

Molecular signature of kappa-carrageenan mimics chondroitin-4-sulfate and dermatan sulfate and enables interaction with arylsulfatase B[☆]

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Abstract

The common food additive kappa-carrageenan (κ -CGN) is a sulfated polysaccharide that resembles chondroitin-4-sulfate (C4S) and dermatan sulfate (DS). All have a sulfate group on C4 of a glycoside (galactose for CGN and *N*-acetylgalactosamine for C4S), and the sulfate-bearing glycoside is linked in a β -1,4-configuration to an unsulfated, six-carbon sugar (galactose for CGN, glucuronate for C4S and iduronate for DS). The enzyme arylsulfatase B (ARSB; *N*-acetylgalactosamine-4-sulfate) is the highly selective enzyme that removes the four-sulfate group from the nonreducing terminus of C4S and DS, thereby regulating subsequent degradation. In this report, κ -CGN is shown to be a substrate for recombinant human ARSB (rhARSB). Sulfate was generated from both C4S and κ -CGN following incubation with rhARSB. Exposure of human colonic epithelial cells to κ -CGN, but not to C4S, produced reactive oxygen species (ROS) and increased interleukin (IL)-8 secretion. The ROS production from κ -CGN was reduced by exposure to rhARSB, but increased by competition from C4S or DS, but not from chondroitin-6-sulfate. Prior treatment of either lambda- or iota-CGN with rhARSB had no impact on ROS, IL-8 or inorganic sulfate production, demonstrating a specific effect of the molecular configuration of κ -CGN. By mimicry of C4S and DS and by interaction with ARSB, κ -CGN can directly interfere with the normal cellular functions of C4S, DS and ARSB. Since C4S and DS are present in high concentration in tissues, the impact of κ -CGN exposure may be due to some extent to interference with the normal biological functions of ARSB, C4S and DS.

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1. Introduction

Carrageenan (CGN) is widely used in processed foods to improve the texture and solubility of food products, including infant formula and nutritional supplements. Recently, iota (ι)- and kappa (κ)-CGNs were reported to prevent some viral infections, including human papilloma virus and influenza [1,2]. These effects reflect the remarkable biological reactivity of CGN. Derived from several varieties of red algae, CGN has been widely used for decades to induce inflammation in animal models in order to identify the cellular and molecular mediators of inflammation and to test the effectiveness of anti-inflammatory therapies [3].

Three major types of CGN are used in food products, including κ -, lambda (λ)- and ι -CGNs. Carrageenan, like the endogenous sulfated glycosaminoglycans (sGAG), is composed of sulfated disaccharides. The fundamental disaccharide units of κ -, ι - and λ -CGNs differ in their sites and extent of sulfation: κ -CGN has 3,6-anhydro-D-galactose with an α -1 \rightarrow 3 link to D-galactose-4-sulfate; ι -CGN has 3,6-anhydro-D-galactose-2-sulfate with an α -1 \rightarrow 3 link to D-galactose-4-sulfate; and λ -CGN is composed of D-galactose-2,6-disulfate with an α -1 \rightarrow 3 link

to D-galactose-2-sulfate. These disaccharide units are linked by β -1 \rightarrow 4 bonds [4]. The fundamental disaccharide unit of chondroitin-4-sulfate (C4S) is composed of D-glucuronate in β -1 \rightarrow 3 linkage to *N*-D-acetylgalactosamine-4-sulfate, and these disaccharide units are also linked by β -1 \rightarrow 4 bonds.

In experiments with human colonic epithelial cells, λ -, κ - and ι -CGNs initiate inflammatory cascades either by interaction with toll-like receptor (TLR)4 or by production of reactive oxygen species (ROS) [5–7]. The CGN-induced inflammation involves increased interleukin (IL)-8; nuclear factor(NF)- κ B, including RelA, RelB, p50 and p52; and B-cell leukemia/lymphoma (BCL)10 [5–8]. The inflammation induced by CGN in the human colonic epithelial cells appears to constitutively activate NF- κ B due to the presence of an NF- κ B binding site in the BCL10 promoter [8,9].

Carrageenan induction of the TLR4–BCL10-mediated inflammatory pathway involves activation of an innate immune response that may be attributable to the presence of the unusual α -D-Gal(1 \rightarrow 3)D-Gal linkage [10]. Experiments with CGN-digesting enzymes indicated that increased exposure to the α -1 \rightarrow 3 galactoside following treatment with specific carrageenase produced an increase in the TLR4–BCL10-mediated inflammatory response, whereas treatment with α -1 \rightarrow (3,6) galactosidase reduced the response [11]. Here, we report how pretreatment of CGN with arylsulfatase B (ARSB) affects the ROS, IL-8 and BCL10 inflammatory responses produced by CGN, and how CGN exposure affects the ARSB activity.

