In Vitro and *Ex Vivo* Studies of Antioxidant Activity of Carrageenans, Sulfated Polysaccharides from Red Algae

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Antioxidant properties of structurally different sulfated polysaccharides (carrageenans) were studied *in vitro* and *ex vivo*. Ferric reducing antioxidant activity of carrageenans and their inhibitory effects on hydroxyl radicals and superoxide anion radicals were demonstrated *in vitro*. Activity of carrageenans depends on the polysaccharide structure. Carrageenans stimulate catalytic activity of SOD from donor erythrocyte.

Key Words: *carrageenans; red algae; superoxide anion radical; hydroxyl radical; FRAP study*

The problems of chemical regulation of oxidative stress leading to various diseases, including cancer, diabetes, septic and hemorrhagic shock, Parkinson's and Alzheimer's diseases, atherosclerosis, aging, *etc.* [13], and the search for bioactive substances with antioxidant activity remain in the focus of attention of scientists engaged in experimental biology and biomedicine [9]. Antioxidant characteristics of substances isolated from aerial plant sources are sufficiently well studied. Studies of the antioxidant effects of sea alga extracts widely used in human diets attracted much recent attention [8,11,12].

Cell walls of red algae contain sulfated polysaccharides (carrageenans); these soluble alimentary fibers play an important role in homeostasis and are used in cooking and food industry. Carrageenans exhibit immunomodulating, antiviral, and anticlotting activities. Biological properties of carrageenans are closely related to their structure characterized by great variety and determined by generic appurtenance of the alga and conditions of its growth [14].

We previously found carrageenan sources among most prevalent red algae of the Russian coastline of the Japan Sea, isolated carrageenans of different types from *Gigartinaceae* and *Tichocarpaceae* families and studied their structure [1,5,15]. Experimental *in vivo* studies showed that carrageenans improve host resistance to bacterial endotoxin and this effect depends on the structural type of the carrageenan and the route of its administration [2].

Here we evaluated antioxidant activity of carrageenans of different structural types by their ferric reducing antioxidant power (FRAP), inhibitory effects towards superoxide anion radicals ($O_2^{\bullet-}$) and hydroxyl radicals ($OH^{\bullet}$), and their effects on SOD activity in donor erythrocyte.

MATERIALS AND METHODS

Carrageenans were isolated from *Gigartinaceae* and *Tichocarpaceae* algae collected in the Sivuchiya Bay (the Japan Sea) and their structures were evaluated as described previously [1,5,15]. Carrageenans of the following structural types were used in the study: kappa, kappa/beta, kappa/jota, lambda1, and lambda2, differing by the number and location of sulfate groups.

In vitro experiments. *FRAP assay* was performed by a modified method [12] using ascorbic acid as the control. The results were expressed in mg ascorbic acid equivalents (AAE) per g specimen.

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Deoxyribose method (non-site-specific formation of hydroxyl radicals). The inhibitory effect of the test samples on hydroxyl radicals was studied as described previously [7] with mannitol for positive control.

Inhibition of superoxide anion radicals. The capacity of trapping superoxide anion radicals was measured by a previously described method [10] in a 96-well plate with ascorbic acid for positive control.

The inhibitory activity of the samples was calculated by the formula: inhibitory activity (A, %)= $(1-(A_{SAMPLE}/A_{BLANK})\times 100$.

Ex vivo experiment. *Measurement of SOD activity.* The method is based on SOD inhibition of nitroblue tetrazoleum reduction by superoxide anion radical generated *in vitro* in the phenazine methosulfate–nicotinamide adenine nucleotide (reduced form) system by a method used in clinical practice [3].

The effects of carrageenans on SOD activity were studied on a pool of venous blood from 3 donors collected from the ulnar vein into sterile silicon-coated tubes with heparin (150 U/ml). Carrageenans were added to the whole blood, the mixture was incubated (1 h, 37°C), and enzyme activity in the resultant erythrocyte suspension was measured spectrophotometrically.

All measurements were carried out in 3 parallel samples. The data were statistically processed by parametric and nonparametric methods using Microsoft Excel software. The deviations were considered significant at $p<0.05$.

RESULTS

FRAP studies. Studies of carrageenans FRAP showed that it was directly proportional to their concentration and duration of the reaction. The exclusion was kappa/jota carrageenan (3.49±2.06 mg AAE/g after 4 min of the reaction).

Lambda1 and lambda2 carrageenans exhibited maximum activities (13.73±5.54 and 11.51±4.39 mg AAE/g, respectively, after 4 min of the reaction), which can be explained by high level of sulfate groups in these carrageenan types [6]. A similar relationship of the reducing power and degree of the polysaccharide sulfation was described previously for alginic acids and fucoidanes isolated from brown alga [12]. However, it is noteworthy that in our study kappa/jota carrageenan characterized by higher content of sulfate groups than kappa (9.21±4.35 mg AAE/g) and kappa/beta carrageenans (6.09±0.97 mg AAE/g) exhibited low activity in this test (3.49±2.06 mg AAE/g). Presumably, the reducing power of carrageenans depends not only on the content of sulfate groups, but also on their location and distribution along the polymer chain.

Deoxyribose method (OH•). Hydroxyl radicals are the most reactive of active oxygen metabolites and induce complex damages in biological macromolecules.

All carrageenan specimens exhibit a moderate suppressive effect on hydroxyl radicals. Kappa/beta carrageenan exhibits the least effect in this test (Fig. 1). Inhibition of hydroxyl radicals by all carrageenans is largely determined by polysaccharide concentration (Fig. 1).

