

# Heparinoid-active sulphated polysaccharides from marine algae as potential blood anticoagulant agents

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**Anticoagulant properties of marine algae have been extensively studied for the last 60 years. Sulphated polysaccharides (SPS) of three major divisions of marine algae, viz. Rhodophyta, Phaeophyta and Chlorophyta are reported to have such properties. Some of the active components have been chemically well characterized. Sulphated galactans and fucoidan sulphates from red and brown algae, respectively, and different sugar sulphates like arabinan, rhamnan sulphates, etc. from green algae are the active molecular species identified. Activity is related to the molecular size, type of sugar and sulphate content of the active component. Sulphate position, type of linkage and molecular geometry are also known to have a role in activity. The proposed mechanisms of action are predominantly on HC-II mediated anti-thrombin activities, direct antithrombin action (thrombin–fibrinogen complex) and minor AT-III involvements. Little anti-factor Xa and fibrinolytic activities are also proposed. Therapeutic interest of algal SPS as anticoagulant has recently been in focus. In future, algal SPS can be developed as anticoagulant/antithrombotic agents or could be used as a model for the same.**

MARINE organisms are found to be very rich sources of food, feed, medicines and energy. They have also proven to be rich sources of structurally diverse bioactive compounds with valuable pharmaceutical and biomedical potential. Marine algae are the only sources for industrially important phycocolloids like agar, carrageenan and alginates. Apart from industrial uses, in recent years, polysaccharides of plant origin have emerged as an important class of bioactive natural products. They are reported to have blood anticoagulant, anti-tumour, anti-mutagenic, anti-complementary, immunomodulating, hypoglycemic, antiviral, hypolipidemic and anti-inflammatory activities<sup>1</sup>.

Sulphated polysaccharides (SPS) are a class of compounds containing hemi-ester sulphate groups in their sugar residues. These are commonly found in marine

algae and higher animals, scarcely present in microbes and absent in higher plants. SPS are found in varying amounts in three major divisions of marine algal groups, viz. Rhodophyta, Phaeophyta and Chlorophyta. SPS found in Rhodophyta are galactans consisting entirely of galactose or modified galactose units. They are known commercially as agar and carrageenan. Agar is composed of a backbone of alternating units of 3,6-anhydro- $\alpha$ -L-galactopyranosyl-(1 $\rightarrow$ 4) and O- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 3) and carrageenan is composed of only D-galactose units (1,3-linked  $\beta$ -D-galactose and 1,4-linked  $\alpha$ -D-galactose). The general SPS of Phaeophyta are called fucans. This includes the compounds fucoidin, fucoidan, ascophyllan, sargassan and glucuronoxylifucan. They comprise families of polydisperse heteromolecules based on L-fucose, D-xylose, D-glucuronic acid, D-mannose and D-galactose. All the main fucan fractions contain a significant minority of proteins which cannot readily be removed, suggesting that they exist *in vivo* as proteoglycans. The major polysaccharides in Chlorophyta are polydisperse heteropolysaccharides, three main groups occur in green algae, viz. glucuronoxylorhamnans, glucuronoxylorhamnogalactans or xyloarabinogalactans. These molecules are highly branched and do not appear to contain a backbone or simple repeating units<sup>2</sup>. Some of the green algal SPS are found covalently attached to the protein, i.e. they are characterized as proteoglycans<sup>3–6</sup>. Biosynthesis of polysaccharides in marine algae involves incorporation of a number of neutral and acidic monosaccharides into the polymer. These include the ubiquitous D-glucose and also D-galactose, D-mannose, L-galactose, L-rhamnose, L-fucose, D-xylose, D-mannuronic acid, D-guluronic acid and L-guluronic acid, as well as some other sugars. It is assumed that the above-mentioned modifications occur by sequential steps<sup>7</sup>.

Various animal glycosaminoglycans/proteoglycans perform powerful blood anticoagulant activity. The most widely known and therapeutically used glycosaminoglycan is heparin, comprising variously sulphated, alternating, 1,4-linked residues of uronic acid and D-glucosamine<sup>8,9</sup>. Heparan sulphate and dermatan sulphate are other endogenous SPS which are believed to play a

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physiological anticoagulant role<sup>10</sup>. Chemically related polysaccharides have also been identified in numerous living organisms<sup>11</sup>.

Anticoagulation occurs predominantly by inhibiting the key coagulation serine proteases, thrombin and factor Xa. This is facilitated by accelerating the activity of major physiological serine protease inhibitor-SERPIN-antithrombin III (AT-III)<sup>12</sup>. There is lesser inhibition in the case of IXa, XIa, XIIa and kallikrein. Another SERPIN heparin co-factor II (HC-II) has been identified that exclusively inhibits thrombin, but has no significant activity against other coagulation or fibrinolytic proteases<sup>13</sup>. The anti-haemostatic properties of heparin and other sulphated polysaccharides extend beyond anticoagulation and include fibrinolytic potentiation and anti-lipemic effects<sup>14</sup>. Though heparin is a primary anticoagulant drug, it has some disadvantages like it is extracted from internal organs of higher animals and purified; hence its production is difficult and it exhibits haemorrhagic-like side effects. These disadvantages associated with heparin have opened up a new area of antithrombotic research for discovering novel anticoagulant agents. Recently few drugs have been introduced and many are in clinical trials.

This review article is an emphasis on the blood anticoagulant activity of SPS from 3 major divisions of marine algae, Rhodophyta, Phaeophyta and Chlorophyta.

### Distribution of anticoagulant SPS

The first report on marine algal extracts possessing blood anticoagulant properties was by Chargaff *et al.*<sup>15</sup>, demonstrating anticoagulant activity in an extract of a red alga *Iridaea laminarioides*. This material, a 'galactan sulphuric acid ester', was shown to possess 30 U/mg of heparin equivalence. Subsequent studies described similar anticoagulant properties in agar and carrageenan<sup>16,17</sup>. A group of 19 species belonging to red, brown and green algae were screened by Elsener in 1938, but anticoagulant activity was found only in *Delesseria sanguinea* (red alga)<sup>17</sup>. Since these studies, there have been many reports relating to the anti-haemostatic properties of extracts and purified fractions from red, brown and green algae. There is a greater incidence of anticoagulant activity in extracts from the brown algae compared to red and green algae<sup>18</sup>. So far, about 150 species representing three major divisions of marine algae, Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae), have been reported to have blood anticoagulant activities. Some species have been studied well and their active molecular species have been characterized, and in some cases mechanisms of anticoagulant action are also well studied.