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2. Methods and materials

2.1. Cell culture

NCM460 cells were grown as recommended using M3:10A media (INCELL, San Antonio, TX, USA) and as previously described [5–7,12]. Confluent cells in T-25 flasks were harvested by EDTA-trypsin and subcultured in 12-well tissue culture plates with $\sim 5 \times 10^5$ cells per well for most of the experiments. Cells were maintained at 37°C in a humidified, 5% CO₂ environment, and following treatments, cells were harvested by scraping. Total cell protein was measured by BCA Protein Assay Kit (Pierce, Rockford, IL, USA) using bovine serum albumin as standard.

κ -, λ - and ι -carrageenans were obtained commercially (Sigma Chemical Co., St. Louis, MO, USA) and dissolved in sterile water. In the majority of experiments, cells were treated with CGN (1 mg/L) for 24 h. Spent media were collected from control and treated wells at specified intervals and stored at -80°C pending analysis. Chondroitin-4-sulfate ($>90\%$, Biocolor Ltd., Newtownabbey, Northern Ireland), dermatan sulfate (DS) ($\geq 90\%$, Sigma), chondroitin-6-sulfate (C6S) (100%, Associates of Cape Cod, Inc., East Falmouth, MA, USA) and C4S:C6S (70:30, Sigma) were used.

2.2. Enzyme treatments

κ -Carrageenan (1 mg/L) was digested by recombinant human (rh)ARSB (1 $\mu\text{g/L}$; R&D, St. Paul, MN, USA), recombinant α -1 \rightarrow (3,6)-galactosidase from *Escherichia coli* (200 U/L; Calbiochem, EMD Chemicals, Inc., Gibbstown, NJ, USA) or κ -carrageenase (κ -CGNase) (a generous gift from Dr. Gurvan Michel). κ -Carrageenase, which cleaves the β -1 \rightarrow 4 linkages of the β -1 \rightarrow 4- α -1 \rightarrow 3 galactans, was used at a concentration of 3 mg/L to digest 1 mg/L CGN, either alone or in combination with other enzymes for 18 h at 37°C. NCM460 cells grown in microwells of 12-well tissue culture plates were exposed to the digested CGN-enzyme mixture in 5% CO₂ environment for 24 h (unless stated otherwise). After 24 h of exposure to the CGN-enzyme mixture, the spent media were collected and assayed for IL-8, and cells were harvested, lysed and assayed for either total cell protein or BCL10.

2.3. Measurement of ROS

Reactive oxygen species generated following exposure to κ -CGN were measured using Hydroethidine (HE) (Invitrogen, Carlsbad, CA, USA) to detect intracellular superoxide anion by the change from blue to red fluorescence in the presence of O₂⁻ upon formation of the oxyethidium derivative [7]. NCM460 cells were plated in 96-well tissue culture plates ($\sim 50,000$ cells/well) and treated with κ -CGN (1 mg/L) that was previously digested for 18 h with rhARSB (1 $\mu\text{g/L}$). After 24 h, the media were removed, and cells were washed with Hank's balanced salt solution (HBSS). Then, the cells were incubated at 37°C for 60 min with 200 μL of HBSS containing 10 μM HE. Next, HBSS containing HE was removed, fresh HBSS (200 μL /well) was added, and the fluorescence emitted by the cells was measured using a microplate fluorescence reader (FLUOstar, BMG Labtech, Inc., Cary, NC, USA) at 488-nm excitation with a 610-nm emission filter. For the dose-response curve, different concentrations of κ -CGN (0.1, 1 and 5 mg/L) were digested with rhARSB (1 $\mu\text{g/L}$) prior to treatment of the NCM460 cells.

To determine whether ROS were generated from the sulfate (<1 kD) or the κ -CGN- or C4S-containing fraction (>1 kD), 1 mg of κ -CGN or 1 mg of C4S was digested with 1 μg of rhARSB in 1 ml of 0.05 sodium acetate buffer, pH 5.6, for 18 h at 37°C and separated through a 1-kD molecular weight (MW) cutoff filter. The <1 -kD filtrate (sulfate-containing) and the >1 -kD (κ -CGN- or C4S-containing) fractions were lyophilized and reconstituted in 1 ml phosphate-buffered saline (PBS), pH 7.4, and 1- μL aliquots were added to 1 ml of media to expose NCM460 cells in a 96-well culture plate ($\sim 50,000$ cells/well) to a concentration of ~ 1 mg/L digested κ -CGN or C4S.