Inhibition of superoxide anion radicals ($O_2^{\bullet-}$). Inhibitory activity of all specimens towards $O_2^{\bullet-}$ was similar (lambda1) or higher than that of ascorbic acid (inhibitory activity of ascorbic acid was about 30%,

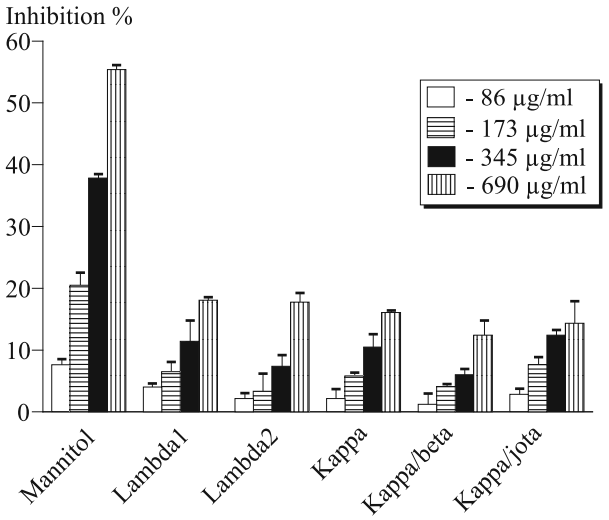

Fig. 1. Inhibitory effects of carrageenans of different structural types towards hydroxyl radicals. Control: mannitol.

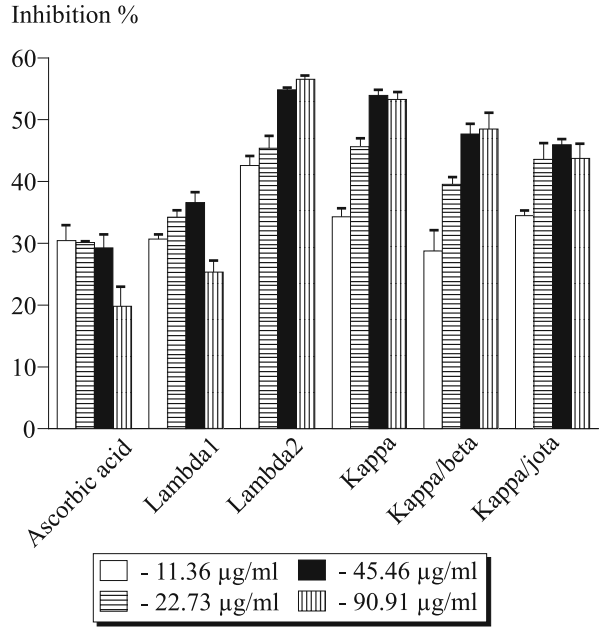

Fig. 2. Inhibitory effects of carrageenans towards superoxide anion radicals. Control: ascorbic acid.

Fig. 2). The concentration dependence of the test samples was but slightly expressed, and activities virtually did not change at polysaccharide concentrations >45.46 μg/ml. Brazilian scientists previously revealed antioxidant activity of sulfated polysaccharides from brown and red algae and showed higher inhibitory effects of fucoidane and lambda carrageenan towards superoxide anion radicals in comparison with kappa and jota carrageenans, while jota carrageenan exhibited maximum activity towards hydroxyl radicals [11]. In our study, the inhibitory activities of carrageenans increased in the following series: kappa/jota<kappa/beta<kappa<lambda2. It seems that sulfation degree is not the only parameter essential for manifestation of this property. Studies of other polyionic polysaccharides showed that antioxidant activity is determined by the location of ionic group in the carbohydrate chain and by the molecular weight of this chain [6,4]. Moreover, even orientation of the hydroxyl groups in the C-4 position for D-glucuronic and D-galacturonic acids is essential for manifestation of their antioxidant effects [4]. The relationship between fine structural characteristics of carrageenans and their activity deserves further studies.

Measurement of SOD activity. The effects of carrageenans on catalytic activity of SOD, an antioxidant defense enzyme, were studied on donor blood erythrocytes. Two polysaccharide specimens were used in *ex vivo* experiments: kappa carrageenan active towards superoxide anion radicals and lambda1 carrageenan with an effect comparable to the inhibitory effect of ascorbic acid.

The results of labda1 and kappa carrageenans on activity of SOD catalyzing the reaction of superoxide anion radical disproportioning are presented in Figure 3. In high concentrations (100 μg/ml), kappa and lambda1 carrageenans stimulate catalytic activity of this enzyme, which is a result of their antioxidant properties.

Hence, preliminary studies of carrageenan antioxidant effects showed that all the studied carrageenans exhibit potential antioxidant activity depending on their structure. Sulfated carrageenans lambda1 and lambda2 exhibited higher reducing power and inhibition of hydroxyl radicals.

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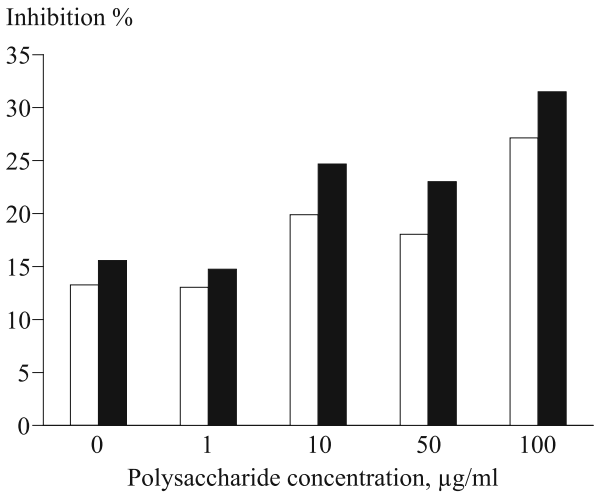

Fig. 3. Effects of lambda1 (light bars) and kappa carrageenans (dark bars) on erythrocyte SOD activity.

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