### Extraction, purification and structure determination

Neutral polysaccharides are usually extracted with water. Low molecular weight and very high molecular weight SPS are extracted with cold water and hot water (>90°), respectively<sup>2</sup>. Mild acid, CaCl<sub>2</sub>, cold and hot water are used for the extraction of anticoagulant SPS from brown algae<sup>3</sup>. The most active fraction of *Codium* spp. was found in cold water extract whereas *Udotea* spp. exhibited higher anticoagulant activity in the hot water extract<sup>19</sup>. Polysaccharides are usually isolated by precipitation with alcohol from a non-dialysable fraction of water extract of the source material. The precipitate is digested with water, filtered, dialysed and freeze-dried. Fractional precipitation is achieved with cetyl trimethyl ammonium hydroxide (CTA-OH)<sup>20</sup>. Fractional solubilization with acetic acid is used to free the polysaccharide from its salt, followed by precipitation with ethanol. Sulphated arabinan, the most active anticoagulant fraction from *Codium* spp. was obtained by fractional precipitation with KCl<sup>21</sup>. Complex formation with copper salts or boric acid has also been utilized for purification of polysaccharide fraction<sup>22,23</sup>. The crude polysaccharide fractions obtained by precipitation are usually further purified by ion-exchange, gel permeation and affinity chromatography. Molecular weight of polysaccharides is determined by low angle laser light-scattering and gel permeation methods.

In complex carbohydrates, neutral sugars are identified by their derivatives, alditol acetates by GC<sup>24</sup> or alditols by HPLC<sup>25</sup>. Glycosidic linkages are usually determined by methylation analysis<sup>26</sup>, which is either performed by Hakomori's method<sup>27</sup> or modifications thereof<sup>28</sup>, carried out by GC-MS. A procedure has been developed by which the glycosidic linkages of complex carbohydrates can be determined at 1–5 µg level<sup>29</sup>. In this technique, saccharides are reduced with sodium borodeuteride prior to per-O-methylation. Identification of microscale partially-O-methylated alditol acetates by GC-MS in the multiple, selected ion-mode (stacked) electron-impact, mass chromatography<sup>30</sup> is found to be about seven times as sensitive as standard GC-MS. FD-MS, SI-MS, FAB-MS and LD-MS are also used for the structural determination of sugars. One- and two-dimensional NMR techniques are used for the identification of individual sugar residues, their configuration, interglycosidic linkages, sequencing and the site of appended groups.

### Evaluation of anticoagulant activity

Methods used to determine the anticoagulant activity are usually commensurate with the historical phase of haemostatic investigation. Thus early studies have used

whole blood clotting times and the United States/British pharmacopoeial assays, whereas more recent studies have used clot end point PT, APTT and TT. Direct anti-Xa, direct anti-thrombin activities, potentiation of AT-III and HC-II inhibition of thrombin were also measured using chromogenic substrates for a precise mechanism of action. Potency of activities is expressed by (a) time delay (second), (b) clotting time ratio, (c) heparin units/mg, and (d) relative anti-thrombin activity to heparin.

## Chemistry and anticoagulant activity

### Red algae

Anticoagulant activity has been studied in over 40 species of red algae. Carrageenan is classified into various types such as  $\lambda$ ,  $\kappa$ ,  $\iota$ ,  $\epsilon$ ,  $\mu$ , all containing 22–35% sulphate groups. This classification was made based on its solubility in potassium chloride. However, this classification system has been considered somewhat unsatisfactory, as solubility characteristics may vary between different samples of the same source material<sup>4</sup>. Many reports exist on anticoagulant activity of carrageenan<sup>31–35</sup>. *Chondrus crispus* is the primary source of  $\lambda$ -carrageenan, whereas *Eucheuma cottoni* and *E. spinosum* are the sources of  $\kappa$ - and  $\iota$ -carrageenans, respectively. Between the carrageenan types,  $\lambda$  carrageenan has approximately twice the activity of unfractionated carrageenan and four times the activity of  $\kappa$ -carrageenan. However, the most active carrageenan has approximately one-fifteenth the activity of heparin<sup>31,32</sup>. The principal basis of the anticoagulant activity of carrageenan appeared to be an anti-thrombic property<sup>31,32</sup>.  $\lambda$ -carrageenan showed greater anti-thrombic activity than  $\kappa$ -carrageenan probably due to its higher sulphate content, whereas the activity of the unfractionated material remained between the two.  $\lambda$ -carrageenan consistently prolonged the clotting time and was more toxic than  $\kappa$ -carrageenan. The difference in sulphate content between the two carrageenans did not correspond directly to differences in anticoagulation action and toxicity<sup>33</sup>. It was found that toxicity of carrageenans depended on the molecular weight<sup>34</sup>. Similar results were obtained with  $\lambda$ -carrageenan of *Phyllophora brodiaei*, which gave the highest blood anticoagulant activity<sup>36</sup>. Carrageenan of *Grateloupia turuturu* also showed anticoagulant activity<sup>36</sup>. Sulphated galactan from *G. indica* of Indian waters exhibited anticoagulant activity as potent as heparin. Its SPS is predominantly a 1→3 linked D-galactan, where some of the galactose moieties bear sulphate ester groups at position 2 only, and it does not contain 1→4 linked galactosyl moieties or alkali-labile sulphate groups at position 3 or 6. Thus, it is different from the carrageenan class of polysaccha-

rides<sup>37</sup>. In an experiment with various carrageenans for anticoagulant activity, the carrageenan of *G. dichotoma*<sup>38</sup>,  $\lambda$ -carrageenan (Sigma),  $\iota$ -carrageenan (Sigma) and carrageenan type LC (Copenhagen Pectin Factory) were placed in decreasing order<sup>39</sup>.

