Fucoidans: Pro- or antiangiogenic agents?

Nadezhda E Ustyuzhanina2, Maria I Bilan3, Natalia A Ushakova4, Anatolii I Usov3, Mikhail V Kiselevskiy2, and Nikolay E Nifantiev1,2

1Laboratory of Glycoconjugate Chemistry; 2Laboratory of Plant Polysaccharides, N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky prospect 47, Moscow 119991, Russia; 3Laboratory of Plant Polysaccharides, N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky prospect 47, Moscow 119991, Russia; 4Russian Academy of Medical Sciences, V.N. Orekhovich Research Institute of Biomedical Chemistry, ul. Pogodinskaya 10, 119121 Moscow, Russia; and 5N.N. Blokhin Russian Cancer Research Center, Russian Academy of Medical Sciences, Kashirskoe shosse, 24, Moscow 115478, Russia

Received on May 15, 2014; revised on June 18, 2014; accepted on June 20, 2014

Sulfated polysaccharides of brown algae (fucoidans) attract great attention due to their high and strongly diversified biological activity. This review summarizes recent data on the structural variability of these polysaccharides and reports their anti- and proangiogenic properties. Recent publications have revealed that fucoidans isolated from different algal species may differ considerably in the structures of their backbones and branches, in both monosaccharide composition and sulfate content. It was found that the degree of sulfation significantly influences the biological properties of fucoidans. Additionally, fucoidan action in angiogenesis is highly dependent on molecular weight: antiangiogenic activity is connected with the high-molecular weight of polysaccharide molecules, whereas the low-molecular-weight fractions may act as proangiogenic agents. The influence of other fine structural details of fucoidans on angiogenesis remains to be established.

Keywords: angiogenesis / FGF / fucoidan / structure / VEGF

Introduction

Angiogenesis, the formation of new blood vessels, occurs under normal conditions during growth and tissue repair, but this process could also be regarded as a hallmark of pathologic al states such as tumor progression and various diabetic and inflammatory diseases (Carmeliet and Jain 2000; Potente et al. 2011). A number of signal molecules are known to be involved in the regulation of angiogenesis. The key players are growth factors, mainly vascular endothelial growth factor (VEGF) and fibroblast growth factors (FGF), which activate cell migration and tube formation (Ribatti 2005; Stringer 2006). Different in vitro and in vivo experimental methods have been developed for the study of certain steps of angiogenesis, as well as for the estimation of the influence of various compounds on this process (Tahergorabi and Khazaei 2012). Among the in vitro models, cell experiments for assessment of the ability of cell migration, new tube formation (Matrigel assay), growth factors and other cytokines secretion (ELISA) and growth factors expression (western blot) are very common. Some in vivo models require Matrigel application as a matrix for cell growth, and also hindlimb ischemia and corneal angiogenesis assays are used.

Heparan sulfate proteoglycans (HSPG), components of the cell surface and extracellular matrix, were shown to play an important role in angiogenesis (Stringer 2006; Casu et al. 2010; van Wijk et al. 2013; Knelson et al. 2014). Different fragments of the polysaccharide chains of HSPG are responsible for interactions between a number of growth factors and chemokines, promoting the formation of ternary complexes with their receptors (Mulloy and Rider 2006; Brown et al. 2013). Thus, it was shown that the presence of N-sulfated glucosamine and 2-O-sulfated iduronate units are essential for the bFGF binding to HSPG, while 6-O-sulfated glucosamine residues are required for mitogenic activity of the complex (Pye et al. 1998; Gooder et al. 2008). For the interaction with VEGF, heparan sulfate (HS) chains should contain two highly sulfated oligosaccharide domains, which are linked through a partially sulfated fragment (Robinson et al. 2006).

Exogenous anionic polysaccharides are known to influence angiogenesis via the mechanism of competitive inhibition of HSPG. The highly sulfated polysaccharides heparin and HS derived from animal tissues have been shown to be potent inhibitors of angiogenesis (Borsig 2010; Casu et al. 2010). The availability of synthetic oligosaccharides related to these biomolecules led to determination of the pharmacophore fragments and elucidation of the mechanism of action of heparin and HS polysaccharide chains (Cole et al. 2010). Parameters such as a chain length and pattern and degree of sulfation were found to be important for a biological effect. In this series, highly sulfated dodecasaccharide was shown to be the most potent antiangiogenic agent due to efficient binding both to VEGF and FGF1. Other examples of efficient inhibitors of angiogenesis are low-molecular-weight heparin, pentosan polysulfate and phosphomannopentaose sulfate (PI-88), which were also shown to interact with the growth factors (Cole and Jayson 2008; Kudchadkar et al. 2008).