2.4. Measurement of sulfate content

Sulfate was assayed using the QuantiChrom kit (BioAssay Systems, Hayward, CA, USA) that measures inorganic sulfate in the linear range (0.96–115 mg/L). The assay was performed in a 96-well plate with a sodium sulfate standard, and optical density was measured at 600 nm (FLUOstar). κ -Carrageenan (1 mg) or C4S (1 mg) was digested with rhARSB (1 μg) in 1 ml of 0.05-M sodium acetate buffer, pH 5.6, for 18 h at 37°C.

The free sulfate generated due to the action of rhARSB on κ -CGN or C4S was separated from the high-molecular-weight CGN or C4S by passing the digestion mixture through a 1-kD MW cutoff filter (Harvard apparatus, Holliston, MA, USA), yielding a sulfate-containing filtrate of MW <1 kD and a CGN- or C4S-containing retentate of MW >1 kD. To measure free sulfate production, both of the fractions were lyophilized and reconstituted in 0.1 ml PBS, pH 7.4, to get a sulfate concentration 10 times more concentrated than the original digest and within the range of the sulfate measurement assay. Sulfate concentration was expressed as mg/L.

2.5. Competition experiment between κ -CGN and C4S, C6S and DS at different concentrations of rhARSB

The ROS production was measured by the HE method, as described above, in the NCM460 cells following exposure to κ -CGN (1 mg/L \times 24 h) that was pretreated with

increasing concentrations of rhARSB (0, 0.05, 0.1, 0.2, 0.5, 1 and 2 $\mu\text{g/L}$) for 18 h at 37°C. Chondroitin-4-sulfate:C6S, C6S or DS (1 mg/L) was included in the digestion mixture with κ -CGN (1 mg/L) and rhARSB of varying concentrations for possible competition.

2.6. Measurements of IL-8 and BCL10 by enzyme-linked immunosorbent assay

Secretion of IL-8 in the spent media of control and κ -CGN-treated NCM460 cells was measured using the DuoSet enzyme-linked immunosorbent assay (ELISA) kit for human IL-8 (R&D Systems Inc., Minneapolis, MN, USA), as described previously [5–7]. Sample values were normalized by total protein content (BCA Protein Assay Kit, Pierce) and expressed as pg/mg protein. Cellular BCL10 was measured by ELISA, as previously reported [13].

2.7. Statistical analysis

Differences in results were evaluated by InStat software (GraphPad Software, Inc., La Jolla, CA, USA) using 1-way analysis of variance (ANOVA) with Tukey–Kramer posttest, unless stated otherwise. $P<.05$ was considered statistically significant. In figures, * represents $.01<P<.05$, ** represents $.001<P<.01$, and *** represents $P<.001$. All experiments were performed with at least three independent samples and technical replicates of each determination.

3. Results

3.1. Structural homology between κ -CGN and C4S and DS

The similar configuration of the 4-SO₄ group between κ -CGN and C4S (Fig. 1) suggests how κ -CGN can mimic C4S and DS. The 4-SO₄ containing galactose or NAcGal is linked by a β -1 \rightarrow 4 bond to an adjacent unsulfated hexose, galactose in the case of κ -CGN, glucuronate for C4S and iduronate (or glucuronate) for DS. Chondroitin-4-sulfate and DS have β -1 \rightarrow 3 bonds alternating with β -1 \rightarrow 4 linkages, in contrast to the α -1 \rightarrow 3 bonds alternating with β -1 \rightarrow 4 bonds of CGN.

3.2. Effects of rhARSB on CGN and C4S and on ROS production

When NCM460 cells were exposed to κ -, ι - and λ -CGN (1 mg/L \times 24 h), the production of ROS, as measured by HE assay, markedly increased (to 9.62 ± 0.46 , 8.96 ± 0.20 and 9.94 ± 0.52 times the baseline levels, respectively; $P<.001$ for each) (Fig. 2A). Following κ -CGN pretreatment with rhARSB (1 $\mu\text{g/L}$ for 18 h), the ROS production declined to 5.54 ± 0.9 times the baseline. However, pretreatment of λ - or ι -CGN with rhARSB did not reduce the CGN-associated ROS production.

The ROS production from NCM460 cells exposed to different concentrations of κ -CGN (0.5, 1 or 5 mg/L) increased with higher concentrations of κ -CGN (Fig. 2B). When exposed to κ -CGN pretreated with rhARSB (1 $\mu\text{g/L}$ for 18 h), significant declines in the CGN-stimulated ROS production were evident at each concentration ($P<.001$, 1-way ANOVA with Tukey–Kramer posttest).