Agar type of SPS were isolated from the five species of genus *Laurencia*, and their activities range from marginal to control value<sup>40</sup>. Carrageenophytes like *Furcellaria fastigiata*, *E. spinosum*, *Gigartina acicularis*, *G. pistillate*, *G. radula* and *Iridaea* have been studied and it was found that only *G. acicularis* showed very potent anticoagulant activity<sup>41</sup>. However, other studies on *Iridaea*<sup>15,16</sup> have confirmed the presence of anticoagulant activity. Similarly, carrageenan of *E. spinosum* was reported to have anticoagulant activity<sup>42</sup>. Polysaccharide extract of *G. acicularis* was checked for its *in vivo* effect on dogs. When the extract was administered intravenously at a dose of 1 mg/kg and at a concentration of 2 mg/ml, it showed maximal anticoagulant activity after 4 h, which was monitored by whole blood clotting time estimations<sup>41</sup>. Effect of carrageenan on human platelets has also been studied<sup>43–46</sup>. It has been reported in *ex vivo* study that carrageenan had anticoagulant effect and inhibited platelet aggregation. Among the carrageenans,  $\lambda$ -type was the most potent anticoagulant at low concentration<sup>35</sup>. In another study, carrageenan prolonged the clotting time in partial thromboplastin time assays and inhibited amidolytic activity of thrombin<sup>43</sup>. It was later reported that pretreatment of platelets with AT-III prevented subsequent aggregation initiated by carrageenan<sup>44</sup>. The influence of carrageenan on the clotting mechanism of blood has also been studied *in vitro* and *in vivo* in the rabbit<sup>47,48</sup>. Carrageenan also activates Hageman factor<sup>49</sup>. Contrary to earlier findings, Schimpf *et al.*<sup>47</sup> reported that carrageenan had anti-thrombin effect. It inhibited fibrin aggregation or polymerization. In the presence of carrageenan the clotting time was lengthened in tests in which the fibrin polymerization phase was involved<sup>47</sup>. Schwartz and Kellermeyer<sup>49</sup> found that carrageenan activated the factor XII in human plasma and thus promoted blood coagulation. *Polysiphonia denudata*, *Corallina granifera*<sup>50</sup> and *Phyllophora nervosa*<sup>51</sup> were tested but none of them showed anticoagulation activity. *C. rubens*<sup>52</sup> and *Pterocladia capillaceae*<sup>53</sup> showed anticoagulant activity which was determined by recalcification, thrombin and euglobulin lysis time assays, and the active compound was identified as polypeptide. Some British marine algae have been reported to have anticoagulant activity<sup>54–56</sup>. Anticoagulant activity of some Indian marine algae is reported and heparin activity was calculated as USP units/mg, using Azure A assay. *Acanthophora spicifera*<sup>57</sup> and *G. filicina*<sup>58</sup> had activity of 28.5 and 30 USP/mg, respectively. Polysaccharides from tetrasporic plants ( $\lambda$ -carrageenan) of *Stenogramme interrupta* showed higher activity than

those isolated from cystocarpic ( $\kappa$ /I-carrageenan) plants in TT test<sup>59</sup>.

Carrageenans are chemically modified or depolymerized to exert anticoagulant activity. Low molecular weight  $\lambda$ -,  $\kappa$ -, and I-carrageenans and their sulphated derivatives were prepared and tested for their APTT activity<sup>60</sup>. It was found that  $\lambda$ -,  $\kappa$ -, and I-carrageenans which had a high sulphur content (5.1, 4.3 and 4.3 moles per mole of disaccharide, respectively) and higher molecular weight (MW) ( $105\text{--}189 \times 10^4$ ) showed higher activity than those with low sulphur content (1.2–1.9 moles per mole of disaccharide) with low MW ( $1.0 \times 10^4$ ). Among all three types,  $\lambda$ -carrageenan showed maximum activity (67 units/mg)<sup>60</sup>. O-acetylated low molecular weight carrageenans that were prepared had low anticoagulant and potent anti-HIV activities<sup>61</sup>. The butanoylated derivatives of  $\lambda$ -carrageenan with degree of substitution of 1.1–1.5 had anticoagulant activities of 6.7–8.5 units/mg and it was observed that activity was decreased with substitution of acyl groups<sup>61</sup>.

**Mechanism of action:** Initial work on mechanism of anticoagulant activity of carrageenan has been reported<sup>62</sup>. The therapeutic potential of carrageenan component is limited in undegraded materials, by its propensity to form insoluble complexes with plasma proteins such as fibrinogen. Degraded forms of carrageenan produce soluble complexes with fibrinogen at physiological pH<sup>12</sup>. Platelet aggregation is induced by carrageenan preparations at concentrations that also demonstrate significant anticoagulant activity<sup>33</sup>. However, because of the mode of this anticoagulant activity, platelet aggregation by thrombin is inhibited by carrageenan<sup>35</sup>. The mechanism of anticoagulant activity of carrageenan is exhibited via thrombin inhibition. Amidolytic studies, using chromogenic substrates, initially indicated that the anti-thrombin activity might be mediated via AT-III<sup>35</sup>, the major mechanism by which heparin acts. In these studies carrageenans appeared to inhibit amidolysis of thrombin directly and via AT-III; however, only AT-III potentiated Xa amidolysis was observed. These interactions may be influenced by certain critical qualities of the polyanionic polymers, i.e. sulphation, size, pattern of ionic substitution and polymer rigidity<sup>35</sup>. However subsequent studies using AT-III depleted plasma, showed residual anti-thrombin activity in the presence of carrageenans<sup>63,64</sup>.  $\lambda$ -carrageenan has been shown to potentiate the inactivation of thrombin by 'anti-thrombin BM'<sup>65</sup>. These observations would therefore imply that there is either anti-thrombin potentiation via HC-II and/or a direct anti-thrombin effect. Therefore, it can be concluded that anticoagulant activity of galactan sulphate of red algae is the inhibition of thrombin and it may occur either directly (mechanism unknown) or indirectly via HC-II.

### Brown algae

Presence of anticoagulant activity in brown algae was first reported in 1941, where *Laminaria* showed anticoagulant effect, its active compound being located in hold fasts<sup>66</sup>. Around 60 brown algal species are identified to have blood anticoagulant properties. The anticoagulant components of brown marine algal extracts are found in a group of polysaccharides, more commonly referred to as 'fucans'. In brown algae, fucoidan has been the most extensively-studied fucan. The initial studies described significant *in vitro* and *in vivo* activity of fucoidan from *Fucus vesiculosus*. Furthermore, it was found that no toxic symptoms were displayed. The most active fucoidan fractions predominantly consisted of sulphated fucose residues. Thrombin inhibition activity of these fractions exceeded that of heparin<sup>67</sup>. Bernard and Springer<sup>68</sup> further characterized these active fucoidan fractions and demonstrated that the material was essentially homogeneous, with molecular weight  $7.4 \times 10^4$  daltons and possessed 60–80% of the activity of heparin in the recalcification time tests and 15–18% heparin activity in the whole human blood. Adams and Thorpe<sup>69</sup> found that fucoidin fractions F13 and A showed activity of 8.9 and 9 heparin units/mg, respectively.

