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Polysaccharides from *Gracilaria corticata*: Sulfation, chemical characterization and anti-HSV activities

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ABSTRACT

In this study, we have analyzed water-extracted polysaccharides of *Gracilaria corticata*. The water extract (WE), a galactan-containing sub-fraction (F3) and their hyper sulfated derivatives (WES1, WES2, F3S1 and F3S2) had anti-HSV activity with inhibitory concentration 50% (IC_{50}) from 1.1 to 27.4 μg/ml. Sub-fraction F3, which has a molecular mass of 30 kDa, consists of a backbone of β-(1 → 3) and α-(1 → 4)-linked-galactopyranosyl residues. This linear galactan contained Gal_2Xyl_1, Gal_2AnGal_2, Gal_4 and Me-Gal_3AnGal_2 as oligomeric building subunits. Sulfate group was located at C-4 of (1 → 3)-linked galactopyranosyl residues of the native galactan, and appeared to be very important for the anti-herpetic activity.

1. Introduction

During the last decade, the number of antiviral agents approved for clinical use has been increased from five to more than thirty drugs [1,2]. However, as these compounds are not always efficacious or well tolerated and drug-resistant strains are rapidly emerging, the demand for antiviral drugs with novel mode of action is great. In recent years, screening assays of the antiviral activity of extracts from marine algae have led to the identification of a number of sulfated polysaccharides with potent *in vitro* inhibitory effects against various animal virus including herpes simplex virus (HSV) types 1 and 2 [3–8]. These polysaccharides include predominantly fucoidans, sulfated galactans, sulfated mannans and sulfated heteroglycans.

Many researchers have conducted *in vivo* studies on the bioavailability of sulfated polysaccharides. But the results obtained are controversial. For example, it has been observed that the *in vivo* effectiveness of oral administration of dextran sulfate to HIV was disappointing [9,10]. On the other hand, in an open phase I/II dose-escalation study in which six AIDS patients were treated with dextrin 2-sulfate, there was a significant decrease in viral load [11]. In addition, a more recent clinical study that employed different analytical techniques found that dextran sulfate is absorbed rapidly in humans after oral administration, indicating that this macromolecule has therapeutic potential [12] and merits further study. It was suggested that the main problem encountered with *in vivo* study related to the nature of infection and the manner of treatment [13]. The fact that several polysaccharides, such as cellulose sulfate, dextran/dextrin sulfates and carrageenan have successfully completed phases I and II clinical trials as antiviral candidate and are currently undergoing Phase III trial is noteworthy [14–17]. Thus, evaluation of the potential of sulfated polysaccharides of marine algae as anti-HSV agents will be of considerable interest.

In a preceding paper [18] it was shown that water-extracted polysaccharide-containing fraction of the red seaweed *Gracilaria corticata* possesses *in vitro* anti-herpetic activity. The present study reports structural features of the purified polysaccharides as well as oligosaccharides generated there from by partial acid hydrolysis, and their anti-HSV activity. Sulfation of the crude and purified galactan and their anti-HSV study were also carried out. Using chemical and spectroscopic methods and various forms of chromatography including MALDI-TOF-MS, we have been able to deduce structural features of a sulfated galactan with anti-herpetic activity.

2. Materials and methods

2.1. Isolation of polysaccharide

The polysaccharide-containing fraction investigated was obtained by extraction of the depigmented algal powder (DAP) of *G. corticata* with water (pH 6.5, at 35–40 °C) as

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described [18] except that the extract, instead of purification by freeze–thaw method, was diluted with 4 volumes of 2-propanol and polymeric material was isolated after lyophilization of the precipitate.

2.2. Anion-exchange chromatography

A solution (20 ml) of the crude polysaccharide-containing fraction (WE, 75 mg) in 50 mM sodium acetate (pH 5.5) was applied to a column (25 cm × 1.6 cm) of DEAE-Sepharose FF (AcO^-; Amersham Biosciences AB, Uppsala, Sweden). Elution was carried out successively with 0.2-M (fraction F1), 0.7-M (fraction F2) and 2.0-M NaOAc (fraction F3) buffer (pH 5.5) in a stepwise manner. The flow rate was 1.5 ml/min and elution with each eluant was carried out up to the absence of a positive reaction for carbohydrates with phenol and sulfuric acid. Appropriate fractions were pooled, dialyzed and lyophilized.

