REVIEW

Fucose-binding lectins: purification, characterization and potential biomedical applications

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Abstract

The property of lectins to specifically recognize and bind carbohydrates makes them an excellent candidate in biomedical research. Among them are fucose-binding lectins possessing the capacity to bind fucose are taxonomically, evolutionarily and ecologically significant class of lectins that are identified in a wide range of taxa. Purification of fucose-binding lectins dates back to 1967 when L-fucose binding protein from *Lotus tetragonolobus* was isolated using a dye that contained three α-L-fucopyranosyl residues. Beginning with that, several FBLs were purified from various animals as well as plant sources that were structurally and functionally characterised. This review focuses on fucose-binding lectins, their occurrence and purification with special emphasis on various strategies adopted to purify them followed by molecular and functional characterization. The exclusive ability to recognize and bind to fucose-containing glycans endows these lectins with the potential to act as anti-cancer agents, diagnostic markers and mitogens for immune cells. Though they have been in research focus for more than half a century with their occurrence reported in various taxa, they still need to be explored for their prospective functions to develop them as a biological tool in biomedical research.

Keywords Lectins · Fucose-binding · Molecular characterization · Functional applications · Review

Introduction

From erythrocyte agglutinating proteins to biological recognition molecules that target the saccharine side of the cells, lectins, the carbohydrate-binding proteins of non-immune origin possess a prehistoric legacy. Since the identification of this heterogeneous group of versatile proteins in 1888 by Stillmark [1] from seeds of the castor plant, *Ricinus communis*, the occurrence of lectins became apparent. The dawn of the 20th century marked a significant leap in lectin research. The inhibition of reactions by sugars envisaged that the phenomenon of haemagglutination could be a result of the interaction of plant proteins with carbohydrates present on the surface of red blood cells, a speculation that formed a major milestone in lectinology. Despite plant lectins, the prevalence of lectins in animals (both vertebrates as well as invertebrates) was also firmly established subsequently.

Though there was evidence for the occurrence of haemagglutinins in the body fluids of crustaceans and arachnids, not until the 1960s that, an intricate multicellular vertebrate system was explored. The first mammalian lectin isolated was the galactose-specific asialoglycoprotein receptor by Ashwell and Morell in 1974 [2]. The discovery of macrophage mannan receptors in the early 1980s marked the establishment of the conviction that vertebrate lectins were capable of recognizing specific endogenous ligands [3].

In recent years, there has been a lot of interest in the ability of lectins to discern subtle variations in the carbohydrate structures present on the cell surface that are significant in protein-carbohydrate recognition. Lectins from diverse sources with varied carbohydrate-binding specificities have become increasingly popular as a class of biopharmaceutical products in addition to reagents for drug development and diagnostics [4]. They are known to be excellent anti-proliferative, anti-bacterial, and anti-fungal agents and are discovered to be mitogenic to immune cells. In addition, they are utilised to identify blood types and bacteria, as well as to monitor changes in both normal and cancerous cells [5, 6]. Undoubtedly, their enormous potential is still serving as

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an impetus for their investigation from various sources, followed by their purification for the intended usage.

Due to their ability to recognise sugars, lectins, particularly fucose-binding lectins have been widely utilised to track alterations in the fucosylation of plasma glycoproteins like anti-freeze proteins (AFP), the most effective and widely used cancer marker for the detection of hepatocellular carcinoma [7]. Numerous serum glycoproteins and cell-surface membrane receptors associated with carcinoma are known to be highly fucosylated serving as an essential diagnostic marker [8]. The fucose-binding lectin from *Dicentrarchus labrax* (DIFBL) and *Aspergillus niger* (ANL) are known to possess anti-cancer properties [7, 9]. Fucose-binding lectins are also known to activate B cells since fucosylated epitopes constitute a portion of the B cell receptor (BCR). This is best illustrated by the ability of fucose-binding lectins BambL and LecB from *Burkholderia ambifaria* and *Pseudomonas aeruginosa* respectively, to induce B cell activation [10, 11]. These findings are instrumental in showcasing these saccharide-binding molecules way more than a haemagglutinating protein exposing its unravelled potential that can revolutionize modern medicine.

Purification of fucose-binding lectins dates back to 1965 when a dye that contained three α-L-fucopyranosyl residues was used to isolate L-fucose binding protein from *Lotus tetragonolobus* [12]. Following that, several fucose-binding lectins are isolated from different plant and animal sources, and their structural and functional characteristics were defined. This review focuses on fucose-binding lectins, their occurrence and purification with special emphasis on various strategies adopted to purify FBLs followed by molecular and functional characterization. Despite being the subject of research for decades and having been found in many species, fucose-binding lectins still need to be investigated for their potential activities to be used as biological tools in biomedical research.

Methods

The Science Direct (https://www.sciencedirect.com/) was accessed as a source of literature database and searched for peer-reviewed journal articles with keywords "fucose specific lectin-FSL" or "fucose binding lectins-FBL". Using these terms as search keywords to find the articles, 6556 research articles were retrieved using the keyword, FSL and 6161 articles were retrieved with the keyword, FBL. As the keyword, FSL retrieved all the articles covering the articles searched under FBL, the FSL keyword was used to display the search results of 100 articles per page. Then the total number of 6556 research articles were filtered to categorize articles pertaining to this topic of review. Finally,

the most relevant research articles on the purification, structure and characterization of FSL were filtered as 27 articles between the years 1955 and 1999 and 43 articles between the years 2000 and 2023. The review was mainly concentrated on lectins with fucose specificity from several sources of organisms with emphasis on its detection, various strategies used for purification, and molecular and functional characterizations.

Results

Occurrence and structural features of FBLs

As early as 1967, lectins that bind fucose were identified in the seeds of *Ulex europeus* and *Lotus tetragonolobus* [12]. Subsequently, fucose-binding lectins are identified in a broad range of taxa including Proteobacteria, Cyanobacteria, Actinobacteria, Planctomycetes, Bacteroidetes and others [13–16]. They are also reported to be found in planaria, sea urchins, lobe-finned and ray-finned teleost fishes, horseshoe crabs, insects, *Xenopus* spp., salamanders and so on.