Fucoidans represent an intriguing class of sulfated polysaccharides which are regarded as potential agents for the

© The Author 2014. Published by Oxford University Press. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com
regulation of angiogenesis. These biopolymers evoked interest, as can be seen from the bibliographic statistical records in Figure 1 (cf Ale et al. 2011). Great attention to fucoidans can be related to their availability from brown seaweeds, low toxicity and large broad structural variability, which stimulates studies to assess a relation between their structure and biological activity. In this review, we summarized the available data regarding the influence of fucoidans on angiogenesis in relation to their structural characteristics.

**Structural variations in fucoidans**

All brown algae contain sulfated polysaccharides known as fucoidans (Percival and McDowell 1967; Painter 1983). These polysaccharides are usually composed essentially of sulfated \(\alpha-L\)-fucose residues, but may also contain galactose, mannose, xylose, uronic acids and acetyl groups, sometimes in appreciable amounts. Algal fucoidans are present in natural sources in large amounts and are very interesting biologically active biopolymers, which are virtually devoid of toxicity (Li et al. 2005; Gideon and Rengasamy 2008). The most attractive property is their heparin-like anticoagulant and antithrombotic activity (Nagumo and Nishino 1996; Mourão 2004), but many other activities, such as antiviral, anti-inflammatory, antitumor, antiangiogenic, antiadhesive and others, have also been reported. There are a lot of review papers describing various biological actions of fucoidans and attempting to correlate their biological activities to their chemical structures (Berteau and Mulloy 2003; Cumashi et al. 2007; Kusaykin et al. 2008; Li et al. 2008; Pomin and Mourão 2008; Holtkamp et al. 2009; Ale et al. 2011; Besednova et al. 2011; Fitton 2011; Jiao et al. 2011; Wijesinghe and Jeon 2012). Unfortunately, in most cases, the chemical structures of fucoidan preparations used in biological experiments were not fully characterized. In spite of the great efforts of investigators, the detailed elucidation of algal fucoidan structures remains extremely difficult due to the structural heterogeneity and lack of regularity in fucoidan molecules (Bilan and Usov 2008; Usov and Bilan 2009). As a result, a distinct and reliable conclusion regarding chemical structures responsible for specific biological activities of fucoidans is often difficult to formulate.

Fucoidans isolated from different algal species may differ considerably in their composition and chemical structure. The simplest polysaccharides contain a linear backbone of 3-linked fucose residues with some branches (Figure 2). Such a fucoidan was isolated from *Chorda filum* (Chizhov et al. 1999). The \(^1\)H NMR spectra of its desulfated and deacetylated derivative corresponded to a hexasaccharide repeating unit, which contains five \(3\)-linked \(\alpha-L\)-fucopyranose residues in a linear chain and one \(\alpha-L\)-fucopyranose residue as a branch at position 2. Sulfate groups in the native polysaccharide occupy positions 4 (mainly) and 2, whereas some 3-linked \(\alpha-L\)-fucopyranose residues are acetylated at O-2. Similar fucoidans were found in *Laminaria saccharina* (which has now been renamed as *Saccharina latissima*) (Usov et al. 1998; Bilan et al. 2010), *L. cichorioides* (Zvyagintseva et al. 2003) and *Lessonia vadosa* (Chandia and Matsuiro 2008). A fucoidan from *Analiplus japonicus* (Bilan et al. 2007) contains the same (1\(\rightarrow\)3)-linked backbone but is much more branched. Acetylated fucoidan containing the same 3-linked backbone with single \(\alpha-D\)-glucopyranosyluronic acid residues as branches at position 2 of the main chain was isolated from *Cladosiphon okamuranus* (Nagaoka et al. 1999; Sakai, Ishizuka, et al. 2003). Alga *Punctaria plantaginea* was shown to contain sulfated xylufucan having similar (1\(\rightarrow\)3)-linked backbone, where two of every three fucose residues are sulfated at O-2, giving rise to tri-saccharide repeating units. However, this structural regularity is masked by the random xylosylation of many fucose residues at O-4 (Bilan et al. 2014). The most unusual polysaccharide of this type was found in *Chordaria flagelliformis*, where about one-third of the backbone residues are glycosylated at O-2 by \(\alpha-D\)-glucopyranosyluronic acid residues, and about half of these residues are glycosylated at O-4 by single \(\alpha-L\)-fucofuranose residues or by the disaccharides \(\alpha-L\)-Fuc\(f\)-\(f\)-\(1\(\rightarrow\)2\)-\(\alpha-L\)-Fuc\(f\)-\(1\(\rightarrow\)\) (Bilan et al. 2008).

Fucoidans isolated from the representatives of the order Fucales contain a backbone of alternating 3- and 4-linked \(\alpha-L\)-fucopyranose residues (Figure 2). Thus, the predominant

**Fig. 1.** Statistical data for scientific publications related to fucoidan studies and their biological activities (ca 20% of such papers are dedicated to angiogenesis investigation and antitumor effect). Searched in April, 2014 with SciFinder (CAS) database.