2.3. Size exclusion chromatography

System A: Apparent molecular mass of the sulfated galactan was determined by size exclusion chromatography on a Sephacryl S-300 column (90 cm × 2.6 cm, Amersham Biosciences AB), as described previously [19].

System B: Size exclusion chromatography of the oligosaccharides generated by partial acid hydrolysis of F3 fraction was carried out on Superdex 30 prep grade column (40 cm × 2.6 cm, Pharmacia) using 0.5-M sodium acetate buffer (pH 5.0) as eluent. The fractions eluted at total volume was concentrated and re-chromatographed on Sephadex G-10 column (90 cm × 2.6 cm) using water as eluent. Fractions eluted at K_{av} value 0.2–0.6 was pooled and lyophilized (named as 'oligo').

2.4. Sulfate estimation and desulfation

Estimation of sulfate by IR-spectrometry [20] and solvolytic desulfation by the method of Nagasawa et al. [21] were carried out as described [22].

2.5. Sugar analysis

Total sugars, 3,6-anhydrogalactose and uronic acids were determined by the phenol [23], resorcinol [24] and *m*-hydroxydiphenyl [25] assay, respectively. Neutral sugars were released by hydrolysis in 2 M TFA and analyzed as their alditol acetates [26] by GLC as described [27].

2.6. Sulfation

Polysaccharide-containing fractions (WE and F3) were sulfated using the method as described [28]. Briefly the freeze-dried polysaccharide (100 mg) was soaked in dry DMF (2 ml) and SO_3–pyridine complex dissolved in 2.5 ml DMF mixed with the polysaccharide. For every mol of SO_3–pyridine complex, 1 mol of pyridine was added to the mixture. The reaction was carried out under nitrogen atmosphere at 90 °C. After cooling to room temperature, 25 ml water was added and then the pH of the solution was adjusted to 7 with 1 M NaOH. Finally, the sulfated polysaccharide was purified by passing through Sephadex G-25 column and freeze-drying the eluate between K_{av} 0.0 and 0.8. Fraction WE and F3 upon sulfation with SO_3–pyridine complex yielded WES1 and F3S1, and WES2 and F3S2 when the reaction time was 4 and 8 h, respectively.

2.7. Periodate oxidation

A solution of F3D (10 mg) in 0.25 M formic acid, pH adjusted to 3.7 with 1 M NaOH was treated with of $NaIO_4$ (53.5 mg) as described [29]. Dialysis of the resulting solution and lyophilization of the retentate yielded the oxidized product (F3D-OX).

2.8. Linkage analysis

Two mg each of the sulfated galactan (F3), its desulfated derivative (F3D), and the periodate oxidized material (F3D-OX) were subjected to two rounds of methylation [30], with the modifications suggested by Stevenson and Furneaux [31]. The permethylated materials were converted into their partially methylated alditol acetates and analysed by GLC and GLC/MS (Shimadzu QP 5050A GLC/MS) as described [32].

2.9. NMR analysis

The ^1H NMR spectra of the native and desulfated galactan were recorded on a Bruker 600 spectrometer (Bruker Biospin AG, Fallanden, Switzerland) operating at 600 MHz for ^1H. The ^{13}C NMR was recorded on a Bruker 300 spectrometer.

2.10. Partial acid hydrolysis

Partial hydrolysis of the sulfated galactan (F3; 50 mg) with 100 mM TFA at 100 °C for 75 min was carried out as described [33].

2.11. Matrix assisted laser desorption ionisation-time of flight (MALDI-TOF) mass spectrometry

MALDI-TOF-mass spectrum of the acid-generated oligosaccharides-containing fraction (oligo) was recorded on a Bruker Daltonics flexAnalysis MALDI-TOF mass spectrometer.

2.12. Cells and viruses

Vero (African green monkey kidney) cells were grown as monolayers in minimum essential medium (MEM) (GIBCO, USA) supplemented with 5% inactivated calf serum. For maintenance medium (MM), serum concentration was reduced to 1.5%. Virus stocks of HSV-1 strain F and HSV-2 strain MS were propagated and assayed by plaque formation in Vero cells.

2.13. Cytotoxicity assay

Vero cell viability was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma–Aldrich, USA) method as described [3].

2.14. Antiviral assay

Antiviral activity was evaluated by reduction of virus plaque formation as described [4].

