Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9275
ISSN on-line: 1807-8621
Doi: 10.4025/actascitechnol.v34i2.11201

Iota-carrageenans from *Solieria filiformis* (Rhodophyta) and their effects in the inflammation and coagulation

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ABSTRACT. Biochemical analyses are important tools for discovering new bioactive compounds for medical clinic. This study aimed at isolating *iota*-carrageenans (ι-CARs) from *Solieria filiformis* (Rhodophyta) by enzymatic extraction (EE), refined hot-water extraction (RHWE) and hot-water extraction (HWE), and test (EE, s.c.) their anti-inflammatory effects in the peritonitis model using the Lambda-carrageenan (700 μg cavity^{-1}, i.p.) as an inflammatory stimuli in rats. The activated partial thromboplastin time (APTT) was also evaluated in ι-CARs fractions, obtained by ion-exchange chromatography (DEAE-cellulose), using rabbit plasma and compared to heparin (193 IU mg^{-1}). The results showed that the ι-CARs (EE) (3 or 9 mg kg^{-1}) containing 89.92% total sugars, 29.02% sulfate and absence of contaminant proteins inhibited ($p < 0.05$) the cellular infiltrate in the peritoneal cavity of the animals, but with 27 mg kg^{-1} had no anti-inflammatory effect ($p > 0.05$). Similar chromatography profiles were obtained among the methods; however, with fractions revealing different pattern on charge density by electrophoresis. Fractions had no virtually effects on APTT (1.16, 1.73 and 1.59 IU mg^{-1} for EE, RHWE and HWE, respectively). Further investigations to better understanding the actions of *S. filiformis* ι-CARs (EE) in the inflammatory response are suggested.

Keywords: red alga, sulfated galactans, extraction methods, peritonitis, APTT test.

Iota-carragenanas da rodofícea *Solieria filiformis* e seus efeitos na inflamação e coagulação

RESUMO. Análises bioquímicas são ferramentas importantes para a descoberta de novos compostos bioativos para clínica médica. Neste estudo, *iota*-carragenanas (ι-CARs) da rodofícea *Solieria filiformis* foram isoladas (extração enzimática (EE), extração aquosa a quente refinada (EAQR) ou extração aquosa a quente (EAQ)) e testadas (EE; s.c.) no modelo de peritonite, usando-se a Lambda-carragenana (700 μg cavidade^{-1}; i.p.) como um estímulo flogístico, para averiguar seus efeitos anti-inflamatórios em ratos. O tempo de tromboplastina parcial ativada (TTPA) também foi avaliado em frações de ι-CARs, obtidas por cromatografia de troca iônica (DEAE-celulose), usando plasma de coelho e comparadas à heparina (193 UI mg^{-1}). Verificaram-se que ι-CARs (EE) (3 ou 9 mg kg^{-1}) contendo 89,92% de açúcares totais, 29,02% de sulfato e destituídas de contaminação proteica, inibiram ($p < 0,05$) o infiltrado celular na cavidade peritoneal dos animais, mas com 27 mg kg^{-1} não apresentaram efeito anti-inflamatório ($p > 0,05$). Os perfis cromatográficos mostraram-se semelhantes entre os métodos de extração, porém revelando, por eletroforese, frações com diferenças em termos de densidade de carga. As frações praticamente não alteraram o TTPA (1,16; 1,73 e 1,59 UI mg^{-1} para EE, EAQR e EAQ, respectivamente). Investigações são sugeridas para melhor compreender as ações das ι-CARs (EE) da *S. filiformis* na resposta inflamatória.

Palavras-chave: alga vermelha, galactanas sulfatadas, métodos de extração, peritonite, teste do TTPA.

Introduction

Carrageenans (CARs) represent a generic name of a family of sulfated polysaccharides (SPs) with hydrophilic properties. They are sulfated galactans mainly consisting of alternating 3-linked β-D-galactopyranose (G-units) and 4-linked α-D-galactopyranose (D-units) or 4-linked 3,6-anhydro-α-D-galactopyranose (DA-units), forming disaccharide repeating unit of CARs, and found at high concentrations in the extracellular matrix of red seaweeds. Several additional variations on their chemical structures can also occur from substitutions by *O*-methyl and pyruvate groups, and small quantities of xylose. CARs are naturally divided into six basic forms: Iota (ι)-, Kappa (κ)-, Lambda (λ)-, Mu (μ)-, Nu (ν)- and Theta (θ)-CARs (Figure 1).