**Fig. 2.** Two types of fucoidan backbones.
repeating structure \((\rightarrow 3)\alpha-L-Fucp-2-S-(1 \rightarrow 4)\alpha-L-Fucp-2,3-di-S-(1\rightarrow)_n\) was suggested for fucoids from *Asciophyllum nodosum* and *Fucus vesiculosus* (Chevolot et al. 2001) in contrast to the previous structural evidence (Patankar et al. 1993). A very similar structure was established for the fucoidan from *Pelvetia canaliculata* by enzymolysis studies (Descamps et al. 2006). Fucoidans with similar backbones but differing in the sulfation pattern were isolated from *Fucus evanescens* (Bilan et al. 2002) and *F. distichus* (Bilan et al. 2004), while the fucoidan from *F. serratus* (Bilan et al. 2006) was shown to contain oligosaccharide branches at O-4 of 3-linked residues of the main chain. It should be noted, however, that the isolation of fucoidan fractions, where \((1 \rightarrow 3)\)-linkages predominate over \((1 \rightarrow 4)\)-linkages, was also described either from *A. nodosum* (Marais and Joseleau, 2001) or from *F. evanescens* (Kusaykin et al. 2006).

Some fucoidans may contain fucose and galactose in comparable amounts. Such polysaccharides were extracted from sporophylls of *Alaria fistulosa* (Usov et al. 2005) and *Undaria pinnatifida* (Lee et al. 2004; Hemmingson et al. 2006; Synytsya et al. 2010), as well as from *Laminaria* (Saccharina) *japonica* (Xue et al. 2001; Wang et al. 2010; Vishchuk et al. 2011), *L. cichorioides* (Yoon et al. 2007), *L. gurjanovae* (Shevchenko et al. 2007) and *Sargassum patens* (Zhu et al. 2010). Fucoidan fractions that are rich in galactose and sulfate were obtained also from the very complex mixtures of polysaccharides extracted from *S. stenophyllum* (Duarte et al. 2001) and *Adenocystis utricularis* (Ponce et al. 2003). The careful fractionation of the crude sulfated polysaccharide from *L. angustata* var. *longissima* resulted in the isolation of a small amount of sulfated galactan, differing from the well-known sulfated galactans of the red seaweeds and ascidians (Nishino, Takabe et al. 1994). At the same time, oligosaccharides consisting of both sugars were obtained from galactofucans of *Ecklonia kurome* (Nishino et al. 1991) and *L. gurjanovae* (Shevchenko et al. 2007). A highly sulfated galactofucan fraction from *S. polycystum* was shown to contain a backbone of 3-linked \(\alpha-L\)-fucopyranose 4-sulfate residues, as in many other fucoidans, but rather short sequences of these residues were interspersed by the single 2-linked \(\alpha-D\)-galactose residues also sulfated at O-4 (Bilan et al. 2013) (Figure 3).

Brown algae may contain heterofucans of much more complex composition. Several polysaccharide fractions composed of fucose, xylose, uronic acid, galactose and sulfate in different proportions were isolated from *A. nodosum* (so-called ascophyllan, Larsen et al. 1966; Jiang et al. 2013), *Dictyota menstrualis* (Albuquerque et al. 2004), *Padina gymnospora* (Silva et al. 2005), *Spatoglossum Schroederi* (Leite et al. 1998; Rocha et al. 2005) and *Hizikia (Sargassum) fusiforme* (Li et al. 2006). Residues of \(\beta-D\)-glucuronic acid were suggested to be

![Fig. 3. The main structural elements of recently discovered fucoidans from brown seaweeds.](image-url)
backbone components of these polysaccharides. Fucoidan fractions enriched in glucuronic acid residues were found also in *L. japonica* (Wang et al. 2008) and many other brown algae. The most carefully investigated glucuronic acid-containing polysaccharide from *Kjellmaniella crassifolia* was shown to contain branched trisaccharide repeating units. Its treatment with bacterial lyase gave rise to a series of unsaturated trisaccharides differing in the number and positions of sulfate. Their structures were determined as \( \Delta^1,5\text{-}\text{GlcA-}(1 \rightleftharpoons 2)\text{-}[\alpha\text{-Fucp-3-S-(1 \rightleftharpoons 3)]-}\text{-}\text{d-Man, } \Delta^1,5\text{-}\text{GlcA-}(1 \rightleftharpoons 2)\text{-}[\alpha\text{-Fucp-3-S-(1 \rightleftharpoons 3)]-}\text{-}\text{d-Man-6-S and } \Delta^4,5\text{-}\text{GlcA-}(1 \rightleftharpoons 2)\text{-}[\alpha\text{-Fucp-2,4-di-S-(1 \rightleftharpoons 3)]-}\text{-}\text{d-Man-6-S according to a backbone composed of GlcA and Man with Fuc as side substituents. This finding indicated the existence of a novel polysaccharide in the fucoidan family and a novel endolyase activity in the bacterial extracellular enzymes (Sakai, Kimura et al. 2003).* A quite unusual structure was suggested recently for heteropolysaccharide from *S. fusiforme* containing a backbone built up of alternating \( \alpha\)-galacturonic acid and \( \alpha\)-hexose (galactose, mannose or fucose) residues (Hu et al. 2014).

