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Fucoidans — sulfated polysaccharides of brown algae

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Abstract. The methods of isolation of fucoidans and determination of their chemical structures are reviewed. The fucoidans represent sulfated polysaccharides of brown algae, the composition of which varies from simple fucan sulfates to complex heteropolysaccharides. The currently known structures of such biopolymers are presented. A variety of the biological activities of fucoidans is briefly summarised. The bibliography includes 263 references.

I. Introduction: algal fucoidans and fucan sulfates of echinoderms

Brown algae (Phaeophyceae) constitute a large taxon (division) of lower marine plants counting over 1000 species.[1,2] These algae are prevalent in seas of the ocean. Several species of brown algae are obtained from natural populations on an industrial scale or grown on sea plantations. They are used for food or undergo chemical treatment in order to isolate alginic acids and their salts. The alginic acids are unique polysaccharides the linear molecules of which are made up of β-D-mannuronic and α-L-guluronic acid residues.[3] They find wide application in medicine, food and biotechnological industries and a number of other industry branches.[4]

Apart from the alginic acids all known brown algae contain sulfated polysaccharides called fucoidans.[5,6] α-L-Fucose residues esterified with sulfuric acid are the basic components of the majority of their molecules. Fucoidans frequently include several other monosaccharides — galactose, mannose, xylose, uronic acids and also acetyl groups. Such polysaccharides do not occur in other divisions of algae and in land plants; however, related biopolymers were found in marine invertebrates (sea urchins and holothurians) belonging to the type Echinodermata.[7] Animal polysaccharides contain only fucose and sulfate groups, and in accordance with the accepted nomenclature of carbohydrates these polysaccharides are referred to as fucan sulfates, whereas the term 'fucoidans' denotes polysaccharides with more complex composition obtained from algae.

Algal fucoidans are available from natural sources in large quantities and known as promising biologically active biopolymers that are virtually devoid of toxic properties.[8,9] Their heparin-like (anticoagulant and antithrombotic) action[10,11] is studied in most detail; however, of no lesser interest are their other activities, such as antiviral, antiinflammatory, antitumour, antiangiogenic, antiadhesive, *etc.* In recent years rapid growth in the number of publications devoted to the biological properties of fucoidans has been observed. At the same time the chemical exploration of these polysaccharides develops appreciably more slowly. As follows from recently published reviews,[12–17] a detailed structural analysis of algal fucoidans still represents considerable difficulties. For that reason the structural diversity of fucoidans is not completely investigated until now, while establishing reliable correlations between their chemical structures and biological effects fails.

The first reference to fucoidans in the literature is dated 1913,[18] while the first data on their chemical structures appeared about 60 years ago.[19,20] The research subject of these pioneer works was a fucoidan from *Fucus vesiculosus*, which further was used most often in biological tests. Methods of structural analysis of fucoidans existing at that time allowed the suggestion of several alternative structures (linear and branched, with different types of bonds between fucose residues and different arrangement of sulfate groups),[20] the choice between which proved to be complicated. Subsequently, the structure of this fucoidan was repeatedly revised with the development of methods of the study of polysaccharide structures.[21,22] However, even nowadays, despite the employment of state-of-the-art powerful physicochemical techniques such as NMR spectroscopy, the determination of the chemical structures of fucoidans continues to be a difficult problem. This is accounted for, on the one hand, by the fact that algae

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often contain complex mixtures of sulfated polysaccharides of different chemical nature, the separation of which is no easy task.[23,24] On the other hand, this is associated with pecularities of fucoidan molecular structures, such as high sulfation degree, branching, and the lack of convenient methods of their partial degradation to oligosaccharides. However, the main difficulties arise from the irregular distribution of individual structural units along a biopolymer chain.

The special properties of algal fucoidans can be illustrated by their comparison with fucan sulfates from echinoderms. The fucan sulfates were isolated from the body wall of holothurian,[7,25,26] where they seem to act as analogues of sulfated glycosaminoglycans of the connective tissue of vertebrates, and also from the sea urchin eggs,[7,27–30] where they form an external jelly coat around each egg. In the last case fucan sulfates play an important role in the fertilisation process, contributing to the recognition of an egg by a spermatozoon and inducing the development of an acrosomal reaction.[31] No wonder that the structures of the fucan sulfates of sea urchins are species-specific.

From the chemical point of view, known fucan sulfates are linear polymers of α-L-fucose containing only 1→3 (3-linked) or 1→4 (4-linked) bonds between monosaccharides. Further structural differences between them result from the positions of sulfate groups. In the simplest cases all fucose residues are sulfated in a same manner. Thus, from the sea urchin *Strongylocentrotus franciscanus* a polymer of 3-linked 2-sulfate of α-L-fucose[29] was isolated, while from *S. droebachiensis* was a polymer of 4-linked 2-sulfate of α-L-fucose.[30] In more complex cases sulfate groups are located along the chain in such a way that trisaccharide or tetrasaccharide repeat units differing in the structure for different species of sea urchins are formed (Fig. 1).[7,27–30]

Like similar polysaccharides of other classes, native fucan sulfates give rise to well-resolved NMR spectra, the complexity of which is determined by the composition and structure of oligosaccharide repeat units. These spectra are fully interpretable and serve as a main tool for the structural analysis of fucan sulfates from echinoderms. On the contrary, the NMR spectra of native fucoidans from algae, as a rule, defy direct interpretation and contain either poorly resolved broadened signals or many narrow signals with similar chemical shifts, which are difficult to assign to distinct atoms.[7] Such spectra are characteristic of irregular polymers.

The aim of this review is to discuss the ways of overcoming problems related to the determination of fucoidan structures. In addition, recently published literature data on the structures and biological activities of fucoidans are presented.

II. Methods of structural analysis of fucoidans

1. Detection of fucoidans and determination of their content

L-Fucose is practically the only 6-deoxyhexose present in brown algae. Therefore the presence of a fucoidan can be detected and its quantitative content can be estimated by determination of this monosaccharide in extracts using a specific colour reaction for 6-deoxysugars (concentrated sulfuric acid + hydrochloride of L-cysteine[32]) or by chromatography upon acidic hydrolysis of biomass samples. The spectrophotometric technique recommended in a popular phycology guide,[33] in many cases is not applicable due to the presence of coloured substances extracted along with fucoidans from algal biomass. According to a modified procedure,[34,35] such impurities are destroyed by mild oxidation on treatment with sodium chlorite in a weakly acidic solution. Colorimetric measurements are carried out upon the removal of oxidation products and reactants from the solution by dialysis. In this case there is the risk of a partial loss of the fucoidan owing to incomplete extraction or in the dialysis process, hence, the spectrophotometric method frequently leads to underestimated values of the polysaccharide content as compared to the chromatograpic determination of fucose after biomass hydrolysis. Nevertheless, the spectrophotometric technique can successfully be used in the cases where there is need to obtain comparative results for a large collection of samples.[34] The fucose content in fucoidans averages ~50%, therefore values obtained for fucose and multiplied by 2, as a first approximation, correspond to the content of fucoidans.[33]

Biological experiments often require the monitoring of the behaviour of fucoidans without their destruction. For this purpose in polysaccharide molecules fluorescent or

