°C; C, monolayers treated with 100 μg/ml fucoidin for 2 h at 37°C. ×265.
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Fig. 12. Cell-type specificity of the morphological effect of fucoidin on monolayers. BHK (A, B) and 3T3 (C, D) fibroblast monolayers were incubated in the presence (B, D) or absence (A, C) of 1 mg/ml fucoidin for 2 h at 37°C.

Cell type specificity of fucoidin

We have investigated the effect of fucoidin on another bovine aortic endothelial cell line, BAE, and two lines of fibroblast-like cells, BHK and 3T3. BAE cells were also found to be susceptible to disruption by fucoidin in a manner similar to A14CL-1 cells (Fig. 11). 3T3 cells are particularly appropriate as controls since they have a flattened cobblestone-like morphology similar to endothelial cells (Porter, Todaro & Fonte, 1973). BHK cells are spindle-shaped cells, which become organized in parallel arrays at confluency (Erickson, 1981). The effect of fucoidin incubation on 3T3 and BHK cells is shown in Fig. 12. Fucoidin does not induce the retraction of lateral contacts between adjacent cells with either 3T3 or BHK cells, in contrast to endothelial cells. We have also examined the binding of $[^{125}\text{I}]$fucoidin to these fibroblastic cell types. At 20 μg/ml $[^{125}\text{I}]$fucoidin, 3T3 or BHK cells bind only 5% of the total fucoidin per cell as do endothelial cells. Under the same conditions, binding of $[^{125}\text{I}]$fucoidin to the gelatin-coated dish alone is 1–2% of the binding to endothelial monolayers.

DISCUSSION

Recent interest has been directed toward the investigation of the organization of endothelial cell monolayers in culture (Vlodasky & Gospodarowicz, 1979; Gospodarowicz & III, 1980). Vlodavsky & Gospodarowicz (1979) have identified a protein (CSP-60) whose expression at the cell surface correlates with the formation of a
confluent 'cobblestone' morphology. We have found that the sulphated fucoscontaining polysaccharides, fucoidin and sea-urchin egg jelly fucan, induce a dramatic alteration in the morphology of cultured bovine aortic endothelial cells. After treatment with fucoidin, regions of the lateral surfaces of the cell, formerly in contact with adjacent cells, have retracted, exposing the subendothelial matrix. This effect of fucoidin is not likely to be due to cellular toxicity, since the morphological effect of fucoidin is rapidly reversible. Within 1 h after removal of fucoidin and replacement with fresh media, contact is re-established along a proportion of the cell periphery, and after 24 h incubation the treated monolayers resemble the untreated controls.

The effect of fucoidin is specific. Only fucoidin and a similar sulphated fucan from sea-urchin egg jelly were found to disrupt endothelial monolayers, while a variety of other polysaccharides and glycoconjugates had no effect. Although sulphation appears to be necessary for the activity of fucoidin, it is not sufficient. Other sulphated glycoconjugates including dextran sulphate and heparin, which have higher charge densities than fucoidin, have no effect on the morphology of endothelial monolayers at higher concentrations. The effect of fucoidin on cultured A14CL-1 monolayers also displays cell-type specificity. A cultured line of bovine aortic endothelial cells, BAE, reacts in a similar fashion to A14CL-1 cells upon treatment with fucoidin whereas monolayers of 3T3 and BHK cells show no apparent morphological response.

The morphological effect of fucoidin on endothelial monolayers appears to be due to the binding of fucoidin to a specific site on the endothelial monolayers. The concentration dependence of the morphological effect of fucoidin correlates well with the binding of fucoidin to endothelial monolayers. The concentration required for a maximal morphological effect of fucoidin on A14CL-1 monolayers is in the range of 10–20 µg/ml, while the apparent $K_d$ for fucoidin is $2.3(±0.9) \times 10^{-7}$ M (23 µg/ml). At saturation, there are approximately $2 \times 10^5$ molecules of fucoidin bound per endothelial cell. When endothelial cells are incubated with fucoidin at 37 °C, some of the cell-associated fucoidin is internalized (as defined by resistance to removal by trypsin digestion or displacement by excess fucoidin). It is unlikely that this uptake of fucoidin is responsible for the morphological effect of fucoidin. Uptake by A14CL-1 endothelial cells is greatly reduced at 7 °C, yet cells still retract upon treatment with fucoidin.

The finding that A14CL-1 cells internalize 26% of the total monolayer-associated fucoidin at 37 °C, through a binding interaction, suggests that at least this fraction binds to the endothelial cell surface and not to areas of exposed extracellular matrix. However, it is not known whether the morphological response to fucoidin depends on an interaction with binding sites on the cell surface or with sites on the extracellular matrix. Fucoidin appears to bind to two distinct classes of sites on A14CL-1 monolayers. Approximately 50% of the fucoidin binding can be displaced by heparin, while the remainder cannot. A heparin binding site has been described for cultured human umbilical-vein endothelial cells (Glimelius et al., 1978). Heparin is also known to stimulate the migration of capillary endothelial cells (Azizkhan, Azizkhan, Zetter & Folkman, 1980). It appears that binding of fucoidin to the specific site is responsible for the morphological effect, since the presence of a large excess of heparin does not block the response to fucoidin.
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The mechanism by which fucoidin induces the retraction of adjacent endothelial cells is not clear. One possibility is that the binding of fucoidin to the cell surface produces retraction of the cell periphery by inducing contraction of the cytoskeleton. Another possibility is that fucoidin-binding sites mediate the lateral adhesion between cells or the attachment of cells to extracellular matrix, and that disruption of one or both of these interactions induces retraction of adjacent cell borders. This possibility has been demonstrated in teratocarcinoma stem cells (Grabel, Singer, Martin & Rosen, unpublished data) and sea-urchin fertilization (Glabe et al. 1982), where fucoidin was shown to block cell—cell adhesion by binding to discrete cell surface sites. The later explanation presumes the existence on the endothelial cell surface or in the extracellular matrix of endogenous structures analogous to fucoidin. Exogenous fucoidin would then compete with these endogenous glycoconjugates for the fucoidin binding sites, resulting in the disruption of the monolayer. Jones (1979) has reported the synthesis of extracellular fucose-containing glycoproteins by A14CL-1 endothelial cells. Endothelial cells are also known to synthesize a novel class of sulphated oligosaccharides that are N-linked to protein (Heifetz & Johnson, 1981; Heifetz & Allen, 1982). These structures are not produced or are produced in greatly reduced amounts by smooth muscle cells and a variety of other adult tissues (Heifetz & Johnson, 1981; Heifetz & Allen, 1982; Heifetz, Kinsey & Lennarz, 1980). Whether these sulphated oligosaccharides contain fucose has not yet been reported. Further investigation of the fucoidin binding site and its potential endogenous receptors may yield important information about the organization of the endothelial monolayer.

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