Review

Structure, biological activity, and enzymatic transformation of fucoidans from the brown seaweeds

Mikhail Kusaykin[1], Irina Bakunina[1], Victoria Sova[1], Svetlana Ermakova[1], Tatyana Kuznetsova[2], Natalya Besednova[2], Tatyana Zaporozhets[2] and Tatyana Zvyagintseva[1]

[1] Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch, Russian Academy of Sciences, Vladivostok, Russia
[2] Research Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, Vladivostok, Russia

Recent advances in the study of fucoidans, biologically active sulfated α-L-fucans of diverse structures and synthesized exclusively by marine organisms, are overviewed. Their structure, biological activity, the products of their enzymatic degradation and the different enzymes of degradation and modification are considered.

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1 Introduction

Since ancient times, algae have been used in many cultures owing to their dietary, medicinal and functional food properties. Among them, brown seaweeds dominating in Northern seas are a real treasure-trove of physiologically active polysaccharides. In addition to neutral polysaccharides, such as cellulose and laminaran, and the well-known polyanionic polysaccharide alginic acid, they contain fucoidans, less known highly sulfated polysaccharides. Alginates are the major products manufactured from brown algae for food, pharmaceutical, and technical needs, whereas fucoidans still remain by-products of alginate industry, although these sulfated polysaccharides, along with other components of brown seaweeds, may contribute to the therapeutic efficacy of these algae.

Enzymes that degrade polyanionic polysaccharides are widely used for establishing their structures and structure-activity relationships. Preparations of polysaccharides with standard characteristics as well as medicines and supplements from these biopolymers also often include enzymatic treatments [1–5].

In contrast to alginolytic enzymes, which are relatively well studied [3, 6, 7], literature about fucoidan-degrading enzymes is still sparse. For this reason, besides structural aspects and biological function of fucoidans, this review summarizes our own results and the data of other groups on fucoidan-degrading enzymes (fucoidanases, fucoidan lyases and sulfatases).

2 Structure of fucoidans and their distribution

Fucoidans may constitute up to 25–30% of the algae dry weight, depending on the seaweed species and, to a lesser extent, on season [8–11]. Polysaccharides similar to fucoidans have not been found so far in terrestrial organisms. Fucoidans belong to a family of sulfated homo- and heteropolysaccharides including polysaccharides with high content of glucuronic acid and low content of fucose and sulfates, and polysaccharides built as sulfated homopolymer of fucose (fucans). Galactose, mannose, xylose, and rhamnose moieties have also been found in various fucoidans [8, 10–14].

Although fucoidans have been known since 1913, only few structures have been established. These structures are complex, heterogeneous and

Correspondence: Dr. Tatyana Zvyagintseva, Prospect 100-Letiya Vladivostoka 159, 690022, Vladivostok, Russia
E-mail: zvyag@piboc.dvo.ru
Fax: +7-4232-314050

Abbreviations: TNF, Tumor necrosis factor; **IL**, Interleukin; **IFN**, Interferon

devoid of regularity. Brown algae synthesize highly branched polysaccharides with species-specific sugar compositions [8, 11, 13, 14] and, for this reason, fucoidan structures are extremely diverse. In addition, every species forms several types of fucoidans. For example, commercially available fucoidan from *Fucus vesiculosus* (Sigma) is, in fact, a heterogeneous mixture of more than 15 different fucoidans with varied proportions of individual monosaccharide moieties [13].

Up to 1991, fucoidans were considered as 1,2-α-L-fucans [15]. Recently, a number of new fucoidans has been fully characterized [1, 12, 16–25]. The fucoidans have an α–1,3-backbones or repeating disaccharide units of α–1,3- and α–1,4-linked fucose residues with branchings attached at C2 positions. Depending on the structure of the main chain, fucoidans may be sulfated at C4, C2 or both C2 and C4 positions of fucose units [11, 12, 17, 18, 22, 23]. Some fucoidans may be both sulfated and acetylated [17, 19].