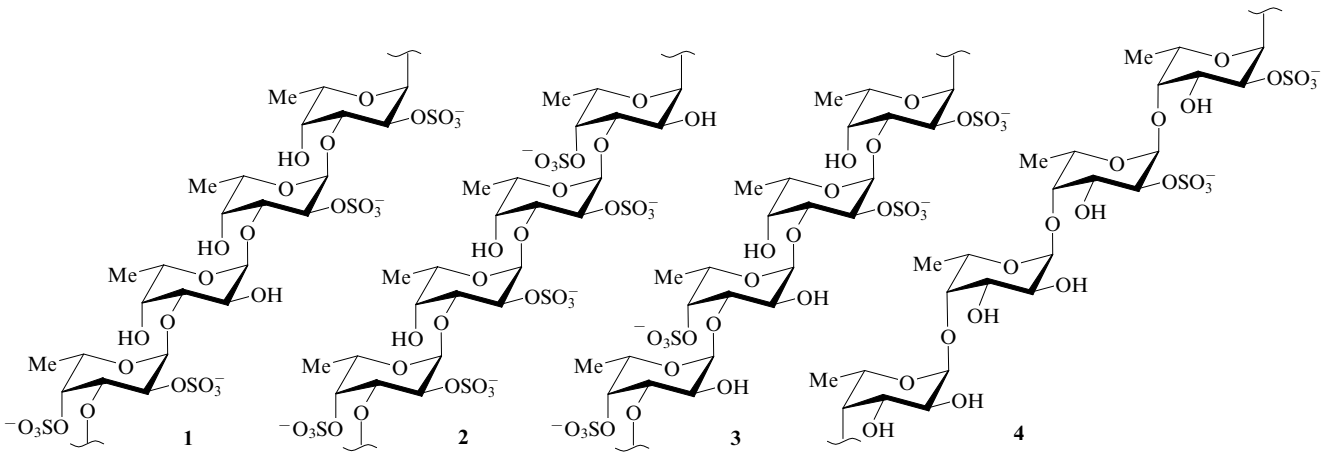


Figure 1. The tetrasaccharide repeat units of fucan sulfates from echinoderms: the holothurian *Ludwigothurea grisea* (**1**)[7] and the sea urchins *Lytechinus variegatus* (**2**),[7] *Strongylocentrotus pallidus* (**3**),[30] *Arbacia lixula* (**4**).[27] The units differ in the number and arrangement of sulfate groups, and also in the position of an interunit bond: (1→3) in compounds **1**–**3** and (1→4) in polysaccharide **4**.

radioactive labels are introduced (it is believed that such a modification at low substitution degrees does not substantially alter the biological properties of polymers [36]). An example can be a conjugate of the fucoidan from *Fucus vesiculosus* with fluoresceinamine[37] which was iodinated with radioactive ^{125}I. The prepared radioactive sample was successfully used for the study of the specific binding of the fucoidan with proacrosin — the basic protein involved in the binding process of sperm–egg interaction in mammals.[38]

Fucoidans can be directly detected and quantitatively determined by immunological methods. Thus, by using specific antibodies it was demonstrated that a fucoidan from *Undaria pinnatifida* is present in the plasma of volunteers to whom the initial seaweed or enriched samples of this polysaccharide were administered with food.[39] In the work [40] a monoclonal antibody was suggested as a reagent for the quantitative determination of a fucoidan from *Laminaria japonica* and for studying its localisation in the algal tissues. The authors revealed that the antibody they obtained reacted not only with the fucoidan isolated from *Laminaria japonica*, but also with that from *Kjellmaniella gyrata*; however, it did not interact with the fucoidan from *Undaria pinnatifida*.

2. Isolation of fucoidans

Fucoidans are well soluble in water, and so they can be extracted from algae with water [41] or dilute acids [42, 43] at room or slightly elevated temperature. In a popular procedure [44, 45] a 2% aqueous solution of calcium chloride, which converts alginate contained in the biomass to an insoluble calcium salt, is used. In order to facilitate the extraction the usage of proteolytic enzymes was suggested.[46, 47] Low molecular weight components can be preliminarily removed by treating the biomass with organic solvents, for instance, a homogeneous mixture of methanol, chloroform and water (4 : 2 : 1), which effectively dissolves both polar and nonpolar substances while leaving biopolymers undissolved.[48] As a pretreatment, the isolation of cell walls was also described.[23]

Apart from fucoidans, which usually are complex mixtures of polyanionic molecules differing in the composition and charge, the aqueous extracts of seaweeds contain laminaran, water-soluble alginates, proteins, polyphenols, *etc*. Fucoidan can be precipitated from the extract as an insoluble salt with a cationic detergent, such as trimethylcetylammonium bromide (cetavlon, cetrimide).[44, 45] In a number of cases a stepwise dissolution of the precipitate allows one to further obtain fractions differing in chemical nature,[45] however, most often the precipitate is converted to a water-soluble sodium or calcium salt, and subsequent fucoidan purification and fractionation procedures are carried out by anion-exchange chromatography. The most popular anion exchangers used for these purposes are DEAE-sephadex,[49] DEAE-sephacel [44] and DEAE-sepharose,[23, 41, 42] more seldom ion-exchange resins are employed;[24] salt solutions of increasing concentration are used as eluents. The separation process can be controlled by electrophoresis in a thin layer of agarose gel [24, 46, 47] or on plates of cellulose acetate.[42] More or less charge-homogeneous fractions are sometimes additionally purified by gel permeation chromatography [47] and are afterwards used for structural analysis.

3. Determination of composition

Methods used for other polysaccharides are also employed for the determination of the composition of fucoidans. Neutral monosaccharides in acid hydrolysates are identfied and quantitatively determined as alditol acetates by GLC using *myo*-inositol as an internal standard.[50] The absolute configurations of monosaccharides can also be established with the use of GLC. For this purpose acetylated or trimethylsilylated glycosides prepared by reaction of monosaccharides with optically active alcohols (*sec*-butanol or *sec*-octanol) are utilised.[51, 52] In some cases an alternative procedure of the reductive amination of monosaccharides with optically active amines appears to be more convenient.[53]

Uronic acids are identified by using anion-exchange chromatography [54] or paper electrophoresis [46, 47] and quantitatively determined by a colour reaction with *m*-hydroxybiphenyl [55] or 3,5-dimethylphenol.[56] The colorimetry is also used for the determination of acetate,[57] whereas sulfate is determined by turbidimetric analysis upon acidic hydrolysis.[58]

4. Preliminary characterisation

For a preliminary characterisation of an isolated fucoidan two physicochemical methods can be used: IR spectroscopy and NMR spectroscopy.

The IR spectrum provides information on the presence of sulfate (an intensive broad absorption band at 1240–1260 cm^{-1}). By analogy to the spectra of sulfated galactans of red algae,[59, 60] less intensive absorption bands at 850 and 830 cm^{-1} are ascribed to axial and equatorial secondary sulfates, respectively. Accordingly, the IR spectrum makes it possible to distinguish a sulfate at the O(4) atom from sulfates at the O(2) and O(3) atoms in the fucopyranose residue.

As already mentioned above, the NMR spectra of native fucoidans are generally too complicated [61] and uninformative for structural analysis. However, in such spectra the signals of the carbon atoms of the CH_3 group of fucopyranose ($\delta \sim 16$), fucofuranose ($\delta \sim 18$) and acetate ($\delta \sim 21$), of the CH_2OH groups of hexoses ($\delta \sim 62$) and of the CO groups of uronic acids and acetates ($\delta \sim 175$) can reliably be interpreted.[54, 62] There is the only instance [62] where the ^{13}C NMR spectrum of a fucoidan (a highly sulfated fraction isolated from *Fucus distichus*) looks like a spectrum of a regular polymer made up of alternating residues of 3-linked α-fucopyranose 2,4-disulfate and 4-linked α-fucopyranose 2-sulfate:† [→3)-α-L-Fuc*p*-2,4-di-S-(1→4)-α-L-Fuc*p*-2-S-(1→]$_n$ (Fig. 2 *a*).

In the study of all other algal fucoidans the interpretation of NMR spectra requires chemical modifications leading to structure simplification, for example, the elimination of non-carbohydrate substituents and removal of side chains of branched molecules.[63]

5. Deacetylation and desulfation

Fucoidans can be deacetylated without giving rise to other changes in the molecular structure by treatment with aqueous ammonium hydroxide at room or slightly elevated

† Here and further on, standard three-letter abbreviations for monosaccharide residues are used for writing polysaccharide formulas, letters *p* and *f* denote pyranose and furanose rings, respectively, letter S means sulfate groups, the Δ character means a double bond.

