



Soluble chitosan–carrageenan polyelectrolyte complexes and their gastroprotective activity

A.V. Volod'ko^{a,*}, V.N. Davydova^a, E. Chusovitin^b, I.V. Sorokina^c, M.P. Dolgikh^c,
T.G. Tolstikova^c, S.A. Balagan^{b,d}, N.G. Galkin^{b,d}, I.M. Yermak^a

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

^b Institute of Automation and Control Processes of Far Eastern Branch of Russian Academy of Sciences, Radio St. 5, Vladivostok 690041, Russia

^c N.N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences, Lavrentjev Ave. 9, Novosibirsk 630090, Russia

^d Far Eastern Federal University, 8 Suhanova Str., Vladivostok 690950, Russia

ARTICLE INFO

Article history:

Received 29 August 2013

Received in revised form 14 October 2013

Accepted 14 October 2013

Available online 22 October 2013

Keywords:

Polyelectrolyte complex

Chitosan

Carrageenan

Supramolecular structure

AFM

Gastroprotective activity

ABSTRACT

The soluble polyelectrolyte complexes (PEC) κ -carrageenan (κ -CG):chitosan was obtained. Binding constant value ($2.11 \times 10^7 \text{ mol}^{-1}$) showed high affinity of κ -CG to chitosan. The complex formation of κ -CG:chitosan 1:10 and 10:1 w/w was shown by centrifugation in a Percoll gradient. Using atomic force microscopy we showed that the supramolecular structure of the complexes is different from each other and from the macromolecular structure of the initial polysaccharides. The gastroprotective and anti-ulcerogenic effect of κ -CG, chitosan and their complexes was investigated on the model of stomach ulcers induced by indometacin in rats. PEC κ -CG:chitosan have gastroprotective properties which depend on their composition. Complex κ -CG:chitosan 1:10 w/w possesses higher gastroprotective activity than the complex 10:1 w/w. These results suggest that the gastroprotective effect of complexes can be associated with their protective layer on the surface of the mucous membrane of a stomach, which avoids a direct contact with the ulcerogenic agent.

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1. Introduction

In recent years, polyionic polysaccharides of marine origin have been used in various composite materials. The sulfated polysaccharides of red algae–carrageenans (CG), are widely applied due to their unique physical properties, structural diversity and biological activity (Yermak & Khotimchenko, 2003). They are composed of alternating 3-linked β -D-galactopyranose and 4-linked α -D-galactopyranose or 4-linked 3,6-anhydrogalactose and classified according to the number and position of sulfate groups and by the occurrence of 3,6-anhydro bridges in α -linked residues. CG is due to its physicochemical properties provides of prolonging the necessary concentration of active substance and enhances the bioavailability of a drug. PECs, based on CG, have been applied to create systems with controlled release of active substances (Velde, Lourenço, Pinheiro, & Bakker, 2002). On the basis of CG, micro-encapsulated and tablet forms of drugs have been produced

(Thommes & Kleinebudde, 2006). It has been shown by Schmidt et al. that CG is effective complexing agent for some tranquilizers and antibiotics (Schmidt, Wartewig, & Picker, 2003).

The combination of physical properties of CG with their strong antiviral, antimicrobial and immunomodulatory activities (Yermak & Khotimchenko, 2003; Yermak et al., 2012), as well as the ability to form complexes with polycations, allows one to create different kinds of bioactive composites.

Chitosan, the cationic (1-4)-2-amino-2-deoxy- β -D-glucan, with degree of acetylation typically close to 0.20, is prevalently produced from marine chitin (Muzzarelli, 2010; Muzzarelli et al., 2012). Chitosan and its partially depolymerized derivatives and oligomers have a wide spectrum of biological activities, and are particularly useful in the area of wound healing, oral delivery, and food industry (Al-Hilal, Alam, & Byun, 2013; Busilacchi, Gigante, Mattioli-Belmonte, Manzotti, & Muzzarelli, 2013; Croisier & Jérôme, 2013; Gamboa & Leong, 2013; Muzzarelli, 1996, 2009). Carrageenan–chitosan PECs can be obtained as films (Yan, Khor, & Lim, 2001), microcapsules (Devi & Maji, 2009, 2010), microspheres (Muzzarelli, Stanic, Gobbi, Tosi, & Muzzarelli, 2004) and gels (Mitsumata et al., 2003) to be used as drug delivery agents (Li et al., 2013), cosmeceuticals (Kamel & Abbas, 2013), nano-layered coatings (Pinheiro, Bourbon, Quintas, Coimbra, & Vicente, 2012),

* Corresponding author at: G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Far-East Branch of the Russian Academy of Sciences, pr. 100-letya Vladivostoka, 159, Vladivostok 690022, Russia. Tel.: +7 4232310719; fax: +7 4232314050.