Such classification has been relevant for the commercial use and to identification of different

weed sources (CAMPO et al., 2009). The genera *Agardhiella*, *Solieria* (MURANO et al., 1997), *Halymenia*, *Hypnea, Chondrus, Eucheuma, Gigartina* and *Kappaphycus* (CAMPO et al., 2009; ROBLEDO; FREILE-PELEGRIN, 2010) of red seaweeds are rich sources in CARs. Also, hybrid CARs have gained more and more attention in the scientific world as well as in industries (VAN DE VELDE, 2008). Perez et al. (1992) reported that the extracted product in a pure form is carrageenin, very instable and hard to obtaining. Also, the carraggenin can bind to one or more divalent cations to form diverse salts of carrageenin: the CARs. Thus, according to Organic Chemistry Division Nomenclature must be used *an* than *ate* because the last one is respect to crystal salts, whereas the carrageenin salts never form crystals.

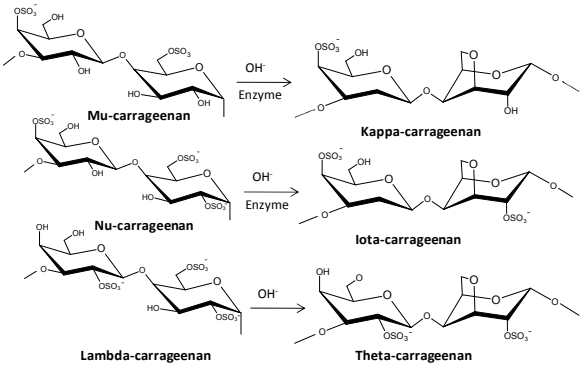

Figure 1. Chemical structures of carrageenans (CAMPO et al., 2009).

In the food industry, CARs are widely used as thickening, gelling and stabilizing agents as well as in various non-food products, such as pharmaceutical, cosmetic, printing and textile formulations (CAMPO et al., 2009; ROBLEDO; FREILE-PELEGRIN, 2010). The increase of demand by algal products (agar, alginate and CARs) combined to lack of knowledge of biological aspects for the cultivation of marine seaweeds has resulted in aquaculture of some species of economic impact in Brazil, such as *Gracilaria birdiae* (MACIEL et al., 2008) and *G. domingensis* (SALLES et al., 2010). These studies have encouraged the production of seaweeds capable of biosynthesizing significant quantities of hydrocolloids for international market, being thus independent of the importation and overexploitation of natural seaweeds banks.

The international market of CARs is estimated as US $450 million per year (ROBLEDO; FREILE-PELEGRIN, 2010). Additionally, red seaweeds CARs are recognized by presenting several biological activities. Antitumor, immunomodulatory, antihyperlipidemic, anticoagulant, antiviral and proinflammatory are properties previously reported (CAMPO et al., 2009; STEPHANIE et al., 2010). Climatic factors of the environment, the employment of different techniques for obtaining and/or the use of different species when collected at different period of year can also influence the chemical and structural characteristics and bioactivity of these compounds (FARIAS et al., 2000; MARINHO-SORIANO; BOURRET, 2003; RODRIGUES et al., 2009).

The search for alternative sources of bioactive compounds is justified due to the incidence of cardiovascular diseases worldwide and the risks of contamination and other several complications (bleeding, thrombocytopenia, etc) of heparin (HEP), a SP commercially obtained from animal tissues and widely utilized on thromboembolic disorders on coagulation and anticoagulant therapy, for example, to safety in cardiac surgeries with extracorporeal circulation, and treatment in the post-surgery of patients subjected to aging (MOURÃO; PEREIRA, 1999).

The side effects (bleeding, gastric perforation, stomach ulcer, etc) associated to therapeutic use of non-steroidal anti-inflammatory drugs for intervention in the exaggerated and uncontrolled inflammatory response in patients subjected to chronic and acute diseases in medical clinic are also reported (RANG et al., 2007).