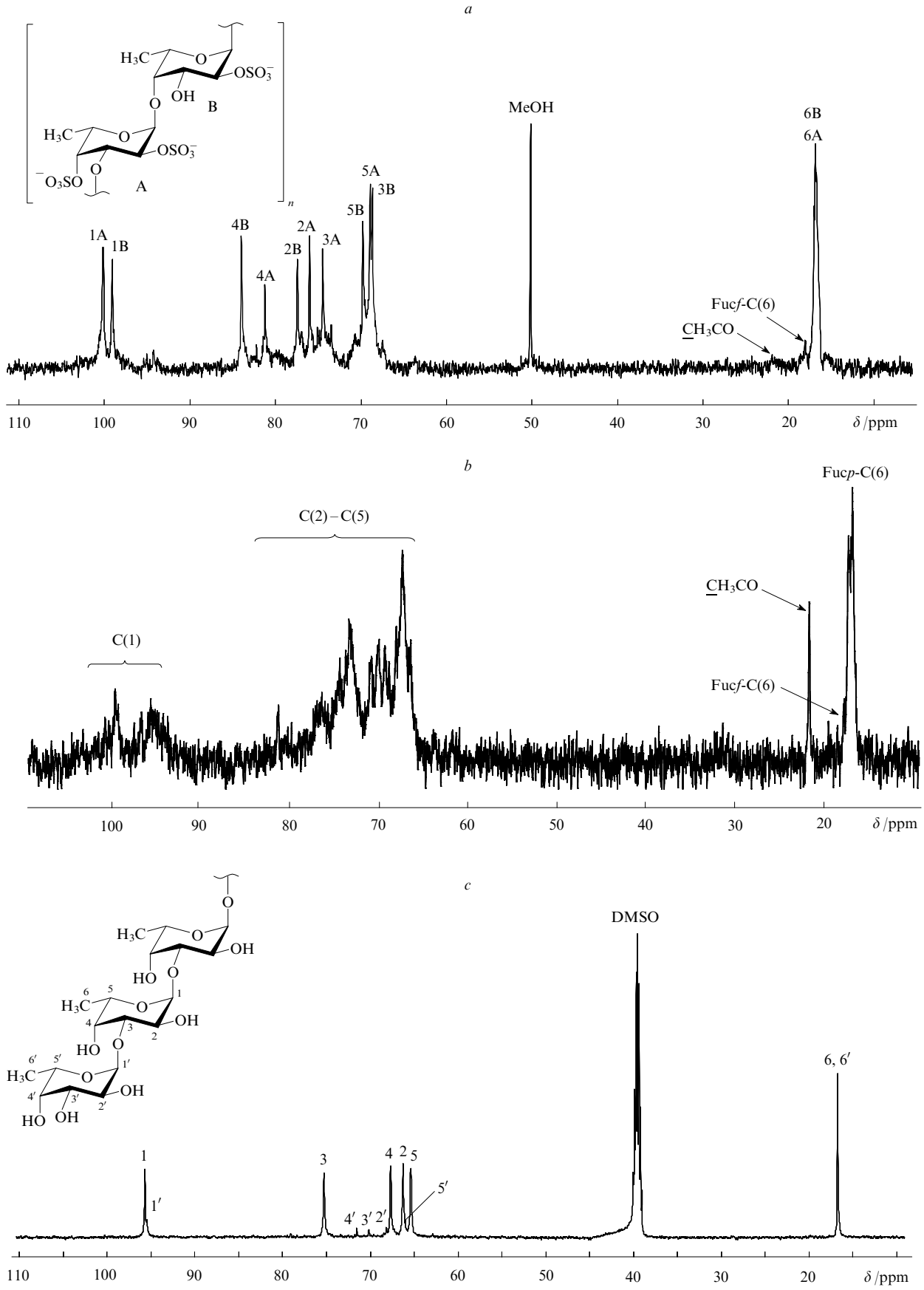


Figure 2. The ^{13}C NMR spectrum of a fucoidan of regular structure from *Fucus distichus*[62] (the signals corresponding to possible insignificant deviations from the regular structure are indicated with arrows) (*a*), a typical ^{13}C NMR spectrum of a native fucoidan from algae (a polysaccharide from *Analipus japonicus*)[63] (*b*) and the ^{13}C NMR spectrum of a polysaccharide sample resulting from desulfation followed by the Smith degradation of a polysaccharide from *Analipus japonicus* (*c*).[63]

temperature.[49] The complete desulfation of the polysaccharide represents a much more complex problem.[64] The acidic methanolysis conditions recommended for sulfated glycosaminoglycans of the connective tissues of animals, the glycosidic bonds of which are quite stable on treatment with acids,[65] are too severe for fucoidans.[54] Most often the so-called solvolytic desulfation[66] is used where a polysaccharide as a pyridinium salt is heated in dimethyl sulfoxide, sometimes with the addition of small amounts of water, methanol or pyridine.[67] Unfortunately, desulfation usually results in a loss of a significant amount of starting material, and therefore the information on the structure of the degraded part of a polymer becomes unavailable. Other desulfation procedures such as solvolysis under microwave irradiation conditions[68] or in the presence of an organic acid and an agent giving rise to stable complexes with SO_3,[69] treatment with trimethylchlorosilane in pyridine[70] or partial hydrolysis in acid medium,[71,72] were suggested mainly for other polysaccharides. Now it is difficult to estimate how well they are applicable to the fucoidans from algae. However, it should be noted that the authors[45] could desulfate a polysaccharide from *Adenocystis utricularis*, which is stable under solvolytic desulfation or even under acidic methanolysis, by heating with trimethylchlorosilane in pyridine.

The enzymatic desulfation of polysaccharides is extremely attractive for gaining stuctural information. Unfortunately, sulfatases affecting fucoidans are in practice not readily accessible. As possible sources of enzymes degrading fucoidans, including sulfatases, marine bacteria or marine invertebrates are usually considered. By using a special procedure based on the determination of the released sulfate anion by capillary electrophoresis,[73] it was found that the digestive glands of the sea mollusc *Pecten maximus* contain a sulfatase affecting a fucoidan from *Ascophyllum nodosum* and regioselectively removing sulfate from position 2 of fucopyranose residues.[74] The action of this enzyme on other fucoidans has not been studied. Other molluscs both bivalves and gastropods and also many representatives of other types of marine invertebrates often contain highly active arylsulfatases,[75,76] however, the latter are normally unable to desulfate fucoidans. Marine bacteria are more famous as sources of fucoidanases (see below).

6. The methylation method

In the case of desulfated polysaccharides methylation is used for the determination of positions of linkages between monosaccharides, whereas the comparison of the methylation products of desulfated and native fucoidans provides information on the positions of sulfate groups. For the methylation of a polysaccharide a simple procedure,[77] according to which a polysaccharide dissolved in dimethyl sulfoxide is treated with powdered sodium hydroxide and methyl iodide, is preferred. In these conditions both free and acetylated hydroxyl groups are transformed to methyl ethers, whereas sulfate groups are retained. Unfortunately, the stability of sulfates is not generally controlled, although examples of an unpredictable loss of a part of sulfate groups upon methylation can be found in the literature.[78] It should be noted that frequently a complete methylation of highly sulfated polysaccharides is hard to achieve, hence for native fucoidans this method, as a rule, gives only approximate quantitative results. For the identification of methylation products standard gas chromatography/mass spectrometry procedures are used.[79]

7. Periodate oxidation

The majority of fucoidans practically do not undergo oxidation with periodate due to the predomination of 1→3 bonds between fucose residues and a high substitution degree of hydroxyl groups. At the same time branches are effectively removed from desulfated molecules by the Smith degradation.[54,63] A polysaccharide resulting from the Smith degradation of a desulfated fucoidan from *Analipus japonicus* contained linear molecules made up of (1→3)-linked α-L-fucopyranose residues (see Fig. 2*c*). It is interesting to note that the polysaccharide was virtually insoluble in water despite its low molecular weight, presumably in consequence of a strongly pronounced tendency of such molecules to intermolecular association.[63]

8. The preparation of oligomeric fragments of fucoidans

The elimination of sulfate groups and the hydrolysis of glycosidic bonds on treatment with acids occur simultaneously and at similar rates, hence, partial acidic hydrolysis of fucoidans results in sulfated fucose derivatives and oligosaccharides, though in low yields. Separation methods for mixtures of isomeric fucose monosulfates with the use of capillary electrophoresis followed by mass-spectrometric identification have been proposed.[80] For the isolation of sulfated oligofucans centrifugal partition chromatography was employed,[81] while electrospray ionisation mass spectrometry was used for the characterisation.[82] However, in this case the conditions were not optimised in order to increase the yield of oligosaccharides.