2.15. Anticoagulant activity

Anticoagulant activity of the galactan fractions was determined using the activated partial thromboplastine time (APTT) assay as described [6].

3. Results and discussion

3.1. Chemical characterization of sulfated galactans from G. corticata

3.1.1. Purification by anion-exchange chromatography

To investigate the structural features of the crude extract polysaccharide (WE) of *G. corticata* having *in vitro* activity against HSV we have extracted the depigmented algal powder (DAP) with water as described [18]. Anion-exchange chromatography on DEAE-Sepharose FF column separated the WE fraction into two major peaks, named as F2 and F3, eluted from the column with 0.7 and 2.0 M NaOAc, respectively. Minor amounts of sulfated polysaccharides were also eluted with 0.2 M NaOAc, 6.0 M Urea and 0.2 M NaOH but these fractions were not further analyzed. The sugar compositions of the sub-fractions are given in Table 1. Fraction F2, which accounted for 20% of the total polymers recovered from the column, contained higher amount of 6-*O*-methyl galactose residues (Table 1). The major sub-fraction F3 contained galactose, 6-*O*-methyl galactose, 3,6-anhydrogalactose and xylose residues as neutral sugars, characteristic of agar type molecule. The presence of agar in *Gracilaria* species had been established [34]. Sub-fraction F3 amounted to 61% of the total carbohydrates recovered from the column and contained 3% uronic acid. It is, therefore, essentially a galactan that might contain high amount of sulfate group, as indicated by its late elution. Indeed, the high charge density of this galactan was confirmed by its degree of sulfation (DS, 0.9), i.e. number of sulfate group per monosaccharide residue. The major fraction (F3) was subjected to further chemical analysis. Because uronic acid is only a minor constituent of the polysaccharide, it was not included in the structural hypothesis.

3.1.2. Molecular mass

Size exclusion chromatography of F3 on Sephacryl S-300 suggests that the polymer is homogeneous (Fig. 1). Based on calibration with standard dextrans, the apparent molecular mass of the polysaccharide would be 30 kDa.

3.1.3. Desulfation

The purified sulfated galactan F3 upon solvolytic desulfation yielded compound F3D in 41% yield. Sugar composition did not change significantly after desulfation. The FT-IR spectrum of F3 showed a peak at 1252 cm^{-1} related to >S=O stretching vibration of the sulfate group and a band at 930 cm^{-1} characteristics of 3,6-anhydrogalactosyl residues [35]. In the spectrum of desulfated polymer F3D band characteristics of sulfate groups (1240–1260 cm^{-1}) was very weak.

3.1.4. Linkage analysis

The glycosidic linkages and the position of sulfate groups in this galactan were determined by methylation analysis of F3 and its desulfated derivative F3D. Methylation analysis of F3D revealed the presence of 2,4,6-tri-*O*-methyl galactose and 2,3,6-tri-*O*-methyl galactose, indicating the presence of (1 → 3)- and (1 → 4)-linked galactopyranosyl residues. On the other hand, permethylated sulfated galactan F3 demonstrated the presence of (1 → 3)- and (1 → 3,4)-linked galactose together with some (1 → 4)-linked and terminal galactose residues. The increase in the proportion of (1 → 3)-linked galactose residue after desulfation and the consequent disappearance of (1 → 3,4)-linked galactose residue in F3D suggests that the sulfate group, if present, located at C-4 of (1 → 3)-linked galactose residues. Although 3,6-anhydrogalactose residues are present as constituent sugar, the presence of small, but significant amount of (1 → 4)-linked galactose residues suggest that all the (1 → 4)-linked galactose residues are not present as 3,6-anhydrogalactose units.