3.3. ROS and sulfate production from κ -CGN and C4S

The ROS production was measured in the NCM460 cells following exposure for 24 h to the retentate and filtrate that were obtained following separation through a 1-kD filter of κ -CGN (1 mg/L) pretreated with rhARSB (1 $\mu\text{g/L}$) for 18 h. The ROS production in the κ -CGN/rhARSB-treated cells was associated with the >1 -kD reaction product, rather than with the <1 -kD filtrate (Fig. 3A). In contrast to the findings with κ -CGN, exposure of NCM460 cells to C4S (1 mg/L \times 24 h) or to DS (1 mg/L \times 24 h) did not lead to any increase in ROS production, and pretreatment with rhARSB had no impact on ROS generation from C4S-treated cells (Fig. 3A).

In the <1 -kD filtrate reaction product obtained following κ -CGN (1 mg/L) pretreatment with rhARSB (1 $\mu\text{g/L}$), the inorganic sulfate content was 0.98 mg/L. The sulfate content following treatment of a similar quantity of C4S (1 mg/L) with similar amount of enzyme

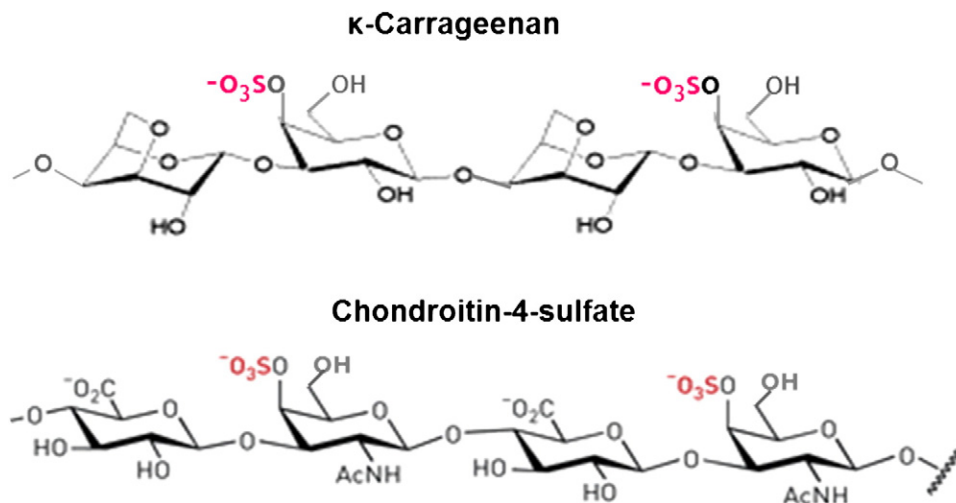


Fig. 1. Structures of κ -CGN and C4S. κ -Carrageenan has alternating galactose-4-sulfate residues and anhydrogalactose residues and α -1,3 and β -1,4 linkages. Chondroitin-4-sulfate has alternating *N*-acetylgalactosamine-4-sulfate residues and glucuronate residues with corresponding β -1,3 and β -1,4 linkages. AcNH=*N*-acetyl; $^{-}\text{OSO}_3$ =sulfate.

(1 $\mu\text{g/L}$) was 1.27 mg/L . Sulfate content present in the >1 -kD retentates was negligible (Fig. 3B). These findings indicate that κ -CGN, like C4S, acted as a substrate for rhARSB. However, exposure to κ -CGN, unlike exposure to C4S, generated ROS, and removal of the

sulfate group from κ -CGN by rhARSB reduced the ROS production, suggesting an involvement of the sulfate group of κ -CGN in the interaction that generated the ROS.