The partial acidic hydrolysis of a fucoidan from *Ascophyllum nodosum* allowed the preparation of low molecular weight fractions which were analysed by mass spectrometry and NMR spectroscopy and also used for biological tests.[82–84] However, individual oligosaccharides were not isolated from the hydrolysate. Recently it was found that the hydrolysis of regular fucan sulfates from two species of sea urchins *Lytechinus variegatus* and *Strongylocentrotus pallidus* on treatment with a dilute acid proceeds in two steps. First, sulfate groups are eliminated from the 2 position of those residues of fucose 2-sulfate which are bound to the residues of fucose 4-sulfate or to which such residues are attached. Then the glycosidic bonds of desulfated fucose residues are cleaved to produce oligosaccharides of certain sizes.[71,72] The applicability of such an approach to the specific desulfation of algal fucoidans, followed by fragmentation of molecules is to be cleared up in future.

Radical depolymerisation on treatment with hydrogen peroxide in the presence of bivalent copper ions was used for the partial degradation of fucoidans from *Ascophyllum nodosum*[85] and *Lessonia vadosa*.[86] Unlike partial acidic hydrolysis this technique enabled the preparation of fractions with a moderate molecular weight and fairly narrow molecular weight distribution virtually without desulfation or a composition change and also in higher yield. The reaction products were used in the study of the biological activity of the depolymerised fucoidan,[86] however, the isolation of individual oligosaccharides important for the structural analysis of the starting polysaccharide failed.

The enzymatic hydrolysis of fucoidans is the most promising approach yielding oligosaccharides. Marine invertebrates and bacteria act as possible sources of corresponding enzymes.[14,87,88] For instance, a fucoidanase

degrading fucoidan to form sulfated oligosaccharides was isolated from the hepatopancreas of the marine bivalve mollusc *Patinopecten yessoensis*.[89] Fucoidanase activity was also found in the digestive glands of the closely related mollusc *Pecten maximus*.[90] However it turned out that the purified enzyme was an exo-α-L-fucosidase which was capable of eliminating only terminal α-L-fucopyranose residues from polysaccharides. Using this enzyme made it possible to decrease the molecular weight of a fucoidan from *Ascophyllum nodosum* and analyse the degraded polysaccharide by NMR spectroscopy.[91]

By studying a large number of marine invertebrates fucoidanase activity was detected in the gastropod mollusc *Littorina kurila*.[75] The partially purified enzyme hydrolysed polysaccharides from *Laminaria cichorioides* and *Fucus evanescens*[92] to produce several oligosaccharides and fucose. However, when a highly regular fucoidan from *Fucus distichus* was employed as a substrate, neither fucose, nor low molecular weight oligosaccharides were found among reaction products. Apart from a fraction looking like the unchanged starting material, a polymer fraction with a lower molecular weight was obtained in low yield. The composition of this fraction was found to have an increased content of units deviating from regular structure (acetylated and incompletely sulfated).[93]

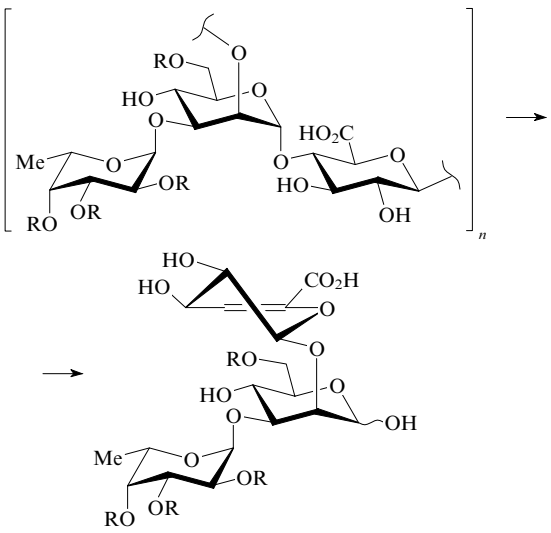
For the structural analysis of fucoidans bacterial enzymes are regarded more promising than mollusc enzymes. Many marine bacteria exert fucoidanase activity,[87,94] for example, *Pseudoalteromonas citrea*.[88,95] As a rule, in order to utilise a bacterial enzyme for the partial degradation of a particular fucoidan, one has to start with a search for an appropriate bacterial strain. This strain is then grown on a selected fucoidan as a substrate, after which the enzyme can be isolated from bacterial cells or culture broth.

Thus, a bacterium from the family Flavobacteriaceae, secreting endo-fucoidan hydrolase, was found in sediments of a waste water treatment facility in alginate manufacturing. A sample of the extracellular enzyme was used for degrading a fucoidan from the brown alga *Pelvetia canaliculata*.[96] As a result, a tetrasaccharide and a hexasaccharide containing repeat disaccharide units →3)-α-L-Fuc*p*-2-S-(1→4)-α-L-Fuc*p*-2,3-di-S-(1→ were obtained. This enzyme was further purified to an electrophoretically homogeneous state, the corresponding gene was successfully cloned and sequenced and the protein was produced with the use of *Escherichia coli*. It was established[97] that the fucanase cleaves only 1→4 bonds in the regular polysaccharide molecule [→4)-α-L-Fuc*p*-2,3-di-S-(1→3)-α-L-Fuc*p*-2-S-(1→]$_n$.

Two more marine bacteria utilising fucoidan were successfully applied to the structural analysis of polysaccharides. One of them, *Fucophilus fucoidanolyticus*, was isolated from the digestive organs of the trepang *Stichopus japonicus* and grown in a medium containing a fucoidan from *Cladosiphon okamuranus*.[98] This fucoidan was then degraded by treatment with the intracellular bacterial enzyme to yield a series of oligosaccharides with the general structrural formula {→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-4-S-(1→3)-α-L-Fuc*p*-4-S-(1→3)-[α-D-Glc*p*A-(1→2)]-α-L-Fuc*p*-(1→}$_m$-3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-4-S-(1→3)-α-L-Fuc*p*-4-S-(1→3)-L-Fuc (m = 0, 1, 2, 3) established with the aid of NMR spectroscopy. Based on these data, a regular structure with branched pentasaccharide repeat unit →3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-4-S-(1→3)-α-L-Fuc*p*-4-S-(1→3)-[α-D-Glc*p*A-(1→2)]-α-L-Fuc*p*-(1→ was suggested[99] for the fucoidan from *Cladosiphon okamuranus*.

An enzyme with another activity type, fucoglucuronomannan lyase, has been found in the bacterium *Fucobacter marina*.[100] When treated with extracellular enzymes of this bacterium, a fucoidan from the brown alga *Kjellmaniella crassifolia* produced three trisaccharides: $\Delta^{4,5}$Glc*p*A-(1→2)-[α-L-Fuc*p*-3-S-(1→3)]-D-Man, $\Delta^{4,5}$Glc*p*A-(1→2)-[α-L-Fuc*p*-3-S-(1→3)]-D-Man-6-S and $\Delta^{4,5}$Glc*p*A-(1→2)-[α-L-Fuc*p*-2,4-di-S-(1→3)]-D-Man-6-S. They bore a residue of the 4,5-unsaturated derivative of glucuronic acid at the nonreducing end and differed only in the number and arrangement of sulfate groups. Thus, a new type of polysaccharides was discovered in the fucoidan family, and among extracellular bacterial enzymes a catalyst of a new type was found. The enzyme represented an endolyase and catalysed the cleavage of α-D-mannoside bonds between α-D-Man and 4-linked β-D-GlcA in the main polysaccharide chain by β-elimination. These data along with chemical analysis results made it possible to establish[101] that the basic structural element in the polysaccharide is the trisaccharide repeat unit {→4)-β-D-Glc*p*A-(1→2)-[α-L-Fuc*p*-3-S-(1→3)]-α-D-Man*p*-(1→}$_n$. The enzymatic cleavage of sulfated fucoglucuronomannan from *Kjellmaniella crassifolia* on treatment with the bacterial endolyase is presented in Scheme 1.[101]

Scheme 1



R = H or SO_3Na.