The biological activities that glycans elicit are typically reliant on the recognition of specific glycan characteristics by the proteins with which they interact. Proteins rely on stereospecific placement of glycan hydroxyl groups at chiral centres, the use of different linkage sites, and extensive branching to achieve specificity in glycan recognition. Generating a three-dimensional understanding of how proteins recognise glycans is thus critical if we are to understand how glycans are synthesised and recognised in the various physiological and pathological processes they regulate. The quest for the structural basis for protein glycan recognition is not new. Protein crystallography has now advanced to a high level of complexity, accounting for the vast bulk of more than 170,000 structures deposited in the Protein Data Bank (PDB), nevertheless, predicting the structure of protein with ligands is still challenging [16].

There have been numerous reports of the three-dimensional structures of lectins that bind fucose. Based on the PDB structures, the fucose-binding lectins whose structure have been determined thus far were gathered, compared and classified by "pfam", a protein family database which resulted in six different pfam families [17]. The structure of fucose-binding lectins including *U. europeus*, *Anguilla anguilla*, and *Pseudomonas aeruginosa* were solved in complex with fucose [18–22]. All three proteins demonstrated different modes of binding towards the same monosaccharide [18]. The fucose-binding lectin from *Pseudomonas aeruginosa* (PA-IIL) in complex with fucose demonstrated a tetrameric structure with a nine-stranded, antiparallel-β-sandwich arrangement with two adjacent calcium cations that mediate

fucose binding whereas the structure of *A. Anguilla* agglutinin (AAA) in complex with fucose displayed no similarity to any other fucose-recognising proteins that are structurally characterised [21]. A new lectin fold and amino acid residues in the carbohydrate recognition domain (CRD) that can recognize the non-reducing terminal fucose and sub-terminal sugar units present in an oligosaccharide ligand was found in the structure of AAA/α-L-fucose. This led to the recognition of the novel 'F-type' structural fold that folds as a β-barrel with a jelly roll topology with the bulk of the fold consisting of eight major antiparallel β-strands [20, 22]. Structurally, the cleft that binds αL-Fuc is surrounded by five loops formed by connecting β strands on the top face of the barrel whereas the bottom of the barrel is closed by two short anti-parallel strands [20]. Nevertheless, this jelly roll topology is also found to be present in L-type lectins [23]. Three other proteins are also identified as members of this F-type lectin family (FTL) including spGH98 from *Streptococcus pneumoniae*, MsaFBP32 from striped bass (*Morone sazatilis*) and lectinolysin from *Streptococcus mitis*. The CRD of these proteins demonstrated similarity in the overall structure displaying β-barrel structure with a jelly roll topology [17]. Among closely related organisms, the domain organization of the FTLs is found to be consistent with some exceptions of unusual organization demonstrating its versatility [13] It is also found that bacterial FTL domain-containing proteins differ significantly from their eukaryotic counterparts, implying that FTL domains have evolved to suit distinct niches demonstrating the adaptability of this lectin family [15].

The crystal structure of *A. aurantia* lectin (AAL), a fungal protein in complex with fucose solved using single-wavelength anomalous diffraction (SAD) revealed that each monomer consisted of a six-bladed-β propeller fold and a small antiparallel two-stranded-β sheet [18]. A six-bladed β propeller architecture very similar to that of the previously mentioned *A. aurantia* is displayed by the fucose-binding lectin from the bacterium *Ralstonia solanacearum*. The overall fold of putative fucose binding lectin from *Burkholderia ambifaria* is also a six-bladed-β propeller formed through oligomerization. These bacterial and fungal fucose-binding six-bladed-β propellers are evolutionarily related although these structures do not display any similarities in the sequence [16].

Another important class of lectins are the C-type lectins that exhibit broad carbohydrate specificity. One group of C-type lectins with EPN (Glu-Pro-Asn) motif can bind L-fucose along with other carbohydrates with a calcium ion directly contributing to the binding of the fucose moiety. Six structures of proteins belonging to the L-type lectin family, a well-studied lectin family is also identified with a fucose moiety which consisted of two antiparallel β-sheets. A lectin from *Sambucus nigra* is known to bind fucose moiety with two identical β- trefoil domains consisting of an arrangement of three subdomains containing four β-strands [25].

Coprinopsis cinerea lectin 2 (CCL2) is a fucoside-binding lectin which also assumes a β-trefoil dimeric structure. Some of the lectins are found to be metal ion-dependent for fucose binding. For instance, both magnesium and calcium are required for carbohydrate binding in the case of fucose-binding lectin from the L-type lectin family. By selecting for precise stereochemistry of hydroxyl groups, calcium ions are known to contribute to lectin's specificity [16]. In contrast to this, no metal ions are needed for fucose binding to the members of FBL belonging to the R-type lectin family. The structures of fucose-binding lectins are composed mainly of β-strands and the diversity in the structural features arises from the arrangement of β-strands. The relationship between the structure of fucose-binding lectins and glycan recognition preference thus remains obscure.

Purification and characterization of FBLs

Purification of lectins began decades back to decipher the carbohydrate constituents of cell surface glycoproteins with their broad usage in the affinity purification of glycoproteins (Table 1). Goldstein and his colleagues pioneered affinity chromatography for the capture of lectins using crosslinked dextran gels, which is contemplated to be a breakthrough in lectin purification [26]. Specifically, the purification of FBLs though referred to as fucose binding protein can be traced back to 1965 when L-fucose binding protein from *L. tetragonolobus* demonstrating haemagglutinating (HA) activity against human-O red blood cells was isolated using a dye that contained three α-L-fucopyranosyl residues with a yield of 45 mg per 100 g of seeds [12]. Here, the inhibition of HA activity by L-fucose served as the basis for the use of the dye with three α-L-fucopyranosyl residues. Further, it was found that agglutinating activity was inhibited only by L-fucose and not by D-fucose establishing the stereospecificity exhibited by the lectin. Following this, Blumberg et al. [27] instead adopted the method of affinity chromatography on a column of N-(c-aminopropyl)-P-D-fucopyranosyl-amine coupled to agarose gel to isolate the lectin. Despite this, insoluble polyleucyl hog A + H blood-group substance was also used for the lectin isolation [28]. Apart from the aforementioned strategies, lectin specific to L-fucose was purified using affinity chromatography from the seeds of *U. europeus* and *L. tetragonolobus*. Here, to isolate L-fucose binding lectin by affinity chromatography, divinyl sulfone is utilized to crosslink L-fucose to Sepharose 6B. This newly developed method could bypass multi-step procedures that include organic syntheses for preparing various carbohydrate-containing ligands appropriate for coupling