Fucose-containing SPS which were mixtures of more than two SPS were isolated from nine species<sup>70</sup>. The fractions have a wide range of composition, with a high content of fucose and sulphate and low content of uronic acid to a high content of glucuronic acid and a low content of fucose and sulphate. The fractions were examined for anticoagulant activity with TT and APTT tests. It was found that sulphate-rich and uronic acid-poor fractions showed relatively high anticoagulant activities compared to fractions with high uronic acid and poor sulphate contents. Some of the fractions showed anti-factor Xa activity<sup>70</sup>. An anticoagulant fucoidan fraction from *Ecklonia kurome* was extensively studied. It consisted of mainly 3-linked and 3,4-disubstituted fucopyranosyl residues, in addition to non-reducing terminal fucofuranosyl and fucopyranosyl residues, 2,3-di and 2,3,4-*tri*-substituted fucopyranosyl residues and galactopyranosyl residues with various glycosidic linkages<sup>71</sup>. Correlation between the sulphate and uronic acid contents and the anticoagulant activity of the fucan sulphates was confirmed by a study on fucan sulphates from *E. kurome*<sup>72</sup>. Further fractionation of the SPS fraction of *E. kurome* yielded highly purified sulphated polysaccharide fractions, B-I and B-II, which were composed of fucose, galactose, mannose, xylose, glucuronic acid and ester sulphate in approximate molar ratio of 1.00:0.36:0.48:1.08:1.85:2.35 and 1.00:0.81:0.18:0.45:0.61:2.00, respectively, indicating that B-I was closely similar in composition to sargassan, except for its lower galactose content, whereas B-II was an

ascophyllan-like polysaccharide in composition. On the other hand, fractions C-I and C-II comprised fucose, galactose, glucuronic acid and ester sulphate in the respective approximate molar ratios of 1.00:0.03:0.03:0:1.61 and 1.00:0.19:0.07:1.48, indicating that they were fucoidans (pure fucans). Anticoagulant activity with respect to APTT using human plasma was approximately 24, 19, 81 and 85% that of heparin for B-I, B-II, C-I and C-II, respectively. All of them showed slight anti-thrombin activity, but none of them showed anti-factor Xa activity<sup>72</sup>. Similar results were obtained for the purified SPS from *Undaria pinnatifida*<sup>73</sup>. Three SPS, A, B, C which were isolated were composed of fucose galactose, mannose, xylose, glucuronic acid and sulphate in approximate molar ratio of 1.00:0.77:0.04:0.03:0.33:0.32, 1.00:0.87:0.03:0.04:0.12:1.12 and 1.00:1.76:0.04:0.08:0.17:3.37, respectively. This suggests that they all may be a group of fucogalactan sulphates. The respective anti-thrombin activities of A, B and C were 1/27, 1/3 and 2 times that of heparin<sup>73</sup>. It has been reported that fucoidans (pure fucans) from *Eisenia bicyclis*<sup>74</sup>, *Hizikia fusiforme*<sup>75</sup>, *Laminaria angustata*<sup>76</sup>, *L. religiosa*<sup>77</sup>, *Pelvetia canaliculata*<sup>78</sup> and *P. wrightii*<sup>79</sup> showed considerably high anticoagulant activity. *Sargassum cinctum* from Indian waters was studied for its SPS content and anticoagulant activity. It was observed that all the fractions contained mainly galactose and a trace of fucose with sulphate (29.5%) and hence activity was also poor<sup>80</sup>. Sixteen species of British marine algae were screened, of which four species, i.e. *Laminaria digitata*, *L. hyperborea*, *L. saccharina* and *Fucus spiralis* showed potent anticoagulant activity<sup>56</sup>.

Sargassan is composed of glucuronic acid, galactose, mannose, xylose and fucose in the molar ratio of 4.57:8.40:1.00:2.48:2.53 and of sulphate (18%)<sup>81</sup>. In spite of the fact that sargassan has high uronic acid content, the polysaccharide was shown to have much higher anticoagulant activity than heparin<sup>81</sup>. Similar high anticoagulant activity has been reported for purified fucan sulphates from *Padina pavonia* (xylofucomannoglucuronan)<sup>82</sup>, *P. tetrastratica* (xylofucogalactomannoglucuronan)<sup>83</sup> and *Dictyota dichotoma* (glucuronoxylomannogalactofucan)<sup>84</sup>. These results indicate that the relationship between the sugar composition and the anticoagulant activity of fucan sulphates is very complex. Commercial crude fucoidan (Sigma) from *F. vesiculosus* was fractionated to clarify the structure-anticoagulant relationship<sup>85</sup>. The products comprised a wide spectrum of fucans ranging from typical fucoidans (major components) containing mainly fucose, sulphate and no uronic acid to low sulphate containing SPS-like fucans (minor components), composed of neutral sugar other than fucose and high uronic acid content. Typical fucoidans initially showed a potent anticoagulant activity, whereas later had no or only slight activity<sup>85</sup>. Pro-

teoglycan-like amino sugar-containing fucan sulphate composed of fucose, galactose, glucose, mannose, xylose, uronic acid, glucosamine and sulphate in the molar ratio of 1.00:0.04:0.01:0.48:0.24:0.18:0.56:1.90 was also isolated from crude fucoidans for the first time from brown seaweed; however, this SPS showed no anticoagulant activity<sup>85</sup>. A new type of sulphated  $\beta$ -D-galactan having backbone of 3- $\beta$ -D-galactosyl-(1 $\rightarrow$ 6)- $\beta$ -D-galactosyl-(1-from *Laminaria angustata* var. *longissima* was reported for the first time from brown seaweed. This is different from well-known galactans of red algae and ascidians and it showed no anticoagulant activity<sup>86</sup>.

An acidic polysaccharide (MW 21 kD) containing fucose, xylose, glucuronic acid and sulphate was isolated from a brown alga *Spathoglossum schroederi*, which was found to be xylofucoglucuronan, showing anticoagulant properties without any haemorrhagic activity<sup>87</sup>. This polymer is composed of  $\beta$ (1 $\rightarrow$ 3) glucuronic acid containing oligosaccharide of 4.5 kD with branches at C-4 of the fucose chains  $\alpha$ (1 $\rightarrow$ 3) linked. The fucose was found to be mostly substituted at C-4 with chains of  $\beta$ (1 $\rightarrow$ 4), xylose, which in turn is also partially sulphated<sup>87</sup>. *Ecklonia kurome* exhibited potent activity and the active fraction was found to contain mainly 3- and 3,4-O-linked  $\alpha$ -L-fucopyranosyl residues, in addition to small proportions of terminal fucopyranosyl and fucufuranosyl groups and 2,3-di and 2,3,4-tri substituted fucopyranosyl and galactosyl residues substituted at various locations<sup>88</sup>, and molecular weights ranging from about 10,000 to 30,000 (ref. 89). Change in the anticoagulant activity and composition of fucan sulphates of *E. kurome* during refrigeration for 3 years was studied. Fucose content and activity were less in stored plant compared to fucan sulphates from freshly collected plants. It was observed that during storage, fucose changed into galactose<sup>90</sup>.