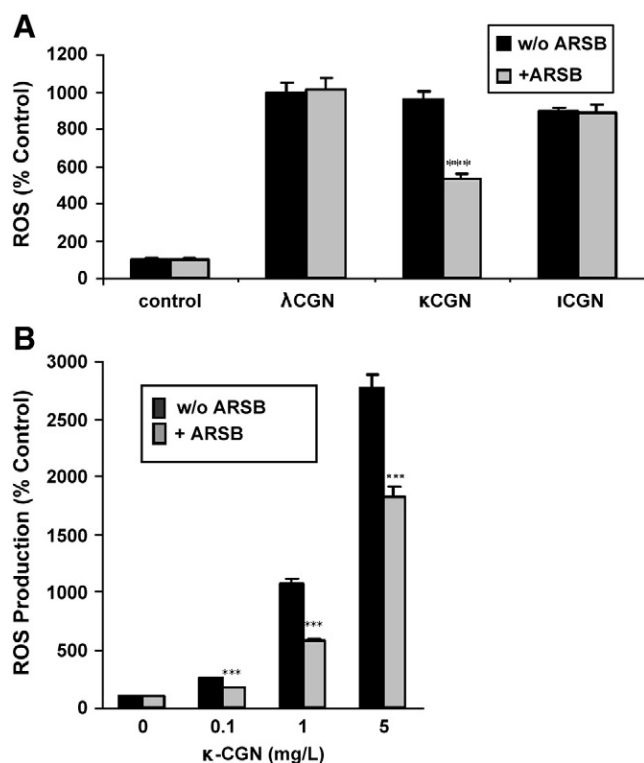


Fig. 2. Production of ROS by κ -CGN in NCM460 cells by is reduced by rhARSB. (A) The ROS production in NCM460 cells was increased by exposure to κ -, λ - and ι -CGN (1 $\text{mg/L} \times 24$ h). In contrast to λ -CGN and ι -CGN, the production of ROS from NCM460 cells by κ -CGN was reduced following pretreatment of κ -CGN with rhARSB (1 $\mu\text{g/L} \times 18$ h) ($P < .001$; 1-way ANOVA with Tukey–Kramer posttest). (B) When NCM460 cells were exposed to increasing concentrations of κ -CGN (0.1, 1 and 5 mg/L), ROS production increased to 2.5, 10.8 and 27.7 times the control. When the κ -CGN was predigested with rhARSB (1 $\mu\text{g/L} \times 18$ h) prior to treatment of the cells, the cellular production of ROS declined significantly to 1.7, 5.7 and 18.2 times the baseline levels ($P < .001$; 1-way ANOVA with Tukey–Kramer posttest).

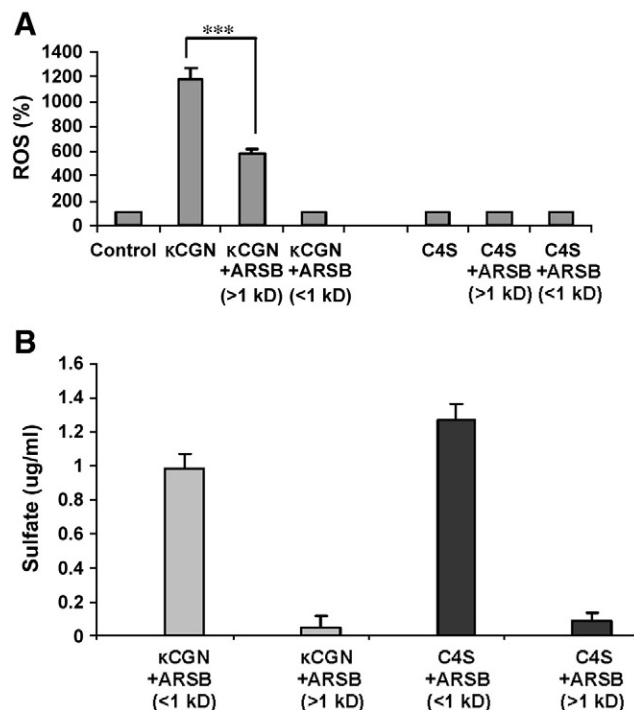


Fig. 3. Reactive oxygen species and sulfate following treatment of κ -CGN by rhARSB. (A) In contrast to the responses to κ -CGN, C4S exposure did not produce an increase in ROS production by the NCM460 cells, and there was no effect of pretreatment with rhARSB (1 $\mu\text{g/L} \times 18$ h). The ROS production in the NCM460 cells following exposure to κ -CGN was associated with exposure to the >1 -kD retentate of the CGN digest. No increase in ROS production occurred when the cells were exposed to the <1 -kD sulfate-containing filtrate. (B) Inorganic sulfate was generated by both κ -CGN and C4S when treated by rhARSB (1 $\mu\text{g/L} \times 18$ h) and was present in the <1 -kD filtrate, but not in the higher MW fraction.