Table 1 Methods employed for purification of fucose-binding lectins (FBL) from diverse sources of organisms

Organism	FBL	Detection of HA activity	Method of isolation	Chromatographic matrix used	Elution strategy	Yield (%)	Specific activity (Au/mg)	Citation
Aleuria aurantia (Orange peel fungus)	AAA	Human ABO; Lewis types; Bombay phenotype; group O cells treated with α (1–2)-fucosidase	Affinity chromatography; Gel filtration; ion exchange chromatography	H-active glycopeptide of desialyzed porcine submaxillary mucine coupled Sepharose 4B; Sephadex G-100; CM-cellulose	20 mM fucose		487.6	[61]
Anguilla japonica (Japanese eel)			Affinity chromatography; Gel filtration	L-fucose agarose; Superdex 75	50mM L-fucose			[62]
Aspergillus niger (Black mold)	ANL	Trypsinized Human A, B, AB, O and rabbit erythrocytes	Affinity chromatography	Mucin-coupled Sepharose-4B	100 mM Glycine-HCl		25.0×10^3	[59]
Boar spermatozoa			High performance gel filtration; Reversed phase chromatography	Superose 12 HR10/30				[63]
Bryothamnion triquetrum (Macroalgae)	BTL	Trypsin treated rabbit erythrocytes	Ion exchange chromatography; RP-HPLC	DEAE-cellulose; Lichrospher RP100 C18 column				[64]
Cerithidea cingulata (Girdled horn shell)	Cc	Miracidial extract-coated sheep RBC	Affinity chromatography	L-fucose agarose	0.3 M L-fucose	100	1.20×10^3	[65]
Dicentrarchus labrax (European seabass)	DlFBL	Rabbit erythrocytes	Affinity chromatography	Sepharose CL6B column; fucose–agarose	0.2 M galactose; 50 mM L-fucose			[66]
Enteromorpha prolifera (Green alga)	EPL-1; EPL-2	Trypsin-treated rabbit erythrocytes	Affinity chromatography	Mannan agarose	100 mM mannose			[34]
Lotus tetragonolobus (Winged pea)			Specific precipitation; Ion exchange chromatography	L-fucosyl dye; DEAE-Cellulose	0.01 M Sodium Phosphate			[67]
Lotus tetragonolobus			Affinity chromatography; Ion exchange chromatography	N- (ε -aminocaproyl)-β -Lfucopyranosyl- amine coupled Sepharose 4B; DEAE cellulose	40 mM L-fucose			[27]
Lotus tetragonolobus		Human O	Affinity chromatography; Gel filtration	L-fucose coupled Sepharose 6B; Sephadex G-200	0.1 M L-fucose	394 mg/100 g		[29]
Lotus tetragonolobus		Human O	Affinity chromatography	L-fucose coupled Sepharose; Porcine gastric mucin-Sepharose	0.05 M L-fucose			[32]
Lotus tetragonolobus		Human O	Affinity chromatography; Ion exchange chromatography	L-fucose immobilized agarose; DEAE cellulose	0.2 M L-fucose			[68]

Table 1 (continued)

Organism	FBL	Detection of HA activity	Method of isolation	Chromatographic matrix used	Elution strategy	Yield (%)	Specific activity (Au/mg)	Citation
Morone saxatilis (Striped bass)	MsaFBP32	Human ABO	Affinity chromatography; Size exclusion chromatography	Divinyl sulfone-conjugated L-fucose-Sepharose-6B; Superose 12 10/30 HR column	200 mM L-fucose	89; 97	50,343; 72,296	[14]
Neoditrema ransonnetii (Surfperch)			Affinity chromatography	L-fucose agarose	0.2 M L-fucose			[69]
Oncorhynchus mykiss (Rainbow trout)	ladderlectin		Affinity chromatography; Size exclusion chromatography	Sepharose CL-4B; TSK G400	Low calcium containing buffer			[70]
Oreochromis niloticus (Nile tilapia)	TFBP	Human O	Affinity chromatography	L-fucose-BSA Sepharose CL 6B	200 mM L-fucose		–	[71]
Pholiota squarrosa (Shaggy scalycap)	PhoSL	Rabbit, horse, pig, goose and guinea pig erythrocytes	Ion exchange chromatography; Affinity chromatography	DEAE-Sepharose; Thyroglobulin-agarose	Linear gradient elution of NaCl; 0.2 M ammonia		–	[72]
Photorhabdus luminescens (Gram-negative bacterium)	PLL	Human A	Affinity chromatography	L-fucose sepharose	0–20 mM L-fucose			[73]
Rhizopus stolonifer (Black bread mold)	RSL	Rabbit erythrocytes	Affinity chromatography	Porcine stomach mucin-sepharose	0.1 M L-fucose		–	[39]
Sparus aurata (Gilt-head bream)	SauFBP32	Rabbit erythrocytes	Affinity chromatography	L-fucose agarose	200 mM L-fucose			[74]
Streptomyces sp.	SFL	Human O	Affinity chromatography; Gel filtration	Affinity absorbent; Sephadex G-25	0.2 M L-fucose		1.1×10^5	[75]
Tachypleus tridentatus (Horseshoe crab)	Tachylectin-4	Human A	Affinity chromatography	Dextran sulfate-Sepharose CL-6B chromatography; ConA-Sepharose chromatography; fucose-agarose chromatography				[76]
Trematomus bernacchii (Emerald rockcod)	TbFTL	Rabbit erythrocytes	Affinity chromatography	L-fucose-agarose	50 mM L fucose		–	[41]
. *Turbo coronatus* (Crown moon turban snail)	Tc	Miracidial extract-coated sheep RBC	Affinity chromatography	L-fucose agarose	0.5 M L-fucose	100	6.10×10^3	[65]
Ulex europaeus (Common gorse)		Human O	Affinity chromatography; Gel filtration	L-fucose coupled Sepharose 6B; Sephadex G-200	0.1 M L-fucose	45 mg/100 g		[29]
Ulva lactuca (Sea lettuce)	Ulva lactuca lectin	Human ABO	Affinity chromatography; Gel filtration	Porcine stomach mucin-Sepharose 6B; Sephadex G-50	0.15 M NaCl and 0.01 M EDTA			[35]