All these results indicate that fucan sulphates with high sulphate and low uronic acid content showed higher anticoagulant activity than those with high uronic acid and low sulphate content. Scientists also suggest that potent anticoagulant fucan sulphates may be highly sulphated fucoidan and galactofucans. The size of the most active components is approximately 50–100,000 daltons<sup>75,78</sup>, whereas fractions with a high molecular size (>850,000 Da) tend to demonstrate lower activity<sup>78</sup>.

Polysaccharides other than fucans from brown algae have been tested, however they were chemically modified in order to impart anticoagulant characteristics. Laminaran<sup>91–93</sup> and alginic acid<sup>94,95</sup> are examples which exerted anticoagulant activity after structural modification like sulphation, reduction or oxidation. Laminaran is a polysaccharide which does not naturally contain sulphate groups. Laminarin sulphates (by sulphation) containing 0.6–2.2 sulphate groups per glucose unit

were examined and it was found that the product containing two sulphate groups per glucose unit gave maximum anticoagulant activity, i.e. about 40 heparin units per mg<sup>96</sup>. Laminarin sulphates of K, L, M and N types (containing 16.8, 14.5, 8.84 and 5.97% sulphate, respectively) were examined and they had activity 35.0, 9.4, 1.4 and less than 1.3 heparin units per mg in tests on rabbits, respectively. Only laminarin sulphate-K showed chronic toxicity<sup>69</sup>. Hawkins and Leonard<sup>97</sup> reported that laminarin sulphate had anticoagulant activity one-third as potent as heparin when given via injection to a dog. In an *in vitro* test, the anticoagulant activity potency of laminarin sulphate was about one-third that of heparin, but during *in vivo* tests on rabbits it exerted a more prolonged anticoagulant effect than heparin<sup>98</sup>. Later, Adams *et al.*<sup>99</sup> prepared sulphated laminarins in degraded form. These compounds possessed reduced anticoagulant effect. The amount of sulphate groups in laminarin affected its anticoagulant activity; this finding agreed with the reports of Gollin *et al.*<sup>100</sup>. Laminarin sulphate and its derivative of sulphamic acid were prepared and the activities of laminarin containing only O-sulphate and both O-sulphate and N-sulphate groups were compared. Laminarin sulphate containing high sulphate groups per glucose had anticoagulant activity 25–30% that of heparin, whereas its sulphamic acid derivative which contained 7.28% nitrogen and half ester sulphate group had a heparin activity of 35–40% (ref. 91). Hawkins and O'Neil<sup>92</sup> tested the same derivative of laminarin. It contained 1.7 and 1.6 sulphate groups per glucose unit, of which approximately 1 and 0.5 were, respectively, in sulphamic acid group. This derivative was more active than its simple sulphate ester and derivatives containing high sulphate and sulphamic acid groups showed higher activity than low derivatives. Sulphated  $\beta$ -aminoethyl ether derivatives of laminaran, a 1,3-linked glucan of *Laminaria digitata*, containing both O-sulphate and N-sulphate groups were found to display anticoagulant activity, but it was not evident in the native polysaccharide. The preparations with the highest sulphate content proved the most potent anticoagulant. When preparations with the same total sulphate content were compared, the most active were those that contained both O-sulphate and N-sulphate groups rather than O-sulphate groups in isolation<sup>92</sup>. Partially oxidized laminaran with sulphate content 15.9% (sulphate groups 1.3, d.s.) gave activity of 26 IU/mg in APTT test. Partially reduced sulphated alginic acid with maximum sulphate (13.4%) and the lowest uronic acid content (20%) gave the highest activity at APTT (55 IU/mg) than low-sulphated ones<sup>93</sup>.

Alginic acid is a polymer of D-mannuronic and L-guluronic acids. Sulphated derivative of alginic acid had a much lower anticoagulant activity and a much higher toxicity than heparin. The activity of the derivative containing 9.6% sulphur was negligible<sup>95</sup>. Both

partially reduced sulphated alginic acid and partially reduced aminated and sulphated alginic acid are chemically modified derivatives of alginic acid. They displayed anticoagulant activity in APTT assays, but not in chromogenic anti-Xa assays. A fraction from the aminated derivative was shown to bind to immobilized AT-III, and both derivatives demonstrated AT-III-dependent inhibition of thrombin<sup>94</sup>.

Chemical modifications like sulphation, oversulphation and desulphation of fucoidan fraction and their anticoagulant activities were also reported. Three oversulphated fucoidans (S-1, S-2, S-3) from purified fucoidan (C-II) were prepared<sup>101</sup>. The molar ratios of sulphate to total sugar residues of C-II, S-1, S-2, and S-3 were 1.28, 1.38, 1.64 and 1.98, respectively. Among the oversulphated fucoidans, the hydroxyl groups of L-fucose residues in S-3 were considered to be almost completely sulphated, because C-II consisted<sup>88</sup> of a backbone of (1  $\rightarrow$  3)-linked  $\alpha$ -L-fucose having sulphate groups attached mainly to position 4. The molecular weight of C-II and the oversulphated fucoidans was between 23,500 and 26,500. All the fractions were examined for anticoagulant activity by APTT and TT, using normal human plasma. For APTT, the activity of S-1, S-2, and S-3 increased to 110, 114 and 119%; for TT 108, 129 and 140%, respectively of the original activity (C-II taken as 100%). These results indicate that the anticoagulant activity of C-II increased with the increase in its sulphate content up to a certain concentration and then gradually decreased with increasing sulphate content. Thus, the anticoagulant activity of fucan sulphate is strongly dependent on its sulphate content. However, the activity became constant over a ratio of sulphate to total sugar of the polysaccharide<sup>101</sup>.

In general, it is known that the anticoagulant activity of polysaccharides is improved chemically by increasing the degree of sulphation<sup>102,103</sup>. However, Uchiyama *et al.*<sup>104</sup> reported that the anticoagulant activity of oversulphated heparin was notably decreased compared with that of the parent heparin as a result of probable occurrence of sulphation at unnatural positions along with natural positions of the polysaccharide. All these results suggest that anticoagulant activity of polysaccharides is dependent not only on their sulphate content, but also on the position of the sulphate groups<sup>104</sup>.

In addition to anticoagulant activity, some oversulphated fucoidans are reported to have fibrinolytic activities<sup>105–107</sup>. Fucoidans and their derivatives were examined for their abilities to stimulate tissue plasminogen activator (t-PA)-catalysed plasminogen activation and clot lysis. Aminated derivative of fucoidan was prepared and examined for its fibrinolytic activities and it was found that the aminated derivative was more potent than native fucoidan as a stimulator of tissue plasminogen activator-plasma clot lysis<sup>106</sup>. A series of fucoidan derivatives was prepared by chemical sul-

phation and desulphation and tested for fibrinolytic and anticoagulant activities. It was found that the activities were dependent on the degree of sulphation<sup>107</sup>.