Since various algal species may differ considerably in the structure of their sulfated polysaccharides (e.g. see Table I in Cumashi et al. 2007), it is not surprising that fucoidans of different origins have different biological activities. Even crude fucoidan obtained from a single algal species may contain fractions which are quite different in their chemical structure and biological properties. A good example is the comparison of the action of ascophyllan and fucoidan isolated from the same *A. nodosum* on various cell lines (Jiang et al. 2010). Reinvestigation of a fucoidan preparation from *S. latissima* (Bilan et al. 2010) showed that the main fucan sulfate described previously (Usov et al. 1998) is accompanied by at least three other types of sulfated polysaccharides, namely (i) branched fucogalactan, (ii) fucogluconoromannan resembling the polysaccharide found earlier in *K. crassifolia* (Sakai, Kimura et al. 2003; see above) and (iii) fucoglucomannan with a backbone of 3-linked \( \beta\)-D-glucopyranosyluronic acid residues with \( \alpha\)-L-fucopyranose residues as single branches at O-4. The main fucan sulfate component was markedly more active in several biological tests compared with other structures (Croci et al. 2011).

For a long time, the alga *F. vesiculosus* was the most popular source of fucoidan for chemical and biological studies. The first chemical structure of this polysaccharide was suggested in 1950 (Conchie and Percival 1950), reinvestigated in 1993 (Patankar et al. 1993) and corrected once again in 2001 (Chevolot et al. 2001). A commercial fucoidan preparation isolated from *F. vesiculosus* is distributed by Sigma-Aldrich Corporation as a brownish powder and is typically used in a number of biological experiments without any purification or fractionation. At the same time, it should be emphasized that this preparation is highly heterogeneous. According to careful fractionation by gel filtration and anion-exchange chromatography (Nishino, Nishioka, et al. 1994), this crude preparation comprises a wide spectrum of polysaccharides ranging from typical fucoidans mainly containing fucose sulfate and no uronic acids to low-sulfated components with a high content of uronic acids and neutral monosaccharides other than fucose. These polysaccharides also had a wide range of molecular weight and differed considerably in their anticoagulant activity.

Hence, according to these findings, the careful fractionation of crude fucoidans and chemical characterization of individual fractions are extremely important for a correlation of chemical structures and biological activities of fucoidan components.

**Influence of fucoidans on angiogenesis**

In spite of the many and various in vitro and in vivo studies of fucoidans, there is no rational concept to predict which certain fucoidans would exhibit pro- or antiangiogenic properties. This confusing situation is explained by the fact that too different and incomparable fucoidan preparations were used in biological experiments in different research groups. Unfortunately, in many biological experiments described in the literature, only crude fucoidans rather than the fractionated and structurally well-characterized samples were used. Earlier, there were attempts to review studies of the fucoidan effect on angiogenesis regarding the mechanism of action (Boisson-Vidal et al. 2007; Wang and Miao 2013; Kwak 2014). Below, we discuss the described data about anti- and proangiogenic effects of fucoidans separately, paying particular attention to the structure of tested polysaccharides. Isolation protocols, structural features and biological effects of the studied fucoidans are summarized in Table I.

**Antiangiogenic effects of fucoidans**

Soeda et al. (1997) investigated the fucoidan from *F. vesiculosus*, which was isolated from the commercially available crude seaweed extract (product of Sigma-Aldrich) by gel chromatography (Table I). The polysaccharide obtained, namely “NF”, was further chemically sulfated with the formation of the over-sulfated derivative “OSF”. As described in the section Structural variations in fucoidans, the polysaccharide from *F. vesiculosus* was shown to contain \((1 \rightleftharpoons 3)(1 \rightleftharpoons 4)\)-backbone. The sulfate content was 31.2% for “NF” and 52.4% for “OSF”. The influence of the polysaccharides on angiogenesis was assessed by in vitro experiments with human umbilical vein endothelial cells (HUVeCs) using basement membrane preparation (Matrigel) as a matrix for cell growth. The sample “OSF” was found to significantly inhibit tube formation by endothelial cells, while “NF” demonstrated only a moderate effect. A detailed study of the mechanism of action has shown that “OSF” inhibited cell migration and stimulated the secretion of the plasminogen activator inhibitor (PAI-1) antigen.