-: No significant results or not determined

to insoluble matrices that were adopted earlier. A yield of 45 mg/100 g of *Ulex* seeds and 394 mg/100 g of *Lotus* seeds were obtained from the affinity column. The yield per 100 g of the protein obtained by affinity chromatography here was much higher than the previously adopted methods [29]. The method according to Fornstedt and Porath [30] was adopted to couple L-fucose to Sepharose 6B instead of Vretblad [31] which utilises epoxy-activated Sepharose 6B. Further, in 1985, Ogata et al. [32] re-examined the isolectin components of fucose binding proteins of *L. tetragonolobus.* The binding (FBP-M) and the non-binding components (FBP-F) obtained by affinity chromatography on porcine gastric mucin Sepharose differed both in their structure and activity.

With very few exceptions, fucose-binding lectins often agglutinated human-O RBC type and can be attributed to the carbohydrate chemistry of human-O red blood cells with exposed fucose residues on its surface (Table 1). In some cases, instead of native erythrocytes, enzyme-treated erythrocytes were used as indicator RBCs. Papain-treated human-O erythrocytes were used as indicator RBCs for the isolation of *Ulva* lectin from *Ulva lactuca* [33]. The indicator RBC used for the isolation of EPL-1 (*Enteromorpha prolifera* Lectin 1) and EPL-2 (*Enteromorpha prolifera* Lectin 2) were trypsin-treated rabbit erythrocytes. Enzyme treatment of erythrocytes demonstrated an increased degree of agglutination [34].

Among the L and D enantiomers of fucose, most of the lectins isolated were found to be L-fucose specific (Table 1). The configurational stereochemistry where the L isomers with hydroxy group positioned on the left of the asymmetric carbon and D isomers with hydroxy group positioned on the right side is an important feature that can ascertain the specificity. In the case of AAA, the primary binding site was found to be the 4-OH group of L-fucose [20]. Owing to the high selectivity and high resolution of separation, affinity chromatography was adopted as the method of isolation of fucose-binding lectins. And in most of the cases L-fucose, which inhibited the haemagglutinating activity was coupled with Sepharose. For instance, L-fucose coupled with Sepharose 4B was used to isolate FBP-M (porcine gastric mucin-Sepharose binding protein) and FBP-F (porcine gastric mucin-Sepharose non-binding protein) from *L. tetragonolobus*. The molecular characterization by polyacrylamide disc electrophoresis revealed the presence of isolectins, with a major lectin fraction having an apparent molecular weight of 35,000 [29]. In some cases, gel filtration was also combined with affinity chromatography. *U. lactuca* as a source of anti-H lectin capable of agglutinating papain-treated erythrocytes was indicated by Gilboa-Garber et al. in 1988. Later, this lectin was isolated by integrating affinity chromatography on porcine stomach mucin-Sepharose 6B and gel filtration method using Sephadex G-100 column with a

yield of 69% and a specific activity of about 34,717 units/mg protein (Table 2). This divalent cation-dependent lectin was found to be stable over a broad range of temperatures and pH. The SDS-PAGE revealed only one single band. Acidic and hydroxyl amino acids were found to be predominant [35].

The haemagglutinin from the culture filtrate of a strain of *Streptomyces* sp. strongly agglutinated human-O erythrocytes and the activity was inhibited by L-fucose. This agglutinin with an estimated molecular weight of 70,000 was isolated using affinity chromatography and affinity absorbent was prepared according to the method put forth by Matsumoto and Osawa [36]. Accordingly, the high specificity rendered by affinity chromatography formed the impetus for the isolation of fucose-binding lectins from variegate sources (Table 1). A highly thermostable fucose-binding lectin with a molecular weight of about 121,000 consisting of four sub-units from the serum of *A. anguilla* was isolated by affinity chromatography on A-peptone-Sepharose column integrated with affinity chromatography using α-L-fucose-agarose (Table 2). Further, it was characterized using isoelectric focusing which identified 17–20 bands between pH 5.5 and 6.2. Interestingly, the fucose-binding lectin isolated was found to be more thermostable than the MBL (mannan-binding lectin) that was isolated from the same serum [37]. Later studies on AAA/α-L-fucose demonstrated the presence of a novel F-type fold. Fucose-binding lectins are also found to be prevalent among different fungal species including *Aleuria aurantia* and *Rhizopus stolonifera* and so on [38, 39]. The fucose-binding lectin from *A. aurantia* was isolated using the H-active glycopeptide of desialyzed porcine sub-maxillary mucine coupled to Sepharose 4B and characterized using PAGE which revealed a dimer with a molecular weight of 72,000 [38]. Besides, MsaFBP32, a lectin isolated using affinity chromatography from the liver extracts of *M. saxatilis* did not possess signature motifs of the CTLs or any lectin families reported at that time but demonstrated two 140-amino acid tandemly arrayed domains on sequencing. A crucial outcome of the study led to the recognition of a novel lectin type (F-type lectins). Apart from the above-mentioned sources, fucose-binding lectins were isolated from various sources with affinity chromatography as the major method of isolation (Table 1). Ion exchange chromatography was employed for the isolation of fucose-binding lectin from the mycelial mats of *Aspergillus fumigatus* [40]. The source, method of isolation, column/matrix used and elution strategy adopted for the purification of reported FBLs are provided in Table 1. Most recently, Ca $^{++}$ - independent fucose binding protein was isolated from the Antarctic fish *Trematomus bernacchii* by affinity chromatography. The molecular weight was found to be 32 and 30 kDa under reducing and non-reducing

Table 2 Molecular characterization of purified fucose-binding lectins (FBL) from diverse sources of organisms