Solieria filiformis (Kützing) P. W. Gabrielson (Gigartinales, Solieraceae) has been experimentally cultivated along the Brazilian coast (Flecheiras Beach, Trairí, Ceará State) (RODRIGUES et al., 2011). Murano et al. (1997) previously isolated the SPs from this species collected at Mar Piccolo, Italy, and observed that their phycocolloids consist of a dominant *ι*-CARs repeating structure, but with some irregularities when were characterized by NMR due to presence of 6-sulfate 4-linked precursor units. It is believed that this native Brazilian species can represent a source of hydrocolloid to be explored for diverse Biotechnological applications (ASSREUY et al., 2010; PONTES et al., 2009). Considering that studies with native seaweeds cultured have been scarcely conducted in Brazil, and that this activity promote social inclusion of people considered in poverty situation (BEZERRA; MARINHO-SORIANO, 2010; MACIEL et al., 2008; SALLES et al., 2010), and few descriptions of biomedical properties of CARs are still reported, we intend to evaluate the anti-inflammatory activity of a crude

extract of ι-CARs obtained from *S. filiformis*, separate ι-CARs fractions, and assay the anticoagulant activity of these hydrocolloids obtained. In this study, the *S. filiformis* ι-CARs obtained by different methods of extraction were also compared.

Material and methods

Preliminary treatment of alga and isolation of *i*-CARs

S. filiformis was collected in October 2009 on the Atlantic coast of Brazil (Flecheiras Beach, Trairí, Ceará State). Algae were cultured in the sea using *long line* structures located at 200 m from costal line (03°13'06"S, 39°16'47"W). After collection, specimens were conducted to Carbohydrates and Lectins Laboratory (CarboLec), Department of Biochemistry and Molecular Biology, Federal University of Ceará, and then cleaned of epiphytes, washed with distilled water and stored at -20°C until use. The ι-CARs were extracted through three different protocols, from dehydrated tissue (5 g, 25°C), and then macerated with liquid nitrogen.

Enzymatic extraction (EE)

The ι-CARs were extracted by papain digestion (6h, 60°C) (Vetec Química, Rio de Janeiro, Rio de Janeiro State) in 0.1 M sodium acetate buffer (pH 5.0) containing 5 mM cystein and 5 mM EDTA as previously described elsewhere (RODRIGUES et al., 2010a).

Refined hot-water extraction (RHWE)

Precursors and other non-gel promoting structural elements were previously extracted at 25°C for 24h in water at 1.5% (w v^{-1}) and then discarded. After that, the algal tissue was re-submitted to an incubation for 6h at 80ºC (MARCONI, model MA 159) in the same solvent at 1.5% (w v^{-1}). The residue was removed by centrifugation (2.725 × g for 30 min. at 4°C), and then the supernatant was precipitated with commercial EtOH (1:3, v:v) for obtaining the ι-CARs.

Hot-water extraction (HWE)

The ι-CARs were extracted by incubation for 6h at 80°C (MARCONI, model MA 159). The residue was removed by centrifugation (2.725 × g for 30 min. at 4°C), and then the supernatant was precipitated with commercial EtOH (1:3, v:v).

The ι-CARs obtained by all methods were centrifuged, redissolved in distilled water, dialyzed against distilled water for 24h (47 × 27 mm cellulose membrane, Sigma-Aldrich, St. Louis, MO, USA), and then freeze-dried (RODRIGUES et al., 2010b). The ι-CARs yields (%) were calculated as the percentage of dry matter.

Chemical composition

The total sugars (TSs) content was estimated by phenol-sulfuric acid analysis using D-galactose as standard (DUBOIS et al., 1956). After acid hydrolysis of the soluble polysaccharides (1 mL of HCl for 5h at 100°C), the free sulfate (FS) was measured by the $BaCl_2$/gelatin method (DODGSON; PRICE, 1962). The content of contaminant proteins (CPs) was measured by the Bradford's method (BRADFORD, 1976), using bovine serum albumin as reference.

Animals

Wistar rats (180-220 g) were randomly used from the Animal House of the Federal University of Ceará, kept under a 12 h light/dark cycle, in temperature-controlled rooms and received water and food *ad libitum*. All the procedures and animal treatments applied in this study were approved (protocol number 80/10) by the Institutional Animal Care and Use Committee of the Federal University of Ceará, Fortaleza, Ceará State, Brazil, in accordance with the international guidelines (NIH publications no. 85-23, revised 1985).

Peritonitis model

One milliliter of λ-CARs (700 μg) or sterile saline (0.9%, w v^{-1}) was injected i.p., 4h later animals (n = 6 per group) were sacrificed, and the peritoneal cavity was washed with 10 mL of saline containing 5 IU mL^{-1} heparin. The peritoneal fluid was recovered and total and differential leukocyte counts were performed. Animals received (s.c.) ι-CARs obtained from EE (3, 9 or 27 mg kg^{-1}) or sterile saline (0.9%, w v^{-1}) 30 min. before inflammatory stimuli (VANDERLEI et al., 2010). Dexamethasone (1 mg kg^{-1}, s.c.) was administered 1h before λ-CARs, as reference. Results were expressed as mean ± S.E.M. of the number of cells × 10^3 mL^{-1} in peritoneal fluid (SOUZA; FERREIRA, 1985).