Generally, brown seaweeds belonging to the orders *Chordariales* and *Laminariales* (*Phaeosporophyceae*) produce polysaccharides built of 1,3-α-L-Fuc*p* residues in the main chains [11, 19, 21, 22, 25] (Table 1). Side chains consist of D-glucuronate (*Cladosiphon okamuranus*) or fucose residues (*Chorda filum*) and are attached to the C2 position of the main chain fucose residues by a regular manner. However, this regularity is masked by random sulfation and acetylation. An exception is fucoidan from *Laminaria gurjanovae* [26], which is, in fact, a galactofucan similar to fucoidan from *Undaria pinnatifida* [27]. Main chain of the fucoidans from brown seaweeds of the order *Fucales* (*Cyclosporophyceae*) is built of alternating 1,3- and 1,4-linked α-L-fucosyl residues [12, 17, 18, 20, 28, 29] (Table 1). Structural differences of the main chains of fucoidans apparently result from different biosynthetic pathways in the seaweeds of families *Phaeosporophyceae* and *Cyclosporophyceae* [17]. Sulfated Fulcans have also been isolated from some marine animals, *e.g.*, echinoderms. They represent linear structures of repeated oligosaccharide units [1, 16, 24] (see Table 1).

3 Biological properties of fucoidans

Fucoidans have been consumed for a long time in such Asian countries as Japan, China and Korea as whole seaweed. Fucoidans from some seaweed species have recently started to be used as nutraceuticals in Australia and the USA. They also display diverse biological activities including antitumor [30–32], immunomodulatory [33, 34], antibacterial [35, 36], antiviral [27, 37, 38], anti-inflammatory [39–43], anticoagulant, and anti-thrombotic [5, 39, 44–50] effects.

Anticoagulant and anti-thrombotic effects were the first described biological activities of fucoidans [51]. Currently, anticoagulants of direct action are almost exclusively represented by heparin preparations. However, clinical applications of heparins are accompanied by side effects [52–55] and an increased risk of prion contamination [56–58]. For this reason, other sulfated polysaccharides including fucoidans have been tested as anticoagulants of direct action. In addition to anticoagulant effect, fucoidans show fibrinolytic activity potentiating tissue- and urokinase plasminogen activators [46, 59]. This allows fucoidans to be considered potential agents to resorb thromboses. Fucoidans from brown seaweeds *Fucus evanescens* and *Laminaria cichorioides* [102], which are widespread in the Far Eastern seas of Russia, reveal properties of direct-action anticoagulants and show inhibitory activity towards thrombin (factor IIa) and factor X. Anticoagulant activity of fucoidan from *F. evanescens* has been found to be connected with antithrombin III. Like heparin, this fucoidan strongly accelerates development of antithrombin III inhibitory effect against thrombin. Both *in vitro* and *in vivo* experiments have demonstrated activation of blood fibrinolytic system by fucoidan from *F. evanescens* [60, 61].

Fucooligosaccharides obtained by enzymatic hydrolysis of *F. evanescens* fucoidan display inhibitory activity mainly towards thrombin factor IIa [44]. Sulfated polysaccharides, in particular fucoidans, are also known as potent anti-inflammatory agents [39–41, 43]. This results from the ability of fucoidans to handicap adhesion of lymphocytes and neutrophil leukocytes to endothelial cells of blood vessels, thus preventing their migration to the locus of inflammation [43]. The anti-inflammatory effect of fucoidan is dose dependent [41]. These authors suggest that the blockage of inflammation at its early stage is due to interaction of fucoidan with P-selectin. Zen *et al*. [42] have shown that fucoidan blocks the adhesion of neutrophil leukocytes to epithelial cells of the intestine by contacting with CD11b/CD18 receptors. Inhibition of leukocytes migration towards standard chemoattractants and the partial adhesion of neutrophils to endothelial cells due to sulfated polysaccharides from red microalgae has been described by Matsui *et al*. [40]. Daily intravenous injections of fucoidans to mice with experimental colitis have even decreased the inflow of leukocytes and the damage of mucosa [43].

