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Influence of structural features of carrageenan on the formation of polyelectrolyte complexes with chitosan

A.V. Volod'ko*, V.N. Davydova, V.P. Glazunov, G.N. Likhatskaya, I.M. Yermak

G.B Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch of Russian Academy of Sciences, Prospect 100 let Vladivostoku 159, Vladivostok 690022, Russia

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ABSTRACT

The polyelectrolyte complexes (PEC) of carrageenans (CG)-κ-, κ/β-, λ-and x-CG with chitosan were obtained. The formation of PEC was detected by Fourier-transform infrared (FTIR) spectroscopy and by centrifugation in a Percoll gradient. The influence of the structural peculiarities of CG on its interaction with chitosan was studied. The results of centrifugation showed that x-CG with a high degree of sulphation (SD) was completely bound to chitosan, unlike low SD κ-CG and κ/β-CG. Binding constant values showed there was a high affinity of CG for chitosan. CG with flexible macromolecule conformation and high SD exhibited the greatest binding affinity for chitosan. The full-atomic 3D-structures of the PEC κ-CG: chitosan in solution have been obtained by the experiments *in silico* for the first time. The amino groups of chitosan make the largest contribution to the energy of the complex formation by means of hydrogen and ionic bonds. The most probable complexes have stoichiometries of 1:1 and 1:1.5.

1. Introduction

The development of new composite materials with a wide spectrum of biological activity is one of the urgent problems of modern biology and medicine. For this development, polymeric systems employing polyionic natural polysaccharides, which play an important role in biological processes are used. They are nontoxic, biocompatible and biodegradable substances and exhibit high biological activity. They can be used for scientific and practical purposes, particularly, for the construction of various biomaterials by complexation with each other and with other oppositely charged biomolecules. Depending on the structure of the polymers and the conditions of formation soluble and insoluble, non-stoichiometric or stoichiometric composites can be formed as solution, gel, nano- and microparticles, films, membranes, and liquid-crystalline dispersions [1–3]. In recent years, various composite materials have used polyionic polysaccharides of marine origin. Chitosan and carrageenan are the most interesting and promising polysaccharides for production using such polymeric systems.

Chitosan, the cationic (1–4)-2-amino-2-deoxy-β-D-glucan, with degree of acetylation typically close to 0.20, is predominantly produced from marine chitin [4]. Chitosan have a wide spectrum of biological activities, and are particularly useful in the medical fields of wound healing and oral delivery and in the food industry [1,2,5–7].

Among polyanions, a special place is occupied by the sulphated polysaccharides of red algae—carrageenans (CG), because of their structural diversity and unique physical and chemical properties [8]. Carrageenans are a family of natural water-soluble linear sulphated galactans extracted from numerous species of red algae. They are composed of alternating 3-linked β-D-galactopyranose and 4-linked α-D-galactopyranose or 4-linked 3,6-anhydrogalactose forming a disaccharide repeating unit. CGs are classified according to the number and position of sulphated esters (S) and by the occurrence of 3,6 anhydro-bridges in the α-linked residues (DA unit) found in gelling galactans [9,10]. Currently, the most studied CGs have a regular structure, such as κ-, λ-, ι-CG. They are distinguished by the presence of 1, 2 and 3 ester-sulphate groups per repeating disaccharide unit, respectively and are widely applied in various fields. However, natural polysaccharides often have irregular hybrid structures, and are determined by the species affiliation of the algae and the condition of their habitat [8]. The importance of CGs in pharmaceutical development in

* corresponding author. Fax: +7 4232314050.
E-mail address: morskaia@list.ru (A.V. Volod'ko).

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recent years has been shown. These pharmaceutical applications depend on the biological and physical-chemical properties of the polysaccharides, which in turn depend on their polysaccharide primary structure [11–15].

Polyelectrolyte composites based on chitosan and CG can be obtained in different forms. Hydrogel beads based on chitosan and CG have been studied as controlled release devices for colon-specific delivery of sodium diclofenac [16]. Tomida et al. [17] have suggested that κ-CG-chitosan membrane spherical capsules can release theophylline as a model drug from the capsules. Tapia et al. [18] evaluated the possibility of using mixtures of chitosan and/or polyelectrolyte complexes of κ-CG and chitosan in tablet form as a prolonged release system, using diltiazem hydrochloride as a model drug.

A soluble form of these complexes could enhance the application by combining the useful properties of its polysaccharide constituents. The complex formation process depends on the chitosan molecular weight and the concentrations and ratios of the starting polysaccharides [19]. However, the relationship between the chemical structure of CGs and the formation process of PEC has still not been thoroughly studied. The primary goal of this study was to examine the influence of the structural peculiarities of carrageenan on its interaction with chitosan and PEC formation.

2. Materials and methods

2.1. Chitosan

A chitosan sample with molecular weight of 115 kDa and 6% degree of *N*-acetylation was obtained by alkaline treatment of crab chitin according to the published protocol [20]. It was deacetylated with the mixture of 40% aqueous solution of NaOH with isopropyl alcohol (1:16 v:v) under heating for 7 h at 100 °C. The pellet was filtered and dissolved in water acidified with hydrochloric acid (pH 3.5), dialyzed against water, and lyophilized [21].