An enzyme attached to cellular surface and capable of degrading a fucoidan from the sporophylls of *Undaria pinnatifida* was found in the marine bacterium *Sphingomonas paucimobilis*.[102] The enzyme did not affect *p*-nitrophenyl-α-L-fucopyranoside, and among products of the enzymatic hydrolysis of the fucoidan several oligosaccharides of various molecular weight and composition were found, however, free fucose was absent. These data indicate that the bacterium produces endofucoidanase, which can find successful application in the structural analysis of fucoidans.

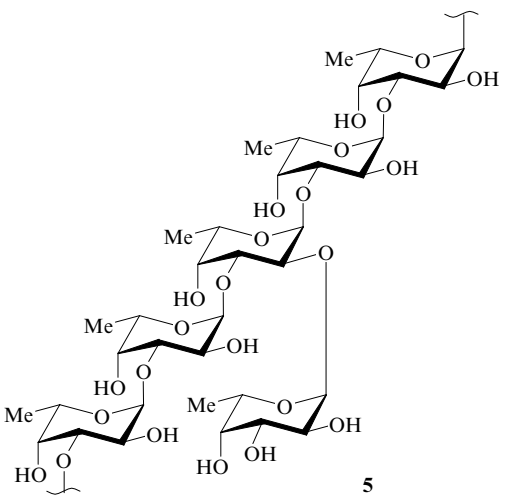
Recently, a new bacterium species has been reported. *Mariniflexile fucanivorans* belongs to the Flavobacteriaceae family and is able to cleave fucoidans from brown algae.[103] The authors perfomed a thorough taxonomic investigation of this bacterium, but did not provide data on the structure of degradation products.

The lack of effective methods of the partial degradation of fucoidans resulted in studies aiming at the alternative synthesis of putative oligosaccharide fragments of their molecules. The oligosaccharides of reliably established structure were to be used as model compounds for the interpretation of NMR spectra, for the study of characteristics of the conformational behaviour of fucoidans and for the identification of the sites of their molecules that are responsible for exhibiting biological activity of one kind or another. In a number of preliminary studies the problems of stereospecific α-fucosylation [104–106] and sulfation of polyhydroxy compounds have been solved.[107] This enabled the synthesis of a large pool of linear and branched oligosaccharides [108–112] containing all possible bonds between α-L-fucopyranose residues with various arrangement of sulfate groups and even with a residue of α-D-glucuronic acid as a single branch out of the (1→3)-linked fucan backbone.[113] For all the synthesised oligosaccharides ^{1}H and ^{13}C NMR spectra were completely interpreted. In order to determine preferable conformations of these compounds in solution the theoretical molecular modelling and experimental determination of transglycosidic vicinal coupling constants ($^3J_{C,H}$) were utilised. It turned out [114] that the conformations of glycosidic bonds in the (1→3)-linked fucan backbone sulfated or glycosylated at the O(2) atoms depend on the remoteness of these bonds from molecule ends, on the chain length and on the presence of a branch at the 2 position. However, in the same chain sulfated at the O(4) atoms the conformations of glycosidic bonds depend only on the presence or absence of a branch at the 2 position. The spectral data obtained for the synthetic oligosaccharides were used for the assignment of signals in complex NMR spectra of fucoidans from some brown algae.[44, 62, 63, 115] The conformational investigations allowed the authors to formulate well-founded concepts of a minimum required size of oligosaccharide molecules used as models for assessing the behaviour of corresponding polysaccharides.[116]

III. Selected representatives of algal fucoidans

1. Fucoidans with the backbone consisting of the 3-linked residues of α-L-fucopyranose

From the brown alga *Chorda filum* a fucoidan of fairly simple composition containing practically only fucose, sulfate and acetyl groups has been isolated.[49] The authors managed to completely interprete the ^{1}H NMR spectrum of its desulfated and deacetylated derivative **5**.

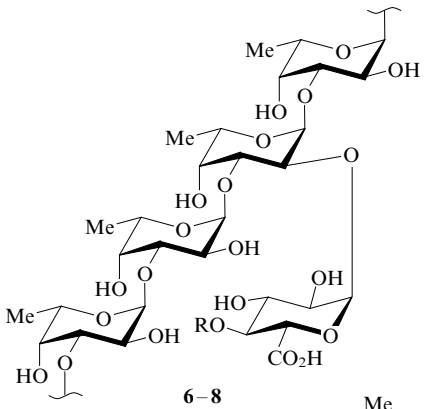


5

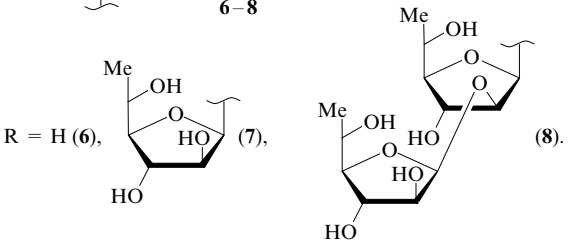
The anomeric region of the spectrum displayed five signals (one of them was of double intensity) corresponding to a hexasaccharide repeat unit consisting of five 3-linked α-L-fucopyranose residues of the backbone and one unsubstituted α-L-fucopyranose residue as a side branch in the 2 position. It is not excluded that slight deviations from regularity, which do not appear in the NMR spectrum, can exist in polymeric molecules. In the native polysaccharide sulfate groups occupy mainly the 4 positions and, to a lesser degree, the 2 positions whereas some 3-linked α-L-fucopyranose residues are acetylated at the 2 position.

Similar fucoidans the structures of which were studied in lesser detail were isolated from *Laminaria saccharina* [54] and *Lessonia vadosa*.[86, 117] A fucoidan from *Analipus japonicus* [63] bears the same (1→3)-linked backbone, but it is more branched (see Fig. 2 *b*,*c*). It has three branches as single α-L-fucopyranose residues at the 4 position and one such a branch at the 2 position on average in every ten residues of the backbone. Sulfate groups in this polysaccharide occupy the 2 positions (predominantly) and 4 (the majority of terminal non-reducing fucose residues are doubly sulfated), whereas acetyl groups are mainly located at the 4 position.

From the seaweed *Cladosiphon okamuranus* an acetylated fucoidan has been isolated. It contains the same 3-linked backbone, but with side α-D-glucopyranosyluronic acid residues attached to the 2 positions of the main chain.[118, 119] The carbohydrate portion of the macromolecule can be represented by the structure **6** (here and in formulas **7**, **8** sulfate groups are omitted).[99, 118]



6–8



R = H (**6**), (**7**), (**8**).

