

# The Structural Peculiarities and Cytokine-Induced Activity of Carrageenan Isolated from Red Alga *Tichocarpus crinitus*

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**Abstract:** *It were investigated the structural peculiarities of  $\kappa/\beta$ -carrageenan from red algae *Tichocarpus crinitus*. A negative-ion MALDI-TOFMS analysis revealed the presence of  $\beta$ -,  $\iota$ - and  $\gamma$ -carrageenan (the precursor of  $\beta$ -carrageenan) blocks. The in vivo cytokine-induced activity of  $\kappa/\beta$ -carrageenan was studied. According to our results  $\kappa/\beta$ -carrageenan under oral administration at dose 100 mg kg<sup>-1</sup> for 7 days significantly stimulates induction of INF- $\gamma$ , IL-12, and IL-4 promoting an increase their levels in mice sera 2, 4 and 2,5 times respectively compared with control group of animals. Moreover  $\kappa/\beta$ -carrageenan stimulates INF- $\gamma$  production more intensively than LPS.*

**Keywords:**  *$\kappa/\beta$ -carrageenan, chemical structure, mass spectrometry analysis, cytokines, cytokine-induced activity.*

## 1. Introduction

Carrageenans are a family of natural water-soluble sulfated galactans extracted from numerous species of red alga and have a wide application in food and medical industry [1, 2, 3]. The basic structural of these polysaccharides units are disaccharide, carrabiose, consisting of alternating  $\beta$ -1,3- and  $\alpha$ -1,4-linked galactose residues. Carrabiose units are classified according to the occurrence of 3,6-anhydro bridges (DA) in the  $\alpha$ -linked galactose residues and the substitution of the free hydroxyl groups with ester sulfate (S), methyl or pyruvate groups [4, 5]. The terms k- and i-carrageenans are used for polysaccharides which contains of  $\kappa$ - (G4S-DA),  $\lambda$ - (G2S-D2S6S) and  $\iota$ -carrabiose moieties (G4S-DA2S), respectively as main components [5, 6]. The large amount of carrabiose structures combined lead to a wide range of possible carrageenan structure [7, 8,]. However natural carrageenans are often hybrid of more than one of these units and made of several carrabiose moieties. The hybrid nature of carrageenans at the molecular level and also their molecular weight are responsible for changes in physico-chemical and biological properties of carrageenans compared with those of their homopolymeric ideal types.

## 2. Results and Discussion

### 2.1 The Structural Peculiarities of Carrageenan Isolated from Red Alga *Tichocarpus crinitus*

The carrageenan sample was isolated from sterile form of red algae *Tichocarpus crinitus* (*T. crinitus*) and used during the current study. The chemical structure of this carrageenan was established earlier and identified as a hybrid structure of  $\kappa/\beta$ -carrageenan [9]. The average molecular weight of studied carrageenan was measured by viscosimetric method and calculated using the Mark-Houwink equation. The  $\kappa/\beta$ -structure of studied in the current work carrageenan sample was confirmed by IR-spectroscopy method. It was shown that the IR spectrum of the isolated polysaccharide contained characteristic absorption bands for  $\kappa/\beta$ -carrageenan [9, 10]. In the

middle range in the spectrum of carrageenan an intensive absorption band at about  $1258\text{ cm}^{-1}$  characteristic to the sulfate ester groups was observed. The FT IR spectrum of examined samples showed absorbance at  $930\text{ cm}^{-1}$  characteristic to 3,6-anhydro-D-galactose residues and also absorption band at  $848\text{ cm}^{-1}$  belong to secondary axial sulfate group of C-4 of galactose residues. By all of these data the presence of  $\kappa$ -carrageenan is demonstrated. In addition an absorption band at  $892\text{ cm}^{-1}$  of non-sulfated galactose residues characteristic to  $\beta$ -carrageenan also clearly revealed in the spectrum of studied polysaccharide. The study of peculiarities of hybrid chemical structure of  $\kappa/\beta$ -carrageenan from *T. crinitus* was conducted using mass spectrometry analysis of carrageenan-derived-oligosaccharides. According the mass spectra of oligosaccharides obtained by mild acid hydrolysis (0.1N HCl, 24 h,  $37^\circ\text{C}$ ) from  $\kappa/\beta$ -carrageenan these components were built up of alternating -DA-G4S-chains with an even number of monosaccharides (even DP), where G4S occupied the reducing end, likely to those observed after enzymatic degradation of the same carrageenan sample [11]. The oligosaccharides with even DP predominated in the mixture due to application of mild acid hydrolysis at  $37^\circ\text{C}$  [12] instead of  $60^\circ\text{C}$  [13] because mild condition causes preservation of 3,6-anhydrogalactose residues resulting to increase of amount of oligosaccharides with even degree of polymerization (DP) [12]. The MALDI-TOFMS analysis of the hydrolysis products of  $\kappa/\beta$ -carrageenan demonstrated the presence of  $\beta$ -,  $\iota$ -, and  $\gamma$ -disaccharide units (the precursor of  $\beta$ -carrabiose) and in addition  $\beta/\iota$ -hybrid structures co-existing in the hydrolysis mixture along with  $\kappa/\beta$ -carratetraose.

## 2.2 Analysis of Cytokine-Induced Activity

The current study was devoted to  $\kappa/\beta$ -carrageenan influence on *in vivo* production of several soluble immune factors, such as interferons and interleukins. It is known the sulfated polysaccharides of red algae - carrageenans have an ability to stimulate of immune mediators biosynthesis including different cytokines, pro-inflammatory as well as anti-inflammatory. Earlier in *ex vivo* experiments we have shown that carrageenan ability to stimulate cytokine synthesis depends on its structural type [14]. In the current study we examined the influence of  $\kappa/\beta$ -carrageenan from *T. crinitus* on the *in vivo* production of a number of immune mediators, in particular, interferon- $\gamma$  (INF- $\gamma$ ) and cytokines, pro-inflammatory (IL-1 $\gamma$ , IL-12) and anti-inflammatory (IL-4) and in addition its action compared with bacterial lipopolysaccharide (LPS) widely known as strong inflammatory agent. Polysaccharide was used orally into mice once a day during 7 days at dose  $100\text{ mg kg}^{-1}$ , LPS (*E. coli*) was injected intraperitoneally at a dose of  $1\text{ mg kg}^{-1}$ . According to our results  $\kappa/\beta$ -carrageenan under oral administration at dose  $100\text{ mg kg}^{-1}$  for 7 days stimulates induction of INF- $\gamma$  and IL-12. The levels of INF- $\gamma$  in mice sera was 2 times more compared with control group of animals, the levels of IL-12 increased 4 times. Also it should be noticed the carrageenan stimulates INF- $\gamma$  production more intensively than LPS. It was shown the carrageenan moderately stimulates of IL-1  $\gamma$  production, so, it levels in mice sera from mice group pretreated with carrageenan was 40% higher than in control group, but 2 times less than after single intraperitoneal injection of LPS. Also, it was shown the carrageenan induces production of anti-inflammatory IL-4 and increases it levels in mice sera more than 2.5 times compared with control.

## 3. Acknowledgements

This work was supported by the Russian Scientific Fund project 14-50-00034.

## 4. References

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