Further investigation of the preparations “NF” and “OSF” together with the desulfated derivative of parent fucoidan “NF”, marked as “desF”, has shown that “OSF” effectively blocks HUVEC migration and the tubulogenesis induced by bFGF at a concentration of 100 µg/mL, while “NF” demonstrated a weak effect (27% inhibition) and “desF” was inactive as the same concentration (Soeda et al. 2000). These results reveal that the degree of sulfation significantly influences the level of antiangiogenic activity of fucoidans.

A subsequent study of the “NF” and “OSF” samples indicated that both polysaccharides prevented the binding of VEGF to its cell surface receptor (Koyanagi et al. 2003). Notably, the highly sulfated polysaccharide “OSF” was shown to be a more potent inhibitor than “NF”. *In vivo* studies of the
Table I. Isolation protocols, structural features and biological effects of brown seaweed fucoidans

<table>
<thead>
<tr>
<th>Seaweed</th>
<th>Isolation and modification methods</th>
<th>Structural features</th>
<th>Biological effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antiangiogenic fucoidans</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. saccharina</td>
<td>Extraction followed by precipitation with Cetavlon and deacetylation with alkali</td>
<td>Homo-(1→3)-backbone with 2-O-α-fucosyl branch as the main component, SO₃Na—29.6%</td>
<td>Potent inhibition of HUVEC tubulogenesis in vitro (100 µg/mL), decreasing of PAI-1 level in HUVEC supernatant</td>
<td>Cumashi et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Extraction followed by precipitation with Cetavlon and deacetylation with alkali</td>
<td>Homo-(1→3)-backbone with 2-O-α-fucosyl branch, SO₃Na—36.8%</td>
<td>Potent inhibition of HUVEC tubulogenesis in vitro (100 µg/mL), decreasing of PAI-1 level in HUVEC supernatant</td>
<td>Croci et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>Product of South Product Corporation</td>
<td>Homo-(1→3)-backbone with 2-O-α-glucuronyl branch, SO₃Na—27.5%</td>
<td>No inhibition of HUVEC tubulogenesis in vitro (100 µg/mL)</td>
<td>Cumashi et al. (2007)</td>
</tr>
<tr>
<td>C. okamuranus</td>
<td>Extraction followed by precipitation with Cetavlon and deacetylation with alkali</td>
<td>Homo-(1→3)-backbone with 2-O-α-fucosyl branch, SO₃Na—34.8%</td>
<td>Potent inhibition of HUVEC tubulogenesis in vitro (100 µg/mL), decreasing of PAI-1 level in HUVEC supernatant</td>
<td>Cumiashi et al. (2007)</td>
</tr>
<tr>
<td>L. japonica</td>
<td>Extraction followed by precipitation with Cetavlon and deacetylation with alkali</td>
<td>Homo-(1→3)-backbone with 2-O-α-fucosyl branch, SO₃Na—15.1%</td>
<td>Potent inhibition of HUVEC tubulogenesis in vitro (100 µg/mL), decreasing of PAI-1 level in HUVEC supernatant</td>
<td>Matsubara et al. (2005)</td>
</tr>
<tr>
<td>F. serratus</td>
<td>Extraction followed by precipitation with Cetavlon and deacetylation with alkali</td>
<td>Homo-(1→3)-backbone with 2-O-α-fucosyl branch, SO₃Na—33.2%</td>
<td>Potent inhibition of HUVEC tubulogenesis in vitro (100 µg/mL), decreasing of PAI-1 level in HUVEC supernatant</td>
<td>Cumashi et al. (2007)</td>
</tr>
<tr>
<td>F. distichus</td>
<td>Extraction followed by precipitation with Cetavlon and deacetylation with alkali</td>
<td>Homo-(1→3)-backbone with 2-O-α-fucosyl branch, SO₃Na—36.8%</td>
<td>Potent inhibition of HUVEC tubulogenesis in vitro (100 µg/mL), decreasing of PAI-1 level in HUVEC supernatant</td>
<td>Cumashi et al. (2007)</td>
</tr>
<tr>
<td>F. vesiculosus</td>
<td>Product of Sigma-Aldrich Corporation</td>
<td>Homo-(1→3)-backbone with 2-O-α-fucosyl branch, SO₃Na—15.1%</td>
<td>Potent inhibition of HUVEC tubulogenesis in vitro (100 µg/mL), decreasing of PAI-1 level in HUVEC supernatant</td>
<td>Lv et al. (2012), Xue et al. (2012), Dithmer et al. (2014)</td>
</tr>
<tr>
<td></td>
<td>Product of Sigma-Aldrich Corporation, purified by gel-permeation chromatography</td>
<td>Homo-(1→3)-backbone with 2-O-α-fucosyl branch, SO₃Na—31.2%</td>
<td>Potent inhibition of HUVEC tubulogenesis in vitro (100 µg/mL), decreasing of PAI-1 level in HUVEC supernatant</td>
<td>Soeda et al. (2000), Koyanagi et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>Product of Sigma-Aldrich Corporation, purified by gel-permeation chromatography and chemically sulfated</td>
<td>Homo-(1→3)-backbone with 2-O-α-fucosyl branch, SO₃Na—52.4%</td>
<td>Potent inhibition of the HUVEC migration and tube formation (100 µg/mL) Suppressing of the mitogenic and chemotactic actions of VEGF on HUVEC by preventing the binding of VEGF to its cell surface receptor Potent inhibition of the HUVEC migration and tube formation, increasing of the PAI-1 antigen accumulation, and decreasing of the collagenolytic activity Potent inhibition of the bFGF-induced HUVEC migration and tube formation (100 µg/mL) Suppressing of the mitogenic and chemotactic actions of VEGF on HUVEC by preventing the binding of VEGF to its cell surface receptor, inhibition of in vivo angiogenesis in mice model with Sarcoma 180 cells, Lewis lung carcinoma and B16 melanoma</td>
<td>Soeda et al. (1997), Soeda et al. (2000), Koyanagi et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>Product of Sigma-Aldrich Corporation, purified by gel-permeation chromatography and chemically desulfated</td>
<td>Homo-(1→3)-backbone with 2-O-α-fucosyl branch, SO₃Na—0%</td>
<td>No inhibition of the bFGF-induced HUVEC migration and tube formation (100 µg/mL)</td>
<td>Soeda et al. (2000)</td>
</tr>
<tr>
<td>F. evanescens</td>
<td>Extraction followed by precipitation with Cetavlon and deacetylation with alkali</td>
<td>Homo-(1→3)-backbone with 2-O-α-fucosyl branch, SO₃Na—23.6%</td>
<td>No inhibition of HUVEC tubulogenesis in vitro (100 µg/mL)</td>
<td>Cumashi et al. (2007)</td>
</tr>
<tr>
<td>F. spiralis</td>
<td>Extraction followed by precipitation with Cetavlon and deacetylation with alkali</td>
<td>Homo-(1→3)-backbone with 2-O-α-fucosyl branch, SO₃Na—36.3%</td>
<td>Potent inhibition of HUVEC tubulogenesis in vitro (100 µg/mL), decreasing of PAI-1 level in HUVEC supernatant</td>
<td>Cumashi et al. (2007)</td>
</tr>
<tr>
<td>A. nodosum</td>
<td>Extraction followed by precipitation with Cetavlon and deacetylation with alkali</td>
<td>Homo-(1→3)-backbone with 2-O-α-fucosyl branch, SO₃Na—25.9%</td>
<td>Weak inhibition of HUVEC tubulogenesis in vitro (100 µg/mL)</td>
<td>Cumashi et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Product of Sigma-Aldrich Corporation</td>
<td>Homo-(1→3)-backbone with 2-O-α-fucosyl branch, SO₃Na—24.4%</td>
<td>Weak inhibition of HUVEC tubulogenesis in vitro (100 µg/mL)</td>
<td>Cumashi et al. (2007)</td>
</tr>
</tbody>
</table>