Ion-exchange chromatography and agarose gel electrophoresis

A sample of each crude extract (50 mg) of ι-CARs from *S. filiformis* was dissolved in 0.05 M sodium acetate buffer (pH 5.0) and then fractionated by ion-exchange chromatography on DEAE-cellulose column (1.0 × 24.5 cm) previously equilibrated and washed with the same buffer, followed by separation of fractions of ι-CARs also using the same buffer containing NaCl at

different concentrations (0.5, 0.75 and 1 M). ι-CARs fractions (5.6 mL) were collected and monitored by metachromatic property using the DMB (RODRIGUES et al., 2010a) using an ELISA reader (Amersham Biosciences, Biotrak II) at 525 nm, and the TS were determined according to the method in plate format (MASUKO et al., 2005), also using ELISA reader at 492 nm.

Then the metachromatic fractions were dialyzed against distillated water (47 × 27 mm cellulose membrane, Sigma-Aldrich, St. Lois, MO, USA), and freeze-dried. The degree of polydispersion and the charge density of ι-CARs fractions were checked by agarose gel electrophoresis as described (DIETRICH; DIETRICH, 1976).

Activated partial thromboplastin time (APTT) test

The APTT test was performed using rabbit plasma following manufacturer's specifications (CLOT, Bios diagnostic, Sorocaba, São Paulo State, Brazil). The anticoagulant activity was expressed as international units per mg of polysaccharide using standard heparin (193 IU mg^{-1}).

Statistical analyses

The data of ι-CARs yields obtained was expressed as mean ± S.E.M., followed by analysis of variance (ANOVA) and Tukey's test, considering $p < 0.05$ as significant. The data obtained from the cell counts were submitted to analysis of variance (ANOVA), followed by Bonferroni test used to compare the means. The $p < 0.05$ was considered statistically significant.

Results and discussion

Our research group evaluated the employment of different extraction methods for obtaining SPs from seaweeds (PEREIRA et al., 2005; RODRIGUES et al., 2010b; RODRIGUES et al., 2011). In the present study, the *S. filiformis* ι-CARs yield was different among the extraction methods used (Table 1). Similar yields of these compounds resulted from the RHWE (29.30 ± 0.47%) and HWE (33.54 ± 1.32%), respectively ($p > 0.05$), but were different compared to EE (19.14 ± 0.48%) ($p < 0.05$) from the dehydrated algal tissue (25°C) and lyophilization procedure.

Few data have been reported for ι-CARs from *S. filiformis*. Sequential extractions for obtaining ι-CARs of this species undertaken by Murano et al. (1997) showed that the major part of ι-CARs were extracted by stirring in water at 25°C (78%, w w^{-1}, six times) compared to 85°C (45 min., three times) and 120°C (1h, pressure, twice).

Table 1. Total yield and chemical composition of ι-CARs from *Solieria filiformis* in the different the extraction methods.

Method	Yield (%)	TSs (%)	FS (%)	CPs (%)
EE	19.14±0.48*	89.92	29.02	-
RHWE	29.30±0.47	49.55	26.18	3.94
HWE	33.54±1.32	56.43	35.05	1.44

Mean value of three determinations; * Statiscally different at $p < 0.05$; - Non-detected.

The ι-CARs obtained in water at 25°C were also characterized by NMR and FT-IR analysis, and the results suggested a slightly higher content or different distribution of precursor and other non-gel promoting structural elements. According to the authors, the crude extracts in water at 120°C contain large amounts of floridean starch, whereas no starch was detected at 25°C.

In our case, we extracted the *S. filiformis* ι-CARs using three different methods and noted that the highest yield of these phycocolloids was given in water at 80°C (HWE). This suggests a mixture with floridean starch in this crude extract based on Murano et al. (1997). Also, we performed a previous extraction of algal tissue at 25°C and then eliminated this yield obtained, followed by a re-extraction of ι-CARs from the same algal tissue at 80°C, being thus designated RHWE (Table 1).