3.4. Combined exposure to C4S or DS reduces the decline in ROS produced by pretreatment of κ -CGN with rhARSB

The ROS production was measured in the NCM460 cells following exposure to κ -CGN pretreated with increasing concentrations of rhARSB (range: 0–2 $\mu\text{g/L}$) (Fig. 4A). The ROS production declined as the rhARSB concentration increased, reaching a minimum at exposure

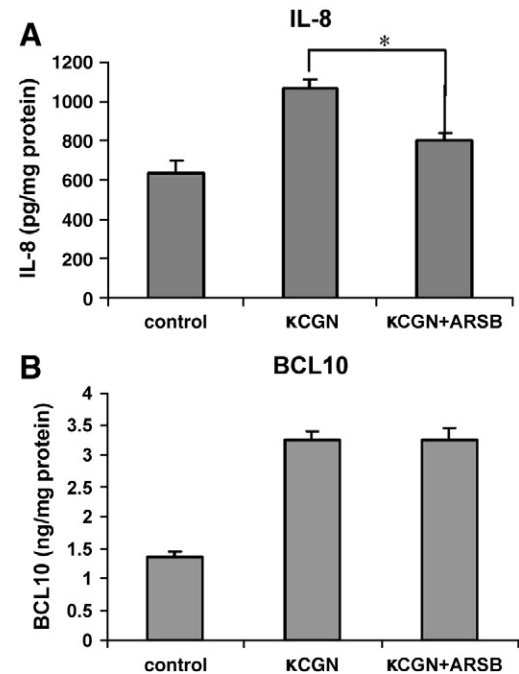
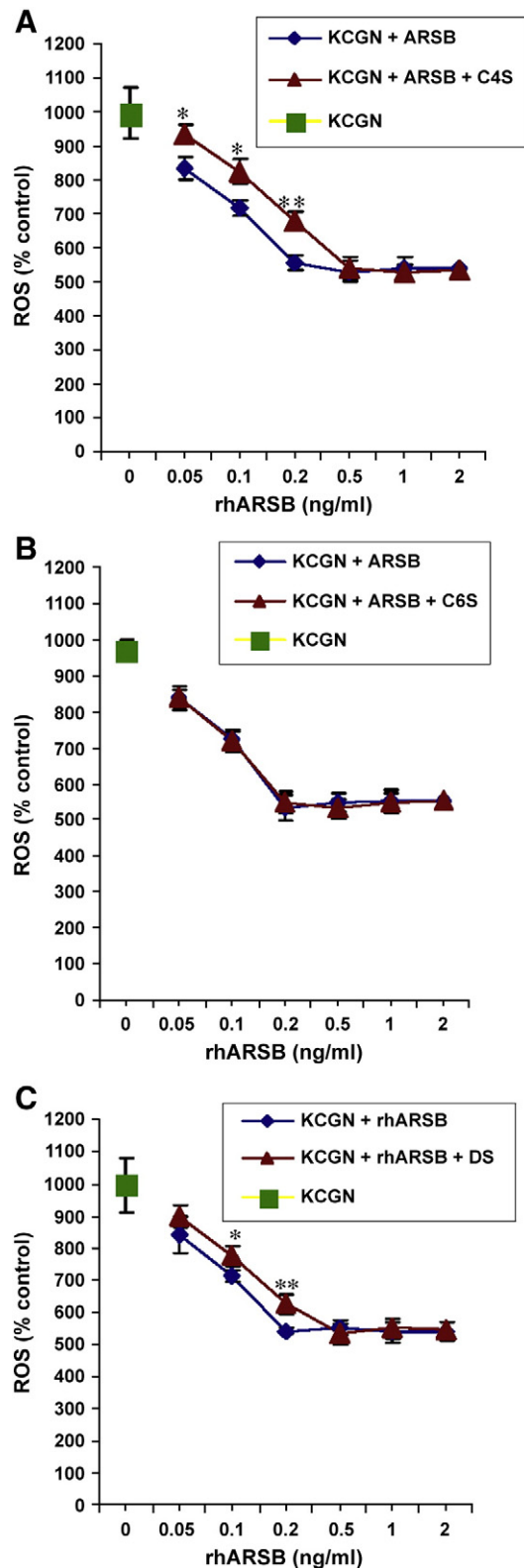


Fig. 5. Arylsulfatase B reduces CGN-induced increase in IL-8 secretion from NCM460 cells, but not the CGN-induced increase in BCL10. (A) Interleukin-8 secretion from NCM460 cells declined following combined exposure to κ -CGN (1 mg/L) and rhARSB (1 $\mu\text{g/L}$) ($P < .05$). (B) In contrast, the cellular BCL10 content was unaffected by combined exposure to κ -CGN and rhARSB. This result is consistent with the CGN-induced effect on BCL10 production proceeding by the TLR4 innate immune-mediated inflammatory cascade, rather than the ROS-mediated pathway.