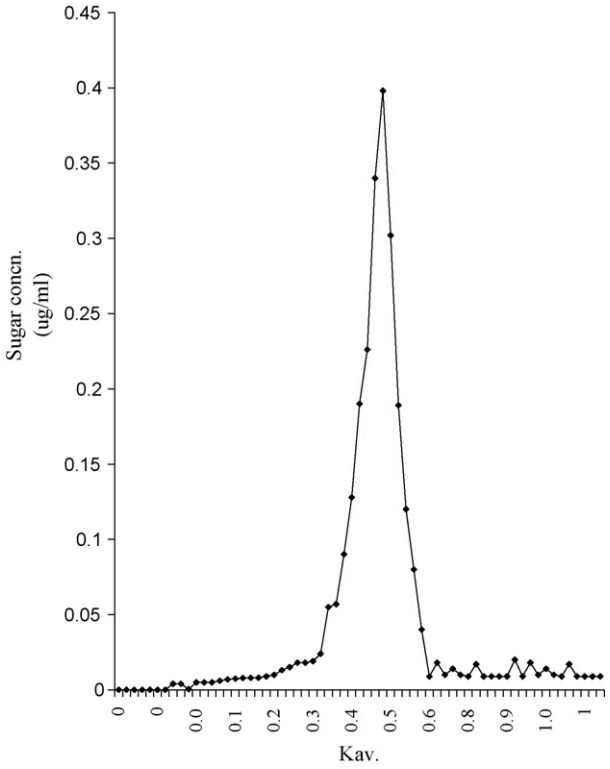

Fig. 1. Size exclusion chromatography of the fraction F3 obtained from *Gracilaria corticata* on Sephacryl S-300 column.

3.1.5. Periodate oxidation

The desulfated polymer F3D was treated with periodic acid. Methylation analysis of the oxidized material (F3D-OX) yielded mainly 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl galactitol,

Table 1
Sugar composition of the crude water extract (WE) of *Gracilaria corticata*, fractions obtained there from by AEC as well as their sulfated and desulfated derivatives (see text for the identification of fractions)

Sugar	WE	WES1	WES2	WED	F1	F2	F3	F3S1	F3S2	F3D
NS[a]	34	32	31	32	21	17	27	26	24	52
DS	1.0	1.2	1.5	–	–	–	0.9	02	2.1	–
Xyl[b]	Tr	03	02	Tr	–	06	05	02	02	04
6-*O*-Me-Gal[b]	33	40	38	40	91	34	14	16	18	18
3,6-AnGal[b]	20	03	Tr	10	–	17	32	02	Tr	30
Gal[b]	46	54	59	50	09	43	49	80	79	58

NS: neutral sugar. DS: degree of sulfation. Tr: trace. (–): not determined.
[a] Percent weights of fraction dry weight.
[b] Mole percent.

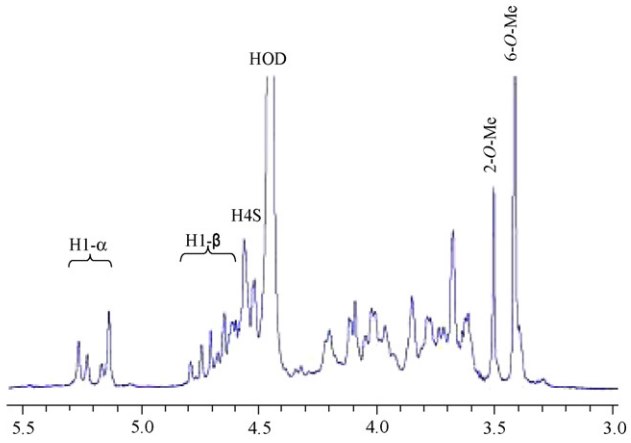

Fig. 2. ^{1}H NMR spectrum at 600 MHz of the sulfated galactan (F3) obtained from *Gracilaria corticata*. The spectrum was recorded at 60 °C for samples in D_2O solution. Chemical shifts are relative to external trimethylsilylpropionic acid at 0 ppm.

which demonstrate the presence of (1 → 3)-linked galactose residues. This suggests that the (1 → 4)-linked galactose residues are oxidizable by periodic acid.