We also observed that the sulfate content found in the HWE (35.05%) and RHWE (26.18%), respectively, characterized both extracts. In the crude extracts obtained in water (HWE and RHWE) were detected high CPs contents (Table 1). These results are in accordance with another study published by our group (RODRIGUES et al., 2011). In contrast, the lowest lyophilized yield of CARs in this experiment was 19.14 ± 0.48% (EE), whereas 46.8 and 25% of crude extracts of ι-CARs (EE) of *S. filiformis* were isolated by Pontes et al. (2009) and Assreuy et al. (2010), respectively, although dried overnight (60°C; 24h). No CPs was detected by the employment of papain digestion also in comparison to others studies (RODRIGUES et al., 2010b; RODRIGUES et al., 2011). These results suggest a selectivity of EE for obtaining of seaweeds carbohydrates. Based on these data, we also examined this crude extract by infrared (data not shown), and the results indicated the presence of ι-CARs in EE (MURANO et al., 1997). Therefore, these results led us to conduct an acute inflammation assay.

Peritonitis model

In order to evaluate the *S. filiformis* anti-inflammatory effect, the ι-CARs (s.c.) isolated by EE (Table 1) were tested in the model of peritonitis in rats. It has been recognized that λ-CARs induce total leukocytes and neutrophil migrations into the rat peritoneal cavity (VANDERLEI et al., 2010) by an indirect mechanism, via activation of macrophages (SOUZA; FERREIRA, 1985). Our present data show that in the λ-CARs-induced peritonitis, the *S. filiformis* SPs (3 or 9 mg kg^{-1}, s.c.) significantly produced $p < 0.05$) anti-inflammatory effects (47.19 and 36.63%, respectively) by a decrease in total leukocytes migration in the peritoneal cavity of the animals (Figure 2A). Dexamethasone (1 mg kg^{-1}, s.c.) reduced the λ-CARs-induced leukocytes migration by 70%.

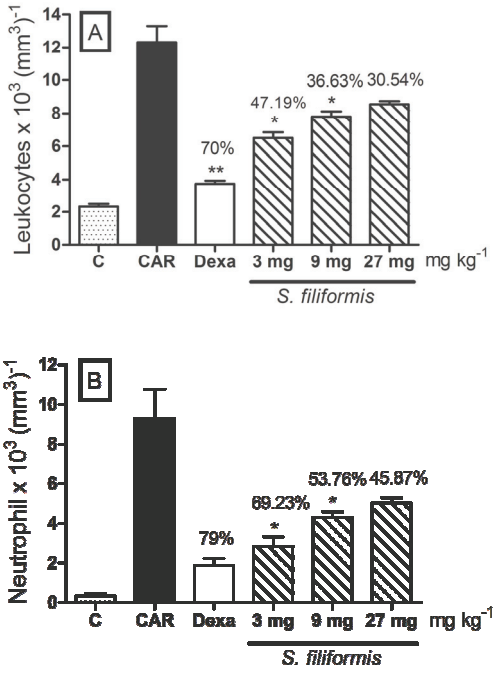

Figure 2. Effect of *ι*-CARs from *Solieria filiformis* on the total leukocytes (A) and neutrophil (B) migrations induced in rats. Data are expressed as mean ± S.E.M. of 6 rats for each group. $^{*}p < 0.05$ indicates significant difference from the λ-CARs group and $^{**}p < 0.05$ indicates significant difference compared to *ι*-CARs (ANOVA; Bonferroni test).

In respect to the effects on the neutrophil migration (Figure 2B), the *S. filiformis* ι-CARs also exhibited similar stimulus in comparison with the same doses tested on the total leukocytes migration (Figure 2A). Dexamethasone (1 mg kg^{-1}, s.c.) decreased the λ-CARs-induced neutrophil migration by 79%. In the coagulation process, slight differences in the proportion and/or distribution of sulfate residues in the polysaccharides chains may be critical for the interaction of proteases, inhibitors and activators of the coagulation system, resulting in a distinct pattern of anti- and procoagulant activities and in anti- and prothrombotic actions (FONSECA et al., 2008). In this line we suggest that the *S. filiformis* ι-CARs may also be acting in the inhibition and induction of inflammatory mediators, but this deserves posterior investigations.

According to the recent study from Assreuy et al. (2010), a SP separated by ion-exchange chromatography (DEAE-cellulose) from *S. filiformis* demonstrated that the compound tested (1 mg kg^{-1}) in the paw edema model was not anti-inflammatory, but capable of inducing acute edema at 30 min. Although our data reported here are not in accordance with studies of red seaweeds sulfated galactans in the literature (ASSREUY et al., 2008; ASSREUY et al., 2010; SILVA et al., 2010), this may be responsible for the anti-inflammatory effects exhibited in the peritonitis model (Figure 2).