Table 1. Structural characteristics of some fucoidans

Source	Structure	References
Seaweeds	[→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(4SO^-_3)-(1→3)-α-L-Fuc*p*-(4SO^-_3)-(1→3)-α-L-Fuc*p*-(1→]n	[21]
Chordariales	2	
Cladosiphon okamuranus	↓	
	GlcA	
Fucales	[→3)-L-α-Fuc*p*-(2SO^-_3)-(1→4)-α-L-Fuc*p*-(2,3SO^-_3)-(1→]n	[18]
Ascophyllum nodosum		
Fucus vesiculosus	[→3)-L-α-Fuc*p*-(2SO^-_3)-(1→4)-α-L-Fuc*p*-(2,3SO^-_3)-(1→]n	[18]
Fucus evanescens	[→3)-L-α-Fuc*p*-(2SO^-_3)-(1→4)-α-L-Fuc*p*-(2SO^-_3)-(1→]n partly sulfated at C4, acetylated; 1→3:1→4 = 1:1	[17, 20]
	[→3)-α- L-Fuc*p*-(2SO^-_3)-(1→4)-α-L-Fuc*p*-(2SO^-_3)-(1→]n partly sulfated at C4; 1→3:1→4 = 3:1	
Fucus distichus L.	[→3)-α-L-Fuc*p*-(2,4SO^-_3)-(1-4)-α-L-Fuc*p*-(2SO^-_3)-1→]n	[12]
Laminariales	[→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→]n	[22]
Ecklonia kurome	Sulfated groups at C4	
Chorda filum	[→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→]n	[19]
	2	
	↓ sulfated at C4 and C2, acetylated at C2	
	L-Fuc*p*	
Laminaria cichorioides	[→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→]n	[11]
	2	
	↓ Sulfated groups at C2 and C4	
	L-Fuc*p*	
Kjellmaniella crassifolia	[→3)-L-α-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→]n	[25]
	2	
	↓	
	L-Fuc*p*	
Echinoderms	[→4)-α-L-Fuc*p*-(2SO^-_3)-(1→4)-α-L-Fuc*p*-(2SO^-_3)-(1→4)-α-L-Fuc*p*-(1→4)-α-L-Fuc*p*-(1→]n	[16]
Arbacia lixula		
Lytechinus variegates	[→3-α-L-Fuc*p*-(2SO^-_3)-(1→3)-α-L-Fuc*p*-(4SO^-_3)-(1→3)-α-L-Fuc*p*-(2,4SO^-_3)-(1→3)-α-L-Fuc*p*-(2SO^-_3)-(1→]n	[1]
Ludwigothurea grisea	[→3)-α-L-Fuc*p*-(2,4SO^-_3)-(1→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(2SO^-_3)-(1→3)-α-L-Fuc*p*-(2SO^-_3)-(1→]n	[1, 16, 24]

Fucoidans have also been shown to have chemoattractant properties after their intraperitoneal introduction. Nezgovorov *et al*. [62] registered a neutrophil exudation 15 min after intraperitoneal introduction of fucoidan to mice, which reached a maximum next day and retained it for 1 month. Over 72 h after fucoidan introduction into peritoneal cavity, the content of monocytes and macrophages was still above the normal level.

While the ability of fucoidans to induce synthesis of, mainly, pro-inflammatory cytokines *in vitro* is known, the production of anti-inflammatory cytokines does occur, but to a much smaller degree [63, 64]. Fucoidan from brown alga *F. evanescens* induced production of TNF-α, IL-1, IL-8, and IFN [65]. Increased production of pro-inflammatory cytokines can promote development of inflammatory reaction at the early stages of infection process, produce neutrophil inflow into the locus of inflammation and activate neutrophils, macrophages, and natural killer cells. As a result, fucoidans can intensify phagocytosis, proliferation of lymphocytes and other effects, thus exhibiting immunomodulatory activity [34]. Fucoidans from *F. evanescens, L. cichorioides, Laminaria japonica* have also been shown to intensify both humoral and cellular immunity and stimulate functional activity of neutrophil leukocytes [34].

The ability of fucoidans to inhibit alternative and classical pathways of the complement activation also characterizes them as preparations with a future as agents with anti-inflammatory activity [66, 67].

Fucoidans from brown algae proved to be antagonistic to a wide range of pathogens. Fucoidans from brown seaweeds were shown to inhibit the adhesion of *Corynebacterium diphtheriae* to buccal epithelial cells [68] or *Hantavirus* to the cell culture *Vero* E-6 [69]. Anti-adhesive action of these polysaccharides seems to be important with regards to their application in medicine as fucoidans could be used as blocking agents of pathogenic bacteria colonizing on the epithelium of mucosa and other human tissues.

Several publications have described antitumor effects of fucoidans. Authors of some reports [30–32] observed inhibition of the growth of sarcoma and leukemic cells, the cells of fibroblast tumor line and adenocarcinoma under experimental conditions. They attributed the antitumor activity of fucoidans to stimulation of mononuclear phagocytes, production of TNF-α by macrophages [70, 71] and induction of apoptosis of tumor cells [72].

Considering all these findings, the use of brown algae appears to be a good choice for biotechnology. The submitted data show that fucoidans possess wide pharmacological activity, so that they could be successfully used for construction of new medical preventive drugs. The drugs based on fucoidan can be recommended as anticoagulant, anti-thrombotic and fibrinolytic agents, as well as immunomodulatory, and anti-inflammatory medicines for complex therapy of the diseases followed by disorders in immunity and hemostasis.