3.1.6. NMR analysis

NMR spectroscopy is a nondestructive technique for giving valuable structural information on the structure of sulfated polysaccharides. F3 has a complex ^{1}H NMR spectrum (Fig. 2). The anomeric resonances at 5.14–5.27 and 4.60–4.79 ppm suggest the presence of α-(1 → 4)- and β-(1 → 3)-linked galactopyranosyl units, respectively [36–39]. Normally, the (1 → 3)- and (1 → 4)-linked galactosyl residues present in the sulfated galactan of marine red macroalgae are respectively β-and α-linked [34,39–41]. The four α-anomeric signals probably denote non-uniform distribution of substitutents (sulfate and methyl ester groups) along polymeric backbone. The spectrum also included resonances characteristic of signals from ring protons (H-2 to H-6) between 3.61 and 4.56 ppm. The very high intensity sharp signals appearing at 3.50 and 3.42 ppm in the ^{1}H NMR spectrum were tentatively attributed to the methoxy group of 2-*O*-methyl α-(1 → 4)-linked-3,6-anhydrogalactose and 6-*O*-methyl-β-(1 → 3)-linked-galactose residues, respectively [37]. Integration of these signals suggests that 17% of the α-(1 → 4)-linked-3,6-anhydrogalactose units are 2-*O*-methylated and 15% of the β-(1 → 3)-linked-galactose units are 6-*O*-methylated. Based on comparison with the NMR spectra of standard galactan the sharp signal at 1.45 ppm may be assigned to the methyl group of pyruvic acid ketal [18,36]. It remains to be clarified the possible occurrence of 3,6-anhydro-α-galactose in the sulfated galactan from *G. corticata*. The chemical shift of signal at 5.27 ppm is comparable to the anomeric signals of 3,6-anhydro-α-galactose residues of native galactan from *Catenella nipae* [39,40]. In order to check whether the 3,6-anhydro ring of galactose is disrupted during the desulfation reaction with DMSO we submitted the galactan from *G. corticata* for solvolytic desulfation. The α-anomeric region of the NMR spectrum of the galactan was not modified after this reaction therefore assuring that the 3,6-anhydro ring resists the condition used for the desulfation of the polysaccharide.

In conclusion, NMR analysis indicates that the sulfated galactan from *G. corticata* F3 is a linear polysaccharide, containing α-(1 → 4)- and β-(1 → 3)-linked-galactopyranosyl units. A variable sulfation pattern and probably a non-uniform distribution of the methoxyl groups confer high heterogeneity to this polysaccharide.

3.1.7. Partial acid hydrolysis

To investigate the role of molecular weight in inhibition of HSV we have partially hydrolyzed the sulfated galactan with acid. The generated fragments were purified on Superdex-30 into several populations. The major one, eluted at total volume, on re-chromatography with a Sephadex G-10 column yielded a fraction (named as 'oligo') at K_{av} 0.2–0.6.

3.1.8. MALDI-TOF-MS analysis

MALDI-TOF-mass spectrometry, because of its sensitivity and applicability to the analysis of mixtures is a convenient tool for the structural analysis of oligosaccharides [42]. We have applied this technique for the analysis of acid-generated oligomeric fragments (oligo). MALDI-TOF-mass spectrum in the positive mode of 'oligo' fraction shows the presence of four oligosaccharides (Fig. 3). Pseudo-molecular ions at *m*/*z* 497, 656, 688 and 861 could be assigned to Gal_2Xyl_1, Gal_2AnGal_2, Gal_4 and Me-Gal_3AnGal_2, respectively on the basis of their molecular weight and the sugar composition data. Ion at *m*/*z* 656, which is the major peak corresponding to Gal_2AnGal_2 suggests the presence of an oligomeric building block containing two galactose and two 3,6-anhydrogalactose residues. It is well known that agar, the major matrix phase polysaccharide of red seaweeds, is a linear sulfated galactan that contain alternating (1 → 3)-β-D-galactopyranosyl and (1 → 4)-α-L-galactopyranosyl residues [34,43]. Pseudo-molecular ion at *m*/*z* 688 corresponding to Gal_4 suggests the presence of an oligomeric building block containing four galactose residues. The presence of ion at *m*/*z* 497 gives direct evidence that xylose residue, which is linked to galactose, is a component of this galactan. Thus, partial hydrolysis is a useful method in determining the structural features of *G. corticata* galactan.

3.1.9. Sulfation

Degree of sulfation (DS) affects the antiviral activity of polysaccharides [8]. In general, for a particular class of polysaccharide, the higher the charge density, the higher is its activity. To study the effect of charge density, we have hypersulfated the water-extracted fraction (WE) and the major galactan-containing fraction (F3) under various conditions as given in experimental section to yield sulfated derivatives WES1, WES2, F3S1 and F3S2. Their sugar composition and degree of sulfation are given in the Table 1. ^{13}C NMR spectrum of F3S1 revealed the presence of four signals in the anomeric region. The higher intensity of anomeric signals around 101–103 ppm compared to anomeric resonances around 97–99 ppm confirms the presence of large amount of β-(1 → 3)-linked galactopyranosyl residues (Figure not shown). Moreover, it also suggests that the 3,6-anhydro-galactose residues degraded during sulfation. The ^{13}C NMR spectrum also contained a strong signal at 59.1 ppm indicative of methoxyl groups. The fact that no signal was present around 61 ppm suggests that most of the galactose residues are either methoxylated or sulfated at C-6 position.