Continued
fucoidan “OSF” demonstrated its ability to block angiogenesis induced by tumor cells implanted in mice.

Another fucoidan sample, which demonstrated antiangiogenic activity, was prepared from the seaweed Cladosiphon novae-caledoniae (Ye et al. 2005). The studied polysaccharide appeared to be the abalone glycosidase-digested polysaccharide extract that is commercially available as a product named “Power fucoidan”, which was produced by the Daiichi Sangyo Corporation (Osaka, Japan). Unfortunately, there are no data about the structure of such a fucoidan preparation. The polysaccharide was shown to decrease the expression and secretion of VEGF in human uterine carcinoma HeLa cells, and to suppress tubulogenesis induced by tumor cells in vitro.

Matsubara et al. (2005) prepared two fucoidan samples of medium molecular weight (15–20 and ~30 kDa) via the mild acidic hydrolysis of a polysaccharide extract from the seaweed L. japonica. The sample, with a molecular weight of ~30 kDa (SO3Na:33.2%), had similar effects to that of the untreated fucoidan from F. vesiculosus (product of Sigma-Aldrich) and caused the inhibition of angiogenesis in both in vitro and ex vivo experiments. On the contrary, another fucoidan (MW 15–20 kDa) with a low degree of sulfation (SO3Na:8.2%) enhanced HUVEC migration and did not inhibit HUVEC tube formation. The authors suggested that the molecular weight of 20–30 kDa appeared to be a critical point for the fucoidan effect on angiogenesis. Unfortunately, the difference in the degree of sulfation was not considered in explanation of the results of the study.