E-mail address: morskaia@list.ru (A.V. Volod'ko).

dispersants (Tzoumaki, Moschakis, & Biliaderis, 2013) and beads (Saleem, Kotadia, & Kulkarni, 2012).

Obtaining soluble CG:chitosan complexes can expand their application, owing to combination of the useful properties of their constituent polysaccharides or enhancing biological activity. To study the biological effects of CG:chitosan PECs it is necessary to know in detail the complex formation process and supramolecular structure. As it was shown earlier by us, soluble complexes κ -CG:CH may be obtained preferentially by mixing the starting components at given ratios. The formation process of complexes depended on the chitosan molecular weight and the concentrations and ratios of starting polysaccharides (Volod'ko, Davydova, Barabanova, Solov'eva, & Ermak, 2012). This work is devoted to the study of the formation and macromolecular organization of κ -CG:chitosan complexes and to the investigation of their gastro protective and anti-ulcerogenic activities.

2. Materials and methods

2.1. Chitosan

A sample of chitosan was obtained by alkaline deacetylation of chitin (Nabereznykh, Gorbach, Likhatskaya, Davidova, & Solov'eva, 2008). In this work we used sample of chitosan with molecular weight 115 kDa and a degree of *N*-acetylation 6%. The molecular weights of the polycations were determined as published earlier (Davydova, Yermak, Gorbach, Drozdov, & Solov'eva, 2000).

2.2. Isolation of κ -carrageenan

A sample of κ -CG was isolated by extraction with hot water from *Chondrus armatus*. In this work we used sample of κ -CG with molecular weight 311 kDa. The methods of extraction and κ -CG structure determination have been published (Yermak, Kim, Titlynov, Isakov, & Solov'eva, 1999).

2.3. Analytical methods

The content of κ -CG was determined by reaction of the polysaccharide sulfate group with Taylor's blue (1,9-dimethylmethylene blue) (Keler & Nowotny, 1986). 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 trinitrobenzenesulfonic acid (TNBS) (Inman & Dintzins, 1969). The optical density was measured at 410 nm on the μ -Quant spectrophotometer (Bio-Tek Instruments Inc., USA).

2.4. Titration of the tropaeolin 000-II (Orange II)–chitosan complex with κ -CG solution

To 80 μ l of 0.005 M phosphate buffer (pH 5.0), 40 μ l of tropaeolin 000-II (sodium salt of 4-(2-hydroxy-1-naphthylazo)benzenesulfonic acid) solution (0.08 mg/ml) and 20 μ l of chitosan solution (100 μ g/ml) were added. The mixture was incubated for 20 min at 25 °C. Then the resulting complex was titrated with solution (200 μ g/ml) by adding different portions of κ -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 $\Delta D = D_{\text{exp}} - D_0$ was calculated, where D_0 and D_{exp} were absorptions of the solutions before and after the addition of κ -CG, respectively. The values of ΔD_{max} and the binding constant were determined from the Scatchard dependence in $\Delta D/C_{\kappa\text{-CG}}$ versus ΔD coordinates. The chitosan saturation (*Q*) with κ -CG molecules was determined from the ratio $\Delta D/\Delta D_{\text{max}}$.

The number of binding sites on the κ -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 κ -CG concentration corresponding to the chitosan saturation with the κ -CG was determined.

2.5. 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 κ -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 $\times$ 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 κ -CG in the fractions was determined by the reaction of polysaccharide amino group with TNBS (Inman & Dintzins, 1969) and by the reaction of sulfate group with Taylor's blue (Keler & Nowotny, 1986), respectively.

2.6. Atomic force microscopy (AFM)

The samples were dissolved in distilled de-ionized water at concentrations of κ -CG and chitosan of 0.1 mg/mL, 0.01 mg/mL; PECs were prepared at the same concentrations at the ratios κ -CG:chitosan 10:1 and 1:10 w/w. Aliquots (12 μ l) of each sample in an aqueous solution were deposited onto freshly cleaved mica and dried at 37 °C for 24 h. Morphology of polysaccharide and their complexes was studied in air by AFM (Solver P47) in tapping contact mode using a 10 nm radius tip. The topographical parameters of the sample macromolecular structure had been automatically calculated by a self-developed program "Balagan's Grain Analysis". The program allows us to separate objects from the substrate and determine topographical parameters of each object: the lateral sizes, height, volume, occupied area, etc.

The analyzed AFM image is identified with a rectangular two-dimensional matrix where each cell contains a measured height value.

At the first stage of the analysis, the image is smoothed and divided into rows and columns. Then based on each column (row), another two columns (rows) are calculated: in the first one, the local maxima are eliminated, in the second one – the local minima. The calculated four sets of the columns and rows are smoothed out (it is possible to choose the smoothing method) and are interpolated. The interpolation runs as follows: using the method of least squares, by four points, the closest to the interpolated point, a parabola equation is deduced. The interpolated point height is determined from the parabola equation. Then from the interpolated columns and rows an averaged image is constructed.