3.2. Biological activities of sulfated galactans from G. corticata

3.2.1. Antiviral activities

The antiviral activities of crude and purified original sulfated galactans (WE and F3) and their desulfated (WED and F3D) and hypersulfated (WES1, WES2, F3S1 and F3S2) derivatives are summarized in Table 2. Both WE and F3 exhibited a moderate inhibitory effect against HSV-1 and HSV-2 with IC_{50} values in the range 7.8–27.4 μg/ml. The inhibition against HSV-2 (7.8–14.6 μg/ml) was slightly higher when compared with that obtained against HSV-1 (16.2–27.4 μg/ml). When both WE and F3 were further sulfated under different conditions, antiviral activity was altered. For WE, a significant increase of effectiveness was observed only for WES2,

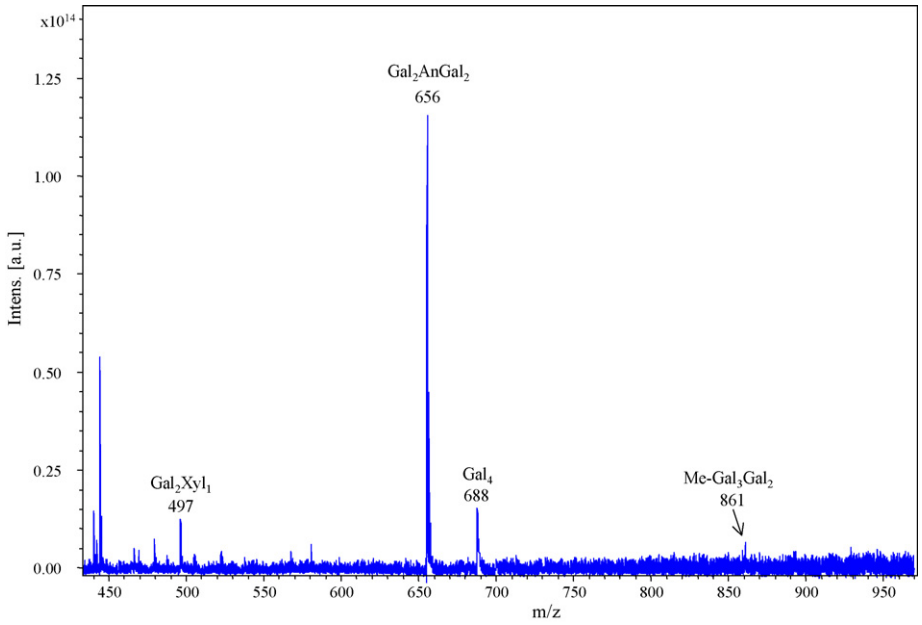

Fig. 3. MALDI-TOF-mass spectrum of oligosaccharides (oligo) generated from the sulfated galactan (F3) of *Gracilaria corticata* by partial acid hydrolysis.

the derivative corresponding to the more prolonged time of sulfation, with IC_{50} values of 3.7 and 1.7 μg/ml against HSV-1 and HSV-2, respectively, whereas the variations in IC_{50} for WES1 were not significantly different from values of WE. With respect to F3, antiviral activity was highly improved for both reaction conditions since the IC_{50} values for F3S1 and F3S2 against both virus serotypes were about 1 μg/ml. As the original and further sulfated derivatives lacked cytotoxicity for Vero cells at concentrations up to 1000 μg/ml, both hypersulfated F3 derivatives were very selective HSV inhibitors with selectivity indices (SI: ratio CC_{50}/IC_{50}) in the range 617.3–877.7 (Table 2). This behavior was in agreement with the corresponding increase of degree of sulfation observed for these fractions, from 0.9 to 2–2.1 (Table 1), although F3S1 and F3S2 also exhibited a significant reduction in the amount of 3,6-AnGal in comparison to F3 (Table 1).

Additionally, the influence of the charge density in the antiherpetic activity of these galactans was corroborated by desulfation of WE and F3. The desulfated compounds WED and F3D reduced drastically their inhibitory activity (IC_{50} 23.7 to >100 μg/ml). The fraction obtained by partial acid hydrolysis (named oligo) was inactive for both types of viruses up to a concentration of 100 μg/ml, showing that the molecular weight is also an important feature in the inhibitory activity of the polysaccharides (data not shown).