In 2007, the results of a comparative biological study of nine structurally different fucoidans were reported (Cumashi et al. 2007). The polysaccharides were isolated from the seaweeds L. saccharina, L. digitata, F. vesiculosus, F. serratus, F. distichus, F. evanescent, F. spiralis and A. nodosum using a combination of extraction and anion-exchange chromatography (see Table I), while the fucoidan from the seaweed C. okamuranus was a commercially available crude extract (South Product Corporation, Japan). The polysaccharides varied in monosaccharide composition, the degree and pattern of sulfation, the types of glycoside bonds and the presence of branches. Their structural features are discussed in the section Structural variations in fucoidans and are summarized in Table I. The fucoidans from L. saccharina, L. digitata, F. serratus, F. distichus and F. evanescent effectively inhibited HUVEC tubulogenesis in vitro at a concentration of 100 µg/mL, which correlated well with the decreasing PAI-1 levels in HUVEC supernatants. The fucoidans from F. spiralis and A. nodosum demonstrated a weak effect, whereas the polysaccharides from C. okamuranus and F. vesiculosus were inactive. These results indicate that the value of biological effect is strongly determined by the structural features of the fucoidans. For instance, the polysaccharides from L. saccharina and C. okamuranus have a similar homo-(1 → 3)-linked α-L-fucosyl backbone, but they vary in the degree of sulfation (1.1 for L. saccharina and 0.4 for C. okamuranus) and type of branches (fucose unit for L. saccharina and uronic acid for C. okamuranus). It could be suggested that the absence of any antiangiogenic activity in C. okamuranus is linked to insufficient levels of sulfation and/or the presence of uronic acid residues as branches in its structure.

The most active sample from the above series, the fucoidan from L. saccharina (“L.s.-P”), was investigated in more detail.
(Croci et al. 2011). First, ion-exchange chromatography of this polysaccharide gave two main fractions: O-sulfated fucogalacturonanomannan L.s.-1.0 and O-sulfated homo-(1 → 3)-linked fucan bearing fucose units at O-2 L.s.-1.25 (Bilan et al. 2010). Then, the study of these polysaccharides in angiogenesis assays demonstrated that only the parent polysaccharide L.s.-P and its fraction L.s.-1.25 were potent inhibitors of bFGF-induced HUVEC tubulogenesis in vitro. Moreover, L.s.-P and L.s.-1.25 significantly reduced angiogenesis in mice bearing Matrigel plugs with melanoma cells. The fucogalacturonanomannan L.s.-1.0 was inactive in all of the performed experiments. Therefore, it could be concluded that sulfated fucans mainly determined the antiangiogenic activity of the crude fucoidan extract.

The antiangiogenic activity of the fucoidan extract derived from the seaweed U. pinnatifida was also studied (Liu et al. 2012). The polysaccharide was prepared by the enzymatic hydrolysis of a crude algal biomass followed by alcohol precipitation and further purification by ion-exchange and gel chromatography. The obtained fucoidan sample consisted mainly of neutral monosaccharides (fucose and galactose, totally 59.2%), sulfates (21%) and uronic acid (9.13%). The MW of the polysaccharide was estimated to be 9.52 × 10^4 Da. There were no data about the configuration of glycoside bonds, modes of linkages between monosaccharide units and their sequences along the polysaccharide chain. This fucoidan was shown to inhibit endothelial cell proliferation and migration. Additionally, at high concentrations (400 μg/mL), this polysaccharide decreased HUVEC tubulogenesis in vitro, and significantly inhibited angiogenesis ex vivo at a concentration of 100 μg/mL.

Commercially available crude fucoidan from the seaweed F. vesiculosus (Sigma-Aldrich) became very attractive, in spite of the absence of sufficient data on its exact structure (see the section Structural variations in fucoidans). A number of researchers used this product without any purification. Thus, it was demonstrated that this preparation inhibited HUVEC angiogenesis induced by both T98G glioma cells and THP1 monocytes (Lv et al. 2012). An increase in the levels of soluble VEGF receptor 1 (sFlt-1) was detected in these experiments. In another investigation, the same commercial fucoidan (Xue et al. 2012) was shown to decrease VEGF expression on mouse breast cancer 4T1 cells in vitro. Experiments on tumor-bearing mice have shown that the fucoidan caused a significant decreasing of microvessel number in the tumors. A general decrease of VEGF expression and secretion in the tumors was observed after fucoidan treatment.

Further studies of this commercial fucoidan were conducted in experiments with retinal pigment epithelium (RPE) cells (Dithmer et al. 2014). The polysaccharide was found to decrease VEGF secretion in RPE/choroid explants and RPE cells and to inhibit angiogenesis of peripheral endothelial cells induced by VEGF and RPE-supernatant. The author suggested fucoidan as an effective agent for the treatment of exudative age-related macular degeneration.