2.2. Carrageenans

The samples of CGs were isolated by extraction with hot water from the algae family Gigartinaceae—*Chondrus armatus* and Tichocarpaceae—*Tichocarpus crinitus.* The algae were collected at The Peter the Great Bay (Sea of Japan) washed with tap water in order to remove excess of salt. Bleaching of the seaweed was achieved by maintaining the specimen in pure acetone for 3 days prior being dried in the air. Dried and milled algae (50 g) were suspended in hot water (1.5 L) and the polysaccharides were extracted at 90 °C for 2 h in a water bath. The polysaccharides were fractionated into gelling KCl- insoluble and non-gelling KCl-soluble fractions and their structures were established according to a published protocol [22,23].

2.3. Complexes chitosan–carrageenan

The complexes of CGs with chitosan were prepared by mixing solutions the initial components at the given ratios. The concentration of CG in the complex for all experiments was 0.5 mg/mL. The mixture was incubated for 15 min.

2.4. Analytical methods

The content of carrageenan was determined by reaction of the polysaccharide sulfate group with Taylor's blue (1,9-dimethylmethylene blue) [24]. The optical density was measured at 535 nm on a μ-Quant spectrophotometer (Bio-Tek Instruments Inc., USA). The content of chitosan was determined by reaction of the amino group with 2,4,6-trinitrobenzenesulfonic acid (TNBS) [25]. The optical density was measured at 410 nm on the μ-Quant spectrophotometer (Bio-Tek Instruments Inc., USA).

2.5. Molecular weight determination

The viscosity of carrageenan and chitosan solutions (0.1–1.0 mg mL^{-1} in 0.1 M NaCl and 2.0–10.0 mg mL^{-1} in 0.2 M NaCl/0.2 M AcOH) was measured in a modified Ubbelohde viscometer (Design Bureau Pushchino, Russia) with capillary diameter 0.3 mm at 25 °C, the time accuracy being with in ±0.1 s. The intrinsic viscosity of the samples was calculated by the extrapolation of the dependence $\ln(\eta) \times C^{-1}$ to infinite dilution using the least square method. Viscosimetric molecular weights of polysaccharide samples were calculated using the Mark–Houwink equation: $[\eta] = K \times M^{\alpha}$, where $[\eta]$ is the intrinsic viscosity and K and α are empirical constants [26,27].

2.6. Determination of solution turbidity

Complexes were prepared by mixing the initial components in the given ratios. The concentration of carrageenan in the complex during all experiments was 0.5 mg mL^{-1}. The absorption of the mixture was determined on the μ-Quant spectrophotometer at 630 nm. The carrageenan solution of corresponding concentration was used as control. The value of its optical density was taken into account when calculating the turbidity.

Solution turbidity (τ) was calculated using the formula

$\tau = (100 - e^{-A}) \times 100,$

where A is the optical density at the given wavelength [19].

2.7. Titration of the tropaeolin 000-II (orange II)–chitosan complex with CG solution

To 80 μL of 0.005 M phosphate buffer (pH 5.0), 40 μL of tropaeolin 000-II (sodium salt of 4-(2-hydroxy-1-naphthylazo) benzenesulfonic acid) solution (0.08 mg mL^{-1}) and 20 μL of chitosan solution (100 μg mL^{-1}) were added. The mixture was incubated for 20 min at 25 °C. Then the resulting complex was titrated with solution (200 μg mL^{-1}) by adding different portions of CG. The absorption was determined with a μ-Quant spectrophotometer (Bio-Tek Instruments Inc., USA) at 483 nm in three parallel samples, and the mean arithmetic value was calculated. The value of $\Delta A = A_{exp} - A_0$ was calculated, where A_0 and A_{exp} were absorptions of the solutions before and after the addition of CG, respectively. The values of ΔA_{max} and the binding constant were determined from the Scatchard dependence in $\Delta A \times C_{CG}{}^{-1}$ versus ΔA coordinates.

The number of binding sites on the CG molecule per amino group of chitosan was assessed from the saturation curve by plotting a tangent to the point that corresponded to the maximal change in the reaction mixture's absorption. Then the CG concentration corresponding to the chitosan saturation with the CG was determined.

2.8. Centrifugation in a Percoll gradient

Percoll (Sigma, 26 mL, 30%) in NaCl solution (0.15 M) was placed into a 28 mL centrifuge tube. A sample of CG, chitosan or their mixture (2 mL) was layered on the Percoll and centrifuged in an angled rotor Heraeus Biofuge Stratos (Germany) at 20,000 × *g* for 60 min. After centrifugation, the tube contents were removed through the top using a peristaltic pump. Fractions (1.5 mL) were collected. The density of the Percoll solution in each fraction was calculated using the refractive index determined on a RF-4 refractometer (LOMO). The presence of chitosan and CG in the fractions was determined