Two over-sulphated fucoidans (sulphate content, 46.2 and 55.0%) were prepared from a purified fraction of *F. vesiculosus* (sulphate content, 34.0%) and evaluated for their anti-thrombin effect. It was found that the inhibitory effect of the over-sulphated fucoidans was greater than that of the parent fucoidan, indicating that both the direct and the HC-II-mediated anti-thrombin activities of  $\alpha(1\rightarrow2)$  fucoidan are dependent on its sulphate content. The higher the degree of polymerization, the greater was the thrombin inhibitory effect. It was also opined that a suitable chain length of fucoidan would be required for forming a ternary complex between the fucoidan, thrombin and HC-II or causing steric hindrance against thrombin by binding to fibrinogen<sup>108</sup>.

**Mechanism of action:** The mechanism of anti-thrombin activity of fucoidan has been investigated. A highly purified fucoidan (C-II, 0–3  $\mu\text{g/ml}$ ) from *E. kurome* was examined for its antithrombin activity by the clotting method using a thrombin–fibrinogen system, in the presence and absence of AT-III<sup>109</sup>. Anti-thrombin activity increased in a fucoidan concentration-dependent manner, in the presence and absence of the protease inhibitor. However, unlike in heparin, the inhibitory activity of C-II was almost the same both in the presence and in the absence of AT-III. This result suggests that C-II directly inhibits the thrombin–fibrinogen reaction<sup>109</sup>. Similar results were reported for purified fucoidans from *P. canaliculata*<sup>78</sup> and *A. nodosum*<sup>78,110–112</sup>. Results indicate that anti-thrombin activity of fucoidan is not mediated by fucoidan–AT-III complex. The direct inhibitory effect of fucoidan on the thrombin–fibrinogen reaction was further examined using C-II (MW, 17,800 and molar ratio of sulphate to total sugar residues [R], 1.08) and its fucoidan derivatives with different molecular weights (MW, 13,000–58,000; R, 1.24–1.28)<sup>89</sup> and with different sulphate contents (R, 0–0.86)<sup>113</sup> by the same clotting method in the presence and absence of AT-III<sup>114</sup>. The anti-thrombin activity of all the fucoidans tested was unchanged, both in the presence and in the absence of the protease inhibitor. The inhibitory activity of fucoidans decreased with decrease in their MW; the fucoidan with the highest MW (58,000) was the most effective inhibitor. However, low molecular weight (LMW) fucoidan fraction (MW, 32,000 and 21,000) showed potent anticoagulant activity<sup>115</sup>. On the other hand, the inhibitory effect of fucoidans diminished extremely compared with that of C-II. Fucoidans with lower sulphate content ( $R < 0.54$ ) did not show any inhibitory effect on the fibrinogen–thrombin reaction. These results suggest that

the inhibitory effect of fucoidans is dependent on both its molecular weight and its sulphate content. Recently, the effect of fucoidan (C-II) on the generation of thrombin and factor Xa was investigated by measuring the amidolytic activities, using the respective specific chromogenic substrates, in both plasma and purified system<sup>116</sup>. C-II inhibited significantly the generation of thrombin in both the intrinsic and the extrinsic pathways, although the intrinsic inhibitory effect was more remarkable than the extrinsic one. On the other hand, C-II was a good inhibitor of factor Xa in the intrinsic pathway, while it was a poor in the extrinsic pathway. In the purified systems C-II also inhibited the formation of prothrombin-activating complex (i.e. prothrombinase), but not its activity. The concentration of C-II required for 50% inhibition of thrombin generation was about one-tenth to one-seventh that of the activity of the generated thrombin in plasma. These results indicate that C-II has an inhibitory effect on the generation of thrombin by blocking the formation of prothrombinase and by preventing the generation of intrinsic factor Xa, in addition to its anti-thrombin activity. Also, the generation–inhibitory effect is more remarkable than its enhancement effect on the antithrombin activity by heparin cofactor-II in plasma. As described previously, several studies<sup>71,105,114,117</sup> indicate that the HC-II-mediated anti-thrombin activity of fucoidan is dependent both on molecular weight and sulphate content, especially on its negative-charge density. Anti-factor Xa of fucoidan fractions from *E. kurome*<sup>78</sup> and *P. canaliculata*<sup>117</sup> was determined by amidolytic activity of the protease using chromogenic substrate, S-222. Fucoidan fraction from other species like *A. nodosum*<sup>78</sup> and *H. fusiforme*<sup>75</sup>, did not exhibit anti-Xa activity. However, fucoidan from *F. vesiculosus* was found to inhibit factor Xa<sup>118</sup>. It is opined that anticoagulation occurs predominantly by inhibiting the key coagulation serine proteases, thrombin and factor Xa<sup>118</sup>. This study clearly indicated that HC-II is the major serine protease inhibitor (SERPIN) involved in the *in vitro* and *ex vivo* activity of fucoidan. The presence of fucoidan enhanced the HC-II thrombin interaction by more than 3500-fold, whereas the enhancement of AT-III–thrombin and AT-III–factor Xa interaction was only 285- and 35-fold, respectively. It was also observed that indirect HC-II and AT-III-mediated interactions are not the only possible anti-thrombin mechanisms exhibited by marine algal polysaccharides. HC-II is activated by fucoidan *in vitro* and in an *ex vivo* plasma system. This suggests that the major anti-thrombin activity of fucoidan *in vivo* is mediated by HC-II and not by AT-III<sup>118</sup>. It is also reported that branched fucans from brown algae are found to be direct inhibitors of thrombin, whereas the linear fucans from echinoderms require the presence of anti-thrombin or HC-II for inhibition of thrombin, as reported for mammalian glycosaminoglycans<sup>119</sup>.

The anticoagulant activity of the most effective fucan fractions is equivalent to or greater than that of heparin and a possible therapeutic role has been envisaged for the active low-molecular weight components. It was reported that the anticoagulant properties of brown algal SPS are not related to a heparin-like mechanism, in which the anticoagulant activity of heparin-like compounds is mediated by a plasma protein AT-III. These heparin-like compounds possess a highly specific binding site for AT-III<sup>73,74,81</sup>. Therefore, the formation of the complex between AT-III and P increases the rate of inhibition of procoagulant enzymes, such as thrombin and IXa, Xa, XIa and XIIa. Grauffel *et al.*<sup>78</sup> concluded from anticoagulant mechanism of fucan sulfates that direct interaction between fucan and thrombin is largely responsible for the kinetic effects of these polysaccharides.