4 Fucoidanases: Distribution and properties

Until now, enzymes degrading fucoidans have only been found in marine organisms: microbes [4, 29, 73–76] and invertebrates [77–79] (Table 2). However, fucoidanase activities produced by these organisms are low. *Proteobacteria* and *Bacteroidetes* associated with brown seaweeds, as well as sea cucumber and sea urchin are the best producers of fucoidanases. The ability of bacteria of the genus *Pseudoalteromonas* from different ecotopes to synthesize fucoidanases is apparently an intraspecific distinctive attribute depending on genomic features of the strain. It can also result from physiological role of bacterium in a certain biotope (Table 3) [74, 75]. Marine bacteria and invertebrates have been shown to decompose structurally different fucoidans from *L. cichorioides* and *F. evanescens* [74, 75]. Bacterial fucoidanases are more specific for the highly sulfated 1,3-α-L-fucan from *L. cichorioides,* whereas fucoidanases of marine invertebrates preferably catalyze degradation of the less-sulfated 1,3;1,4-α-L-fucan from *F. evanescens* [20].

Table 2. Distribution of fucoidanases among marine bacterium and invertebrates of Japanese sea [4, 29, 73–76]

Taxon/type	Total number of samples	Number of active samples			Total number of active samples
		LcF[a)]	FeF[b)]	LcF, FeF	
Bacteria: associants of brown seaweed *F. evanescens*, holoturia *A. japonicus* and sea urchin *Strongylocentrotus intermedius*					
Acinetobacter	1	1	0	0	1
Alteromonas/Pseudoalteromonas	30	2	1	16	19
Bacillus	1	0	1	0	1
Corineforms	6	1	1	3	5
Cytophaga/Flexibacter	14	3	0	5	8
Flavobacterium	20	5	0	8	13
Micrococcus	5	1	1	1	3
Pseudomonas/Halomonas	6	1	0	5	6
Vibrio	6	1	0	2	3
Not identified	8	1	0	4	5
Total	**97**	**16**	**4**	**44**	**64**
Invertebrates					
Cnidaria	1	0	1	0	1
Sipuncula	1	0	1	0	1
Arthropoda	4	1	1	2	4
Mollusca	15	0	4	9	13
Echinodermata	11	1	5	5	11
Chordata	1	0	0	1	1
Total	**33**	**2**	**12**	**17**	**31**

a) Fucoidan from *Laminaria cichorioides*. b) Fucoidan from *Fucus evanescens*.

Table 3. Distribution of fucoidanases among proteobacteria *Pseudoalteromonas* isolated from some ecotopes [74, 75]

Taxon	Source of strain	Amount of strains	
		investigated	Active to FeF[a)]
Pseudoalteromonas citrea	Marine water	5	0
	Marine grass *Zostera marina*	4	0
	Sponges *Plocamia* sp.	1	0
	Halocinthia aurantum	2	0
	Amaroucium translucidum	1	0
	Crenomytilus grayanus	1	0
	Patinopecten yessoensis	3	0
	Apostichopus japonicus	1	1
	Chorda filum	1	1
	Fucus evanescens	1	1
Pseudoalteromonas issachenkonii	*Fucus evanescens*	11	11
Pseudoalteromonas nigrifaciens	Marine water	4	0
	Crenomytilus grayanus	4	0

a) Fucoidan from *Fucus evanescens.*

Studies of fucoidanases face, as a rule, more problems compared to other polysaccharases because of low initial levels of activity as well as heterogeneity and uncertainty of the structures of their natural substrates. In fact, there is no standard fucoidanase assay. This greatly complicates comparison of activities and specificities of different enzymes. Fucoidanase activity can be detected by viscometric assay specific for endodepolymerases [80, 81], or by measurement of an increase in the content of reducing end groups [73, 75, 77, 82], or by carbohydrate-PAGE (C-PAGE) assay of the release of anionic oligosaccharides from the sulfated fucan [28, 29]. Isolation of fucoidanases usually includes, along with precipitation, hydrophobic, ion exchange and/or size-exclusion chromatography, as well as isoelectric and/or chromatofocusing. In most cases, only partially purified preparations of fucoidanases have been obtained; however, some their physico-chemical characteristics have been reported [25, 29, 73, 79–82] (Table 4). The enzymes of invertebrates have, as a rule, acidic pH optimums except for one of the fucoidanases of *Littorina kurila,* which is most active at pH 8.5 [79]. In this respect, the latter is similar to bacterial fucoidanases, which are more active at alkaline pH [29, 73, 80] (Table 4).