Based on chemical analysis data, a heptasaccharide residue containing one branch was proposed as the main repeat structure of the polysaccharide.[118] However, according to enzymatic hydrolysis results (see above), a pentasaccharide repeat unit is more likely.[99]

Another fucoidan with a high content of glucuronic acid was found in the brown alga *Chordaria flagelliformis*.[120] The structural analysis used a fraction of this fucoidan containing L-fucose, sulfate and D-glucuronic acid (the molar ratio 1 : 1 : 0.25) and a small amount of acetyl groups. In order to determine the structure of this polysaccharide several chemical modifications of its molecules had to be carried out, namely, solvolytic desulfation, reduction of

carboxyl groups and partial acidic hydrolysis. The native fucoidan and its modification products were studied by methylation and with the use of 1D- and 2D-NMR spectroscopy. It was established [120] that the fucoidan contains the backbone composed of 3-linked α-L-fucopyranose residues, approximately one third of which is glycosylated at the O(2) atoms by α-D-glucopyranosyluronic acid residues. Around one half of the latter are glycosylated at the O(4) atoms by single α-L-fucofuranose residues (see formula **7**) or by the disaccharide α-L-Fuc*f*-(1→2)-α-L-Fuc*f*-(1→ (see formula **8**). Fucofuranose residues are mono- and disulfated at various positions and some additional amount of sulfate groups occupy the 4 positions of the backbone. Thus, the characteristic of this fucoidan is the presence of fucofuranose residues including sulfated ones which constitute short side chains. The presence of a small amount of unsulfated terminal fucofuranose residues in some fucoidans was also reported earlier,[21, 45, 62, 121] but their positions in polysaccharide molecules remained unknown.

2. Fucoidans with the backbone composed of alternating 3- and 4-linked α-L-fucopyranose residues

The alga *Ascophyllum nodosum* comprises several sulfated polysaccharides.[6, 122] The most sulfated fraction was studied with the use of partial acidic hydrolysis and NMR spectroscopy. For the polysaccharide a regular structure from repeat disaccharide units [→3)-α-L-Fuc*p*-2-S-(1→4)-α-L-Fuc*p*-2,3-di-S-(1→]$_n$ was proposed.[22] The same structural elements were identified in our study of a fucoidan from *Fucus vesiculosus*, as opposed to previous data [21] on its structure. An analagous structure was also established [96] for a fucoidan from *Pelvetia canaliculata* on the basis of its enzymatic hydrolysis (see above).

A fraction of a fucoidan with the same carbohydrate chain, but other arrangement of sulfate groups was isolated from *Fucus evanescens*.[44] The structure of this polysaccharide was determined by using 1D and 2D ^{1}H and ^{13}C NMR spectra of its desulfation and deacetylation products (these spectra could be completely interpreted). In addition, the spectral data were confirmed by methylation. It was demonstrated that the basic structural element of the polysaccharide is the repeating sequence [→3)-α-L-Fuc*p*-2-S-(1→4)-α-L-Fuc*p*-2-S-(1→]$_n$ with some amount of extra sulfate groups in the 4 positions of (1→3)-linked residues, while the remaining hydroxyl groups are acetylated in a random manner.

It should be noted that from the same alga species other authors [88] isolated a polysaccharide in which the amount of (1→3)-bonds between fucose residues was 3.5 times higher than the content of (1→4)-bonds. In a recent work [123] upon the solvolytic desulfation of this polysaccharide a wide assortment of neutral and sulfated lower oligosaccharides containing galactose, xylose and glucuronic acid residues besides fucose was obtained. In spite of rather low yields of these fragments, valuable information on their structure was successfully obtained by using MALDI-TOF mass spectrometry and tandem mass spectrometry with electrospray ionisation. Unfortunately, the content of minor monosaccharides in the parent polysaccharide was not indicated in the article. Neither is a mechanism by which glycosidic bonds are cleaved under solvolytic desulfation known (it is quite possible that small amount of oligosaccharides results from reversion). Hence, so far it is difficult to assess what value the determined oligosaccharide structures have for the refinement of the starting fucoidan structure.

It was already mentioned above that from *Fucus distichus* a fraction of a fucoidan of unusually high regularity degree was successfully isolated (see Fig. 2 *a*).[62] Its ^{13}C NMR spectra appeared as a spectrum of a polymer composed of identical disaccharide repeat units. According to chemical data, the regular structure of the polymer can be insignificantly masked by the partial acetylation or incomplete sulfation of some repeat units.

In a fucoidan from *Fucus serratus* [115] containing fucose, sulfate and acetate in the molar ratio of 1 : 1 : 0.1 the backbone is constructed analogously. However, in contrast to the previous examples the molecules proved to be branched, in particular, around half the 3-linked residues of the backbone are substituted at the O(4) atoms with the trifucose units α-L-Fuc*p*-(1→4)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→. The polysaccharide is mainly sulfated at the O(2) atoms and, to a lesser extent, at the O(4) atoms, though 3,4-diglycosylated and some terminal fucose residues can be lacking in sulfate groups. Acetates are present at the O(4) atoms in 3-linked fucose residues and at the O(3) atoms in 4-linked residues, their ratio is approximately 7 : 3.

The backbone contaning (1→3) and (1→4) linkages between α-L-fucopyranose fragments was also detected in a fucoidan from *Stoechospermum marginatum*, although the authors did not provide evidence for the regular alternation of these two bond types.[41]

3. Sulfated galactofucans

Many fucoidans contain small amounts of other monosaccharides apart from fucose, but the structures of these minor components, as a rule, remain unknown. At the same time polysaccharides containing fucose and galactose in comparable amounts have been described. Fucoidans extracted from the sporophylls of *Alaria fistulosa* [124] and *Undaria pinnatifida*,[42, 125] and from the whole thalli of *Laminaria japonica*,[126] *Laminaria cichorioides*,[43] *Laminaria gurjanovae* [127] and *Sargassum patens* [128] can serve as examples. Fucoidan fractions with a high content of galactose and sulfate groups were obtained from complex mixtures of polysaccharides contained in *Sargassum stenophyllum* [129] and *Adenocystis utricularis*.[45]

A thorough fractionation of the total fucoidan from *Laminaria angustata* var. *longissima* resulted in the isolation of a small amount of a sulfated galactan containing just traces of fucose and glucuronic acid.[130] A structural analysis showed that this polysaccharide had a linear main chain →3)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→. The majority of sulfate groups occupy the 6 positions in 3-linked galactose residues, other sulfate groups can be located at O(3) atoms in 6-linked residues, while galactosyl branches are attached at the 4 positions of monosaccharides. Accordingly, it was demonstrated that brown algae can contain some sulfated galactans differing from the well-known sulfated galactans from red algae and ascidians.

Most polysaccharide samples with a high content of galactose are considered to be galactofucans without special proofs. Reliable evidence for the existence of a bond between galactose and fucose residues was obtained in an early work [121] devoted to a galactofucan sulfate from *Ecklonia kurome*, owing to the isolation of several oligosaccharides containing both monosaccharide residues. The presence of analogous oligosaccharides in the degradation

products of a fucoidan from *Laminaria gurjanovae* was recently confirmed by MALDI-TOF mass spectrometry.[127]

Galactofucan structures were mainly studied by methylation and were only partially determined. Thus, 2,3-disulfated residues of 4-linked α-L-fucose were identified as the main structural elements of a fucoidan from *Laminaria cichorioides*.[43] A wide assortment of methylated derivatives of galactose was detected in methylation products of this polysaccharide, however data on their mutual arrangement were not obtained.

Galactofucans extracted from *Undaria pinnatifida* were studied by several research groups.[42, 125] Their results do not completely coincide, however, it is clear that the pattern of the substitution of both fucopyranose and galactopyranose residues in the main polysaccharide fraction is very complex. The predomination of 3-linked residues points to the fact that both monosaccharides are incorporated in (1→3)-linked chains. In a thorough investigation of a galactofucan from *Adenocystis utricularis* it was demonstrated[45] that the fucan component basically constitutes a backbone composed of 3-linked α-L-fucopyranose residues, normally sulfated at the O(4) atoms and containing branches at the O(2) atoms. Side branches are represented by unsulfated fucofuranose residues and fucopyranose residues partially sulfated at the O(2) atoms. As already noted above terminal fucofuranose residues were earlier identified in some other fucoidans.[21, 62, 121] The galactan portion of the polysaccharide is more heterogeneous, with predominated D-galactopyranose residues bound at the 3 and 6 positions and bearing sulfate groups at the O(4) atoms (including terminal non-reducing residues). As follows from ^{13}C NMR data, at least a portion of galactose residues has α-configuration.

4. Fucoidans of more complex composition.

From the brown alga *Dictyota menstrualis* several polysaccharide fractions containing fucose, xylose, uronic acid, galactose and sulfate in various proportions have been isolated.[131] For two of them a high anticoagulant activity was registered, however no structural information was provided in the study.