to 0.5 $\mu\text{g/L}$ rhARSB. In the presence of C4S and C6S (70:30; 1 mg/L), the decline in ROS production was significantly reduced ($P = .02$ for rhARSB concentration of 0.05 $\mu\text{g/L}$, $P = .02$ for 0.1 $\mu\text{g/L}$ and $P = .0035$ for 0.2 $\mu\text{g/L}$; unpaired t test, two-tailed). Chondroitin-6-sulfate (>90% purity) in combination with rhARSB and κ -CGN had no impact on the ROS production (Fig. 4B), indicating that the decline in ROS from the C4S:C6S mixture was attributable to competition with C4S for rhARSB. Combined exposure of κ -CGN and DS to rhARSB demonstrated significantly less decline in the ROS production ($P = .03$ for rhARSB concentration of 0.1 $\mu\text{g/L}$, $P = .01$ for 0.2 $\mu\text{g/L}$; unpaired t test, two-tailed) (Fig. 4C). These results were consistent with competition between κ -CGN and C4S or DS for rhARSB. In the absence of rhARSB, C4S or DS in combination with κ -CGN had no effect on ROS production.

Fig. 4. Chondroitin-4-sulfate and DS, but not C6S, compete with κ -CGN for rhARSB and lead to less reduction in ROS production. (A) When κ -CGN (1 mg/L) was treated with rhARSB (1 $\mu\text{g/L} \times 18$ h), the ROS production from the NCM460 cells declined as the concentration of the rhARSB increased (from 0.05 to 0.5 $\mu\text{g/L}$) and then stabilized (between 0.5 and 2 $\mu\text{g/L}$). When C4S:C6S (1 mg/L; 70:30) was added to the κ -CGN and rhARSB digestion mixture ($\times 18$ h), it competed with the κ -CGN for the rhARSB. When the digestion mixture was added to the NCM460 cells, the decline in ROS was less than from the κ -CGN and rhARSB digest. The ROS production was significantly greater for rhARSB concentrations ≤ 0.5 $\mu\text{g/L}$ ($P = .02$, $P = .02$ and $P = .0035$ for rhARSB concentrations of 0.05, 0.1 and 0.2 $\mu\text{g/L}$, respectively; unpaired t test, two-tailed) in the presence of C4S:C6S. (B) Chondroitin-6-sulfate (1 mg/L) did not compete with the κ -CGN for the rhARSB (1 $\mu\text{g/L}$), and the rhARSB-induced decline in the κ -CGN-stimulated ROS production was unaffected. (C) Dermatan sulfate (1 mg/L) competed with κ -CGN (1 mg/L) for rhARSB (1 $\mu\text{g/L}$), and the reduction of ROS production in the NCM460 cells was significantly less than when the cells were exposed to κ -CGN pretreated with rhARSB. The ROS production by κ -CGN in the presence of DS was significantly greater for rhARSB concentrations of 0.1 and 0.2 $\mu\text{g/L}$ ($P = .03$ and $P = .01$, unpaired t test, two-tailed).

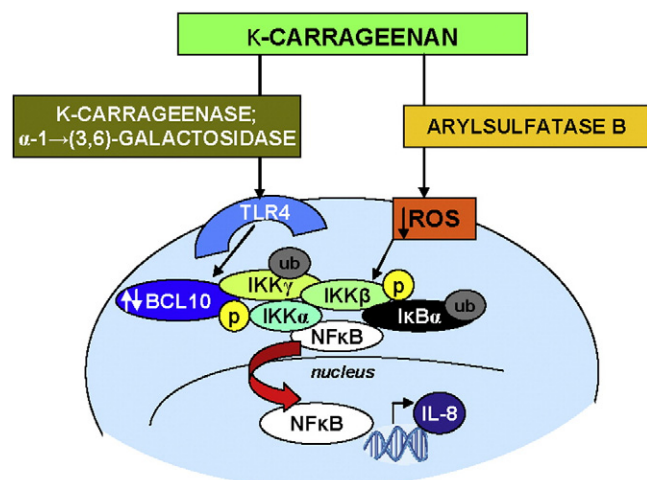


Fig. 6. Overall pathways by which enzymes can modify CGN-induced inflammation. Carrageenan causes inflammation by two major pathways. These include an ROS-mediated pathway, involving IKK β , leading to phosphorylation and ubiquitination of I κ B α , nuclear translocation of NF- κ B (RelA) and increased IL-8 expression. Carrageenan also acts through an innate immune response pathway that involves the TLR4 and BCL10, leading to ubiquitination of IKK γ , phosphorylation and ubiquitination of I κ B α , nuclear translocation of NF- κ B (RelA) and increased IL-8 expression, or leading to phosphorylation of IKK α and nuclear translocation of NF- κ B (RelB). I κ B=I κ B kinase.