Fucoidanases of the marine bacterium *Vibrio* sp. no. 5 and of sea mollusk *Haliotis* sp. were inhibited by Ag^+, Hg^{2+}, Fe^{2+}, and Mn^{2+} ions. Cu^{2+} and Pb^{2+}, however, did not affect their activities, whereas Co^{2+} and Mg^{2+} slightly activated them at comparable concentrations [80, 82]. Fucoidanase from the marine bacterium *"Fucanobacter lyticus"* SN-1009 was inhibited by Ag^+, Zn^{2+}, Cu^{2+}, and Hg^{2+} ions [25]. Fucoidanases from marine mollusk *L. kurila* and the bacterium *"F. lyticus"* SN-1009 were activated by 0.2 and 0.4 M NaCl, respectively [25, 79].

5 Specificity of the fucoidan-degrading enzymes

Known fucoidanases form sulfated fucooligosaccharides [25, 28, 73, 81] or a mixture of sulfated fucose and fucooligosaccharides [79, 80, 82] as the final products of fucoidan degradation. In most cases, only data that compare enzymatic activities towards various fucoidans with undefined structures are available. For example, fucoidanases E1, E2, and E3 from *Vibrio* sp. no. 5 have been shown to effectively split fucoidan from *Kjellmaniella crassifolia,* although they show a minor effect towards fucoidan from *Nemacystus decipiens* [80].

Partially purified fucoidanase from the hepatopancreas of *Pecten maximus* degraded fucoidan from *Ascophyllum nodosum* to low-molecular-weight fragments (3 kDa), in which unsulfated and C2-sulfated fucose residues are connected by α-1,3- and α–1,4-glycosidic bonds [83]. The structures of the products formed by the action of fucoidanases isolated from marine bacteria *"F. lyticus"* SN-1009 [84], *"Fucophilus fucoidanolyticus"* SI-1234 [85] and *Mariniflexile fucanivorans* SW5 [28, 86] have also been determined (Table 5). The extracellular endo-fucoidanase from *"F. lyticus"* SN-

Table 4. Properties and mode of action of fucoidan-degrading enzymes [25, 29, 73, 79–82]

Source	Enzyme	Mode of action	Optimum temperature (°C)	pH optimum	pH stability	pI	kDa
Bacteria	E1	Exo	38–45	6.0	4.0–9.0	5.80	39.5
Vibrio sp. *No.*5	E2	Exo	38–45	6.0	4.0–9.0	5.75	68.0
	E3	Exo	38–45	7.5	4.0–9.0	7.65	68.0
'Fucanobacter lyticus' SN-1009	E1	Endo	30–35	6.5–8.0	n.d.	n.d.	100.0
Pseudoalteromonas citrea KMM 3296, KMM 3297, KMM 3298	E1	Endo	n.d.	6.5–7.0	n.d.	n.d.	n.d.
Mariniflexile fucanivorans SW5	E1	Endo	20–25	7.5	n.d.	n.d.	105.0
Mollusks *Patinopecten yessoensis*	E1	Endo	n.d.	n.d	n.d.	7.4	85.0
Haliotis sp. (hepatopancreas)	E1	Exo	38	5.4	2.0–10.0	n.d.	100.0–200.0
Littorina kurila	E1	Endo	n.d.	5.4	n.d.	n.d.	n.d.
	E2	Endo	n.d.	8.5	n.d.	n.d.	n.d.
Echinoderms *Strongylocentrotus nudus*	E1	Exo	45	3.0–4.0	2.0–5.0	n.d.	130.0

1009 degraded a number of fucoidans from brown algae of the order *Laminariales*: *Lessonia nigrescens*, *L. japonica*, *Ecklonia maxima* (Table 5). Sulfated polysaccharides of the brown seaweeds *F. vesiculosus*, *A. nodosum* (order *Fucales*), *Nemacystus decipiens*, *C. okamuranus* (order *Chordariales*) were, in turn, not split by this fucoidanase [25]. Fucoidans structures of brown seaweeds from the orders *Fucales* and *Chordariales* are known to differ from those from *Laminariales* (Table 1), which are represented by 1,3-α-L-fucans [25]. Hence, the enzyme secreted by "*F. lyticus*" SN-1009 reveals specificity of an endo-1,3-α-L-fucoidanase.