For an analogous polysaccharide from *Padina gymnospora* studied by the same research group[46] a structure with the backbone comprising mainly 3- and 4-linked β-D-glucuronic acid residues substituted at the O(2) atoms with α-L-fucose and β-D-xylose residues was suggested. Sulfate groups were found only at the O(3) atoms in 4-linked α-L-fucose residues. It was shown that it is such sulfation type that is responsible for the anticoagulant activity of the polymer.

Two complex sulfated polysaccharides were also isolated from the seaweed *Spatoglossum schroederi*.[132] One of them containing fucose, xylose, glucuronic acid and sulfate was studied by using methylation, cleavage by glycosidases and sulfatases from the mollusc *Tagelus gibbus*, partial acidic hydrolysis and NMR spectroscopy. It was established that the polymer core consists of 3-linked β-D-glucuronic acid residues containing branches at the O(4) atoms as chains of 3-linked α-L-fucose residues. These residues are, for the most part, substituted with sulfate at the O(4) atoms, and at the 2 positions bear chains of 4-linked β-D-xylose residues which are also partly sulfated. The second polysaccharide practically did not contain uronic acids and consisted of fucose, xylose, galactose and sulfate in the molar ratio of 1 : 0.5 : 2 : 2. Its core was mainly composed of 4-linked β-galactose residues partially sulfated at the O(3) atoms. About 25% of these units bore branches at the O(2) atoms as oligosaccharides (mostly, tetrasaccharides) comprising 3-sulfated 4-linked α-L-fucose residues and terminal unsulfated β-xylose residues or 4-linked β-xylobiose.[47]

A very complex fucoidan lacking fucose in the core was isolated from the brown alga *Hizikia fusiforme* (the correct name of this species is *Sargassum fusiforme*).[133] Its backbone was composed of alternating 2-linked α-D-mannnopyranose residues and 4-linked β-D-glucuronic acid residues with a small amount of 4-linked β-D-galactose. Two thirds of the mannose residues had branches at the 3 positions, the galactose residues were mainly bound at the 6 position, and both 4- and 6-linked galactose residues contained branches at the O(2) atoms. Approximately two thirds of α-L-fucose residues represented non-reducing terminal units, while the other fucose residues were attached at the 4, 3 and 2 positions. Around two thirds of α-D-xylose residues also represented non-reducing terminal units, while the others were 4-linked. Sulfate groups were located at the O(6) atoms of 2,3-bound mannose, at the O(4) and O(6) atoms of 2-linked mannose, at the O(3) atoms of 6-bound galactose and at the all possible positions of fucose (some fucose residues bore 2 sulfate groups), whereas from glucuronic acid and xylose residues sulfates were absent.

A polysaccharide with an analogous glucuronomannose core was earlier found in *Kjellmaniella crassifolia*.[101] Its structure was mainly studied with the use of enzymatic cleavage (see Scheme 1).

IV. Biological activity of fucoidans

1. Anticoagulant properties

The activity of fucoidans preventing blood coagulation was discovered in 1957.[134] Since then this property has in most detail been studied in the hope of finding a convenient analogue of or a substitute for the natural anticoagulant heparin. The latter is currently widely used in medicine,[135] although there is a number of drawbacks to it. The research of this activity type was in large part encouraged by the fact that the activity can be quantitatively measured with the use of comparatively simple techniques in which readily available heparin serves as a reference. Numerous works covering the anticoagulant properties of fucoidans published before 1995 were in detail discussed by Nagumo and Nishino.[10] References to later studies can be found in the recent reviews [13–16, 136].

Heparin isolated from animal tissues is a sulfated polysaccharide, however, its carbohydrate chains differ from fucoidan ones.[137] Hence, it was natural to assume that a high sulfation degree is of paramount importance for exerting anticoagulant properties.[138, 139] Indeed, in a number of studies it was demonstrated that an additional chemical sulfation of fucoidans results in a higher anticoagulant activity,[140, 141] whereas desulfation completely inactivates polysaccharides. At the same time in some studies comparing the anticoagulant activity of fucoidans isolated from different species of brown algae,[142–146] it was established that polysaccharides that are close in sulfate content, but differ in origin can considerably differ in biological effect. It follows that a high sulfation degree is a necessary, but not sufficient structural element lending anticoagulant properties to fucoidans.

The complex process of blood coagulation is controlled by many factors among which an important role is played

by antithrombin III (AT-III) and heparin co-factor II (HC-II), thrombin inhibitors preventing the conversion of fibrinogen to fibrin. Antithrombin III also inhibits other serine proteases of the coagulation cascade. Its activity increases approximately by 3 orders of magnitude upon the formation of a complex with heparin whose molecules have specific active sites for interaction with AT-III. The sites constitute pentasaccharide chain segments with unique sequences of monosaccharide residues and unique arrangements of sulfate groups.[137] It is unlikely that molecule fragments which are similar to these active sites can be found in algal fucoidans. Accordingly, although in the studies [147, 148] the interaction of fucoidans with AT-III has been demonstrated, most of these polysaccharides are supposed to interact with HC-II also resulting in its activation.[43, 149, 150]

It is also not excluded that fucoidans directly interact with thrombin and other proteases leading to the inactivation of the enzymes. In this case the molecular weight of a polysaccharide can be a crucial factor since depolymerisation weakens anticoagulant properties.[86] Interesting observations of what structural peculiarities are important for exerting anticoagulant properties have been made on fucan sulfates from echinoderms,[11, 61, 151, 152] but these data cannot be directly applied to fucoidans. Nevertheless, it can be noted that for the anticoagulant effect the presence of 2,3-disulfated fucose residues in polysaccharides is essential,[84] while the availability of branches as α-D-glucuronic acid residues out of the backbone substantially descreases the activity.[145, 146]

The mechanism of fucoidan action, which differs from that of heparin, is of great practical interest since they can be used in the cases where the application of heparin itself, for some reason or other, is ineffective. The anticoagulant action of fucoidans (as well as that of heparin) can be quickly blocked by the intravenous introduction of biocompatible cationic polymers such as protamine sulfate and VIM-DEMC (a synthetic copolymer of 1-vinylimidazole with diethyl maleate).[153]

Fucoidans frequently exert antithrombotic action which is not directly related to anticoagulant activity.[11, 154, 155] Thus, a sulfated galactofucan from *Spatoglossum schroederi* lacking anticoagulant properties in *in vitro* experiments proved to be an efficacious antithrombotic agent in *in vivo* experiments.[47] Apart from the very antithrombotic action, this polysaccharide was twice more effective than heparin in stimulating the synthesis of antithrombotic heparan sulfate by endothelial cells. Upon desulfation both activities have been lost.

2. Antiinflammatory properties

It is known that leucocytes exit the bloodstream and accumulate in an inflammatory nidus on being treated with specific receptors called selectins.[156] There are three types of selectins (P-, E- and L-selectins), which differ in location and appearance rate upon the induction of inflammation. In particular, P-selectin is expressed on the surface of platelets and endothelial cells at the very beginning of an inflammatory process. Its natural ligands [157, 158] are bioglycan fragments containing tetrasaccharide units of the Sialyl-Lewis^x type in combination with tyrosine sulfate residues.[159] The interaction of P-selectin with these ligands is effectively inhibited by sulfated polysaccharides [160] including heparin [161] and fucoidans.[162–165] It is thought that it is this property that underlies the antiinflammatory action of fucoidans in *in vivo* experiments on a model of experimental peritonitis in rats.[163–165] It is important to note that fucoidans isolated from various algal species exert different antiinflammatory activity which does not correlate with their anticoagulant effect.[145] Thus, in contrast to heparin whose therapeutic application as an antiinflammatory remedy is complicated due to inevitable disorders of blood coagulation, there is hope that antiinflammatory agents practically lacking anticoagulant activity can be found among fucoidans.