3.5. Effects of rhARSB on κ CGN-induced changes in inflammatory mediators

Following exposure for 24 h to κ -CGN pretreated with rhARSB, the IL-8 secretion from the NCM460 cells was significantly reduced, consistent with the decline in ROS production ($P < 0.05$; Fig. 5A). Slight declines in IL-8 secretion also occurred following exposure of the cells to κ -CGN pretreated with rhARSB in combination with either α -1 \rightarrow (3,6)-galactosidase or κ -CGNase, but these were not statistically significant (data not shown). The κ -CGN-induced increase in BCL10 content in the NCM460 cells was unaffected by exposure to rhARSB (Fig. 5B). This result was consistent with the impact of rhARSB on the CGN-induced ROS-mediated pathway of inflammation, rather than on the CGN-induced TLR4–BCL10-immune-mediated pathway, as presented schematically in Fig. 6.

4. Discussion

Carrageenan activates inflammatory cascades through two major pathways [5–7]. These include an ROS-mediated pathway that involves phosphorylations of Hsp27, IKK β and I κ B α , leading to the nuclear translocation of NF- κ B (p65). Pretreatment of CGN by rhARSB reduced the cellular ROS production and the activation of the ROS-mediated inflammatory cascade. In contrast, ARSB had no impact on the TLR4–BCL10-mediated innate immune pathway of inflammation that is also activated by CGN. Previously, treatment of κ -CGN by κ -CGNase was shown to increase the activation of this pathway, and treatment of CGN by α -1 \rightarrow 3,6-galactosidase was shown to inhibit this pathway, but with no change in ROS production [11].

In the presence of rhARSB and C4S or DS, the production of ROS following κ -CGN exposure was significantly reduced, revealing a competition between C4S or DS and κ -CGN for metabolism by the exogenous rhARSB. Since C4S and DS are involved in so many vital cellular reactions in association with proteoglycans, including with versican, syndecan, decorin and biglycan, the potential of κ -CGN to interfere with the normal metabolism of C4S or DS by ARSB poses a complex problem in mimicry, the ramifications of which are beyond the scope of this report.

Competition between κ -CGN and C4S or DS is attributable to resemblance among their molecular signatures due to the presence of a four-sulfate group on a galactose residue in κ -CGN in an orientation that resembles the four-sulfate group of the *N*-acetylgalactosamine residue of C4S and DS. Exposure to C4S or DS did not generate any increase in ROS, indicating that they can seamlessly intercalate into the cell without exciting an electrophile at the cell surface. In contrast, CGN exposure generated ROS, and removal of the four-sulfate group by rhARSB reduced the ROS generation from κ -CGN (Fig. 3A), indicating a critical role of the sulfonyl group in the overall ROS production. The rhARSB has been demonstrated to be critical for the degradation of C4S and DS [14].

Previously, we reported that the enzymes α -1 \rightarrow (3,6) galactosidase and κ -CGNase had no effect on ROS production, but impacted on the activation of the immune-mediated pathway of inflammation mediated through TLR4 and BCL10. This impact was consistent with their enzymatic effects, to either expose or hydrolyze the α -D-GAL(1 \rightarrow 3)-D-GAL epitope and thereby modulate interaction with TLR4 [11]. The high specificity of the rhARSB for the molecular signature of κ -CGN is consistent with the lack of an interaction between ARSB and ι -CGN that has four-sulfate groups, but alternating with two-sulfates.

Since ARSB regulates the subsequent degradation of C4S and DS [15,16], the impact of κ -CGN may be mediated not only by its effects on inflammation and cell cycle progression, but by interference with sGAG and sulfatase-regulated processes [3,5–8,17–26]. Identification of ARSB and of arylsulfatase A (cerebroside-3-sulfatase) in the cell membrane of epithelial and endothelial cells and availability of recombinant ARSB for treatment of mucopolysaccharidosis (MPS) VI present new opportunities for discovery of how sulfatases, formerly considered to be present only in the lysosomes, can affect vital cellular processes, as well as how interference with their function, as by CGN, can alter normal cellular interactions and signaling [17–28]. Since CGN is so widely used in food products, including in infant formula and nutritional supplements, as well as in pharmaceuticals, cosmetics, room air fresheners and toothpaste, the resemblance between κ -CGN and C4S and DS and the interference by κ -CGN with normal ARSB function may have profound ramifications for human physiology and proclivity to disease.

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