"F. fucoidanolyticus" SI-1234 consumes to a certain extent fucoidans of various structures from *N. decipiens*, *L. japonica*, *Kjellmaniella crassifolia*, *Undaria pinnatifida*, *F. vesiculosus,* and *Ascophyllum nodosum* (19, 31, 23, 22, 61, and 61%, respectively) and apparently synthesizes a number of fucoidan-degrading enzymes [87]. One of them (intracellular fucoidanase) was found to split 1,3-α-glycosidic bonds in fucoidan from *Cladosiphon okamuranus* [85]. The structures of the oligosaccharides – the products of enzymatic hydrolysis – are shown in Table 5. In addition, "*F. fucoidanolyticus"* SI-1234 synthesized deacetylase, which was effective towards acetylated fucoidan from *C. okamuranus* [85]. Specificity to the type of glycosidic bond was strictly established only for three fucoidanases. One of them, the extracellular enzyme of marine bacterium *M. fucanivorans* SW5 cleaved randomly 1,4-α-glycosidic bonds in regular 1,3;1,4-α-L-fucoidan from *Pelvetia canaliculata* consisting of [(4)-α-L-fucopyranosyl-2,3-disulfate-(1,3)-α-L-fucopyranosyl-2-sulfate-(1-)] repeats [28]. This conclusion was based on NMR data, which have revealed 1,3-α-L-fucosyl residues at the nonreducing ends of all reaction products. The authors have identified the enzyme as an 1,4-α-L-fucoidan endohydrolase.

The nucleotide sequences of genes encoding for fucoidanases and their deduced amino acid sequences from *M. fucanivorans* SW5 [28] and "*F. lyticus"* SN-1009 [88] have been published. The gene of *M. fucanovorans* SW5 fucoidanase was cloned and expressed in *E. coli* and a protein product (1007 amino acids) was isolated. Truncated recombinant protein retained fucoidanase activity. This was considered as a fucoidanase [28, 86], along with two fucoidanases from "*F. lyticus"* SN-1009, and as the members of a new glycoside hydrolase family 107 (Carbohydrate Active Enzyme (CAZy) http://www.cazy.org). Sulfated 1,3-α- and 1,3;1,4-α-L-fucans with established structures from *L. cichorioides* and *F. evanescens*, respectively, were used to determine the specificity of fucoidanases isolated from marine bacterium *Pseudoalteromonas citrea* 3296 and marine mollusk *L. kurila* [11, 20]. The enzymes were active on both substrates. Among hydrolysis products of 1,3;1,4-α-L-fucan, fragments enriched with 1,4-α-glycosidic bonds prevailed. For this reason, both enzymes were identified as 1,3-α-L-fucoidan endohydrolases [20].

Recently, a new enzyme extracellular fucoglucuronomannan lyase was isolated from "*Fucobacter marina"* SA-0082 (*Flavabacterium* sp. SA-0082) [89–91]. The enzyme had M_r of 67 K and was most active at 43°C and pH 7.5. It retained activity in

Table 5. Structures of the products formed by the action of fucoidanases [28, 84–86]

Enzyme source/type of hydrolyzed bond	Da	Fucoidan source, product structure (Na^+ ions not shown)
"Fucanobacter lyticus" SN-1009/1→3	1914	***Kjellmaniella crassifolia***
		α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3) 2) ↑ α-L-Fuc*p*(3SO^-_3)-(1
	2016	α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3) 2) ↑ α-L-Fuc*p*(3,4SO^-_3)-(1
	2264	α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3) 2) ↑ α-L-Fuc*p*(3SO^-_3)-(1
	3110-3111	[3-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(4SO^-_3)-(1→]$_2$ 3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3) 2) ↑ α-L-Fuc*p*(3SO^-_3)-(1
	3460	[3-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(4SO^-_3)-(1→]$_2$ 3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3) 2) ↑ α-L-Fuc*p*(3SO^-_3)-(1
		Lessonia nigrescens
	718	α-L- Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)
	966	α-L- Fuc*p*(4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)
	1068	α-L- Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)
		Laminaria japonica
	2366	α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(4SO^-_3)-(1→3)-α-L-Fuc*p*(4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3)-(1→3)-α-L-Fuc*p*(2,4SO^-_3) 2) ↑ α-L-Fuc*p*(3SO^-_3)-(1
	n.d.	***Pelvetia canaliculata***
Mariniflexile fucanivorans (strain) SW5/1→4		[4-α-L-Fuc*p*(2,3SO^-_3)-(1→3)-α-L-Fuc*p*(2SO^-_3)-(1→]$_n$ n=2,3
		Cladosiphon okamuranus
"Fucophilus fucoidanolyticus" SI-1234 / 1→3	n.d.	[3-L-α-Fuc*p*-(1→3)-L-α-Fuc*p*(4SO^-_3)-(1→3)-L-α-Fuc*p*(4SO^-_3)-(1→3)-α-L-Fuc*p*-(1→]$_n$ n=1-3 2) ↓ α-D-Glc*p*UA-(1