Mauray *et al.*<sup>120</sup> have recently hypothesized that fucoidan could act like heparin by forming complexes with the inhibitor (although anti-thrombin in the case of heparin and HC-II for fucoidan), while synthetic dextran derivatives could act like hirudin by forming complexes with thrombin. It was observed that the fucans with a high sulphate content presented significantly high anticoagulant activity<sup>120</sup>. Fucoidans catalyse thrombin inhibition by AT-III and HC-II, their affinity for each serpin varies according to the seaweed from which they are extracted as well as their chemical composition and molecular weight<sup>112</sup>. Another study was carried out to investigate the binding affinity of fucoidan, heparin and low MW heparin to AT-III, HC-II and fIXa. It was found that binding of fucoidan to these proteins occurred with low affinities compared to heparin and low MW heparin. Fucoidan had higher affinity for the inhibitor HC-II compared to anti-thrombin and enzymes. These data suggest that binding of heparins and fucoidans to the inhibitor is required for the polysaccharide-dependent enhancement in the rate neutralization of the enzyme by the inhibitor<sup>121</sup>.

### Green algae

Reports on anticoagulant properties of green algae are relatively recent ones. Deacon-Smith *et al.*<sup>56</sup> have reported the presence of anticoagulant activity in aqueous extracts of *Codium* species. This study constituted the first report of such activity present in marine green algae. So far, 45 species are reported to have anticoagulant properties. Anticoagulant activity of *Codium* species studied was detected by prolongation of prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) assays. The extracted material did not inhibit the action of Reptilase® and there was no significant effect on fibrin polymerization<sup>56</sup>. Subsequently, eight Tunisian and Venezuelan

marine green algae<sup>122,123</sup> were screened using Bird's agarose gel fusion technique<sup>124</sup>. A group of 23 species was screened and the hot water extract of *Monostroma nitidum* was found to be the most active<sup>125</sup>; the active fraction was found to contain rhamnan sulphate<sup>125,126</sup>. *Codium latum* from Japanese waters yielded sulphated arabinan ( $\alpha$ -1→5) as an active molecule which gave 12.6 times more anti-thrombin activity when standard heparin was taken as 1 (refs 127 and 128).

Thirteen species of green algae belonging to the family Codiaceae were screened from Indian coasts<sup>19,129</sup>. It was found that *C. dwarkense* and *C. tomentosum* showed promising activity. Bioassay guided purification of both the species yielded sulphated arabinan as the most active component and sulphated arabinogalactan as the relatively less active component<sup>129,130</sup>. It was found that arabinose and sulphate play an important role in eliciting anticoagulant activity<sup>129,130</sup>. Two active components have been identified in fractions obtained by molecular exclusion from *C. fragile* spp. *tomentosoides*<sup>131</sup>. The high molecular weight component (approximately 1700 kDa) exhibited greater anticoagulant activity by prolonging PT, APTT and TT. Anticoagulant activity is also evident when fibrinogen, rather than plasma, is used as the substrate in the TT procedure. However, the anti-thrombin activity is greater while using plasma. When incorporated in the chromogenic substrate assay procedures using Benz-Phe-Val-Arg-pNA, the high molecular weight component had a direct effect against thrombin and potentiated the inactivation of thrombin by HC-II. No direct effect was observed against Xa. However, when chromogenic substrate tosyl-Gly-Pro-Arg-pNA was subsequently substituted in thrombin amidolysis experiments, no direct effect was observed. However, studies have shown unexpectedly that heparin and other glycosaminoglycans exhibit direct anti-thrombin activity, when the former chromogenic substrate (S-2160) is used<sup>10,35</sup>. This anomaly stresses the need for caution in the choice and interpretation of chromogenic assay. A high MW proteoglycan ( $1.8 \times 10^6$ ) from *C. fragile* ssp. *atlanticum* exhibited potent blood anticoagulant activity. It has been observed that the activity is directly proportional to the carbohydrate and sulphate contents of proteoglycan and inversely proportional to the protein content of proteoglycans<sup>132</sup>. High molecular weight sulphated proteoglycan and low molecular weight sulphated polysaccharides from the same species were reported to have strong activity<sup>133</sup>. APTT, PT and TT assays were applied and it was found that an increase in anticoagulant activity was demonstrated with increasing concentration and sulphate content of algal products. The proteoglycans (18.4% sulphate) possess the greatest anticoagulant activity, followed by sulphated polysaccharides with 10.2 and 7.5% sulphate, respectively. The effect of TT using fibrinogen as the substrate was com-



plex and differed from that shown using plasma. Anticoagulant activity using fibrinogen occurred at a lower concentration than using plasma, but declined at higher concentrations. This may be due to complex interactions between the algal components and fibrinogen which is altered when the algal components are present in excess. The greater anticoagulant activity demonstrated in the plasma suggests the presence of a cofactor(s) in the plasma, which potentiates this effect. Measurements of the TT using fibrinogen were hampered to some extent by precipitation of fibrinogen. No effect was demonstrated in the RT using a thrombin-like enzyme by any algal products, demonstrating that fibrin polymerization was not impaired. Therefore, the results suggest that the anticoagulant effect occurs in the common pathway and may be anti-thrombic in nature<sup>133</sup>. This has also been reported by Walton<sup>134</sup> for other sulphated polysaccharides, such as carrageenans and dextran sulphate. Carrageenans precipitate plasma proteins, especially fibrinogen; this characteristic was found to correlate with their toxicity on intravenous administration<sup>32</sup>.

**Mechanism of action:** Anticoagulant activity of green algal SPS has been assigned to the common pathway, primarily HC-II-mediated anticoagulant action. Investigations were conducted employing chromogenic substrates for the major coagulation enzymes, factor Xa and thrombin. No direct activity was demonstrated against the active sites of these enzymes, as colour formation was not impaired by the presence of the algal anticoagulants at various concentrations. These enzymes were inhibited indirectly by the algal anticoagulants, however, via their potentiation of the activity of the serine protease inhibitors AT-III and HC-II. The inhibition of thrombin and factor Xa by AT-III was potentiated by the proteoglycan, but not by the SPS. The inhibition of thrombin by HC-II, however, was potentiated by both proteoglycan and SPS. In summary, the mechanism of action is directed primarily against thrombin by potentiation of HC-II activity. Potentiation of the inhibition of thrombin or factor Xa by AT-III may depend on molecular size and/or critical sulphate content. The involvement of AT-III and HC-II provides an explanation for the greater anticoagulant activity displayed by algal components in the TT, using plasma rather than fibrinogen as a substrate source. Involvement of AT-III is perhaps unexpected, due to the lack of the unique high affinity (to AT-III) pentasaccharide sequence in the *Codium* anticoagulants, which is present in heparin, which greatly accelerates the inactivation of coagulation enzymes by AT-III<sup>135</sup>. A number of other sulphated polysaccharides such as SP54 (ref. 136), dextran sulphates<sup>137</sup>, xylan sulphates<sup>138</sup>, carrageenans<sup>64</sup> and fucoidans<sup>78</sup>, however, have also been found to potentiate AT-III activity. Furthermore, heparin with low affinity to AT-III is able to exert a minor anticoagulant

effect, hence the algal proteoglycan may act in a similar way to low affinity heparin. This activity is relatively minor, however, in comparison to that displayed via HC-II, which occurs at lower concentrations of algal anticoagulant.