Source organism	IEF		Native Protein		Subunit composition		Cita-tion
	No. of fractions	pI value	No. of fractions	MW (kDa)	No. of fractions	MW (kDa)	
Aleuria aurantia (Fruiting bodies) (Orange peel fungus)			1	70	1	31 ± 1 (dimer of identical subunits & disulfide bond is not involved)	[61]
Anguilla japonica (Japanese eel)					1	20	[62]
Aspergillus niger (Black mold)					1	30 (with & without 2-mercaptoethanol)	[59]
Boar spermatozoa					2	53 & 20	[63]
Boar spermatozoa			2	53 & 17			[64]
Bryothamnion trique-trum (Macroalgae)	-	-	-	1	9.0 (recom-binant BTL)		[65]
Cerithidea cingulata (Girdled horn shell)	Many	pI 5.3–5.5			3	SDS-PAGE unboiled fractions – 73	[66]
Dicentrarchus labrax (European seabass)					2 1	Reducing SDS-PAGE 34 (intense) & 70 (minor) Non-reducing SDS-PAGE Mobility of the 34 kDa band increased to an equivalent mass of 30 kDa suggesting the presence of disulfide bonds	[67]
Enteromorpha prolifera (Green alga)			EPL 1 EPL 2	60 ± 6 (homodimers) 59.5 ± 3 (homotetramers)	2	Both lectins are 20- to 22-kDa single chain, non-glycosylated proteins.	[34]
Lotus tetragonolobus (Winged pea)			3	120 58 117			[68]
Lotus tetragonolobus			3	9 5 21			[27]
Lotus tetragonolobus			1	35 (in both Sep-hadex G-200 & Ultragel AcA-34)			[29]
Lotus tetragonolobus	3	5.40, 4.97 & 4.80 (FBP-M) 5.32 (FBP-F)	3 (FBP-M) 1 (FBP-F)	26 for all three proteins (FBP-M) 25 (single- FBP-F)			[32]
Morone saxatilis (Striped bass)	5	SDS-PAGE, denatur-ing IEF 5 bands of pI values of 5.09, 4.97, 4.91, 4.87, & 4.86			1	35	[14]
Neoditrema ransonnetii (Surfperch)					2	32 and 23 in SDS-PAGE under reducing conditions	[70]
Oncorhynchus mykiss (Rainbow trout)					2	35 & 16	[71]

Table 2 (continued)

Source organism	IEF		Native Protein		Subunit composition		Cita-tion
	No. of fractions	pI value	No. of fractions	MW (kDa)	No. of fractions	MW (kDa)	
Oreochromis niloticus (Nile tilapia)			1 + 2	260 (dark band) 362 & 782 (two faint bands)	Reducing SDS-PAGE 1 + 2 Non-reducing SDS-PAGE 1 + 2	Reducing SDS-PAGE 23 (45 & 85 – faint bands) Non-reducing SDS-PAGE 22 (36 & 66 – faint bands)	[72]
Pholiota squarrosa (Shaggy scalycap)	1	4.0			1 + 2	4.5 in SDS-PAGE in the presence and two bands 4.5 and 14 in the absence of 2-mercaptoethanol	[73]
Photorhabdus luminescens (Gram-negative bacterium)					1	41	[74]
Rhizopus stolonifer (Black bread mold)			1	28 (hexamer of non-covalently associated RSL monomers)	1	4.5	[39]
Sparus aurata (Gilt-head bream)					2	35.5 ± 1.2 (without 2-mercaptoethanol) 30.0 ± 2.2 (with 2-mercaptoethanol)	[41]
Streptomyces (culture filtrate)	1	5.2	2	140 (minor) & 70 (major) in Disc-gel electrophoresis	1	70 (5% & 7.5% SDS-PAGE with or without 2-mercaptoethanol)	[76]
Tachypleus tridentatus (Horseshoe crab)			1	470 (tachylectin-4; oligomeric protein)	2 2	23.5 & 26 (without 2-mercaptoethanol) 30 & 31.5 (with 2-mercaptoethanol)	[77]
Trematomus bernacchii (Emerald rockcod)					1	30.0 ± 0.7 under non-reducing conditions 32.0 ± 0.5 under reducing conditions	[41]
Turbo coronatus (Crown moon turban snail)	Many	pIs 5.3, 5.4 and 5.5 for 34 kDa pIs 5.0–5.7 for the 31 kDa pIs 5.0–6.2 for the 8–15 kDa			Many	SDS-PAGE unboiled fractions − 80	[66]
Ulex europaeus (Common gorse)			1	68 (Sephadex G-200) 45 (Ultragel AcA-34)			[29]
Ulva lactuca (Sea lettuce)			1	8.37 ± 1.20	1	17.1 ± 2	[35]

-- Not determined / non-significant results

conditions, respectively (Table 2). The two CRD-FBLs displayed by the lectin further authenticated it as a member of the fucose-binding lectins family [41].

FBLs: an *in-silico* approach

With the advent of bioinformatics in the 1960s, computational tools that could analyse and process biological information became popular. Through *in silico* experiments, FBLs from diverse sources of organisms were characterized functionally. The computational models and simulations produced were used to make predictions regarding the possible interactions of FBLs. The interaction of *U. europaeus* isolectin I specific for fucose-containing oligosaccharide was studied using homology modelling and molecular docking [42]. Legume lectins including *Lathyrus ochrus*, *Lens culinaris* and *Pisum sativum* specific for mannose and glucose showed higher affinity for fucosylated glycan than for glycans that are without fucose whereas ConA was not able to differentiate between the two. The ability of the former to discriminate between the two glycans is attributed to the presence of a secondary binding site [43].

The mode by which fucose binding legume lectins viz., UEA 1 (*U. europeus* agglutinin 1) and LTA.