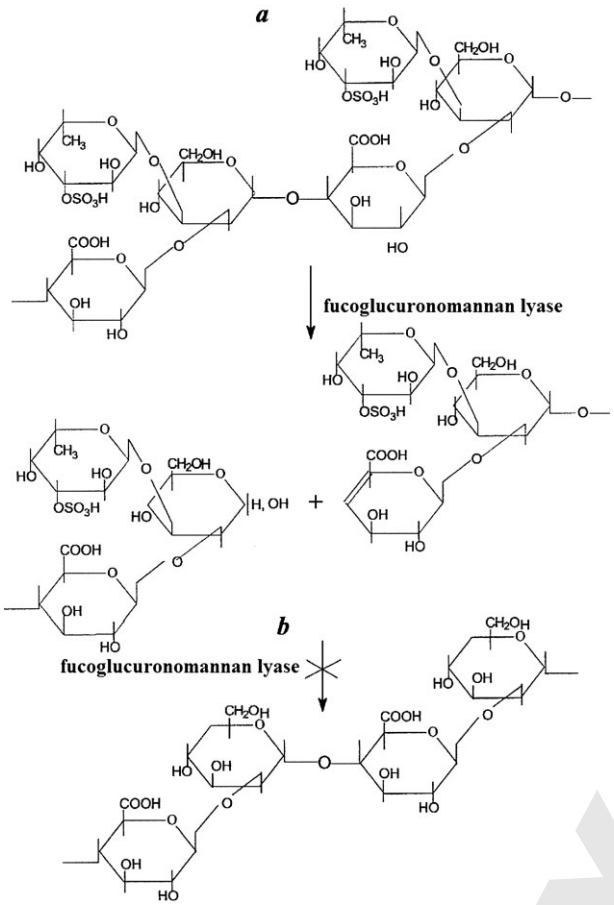

Figure 1. Action of fucoglucuronomannan lyase on fucoglucuronomannan from brown seaweed *Kjellmaniella crassifolia* (a) and on defucosylated polysaccharide obtained by partial acid hydrolysis of the original substrate (b) [90, 91].

0.5 M NaCl but was inhibited by Zn^{2+}, Cu^{2+} and SH-reagents (iodoacetate and *N*-ethylmaleimide). This suggests participation of a thiol group in catalysis [90]. Final products of the action of this enzyme on fucoidan from *K. crassifolia* were trisaccharides of the following structures:

$\Delta^{4,5}$Glc*p*A1-2(L-Fuc*p*(3-OSO_3^-)α1-3)D-Man*p*;
$\Delta^{4,5}$Glc*p*A1-2(L-Fuc*p*(3-OSO_3^-)α1-3)D-Man*p* (6-OSO_3^-);
$\Delta^{4,5}$Glc*p*A1-2(L-Fuc*p*(2,4-OSO_3^-)α1-3)D-Man*p* (6-OSO_3^-).

It follows from these structures that the enzyme cleaved O-glycosidic bond between D-Man and D-GlcA residues in the mannuronane backbone of fucoidan molecule (Fig. 1a). 3-*O* substitution of the D-mannose residue preceding a susceptible bond by sulfated α-L-fucose is a prerequisite of enzyme action (Fig. 1) [91]. Degradation of alginate, hyaluronate, chondroitin sulfates A, B and C, and heparin by this new lyase was not observed. Hence, the enzyme proved to be highly specific for the structural fragments occurring only in fucoidan molecules.

During growth, the bacterium "*F. marina*" SA-0082 utilized 48, 10, 3, and 3% of the fucoidans from *L. nigrescens, U. pinnatifida*, *F. vesiculosus,* and *A. nodosum,* respectively. Apparently, in addition to fucoglucuronomannan lyase, the bacterium synthesizes other fucoidan-degrading enzymes [89]. Thus, at least two types of enzymes (hydrolases and lyases) seem to participate in depolymerization of the fucoidans. Actually, all fucoidans contain different amounts of uronate residues. Some brown algae even synthesize sulfated heteropolysaccharides with the main chain built of uronate moieties [13]. This suggests a possible contribution of lyases to depolymerization of fucoidans. However, whereas lyases are the major enzymes in degradation of alginates – another group of anionic polysaccharides of brown seaweeds – (since the only example of alginate hydrolase has been described so far [3]), fucoidans are mainly decomposed by hydrolases [92, 93].