Proangiogenic effects of fucoidans
The method of radical depolymerization of polysaccharides was developed in the mid-1990s. It was applied to transform a fucoidan from the seaweed A. nodosum into low-molecular-weight fragments (LMWFs) (Nardella et al. 1996). Sulfated polysaccharides of A. nodosum are highly heterogeneous and primarily contain two components, a fucan sulfate and another polysaccharide (ascophyllan) that is rich in uronic acid and xylose (Larsen et al. 1966, 1970). Uronic acid residues were substantially degraded under radical depolymerization conditions, whereas sulfate groups were mainly retained. The procedure provided the possibility of preparing LMWF of ~10 kDa starting from the polysaccharide with a molecular weight of ~600 kDa. Unfortunately, no data about the fine structure of such preparations have been reported.

A number of studies have been performed using LMWF samples. Particularly, the LMWF of 16 kDa was found to stimulate HUVEC proliferation in the presence of fetal bovine serum and FGF-1, while the fucoidan inhibited cell growth in the presence of bFGF (Giraux et al. 1998).

Furthermore, the LMWF effect on endothelial cell differentiation was assessed in experiments of the expression of surface proteins involved in angiogenesis (Matou et al. 2002). LMWF of 16 kDa was shown to increase α6 integrin subunit expression on endothelial cells. The combination of LMWF with bFGF led to a significant enhancement in expression of the α6, β1 and PECAM-1 integrin subunits. As a result, the fucoidan effectively increased bFGF-induced angiogenesis. A similar level of antiangiogenic activity was observed for other LMWF preparations derived from A. nodosum (Chabout et al. 2003; Lake et al. 2006).

A beneficial effect of LMWF also was shown via in vivo experiments in a model of hindlimb ischemia. Here, Luyt et al. (2003) reported that the administration of fucoidan alone or in combination with bFGF at the site of the injury significantly improved revascularization. Sarlon et al. (2012) found that LMWF treatment on endothelial colony-forming cells (ECFCs) increased the cell adhesion to activated endothelium and enhanced extravasation in vitro. In a model of murine hindlimb ischemia in mice, significant decrease of ischemic tissue necrosis was observed after the introduction of ECFC stimulated by LMWF.

Unfortunately, in all of the experiments with LMWF, there was no reference control of the parent fucoidan from A. nodosum. As shown above, the high-molecular-weight fucoidan from this seaweed demonstrated a weak antiangiogenic effect (Cumashi et al. 2007).

Conclusion
Fucoidans isolated from different algal species vary considerably in their composition and chemical structure, including parameters such as the monosaccharide content, the degree and pattern of sulfation, types of glycoside bonds, the presence of branches and molecular weight. The value of the biological effect strongly depends on their origin and the method used for preparation and modification. Due to the fact that the structures of fucoidan preparations have not been elucidated in detail in many cases, it is not possible to unambiguously answer the question of which particular structural characteristics of fucoidans determine the mode of action in angiogenesis. Generally, it can be concluded that high-molecular-weight fucoidans (MW > 30 kDa) with a high degree of sulfation demonstrate an
antiangiogenic effect, whereas low-molecular-weight fucoidans (MW < 15 kDa) promote angiogenesis. This conclusion is correct only in the case of fucoidan derived from *A. nodosum*. It should also be noted that such preparations were mainly characterized according to their molecular weight, but not according to their degree of sulfation and sulfation pattern, both of which may have a stronger influence on their effect on angiogenesis. Thus, the fine structure of fucoidan fragments, which are responsible for the anti- or proangiogenic properties and the mechanism of their action, remains unclear. Unlike the “heparin story,” where most of the structural determinants have already been revealed, the “fucoidan story” is only at the beginning. It can be expected that the use of structurally distinct synthetic oligosaccharides related to different types of fucoidans can be an indispensable approach towards the elucidation of the mechanisms responsible for fucoidan biological activities. The preparation of such molecular probes is currently ongoing (Khatuntseva et al. 2000; Ustyuzhanina et al. 2006; Ustyuzhanina et al. 2009), and includes large oligosaccharides, particularly hexa decasaccharides (Krylov et al. 2011).

**Funding**

This work was supported in part by the Russian Foundation for Basic Research (KOMFI-grant 13-04-40315-K and its parts 13-04-40313-H, 13-04-314-H and 13-04-40315-H).

**Acknowledgements**

We thank Dr Nadezda V. Krukovskaya for assistance in the preparation of Figure 1.

**Conflict of interest**

None declared.

**Abbreviations**

ECFCs, endothelial colony-forming cells; FBS, fetal bovine serum; FGF, fibroblast growth factor; HS, heparan sulfate; HSPG, heparan sulfate proteoglycans; HUVEC, human umbilical vein endothelial cell; LMWF, low-molecular-weight fucoidan; MW, molecular weight; PAI-1, plasminogen activator inhibitor-1; RPE, retinal pigment epithelium; VEGF, vascular endothelial growth factor.

**References**