(*L. tetragonolobus*) recognize L-fucose was studied with the help of structure-based sequence alignment of the different loop regions of legume lectins with distinct monosaccharide specificities and other biophysical data. Four loops (A, B, C & D) form the combining site of legume lectins [44–46]. Aspartate (loop A), glycine or arginine (loop B) and asparagine (loop C) play a crucial role in the recognition of carbohydrates. In addition to these, a residue with a backbone NH group is found to hydrogen bond with the hydroxyl group of the carbohydrate. The conserved triad of residues including asparagine 137, glycine 105, and aspartate 87 was found to contribute invariantly to the binding of L-fucose with all the legume lectins [46]. From the crystal structure of the complex of AAA with α-L-fucose, the presence of a novel fucose recognition fold, a characteristic lectin fold (β-barrel with jelly roll topology) of the entire lectin family was determined at 1.9 Å resolution [20]. Several *in silico* studies were based on the search for fucose-binding domains from diverse sources. MsaFBP32, a fucose-binding lectin, isolated from *M. saxatilis* comprised two tandem domains with an F-type sequence motif [14]. Modelling of CRDs from MsaFBP32 to the crystalline model of AAA suggested the possibility of gene duplication in the bass and stickleback lineage [47, 48]. Similarly, 3-D structure prediction and structure analysis of the interaction between the pentraxin of *Xenopus laevis* and α-L-fucose through homology modelling and molecular docking revealed that the fucose-binding triad (His[52], Arg[79] and Arg[86]) of *A. anguilla*

agglutinin was found to be conserved in the pentraxin of *X. laevis* [49]. In *Xenopus* spp., this lectin is composed of triplicate or quintuple tandem F-type domains [50].

Fucose-binding domains similar to the one present in the bacterial fucolectin of *Pseudomonas aeruginosa* were identified in three hypothetical proteins from bacteria (*Chromobacterium violaceum*, *Burkholderia cepacia* and *Photorhabdus luminiscens*) and in another protein from archaea (*Methanopyrus kandleri*) with conserved calcium binding loops [51]. Calcium appears to play a pivotal role in structural stabilization rather than in direct carbohydrate interactions unlike C-type lectins [50]. These phylogenetically widespread FLDs were identified in diverse domain organizations including prokaryotes, viruses and eukaryotes with a report of 437 FLD sequence clusters [15]. The three-dimensional structures of fucose-binding lectins have been reported and deposited in the Protein Data Bank (PDB). Studies on the recognition of fucosylated human blood group determinants by *Burkholderia ambifaria* lectin (BambL) revealed that Arg[15], Glu[26] and Trp[79] are involved in fucose monosaccharide recognition through hydrogen bonding interactions. The outcome could probably help in the development of fucomimetic molecules that could bind to BambL [52].

Fucose forms a key component of oligosaccharides that are crucial in many biological interactions that include pathogenic bacterial infection of host cells, cell adhesion and glycosylated binding proteins. The S protein of SARS-CoV$_2$ is found to be a highly glycosylated structure. The S protein from ancestral and Omicron strains was found to bind with *Pholiota squarrosa* lectin (PhoSL) a 40 amino acid chemically synthesised peptide and resulted in the inhibition of infection at sub-micromolecular level demonstrated PhoSL as a potent broad-spectrum inhibitor of infection [53].

FBLs: as biological recognition molecules

The 'unusual sugar', fucose (6-deoxy hexose) is ubiquitously present in diverse glycoproteins and glycolipids that are produced by mammalian cells. They are found to be present in 7.2% of oligosaccharides as a common component of glycan modifications that are seen in proteins and lipids. Many serum glycoproteins and receptors found on the cell surface are highly fucosylated and serve as an important diagnostic marker in carcinoma (Fig. 1). This can be attributed to the differential expression of fucosyltransferases in transformed cells leading to the increased fucosylation patterns characteristic of cancer cells [7, 54, 55]. For instance, alpha-fetoprotein (AFP)-L3 fraction, a fucosylated AFP is used as a tumour marker for hepatocellular carcinoma (HCC) clinically [56]. Thus, fucose-binding lectins that could identify aberrant or altered glycosylation
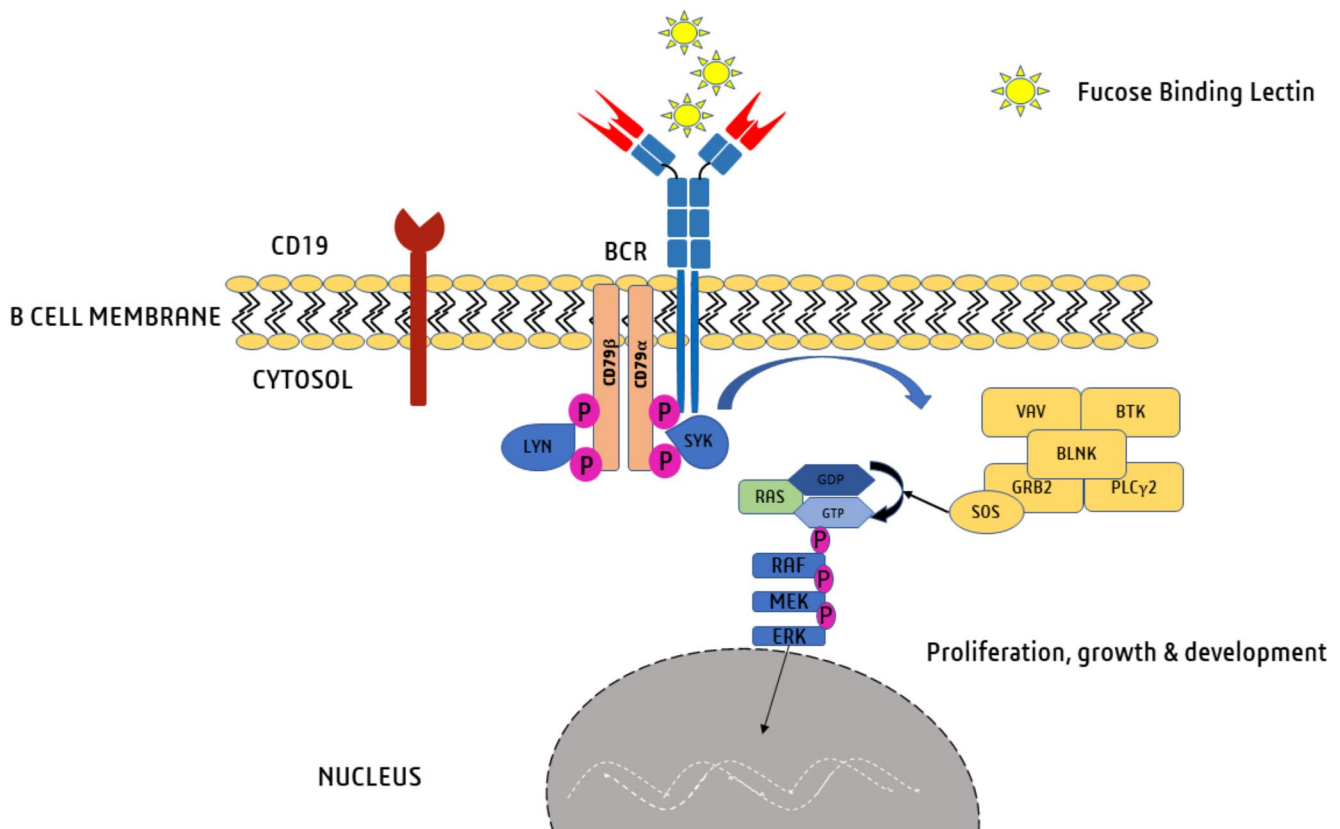
Fig. 1 Schematic illustration of FBL (fucose binding lectin) induced MAPK (mitogen-activated protein kinase) signalling pathway FBL binds to the B cell receptor (fucose residues on the B cell receptor) on the B cell membrane. This binding activates the tyrosine-based activation motifs (ITAMs) found in the CD79, a transmembrane protein that complexes with B cell receptor (BCR). This results in the activation of SYK (spleen tyrosine kinase). This in turn activates the signalosome that comprises Bruton's tyrosine kinase (BTK), phospholipase Cγ2 (PLCγ2), VAV (a group of tyrosine phosphorylation-regulated signal transduction molecules) and the growth factor receptor bound protein-2 (GRB2). This is followed by the activation of genes of RAS (rat sarcoma) which further activates RAF (rapidly accelerated fibrosarcoma). The phosphorylation and activation of RAF is followed by phosphorylation of MEK (mitogen-activated protein kinase), which in turn phosphorylates ERK (extracellular signal-regulated kinase). With numerous targets inside the nucleus, this transmits the mitogen signals required for cell proliferation