The potentiation of HC-II activity that has been described for many SPS and polyanions is thought to be dependent on charge density<sup>139</sup>, although fractions of dermatan sulphate with high affinity to HC-II have been isolated<sup>140</sup>. Although the mechanism of action is directed primarily against thrombin, mediated to a large degree by HC-II, with potentiation of the inactivation of thrombin and factor Xa by AT-III, some direct anti-thrombin activity exists. However, this is not directed towards the active site of thrombin. A possible explanation for this direct activity is that the green algal components occupy the anion binding exosite or fibrinogen recognition site of thrombin—described by Fenton *et al.*<sup>141</sup>—thereby interfering with the binding of fibrinogen and its conversion to fibrin. High MW proteoglycans ( $1.7 \times 10^6$ ) and low MW polysaccharide fraction from *C. fragile* ssp. *tomentosoides* have also been demonstrated to be more potent than low MW ones<sup>142</sup>. The proteoglycans and SPS prolonged the PT, APTT and TT. Both also had a direct effect on the amidolytic activity of thrombin and potentiated the inactivation of thrombin by HC-II. They showed no effect on Atroxin time estimations or the amidolytic activity of factor Xa. The two substances, however, differed in their ability to potentiate the activity of AT-III. The proteoglycan showed no effect whereas the polysaccharide displayed an ability to potentiate the inactivation of thrombin by AT-III. These studies indicate that although some of the anticoagulant activity of these substances is mediated via HC-II, other anticoagulant mechanisms also contribute to the overall inhibitory effect<sup>142</sup>.

## Summary

Algal SPS contain a wide range of chemical compositions. Galactan sulphate in red algae, fucans in brown algae and different sugar sulphates in green algae are present. Their sugar residues exist in various linkages; in red algae, ι-carrageenan (β-1,3-D-Gal-4-SO<sub>4</sub>/3,6-anhydrogalactose-α-1,4-D-Gal-2-SO<sub>4</sub>), κ-carrageenan (β-1,3-D-Gal-4-SO<sub>4</sub>/3,6-anhydrogalactose-α-1,4-D-Gal), λ-carrageenan (β-1,3-D-Gal-2-SO<sub>4</sub>/α-1,4-D-Gal-2,6-di-SO<sub>4</sub>); in brown algae, the most extensively studied fucan sulphate, (α-1,2-L-Fuc-4-SO<sub>4</sub>), Fuc-(1→3)-Fuc, Fuc-(1→4)-Fuc, Fuc-(1→3)-[Fuc-(1→2)-]Fuc, Fuc-(1→4)-Gal; and α-L-arabinan sulphates, rhamnan sulphates, etc. in green algae are reported. Anticoagulant activity is largely dependent on the sugar composition, sulphate content, sulphate position and molecular weight of the compound. The correlation also suggests

that suitable length and/or conformation and moderate extent of negative charge density of the polysaccharide molecule would be required for expression of its effective anticoagulant activity. Algal SPS are, in some cases, demonstrated to have more potent activity than heparin and also differ in the mechanism of anticoagulant action from heparin. Algal SPS show anti-thrombin-mediated anticoagulant activity. Red algal SPS accelerate HC-II/direct anti-thrombin activity. Fucoidan sulphate predominantly accelerates HC-II, direct anti-thrombin activity through thrombin–fibrinogen complex and little anti-factor Xa activity and minor or no AT-III involvement is also reported. Some natural fucoidan sulphates and over-sulphated fucoidan sulphates show fibrinolytic activities. Xyloarabinogalactan of green algae shows anti-thrombin activity indirectly via HC-II, little AT-III and directly by unspecified mechanisms.

### Future prospective of algal SPS as anticoagulant agents

Current research goals are focused on identifying more potent and specific inhibitors of targets playing a crucial role in the coagulation pathway, such as thrombin and factor Xa. Long-term goals are to develop thrombin receptor antagonist or analogues of naturally occurring anticoagulants, e.g. tissue factor pathway inhibitor (TFPI) and protein C. All currently available anti-thrombotic drugs have their own limitations. Heparin-associated thrombocytopenia (HAT) is the most frequent drug-induced immune thrombocytopenia. A study on the structural requirements for a carbohydrate-based anticoagulant with a reduced risk of inducing the immunological type of heparin-associated thrombocytopenia was conducted<sup>143</sup>. Therein some laminaran sulphates (LamS 1–5) have been synthesized and their structures were determined for reduced HAT<sup>143</sup>.

Therapeutic applications of high molecular weight SPS (red algal SPS) have been limited because of their toxic side effects. However, they could serve as models for novel anti-thrombotic agents, or probes for investigating thrombin interactions. However, fucoidan sulphates are found to be very potent anticoagulant agents. Apart from the anticoagulant activity, the fucan sulphates support fibrinolysis by simulating the plasminogen activator-catalysed plasminogen activation. In contrast to heparin, SPS do this even in the presence of high concentrations of plasminogen activator inhibitor. In addition, they also promote clot lysis, possibly by protecting plasmin activity from the  $\alpha$ -2-plasma inhibitor<sup>106</sup>.

Besides the glycosaminoglycans in vertebrates, SPS are widespread in the marine flora. In summary, the fucoidans – the byproducts of alginate production in the

food and cosmetic industries – are easily obtained. They may represent, in the future, a cheap and easy source of a new type of anti-thrombotics. Knowledge of potential heparinoids from marine algae is gaining importance in recent years. Recently, there was a case study on the changes of the haemorrhoeol, plasma cholesterol and albumin and clinical effects in 36 children with refractory nephrosis after treatment with SPS (fucans). It was observed that the indexes of haemorrhoeol and plasma cholesterol decreased obviously compared to the control group. These findings suggest that SPS might be used in the anticoagulation treatment of refractory nephrosis<sup>144</sup>. Fucoidan is being clinically tested for its wide therapeutic use. Algal anticoagulants, in future, may add a new dimension in the management of thrombotic and vascular disorders.

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