Ion-exchange chromatography

A sample of each crude extract of ι-CARs from *S. filiformis* was submitted to ion-exchange chromatography on DEAE-cellulose column, as seen in Figure 3, and similar profiles were found.

The crude extract obtained by EE presented fractions with the higher *ι*-CARs yields compared to those of RHWE and HWE, respectively (Table 2), confirming a previous study that used the papain digestion in the isolation of *H. musciformis* κ-CARs, performed by our group (RODRIGUES et al., 2011). The highest ι-CARs yields were also obtained in the fractions F II, eluted with 0.75 M of salt, compared to all fractions isolated on DEAE-cellulose.

The crude extract (EE) of *S. filiformis* was isolated and fractionated by Assreuy et al. (2010). The chromatographic profile (DEAE-cellulose) separated into four different SPs fractions, eluted with 1.2, 1.4, 1.6 and 1.8 M of salt, respectively. All fractions indicated a relatively low carbohydrate concentration, but with the highest metachromasia verified for fraction eluted with 1.2 M. In contrary of our study, F II, eluted with 0.75 M of NaCl (EE), had a high TSs content (Figure 3A). This suggests that neutral sugars are capable of interacting with the DEAE-cellulose column. This can also be confirmed by the high TSs content present in the crude extract (EE), as already shown in Table 1. All chromatographic profiles revealed here showed two *ι*-CARs fractions (F I and F II), both with 0.5 and 0.75 M of salt, respectively (RHWE and HWE), but three fractions (F I, F II and F III) were indentified in the crude extract of EE, when eluted on DEAE-cellulose (Figure 2). Several fractions were identified in different crude extracts (EE) of SPs obtained from *S. filiformis* by Pontes et al. (2009).