patterns are being extensively studied for their potential clinical applications [57].

A novel recombinant *A. aurantia* lectin with core fucose binding was developed that could identify various glycoprotein markers associated with hepatocellular carcinoma whose native form possesses five binding pockets that can bind to fucose linkages, including α-1,2, α -1,3, α -1,4 and core α-1,6-fucose [18, 58]. Similarly, an L-fucose-specific lectin from the pathogenic fungus, *A. niger* (ANL) displayed strong binding to human pancreatic adenocarcinoma cells (PANC-1) and could be blocked by L-fucose. It also demonstrated a time and dose-dependent inhibitory effect on PANC-1 cells [59]. This lectin was also able to detect tumour-associated glycoproteins linked to human hepatocellular and colon cancer cells. In addition to its role as a diagnostic agent, ANL was also found to increase the apoptosis level in both hepatocellular and colon cancer cell lines indicative of its anti-cancer potential [7]. This effect could be attributed to the capability of ANL to recognize fucosylated glycoproteins associated with various types of carcinomas. Another important lectin with L-fucose binding from *Fenneropenaeus indicus* manifested an antiproliferative effect against breast cancer cells [60]. In addition to its inhibitory effects on cancer cells, ANL also possessed potent antibacterial activity against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Fucose-binding lectins from teleost fishes known to function as opsonins, played a crucial role in the clearance of microbial pathogens. This is evidenced by the fucose binding lectin from Antarctic teleost fish *Trematomus bernacchii* with bacterial agglutination activity underpinning its role in host-pathogen interactions [41]. A novel fucose-specific lectin from the fruiting body of *Coprinopsis cinerea* was found to exhibit nematotoxic activity as a part of their defence of fruiting bodies of agaricomycete against nematodes (Table 3).

In addition to the aforementioned functional characterizations, the fucose-binding lectins can also function as

Table 3 Functional characterization of fucose-binding lectins (FBL) from diverse sources of organisms

Source organism	FBL	Type of functional study	Model organisms used / cell culture experiments	Method of exposure	Impact on target organisms/cell culture experiments	Citation
Aspergillus niger (Black mould)	ANL	Anti-cancer and diagnostic potential on hepatocellular and colon cancer cells	HepG2 (ATCC# HB-8065) and colon epithelial cancer HT-120 29 (ATCC# HTB-38) cell lines	in vitro experiment	Anti-cancer activity	[7]
Aspergillus niger (Black mould)	ANL	Inhibitory effect on PANC-1 cells	PANC-1 cells	in vitro experiment	Dose and time-dependent growth inhibitory effect on PANC-1 cells	[59]
Burkholderia ambifaria (Gram-negative bacteria)	BambL	Cellular responses of human B cells to BambL exposure	Human peripheral blood mononuclear cells (PBMCs)	in vitro experiment	BambL act as superantigen	[10]
Burkholderia ambifaria (Gram-negative bacteria)	BambL	Carbohydrate-dependent B cell activation	murine B cells	in vitro experiment	Polyclonal activation of B cells	[11]
Coprinopsis cinerea (Mushroom - the grey shag)	CML1	Nematotoxic activity	Bacterivorous nematodes	Feeding nematodes with *E. coli* transformants containing empty vectors or plasmids directing the expression of CML1 in the bacterial cytoplasm	Involved in the defense of agaricomycete fruiting bodies against predation by fungivorous nematodes	[78]
Fenneropenaeus indicus (Indian white shrimp)	IFL	Cytotoxic effects in breast cancer cells	MCF-7	in vitro experiment	Antiproliferative effects	[61]
Lotus tetragonolobus (Winged pea)	LTL	Hepatocellular carcinoma diagnosis	Serum sample from HCC patients	Mass Spectrometry	Improved diagnostic sensitivity of AFP-L3 assay	[57]
Pholiota squarrosa (Shaggy scalycap mushroom)	PhoSL	Broad-spectrum effect on SARS-CoV-2 variants	TY-WK-521/2022 (Wuhan strain); TY38-873, BA.1(Omicron Strain)	in vitro experiment	Inhibition of SARS-CoV-2 infection	[53]
Pseudomonas aeruginosa (Gram-negative bacteria)	LecB	Carbohydrate-dependent B cell activation	murine B cells	in vitro experiment	Polyclonal activation of B cells	[11]
Trematomus bernacchii (Ray-finned marine fish)	TbFTL	Bacterial agglutination activity	*Escherichia coli*, *Bacillus subtilis*, *Kocuria rhizophila*	Agglutination assay	Bacterial agglutination by bacterial recognition and crosslinking	[41]

super stimulatory antigens (Fig. 2). The capacity of lectins to bind fucose (fucosylated epitopes in B cells) is known to initiate polyclonal activation of B cells. The bacterial lectins, BambL isolated from *B. ambifaria* and LecB isolated from *P. aeruginosa* are known to activate B cells (Table 3) in a carbohydrate-dependent manner [11]. Doubtlessly, the ability of these lectins to act as mitogens that can stimulate B cells to proliferate has a significant impact on human physiology.