3. Antitumour activity

Following the anticoagulant properties the antitumour action of fucoidans attracted researchers' attention. A long series of works (see, for instance,[166–174]) on this subject was accomplished at the end of the last century, the Japanese authors gave special attention to fucoidans from brown algae traditionally used for food in Japan.[175–183] In more recent studies antitumour activity was registered for fucoidans from seaweeds collected in the coastal waters of Vietnam,[184] Korea [185] and China.[186]

Since fucoidans do not exert cytotoxic activity their action on oncogenesis is mainly accounted for by immunomodulatory effect enabling the mobilisation of host defences.[187–198] At the same time fucoidans can stimulate the apoptosis of tumour cells [199–202] and prevent metastasis by altering the adhesive behaviour of cells [145, 203–206] (perhaps, as a result of selectin blockade as in the case of antiinflammatory action).

Another mechanism of the antitumour activity of fucoidans is the antiangiogenic effect since fucoidans suppress the intensive formation of vessels and so reduce the active supply of blood to tumour tissues.[207–211] The antiangiogenic effect is enhanced upon the additional chemical sulfation of polysaccharide molecules. The 'oversulfated' fucoidans exert an increased activity also in other antitumour tests,[212, 213] however, a comparison of the antiadhesive and antiangiogenic properties of fucoidans of different origin demonstrates that the fine structure of the polysaccharide molecules exerts substantial influence on the activity.[145]

4. Antiviral action

Both natural and synthetic sulfated polysaccharides are capable of inhibiting enveloped viruses pathogenic for the human.[214] Readily available and biocompatible polysaccharides from seaweeds have been thoroughly examined as potential antiviral agents.[215–217] It is thought that fucoidans can effectively prevent the penetration of viruses to cells on account of the modification of properties of cellular surface, although direct interaction of polysaccharides with viral surface proteins or viral enzymes is also possible.

The quest for fucoidans, which can block the herpes virus, has been most poplular. Active polysaccharides were isolated from *Undaria pinnatifida*,[42, 125, 218, 219] *Sargassum horneri*,[220, 221] *Sargassum patens*,[128] *Stoechospermum marginatum* [41] and *Cystoseira indica*.[222] It is also known that fucoidans are active against human immunodeficiency virus.[223, 224] A fucoidan from *Fucus evanescens* was reported to have an inhibiting effect on the plant-pathogenic tobacco mosaic virus.[225] It was demonstrated earlier that sulfated oligosaccharides with alternating (1→3) and (1→4) bonds between fucose residues, which are produced from similar fucoidans upon enzymatic degradation, serve as effective elicitors of defence reactions and can ensure both local and

systemic protection of tobacco plants against a viral infection.[226] In a number of studies it was established [41, 222] that fucoidans practically lacking anticoagulant properties can exert a high antiviral activity.

Interesting observations on the relationship between the structure of a polysaccharide and its activity were made during a study of the interaction of the fucoidan with carbohydrate skeleton **6** isolated from *Cladosiphon okamuranus* [99, 118] with the dengue fever virus.[227] It turned out that the polysaccharide loses its activity not only upon desulfation, but also upon the reduction of the carboxyl groups of uronic acids. Thus, side branches out of the fucoidan backbone, which represent α-D-glucopyranosyluronic acid residues, the presence of which evidently decreases anticoagulant effect,[145] are required for exerting antiviral activity. Out of four known serotypes of the dengue virus the fucoidan inhibits only one by strong binding to its enveloped glycoprotein, weakly interacts with other two serotypes and does not react with the fourth. These distinctions correspond to differences in the composition and arrangement of amino acids in glycoprotein molecules. Thus, the 323 position contains an arginine residue, which is replaced in other two serotypes with a less basic lysine residue. At the same time the glycoprotein of the serotype which is inert to the fucoidan, has at this position a glutamine residue lacking basic properties. The authors suppose [227] that it is this fragment of the glycoprotein molecule that interacts with the carboxyl groups of glucuronic acid residues in the fucoidan. A detailed account of the modern concepts of the mechanisms of the antiviral action of sulfated polysaccharides and the prospects of their clinical use can be found in the recent reviews.[228, 229]

5. Fucoidans and fertilisation

The fertilisation process constitutes a cascade of numerous reactions, in which an important role is played by specific carbohydrate–protein interactions.[230, 231] As already mentioned above, fucan sulfates in the external jelly coats of the sea urchin eggs contribute to the attachment of the spermatozoa of this species and induce an acrosomal reaction.[31, 232] A contributor to the specific interaction of spermatozoa with ova is also bindin, an adhesive protein of spermatozoa, which manifests the features of lectin and possesses a high affinity to sulfated fucose-containing polysaccharides.[233] Algal fucoidans strongly bind to this protein, and, therefore, can inhibit the fertilisation process of sea urchins. Mammals have proacrosin, which has similar properties to those of bindin.[38, 235] It seems that it is the interaction with proteins of the proacrosin type that accounts for the fact that fucoidans at low concentrations block the attachment of spermatozoa to human eggs [236] and prevent the development of further processes aimed at the penetration of a spermatozoon through the external coat of an egg.[237] This feature makes it possible to consider fucoidans as prospective contraceptive agents.[236, 238]

6. Other biological activities of fucoidans

Polysaccharides from brown algae are powerful inhibitors of complement activation.[239–246] Fucoidans also attract attention as antioxidants. Such attributes were registered both for native fucoidans isolated from *Laminaria japonica* [247, 248] and *Fucus vesiculosus*,[249, 250] and for products of the chemical modification of fucoidans, resulting from additional sulfation, acetylation, phthaloylation,[251] phosphorylation and amination.[252] Apart from this, of interest are the antiadhesive properties of fucoidans. It was shown that a fucoidan from *Cladosiphon okamuranus* blocks the adhesion of the bacterium *Helicobacter pylori* to human gastric mucosa [253] and can be used for protection from infections and as an antiulcer agent.[254, 255] A fucoidan from *Undaria pinnatifida* suppresses the attachment of the oocysts of the *Cryptosporidium parvum* parasite to the intestinal epithelium cells of mice [256] and prevents the invasion of erythrocyte with malarial plasmodium.[257] Fucoidans also inhibit the adhesion of *Corynebacterium diphtheriae* and other pathogenic microorganisms to eukaryotic cells.[258, 259] Additional examples of various biological activity of fucoidans are reported in the reviews.[13–16]

V. Conclusion

The high and diverse biological activities of fucoidans imply that these polysaccharides are capable of interacting with a wealth of proteins. This interaction is affected by monosaccharide content and the fine structure and conformations of polysaccharides.[151, 260] It was established that a favourable spatial arrangement of sulfate groups is a prerequisite for the high anticoagulant activity of fucan sulfates,[61, 152] whereas desulfation or additional 'oversulfation' can substantially alter the activity of fucoidans.[141]

The presence of L-fucose as the basic monosaccharide component also appears as a crucial factor for specific polysaccharide–protein interactions. In the case of mammals, glycans containing fucose include blood-group antigens, inhibitors of leucocyte endothelium adhesion controlled by selectins, inhibitors of host cell interactions with microorganisms and molecular members of many pathologic processes including cancer and atherosclerosis.[261] It is obvious that complex fucoidan molecules can contain certain fragments constructed from fucose residues, which mimic corresponding structures of biologically active mammalian glycans.

It must be stressed that fucoidans of different origin, even being very similar in composition, often considerably differ in activity in biological tests due to differences in the fine structure.[145] Different species of brown algae contain structurally different fucoidans, although a direct relationship between a fucoidan structure and the taxonomic position of an alga is not observed. Even different portions of thalli can have distinct polysaccharide composition [124, 262] which is also affected by environmental factors.[92, 221, 263]

Unfortunately, the current level of structural studies provides only averaged data on the structures of fucoidan molecules. Further exploration of the structural diversity of algal fucoidans, the development of novel efficient methods of the specific fragmentation of their molecules and isolation of oligosaccharides of definite structure are required in order to find real correlations between the structural features and biological properties of these complex polysaccharides.

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