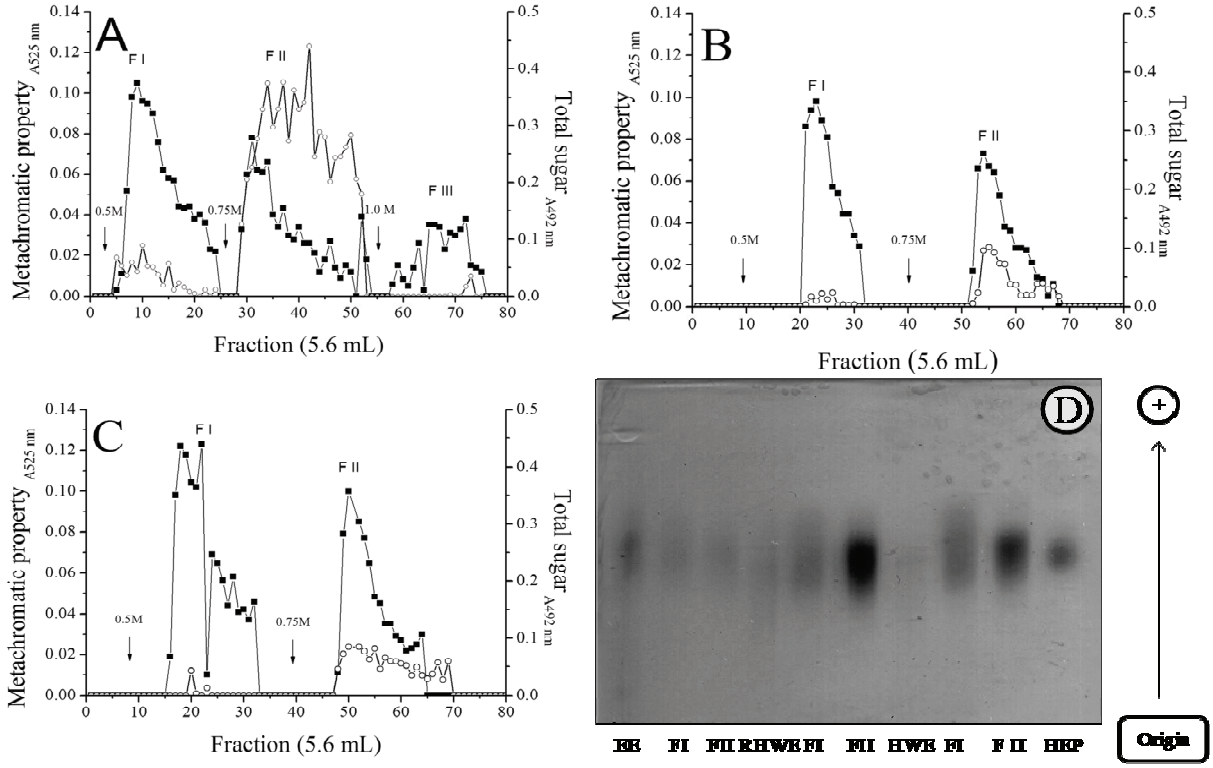

Figure 3. Separation of ι-CARs (EE-A, RHWE-B and HWE-C) from *Solieria filiformis* by DEAE-cellulose. Fractions were collected and checked by metachromasia using 1,9-dimethylmethylene blue (■—■) and phenol-H_2SO_4 (○—○). Arrows represent the NaCl concentration (↓). (D) Agarose gel electrophoresis of *ι*-CARs isolated from *Solieria filiformis*. Extracts (EE, RHWE and HWE), fractions (F I and F II) and heparin (HEP) present on gel were stained with 0.1% toluidine blue.

Table 2. Yield of ι-CARs fractions obtained by ion-exchange chromatography (DEAE-cellulose) from *Solieria filiformis* in the different extraction methods.

Method	Fraction	NaCl (M)	Yield (%)
EE	F I	0.50	4.8
	F II	0.75	6.8
	F III	1.00	-
RHWE	F I	0.50	4.6
	F II	0.75	6.0
HWE	F I	0.50	4.6
	F II	0.75	6.2

Low amount of material for analysis.

Probably, these conflicts of data are justified by the influence of climatic conditions on biosynthesis of different molecules, life cycle of this species, and/or employment of different techniques for obtaining these polymers (MARINHO-SORIANO; BOURRET, 2003; RODRIGUES et al., 2009; RODRIGUES et al., 2011). The occurrence of different SPs in the extracellular matrix of red seaweeds is also reported (RODRIGUES et al., 2009).

According to the agarose gel electrophoresis procedure (Figure 3D), we registered differences in charge density among the isolated fractions. Although polydisperse, F II (obtained by RHWE and HWE methods), had a strong metachromatic band on gel compared to that of EE, and these differences confirmed the results herein obtained (Table 1 and Figure 3). These results may indicate the occurrence of chemical differences in these polymers, although the infrared technique confirms the type of the extracted CAR.

Anticoagulant assays

It has been accepted that there is a strong correlation between coagulation and inflammatory response (RANG et al., 2007). SPs, as negatively charged compounds (AZEVEDO et al., 2009), are macromolecules capable of binding with any basic protein at several levels of specificity, modifying the activity of many biological proteins (PEREIRA et al., 2005). According to Fonseca et al. (2008), differences in patterns of sulfate along polysaccharide chain may confer high affinity for a particular protein.

Supported by these hypotheses, we intended to evaluate the *in vitro* anticoagulant activity of ι-CARs fractions from *S. filiformis*. As can be noted, the fractions had no practical effects in the normal coagulation time (Table 3). F II eluted with 0.75 M of salt showed maximal anticoagulant activity (1.16, 1.73 and 1.59 IU mg^{-1} for EE, RHWE and HWE, respectively), but was less potent than the standard HEP (193 IU mg^{-1}). These data are indicative that

ι-CARs fractions from *S. filiformis* discretely act on the intrinsic and/or common pathways of the blood coagulation system (Table 3), but without effects on the prothrombin test (data not shown) (RODRIGUES et al., 2010b). Similar results were also found for *Caulerpa* SPs of green seaweeds recently investigated by Rodrigues et al. (2010a).

Table 3. Anticoagulant activity of ι-CARs fractions obtained by ion-exchange chromatography (DEAE-cellulose) from *Solieria filiformis* in comparison to HEP.

Method	Fraction	NaCl (M)	APTT test ⋆	
			1.00 mg mL^{-1} ⋆⋆	IU mg^{-1} ⋆⋆⋆
EE	F I	0.50	17.83 ± 0.20 s	1.07
	F II	0.75	19.30 ± 0.65 s	1.16
RHWE	F I	0.50	19.20 ± 0.70 s	1.15
	F II	0.75	28.83 ± 0.80 s	1.73
HWE	F I	0.50	21.53 ± 0.17 s	1.29
	F II	0.75	26.47 ± 0.86 s	1.59

NaCl – Sodium Chloride; ⋆Activated partial thromboplastin time (APTT); ⋆⋆SP concentration to prolong the APTT in seconds; ⋆⋆⋆Anticoagulant activity expressed in international units (IU) per mg of SP (IU mg^{-1}); HEP (193.00 IU mg^{-1}; 0.01 mg mL^{-1}; APTT: 32.10 ± 0.81 s); Control: 17.23 ± 0.08 s.