Conclusion

Among the different monosaccharides relevant for glycan motifs, fucose in particular is found as terminal sugar frequently and is known to be involved in interactions that are pertinent in biological systems. For instance, in the pathological conditions of cancer and inflammation, variations in the expression of fucosylated oligosaccharides are observed with several types of biomarkers associated with them. Owing to the ability of fucose-binding lectins to discern the overexpressed fucosylated epitopes (glycan-based cancer biomarkers) present on the cancer cell surfaces, they are extensively studied for their anti-cancer potential as well as for diagnostic purposes. In addition to this, some of the fucose-binding lectins reported are found to be involved in the polyclonal activation of B cells that are associated with their sole capacity to bind fucose as the receptors of immune cells are found to be highly glycosylated. The property of these lectins to recognize and specifically bind fucose-containing glycans makes them an excellent tool in biomedical research as chemotherapeutic agents, diagnostic agents, mitogens and drug delivery agents. Though FBLs have been in research for more than several decades, most of the identified fucose-binding lectins across various taxonomic groups are still not functionally characterized. The
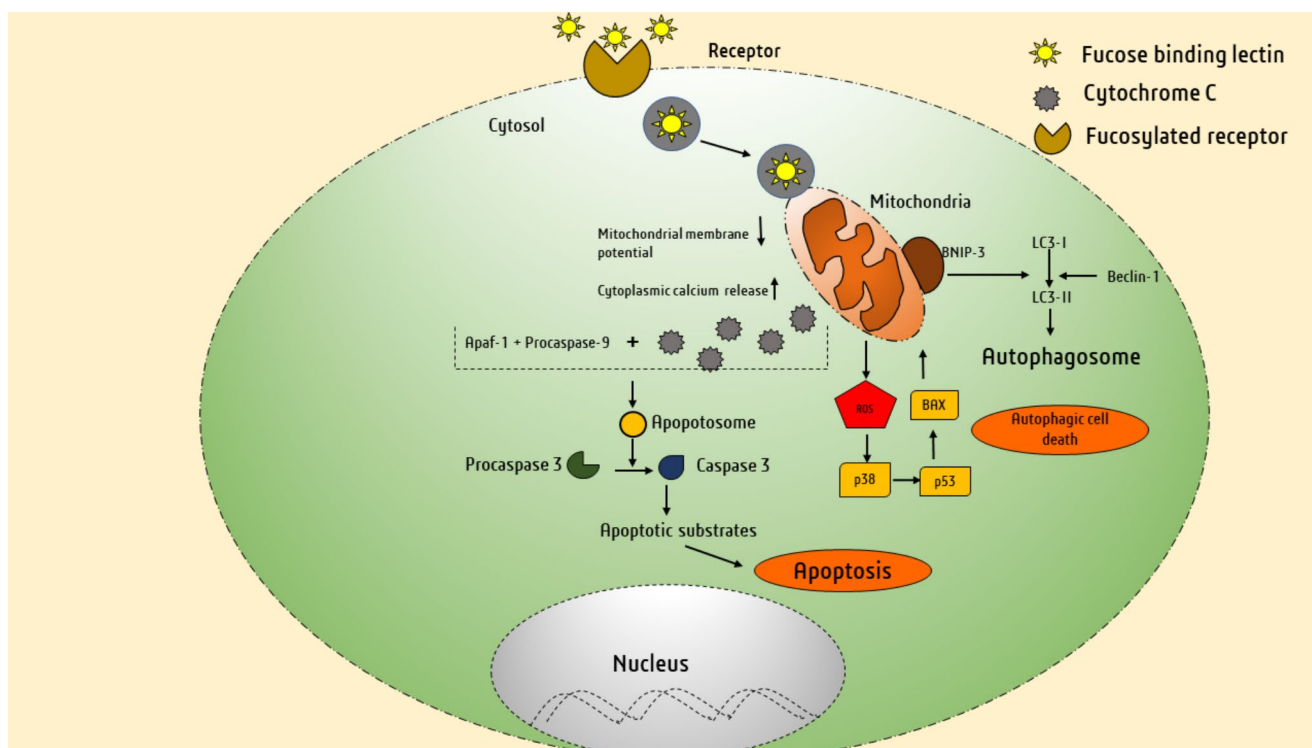
Fig. 2 Schematic illustration of the mechanism of FBL (fucose binding lectin) induced apoptosis or autophagy in cancer cells In lectin-induced apoptosis, the binding of FBL with the fucosylated receptor on the cell surface triggered the loss of transmembrane potential of the cell, disruption of the mitochondrial membrane and release of cytochrome-c through the mitochondrial pathway. It is hypothesized that fucose-binding lectins which are soluble pattern recognition molecules able to recognize mitochondria through the lectin-mitochondrial pathway once internalized. This is speculated to cause a decrease in the mitochondrial membrane potential and an increase in cytoplasmic calcium release resulting in mitochondrial bioenergetic imbalance leading to caspase activation and apoptosis

exclusive property of FBLs to activate immune cells, to be cytotoxic to cancer cells, and to act as a diagnostic tool in identifying cancer cells through various mechanisms needs to be explored further. This review suggests that fucose-binding lectins represent a greater resource to prospect for several therapeutic applications and the enormous potential they endow to develop them as biological tools in biomedical research.

Declarations

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