6 Sulfatases

Sulfatases (EC 3.1.6.-) belong to highly conserved family of enzymes of both pro- and eukaryotes that split sulfate from organic sulfate esters. Bacterial sulfatases apparently remove sulfate groups from sulfated molecules that can be used as carbon source. Sulfatases of mammals participate in transformations of sulfated substrates such as mucopolysaccharides, sulfolipids or steroid hormones [94–97]. Aryl-, steroid-, and glycosulfatases of different substrate specificity have been identified in invertebrates [81, 95].

Desulfation of sulfated polysaccharides with sulfatases greatly simplifies and facilitates structural studies of such complex molecules as fucoidans. However, reports on true fucoidansulfatases are rather sparse. After sulfation of natural polysaccharides with chlorosulfonic acid in the presence of pyridine, sulfated cellulose, sulfated amylose and sulfated glycogen were obtained. Partially purified sulfatase from marine mollusk *Charonia lampas* effectively desulfated only sulfated cellulose, whereas desulfatation of amylose or glycogen, polysaccharides of seaweeds and sea urchin eggs etc. proceeded rather slowly [98]. More active sulfatase was found in some species of marine mollusks collected from a shore in England. It

exhibited activity towards sulfated oligo- and polysaccharides including fucoidans [99]. A partially purified preparation from the bivalve mollusk *P. maximus* effectively desulfated a synthetic substrate *p*-nitrocatecholsulfate, sulfated at C2 L-fucose, and a natural fucoidan from *A. nodosum* of 13 kDa. However, calculations have shown that only approximately 10% of the sulfate content was removed from the fucoidan sample containing 34% of sulfate substituent [100].

Sulfatase hydrolyzing *p*-nitrophenylsulfate rather than natural fucoidan was found in the hepatopancreas of marine mollusks *Haliotis* sp. [81], *L. kurila* [101] and *Patella vulgata* [99]. The same organisms produce fucoidanases. However, only glycosulfatase from *P. vulgata* rapidly desulfated fucooligasaccharides [99]. Presumably this sulfatase eliminates sulfate from sulfated fucose and short fucooligosaccharides formed by fucoidanases [80] since its activity towards native fucoidan was very limited.

7 Conclusion

Fucoidan-degrading enzymes are widespread in sea organisms, although their activity is usually low compared to alginases. By analogy with other sulfated polysaccharides (heparin, chondroitin sulfate, *etc*.) one might expect degradation of fucoidans by both *O*-glycoside hydrolases and lyases specific for hexose-1,4-*O*-uronate bond. Indeed, recent discovery of a lyase that is specific towards branched and sulfated heterofucoidan containing a substituted mannose-1,4-*O*-glucuronate fragment (see Fig. 1) confirms this suggestion. However, an absolute dominance of fucoidan hydrolases over lyases apparently suggests rarity of such fragments in fucoidans compared to other structural elements containing uronates. In any event, enzymatic depolymerization provides an indispensable tool for both structural studies of fucoidans and production of their oligomers, which might display a wide spectrum of biological functions.

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Professor Tatyana N. Zvyagintseva, PhD DSc, graduated from the Department of Chemistry, Far Eastern University in Vladivostok. Her scientific career is tightly bound to the Laboratory of Enzyme Chemistry of the Pacific Institute of Bioorganic Chemistry, Far Eastern Branch of the Russian Academy of Sciences, where she has started to work on the structure and function of laminaran-deploymerases under the guidance of Professor Ludmila A. Elyakova, founder and first director of this laboratory. Under the supervision of Prof. Elyakova Tatyana Zvyagintseva has prepared her PhD and later her DSc thesis. Since the retirement of Prof. Elyakova, Prof. Zvyagintseva has become the head of the Laboratory of Enzyme Chemistry. Major research interests of Prof. Zvyagintseva are in the field of algal polysaccharides and the enzymes of marine organisms involved in their transformation. Professor Zvyagintseva has published more than 100 articles in peer reviewed journals devoted to the structure and function of laminarinases and holds a number of Russian and foreign patents. Under her guidance a number of efficient preparations was developed for the purposes of medicine, cosmetics, agriculture, dietary applications (among them the phytoimmunostimulator ANTIVIR for protection of potato, tobacco, tomato and soybean, TRANSLAM, as well as FUCOLAM-C).

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