No virucidal effects were observed by direct incubation of HSV-1 and HSV-2 virions with either the original polysaccharide fractions WE and F3 or their hypersulfated derivatives (data not shown). This finding is in agreement with a previous work carried out with other sulfated galactans from *G. corticata* [18].

3.2.2. Anticoagulant activity

APTT was measured to evaluate the anticoagulant activity of the galactan fractions from *G. corticata* and results are shown in Table 3. The APTT value of the blood treated with saline as negative control was 36.0 s whereas the value corresponding to heparin, tested as reference positive control, was >180 s at a concentration of 1 μg/ml. Treatment of the blood with the original fractions WE and F3 at concentrations in the range 2–20 μg/ml, near the antiviral IC_{50}, did not produce a significant change in the APTT value respect to the control saline sample. It was required a concentration highly exceeding the IC_{50} for both galactans (200 μg/ml) to produce anticoagulant effect of the same order of that induced by heparin at a concentration 200 times lower of 1 μg/ml. When crude and purified fractions were further sulfated, the values of APTT increased considerably when assayed at a concentration of 20 μg/ml, indicating that the negative

Table 2
Anti-HSV activity and selectivity indices of galactan-containing fractions from *Gracilaria corticata* (see text for abbreviations)

Fractions	IC_{50} (μg/ml)[a]		SI[b]	
	HSV-1 (F)	HSV-2 (MS)	HSV-1 (F)	HSV-2 (MS)
WE	16.2 ± 2.3	7.8 ± 1.3	>61.6	>128.0
WES1	15.0 ± 1.2	7.0 ± 0.3	>66.6	>142.4
WES2	3.7 ± 0.4	1.7 ± 0.1	>273.2	>571.4
WED	55.6 ± 7.8	23.7 ± 1.8	>17.9	>42.2
F3	27.4 ± 2.5	14.6 ± 1.9	>36.4	>68.2
F3S1	1.6 ± 0.03	1.1 ± 0.3	>621.1	>877.7
F3S2	1.6 ± 0.4	1.5 ± 0.3	>617.3	>662.2
F3D	>100	37.6 ± 3.5	Inactive	>26.6

[a] IC_{50} (inhibitory concentration 50%): concentration required to reduce virus plaque number by 50%.

[b] SI (selectivity index): ratio CC_{50}/IC_{50}. CC_{50} (cytotoxic concentration 50%): concentration required to reduce cell viability determined by MTT method by 50%. The values of CC_{50} were >1000 μg/ml for all samples.

Table 3
Anticoagulant activity of fractions from *Gracilaria corticata* (see text for abbreviations)

Fractions	APTT (s)[a]		
	Concentration (μg/ml)		
	200	20	2
WE	103.3 ± 39	42.3 ± 9.7	36.0 ± 2.0
WES1	>180	66.0 ± 4.1	27.0 ± 3.7
WES2	>180	>180	32.3 ± 5.2
F3	54.4 ± 0.9	35.3 ± 1.4	36.0 ± 3.3
F3S1	>180	>180	34.3 ± 1.0
F3S2	>180	>180	32.1 ± 4.1

[a] APTT: Activated partial thromboplastine time (in s). The data are the mean values of two experiments ± standard deviation. APTT for negative control sample with phosphate saline buffer = 36 s. APTT for positive control heparin (1 μg/ml) >180 s.

charge also affected the anticoagulant ability of the polysulfates. However, the antiviral properties of the most active galactans F3S1 and F3S2 still maintained independently of the anticoagulant activity since at a concentration of 2 μg/ml, around the IC_{50}s against HSV-1 and HSV-2 (Table 2), APTT values were as low as that of the control saline (Table 3).

4. Conclusions

In conclusion the high molecular weight galactan sulfates of *G. corticata* efficiently reduced the infectivity of HSV-1 and HSV-2. Here we have intended diverse experimental approaches to corroborate the central role of degree of sulfation for polysaccharide antiviral effectiveness. The sulfation and desulfation reactions performed with the original algal polysaccharides as well as generated oligosaccharides showed that charge density and molecular weight were fundamental determinants for the anti-HSV activity of these compounds.

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