Although the anticoagulant activity of seaweeds SPs is very well known (FARIAS et al., 2000; FONSECA et al., 2008), each polysaccharide may be dependent of a specific structural requirement for its action on coagulation system (PEREIRA et al., 2005). Additionally, due to high complexity and heterogeneity of seaweeds SPs, these molecules may also occur in different regions of algal tissue, revealing different effects on APTT (RODRIGUES et al., 2009).

It was proposed that the sulfated content and/or the position of sulfate on the chemical structure and size molecular of SPs are pre-requisites important for anticoagulant action. So we can discuss that differences exist among the major types of CARs (kappa, iota and lambda) (Figure 1). As a rule, is recognized that the differences among the anticoagulant activity of these CARs mainly occur due to the number of sulfate radicals and the presence or absence of 3,6-anhydro-α-L-galactose residues on chemical structure of these polymers (CAMPO et al., 2009; SILVA et al., 2010). In general, the reduced anticoagulant activity detected for *S. filiformis* ι-CARs fractions isolated by extraction methods (Table 3) may be explained by the low charge density on chemical structure compared to λ-CARs, an important inflammatory inductor in the assessment of the effectiveness of anti-inflammatory drugs (SILVA et al., 2010) (Figure 2).

Assreuy et al. (2010) reported SP fractions with anticoagulant activity from *S. filiformis*. Four different fractions from DEAE-cellulose were eluted with 1.2, 1.4, 1.6 and 1.8 M of salt and were capable of prolonging the normal APTT. However, fraction 1.2 M presented a maximal delay in the coagulation time by 2.5 (97.7 s) higher compared to normal APTT. This 1.2 M fraction (1 mg kg^{-1}) was tested in the acute paw edema model, and also evoked an edematogenic action in rats, suggested by the authors as an inflammatory reaction exhibited by the SP as a defense mechanism from the immune system of the animals. In contrast with our study, when the crude extract (EE) of ι-CARs from *S. filiformis* was applied (s.c) in rats 1h before the λ-CARs administration did not induce pro-inflammatory effects at doses of 3 or 9 mg kg^{-1}, but could be expressing any inflammatory action at dose of 27 mg kg^{-1} (Figure 2); but without anticoagulant effects (Table 3). Although being of different experimental models of acute inflammation, these conflicts of data may be indicative of a biological strategy for the use of these polymers for distinguish systemic events in the inflammatory process.

On the other hand, possibly our results could demonstrate differences in the extraction of constituents by the methods used, and that structural hydrolysis and/or others extracted constituents by EE could became the extracts non-homogeneous (Figure 3D), and inducing or not biological effects, as well as wrong conclusions. Another important data of our study was the solubility of ι-CARs fractions from *S. filiformis* in water. It has also been reported that the water solubility of CARs depends essentially on the levels of sulfate groups and associated cations (sodium, potassium, calcium and magnesium). The proportion of sulfate and the equilibrium of cations in the water solution determine the viscosity of solutions or strength of gels formed by CARs. The gel properties are also greatly dependent on the amount of 3,6-anhydrogalactose and the sulfate contents (CAMPO et al., 2009). In this study, we observed that the addition of calcium chloride to display the APTT did not influence the evaluation by *in vitro* testing (Table 3). Nevertheless, the ι-CARs (EE) produced *in vivo* anti-inflammatory effects (Figure 3).

To κ/ι-hybrids, CARs extracted from a wide range of species showed a linear relationship between their functionality and the κ/ι-ratio (VAN DE VELDE, 2008). Melo et al. (2002) isolated by papain digestion the agar (a SP) from *Gracilaria cornea* (Rhodophyta) and observed that the galactose/3,6-AG ratio was very different than the values considered to ideal agarose ratio. No gelation in aqueous solutions of agar was observed.

Conclusion

The major conclusion of our study is that *ιota*-carrageenans from the red seaweed *Solieria filiformis* isolated by papain digestion suggest chemical differences between these polymers and those from water extractions. Also, *ιota*-carrageenans (papain digestion) from this species have no effect on coagulation cascade, but interfere on acute inflammatory response. Data may be valuable for the design of novel therapeutic agents by pharmaceutical industry.

Acknowledgements

We are funded by CNPq, Renorbio, Capes, MCT, MS and Funcap. Benevides, N. M. B. is a senior investigator of CNPq/Brazil.

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Received on September 17, 2010.
Accepted on April 15, 2011.