At the second stage, the image splits into groups of pixels. To do this, the pixels are sorted by descending of their height. Then, starting from the beginning of the pixels list, each pixel is colored (similar to the numbering) by the following rules:

1. The pixel with coordinates (i_1, j_1) is adjacent to the pixel (i_2, j_2) , if $|i_1 - i_2| \leq 1$ and $|j_1 - j_2| \leq 1$.
2. If near the pixel there are no colored pixels, it is assigned a previously unused color (it will form a new group of pixels).
3. If near a pixel there are colored pixels, it is assigned a color of the nearest local maximum.

On the third stage, pixel groups are combined according to their height and relative position on the plane. The program does the

same actions as on the second stage, but it works with pixel groups instead of single pixels.

On the fourth stage, pixel groups are combined considering pixel groups relative position in space and their size. In addition, the program examines the spatial distribution of pixels within the group, to reduce the effects of distortion in the case of the objects of interest located on the edge of a terrace. When this step finishes, it is possible to filter pixel groups by size and space position.

At the fifth stage, the subtraction of the substrate there occurred. The substrate relief is determined by analysis of the location of the pixel groups in space.

After each object is separated from the substrate, the problem of the object parameters calculation is trivial.

2.7. Investigation of gastroprotective and antiulcerogenic activity

The experiments were carried out in three independent series on Vistar female rats 200–220 g weighing in accordance with the Guidelines for the Care and Use of Laboratory Animals. The animals were given standard granulated food and water ad libitum. Each group consisted of 6 animals.

Model of stomach ulcers. All animals were deprived of food with maintenance of free access to water during 24 h. Then the rats were orally administrated 20 mg/kg indomethacin ("Fluka BioChemica", Italy) suspended in water with Tween-80 ("ICN Biomedicals, Inc.", USA), and 2 h later the animals were fed. One day later the rats were anesthetized with chloroform and necropsied, their stomachs were removed and ulcers of the mucous membrane were counted under a binocular loupe.

The samples of polysaccharides and their complexes were injected intraperitoneally in two modes. When evaluating the gastroprotective effect of κ -CG, chitosan and their complexes, the samples injected once 1 h before indomethacin administration. In the study of anti-ulcerogenic action polysaccharides were injected during three days prior to the introduction of indomethacin (the last injection was 24 h before ulcerogenic agent). Control animals received an equivalent amount of water, the reference group was injected with the gastroprotective drug Phosphalugel (aluminum phosphate gel, "Astellas Pharma", Netherlands) in the same dose and mode as the test polysaccharides. Pauls indexes (PI) were counted in each experiment according to the formula: $PI = A \cdot B / 100$, where A – the average number of ulcers per animal; B – the number of animals with ulcers in the group (%).

Antiulcer activity (AUA) was defined as the ratio of PI of the control group divided to PI of the treatment group. It is considered that the monitoring agent has antiulcer activity on the assumption of $AUA > 2$ units.

PECs κ -CG:chitosan were injected orally at a dose of 5 mg/kg or 0.5 mg/mL/100 g body weight. In this case the dose of the components in the complex were: 0.45 and 4.55 mg/kg. Solutions κ -CG and chitosan were injected at a dose of 4.55 mg/kg. The Phosphalugel was injected at 4.55 mg/kg.

The results were analyzed using Statistica 6 software. The differences were significant at $p < 0.05$.

3. Results and discussion

The method of competitive binding κ -CG with a complex of chitosan/anionic dye was used to determine the binding constants of the polyanion (κ -CG) with a polycation (chitosan). The tropaeolin OOO-II anionic dye (sodium 4-(2-hydroxy-1-naphthylazo)benzenesulfonate) was used as an anionic dye. The method (Glazunov & Gorbach, 1999) is based on the displacement of CG dye from its complex with chitosan. The number of seats of binding was calculated. The number of sulfate groups of κ -CG

associated with one amino group of chitosan was close to stoichiometric. The binding constants were calculated using Scatchard and Hill plots (Table 1). A linear character of the Scatchard plot assumes that no cooperativity during the reaction (Nørby, Ottolenghi, & Jensen, 1980) and that one type of binding sites involves into this process. This binding constant value ($2.11 \times 10^7 \text{ mol}^{-1}$) showed high affinity of κ -CG to chitosan.

The method of centrifugation in Percoll gradient was used for direct detection of the complex formation. Fig. 1 shows the results of Percoll gradient centrifugation of the complex initial components (Fig. 1a) and its mixtures at the ratio of κ -CG:chitosan 1:10 and 10:1 w/w (Fig. 1b and c).

It can be seen from Fig. 1 that the curves of κ -CG:chitosan mixtures (Fig. 1b and c) differed from those of the initial polysaccharides (Fig. 1a). Under the centrifugation conditions chitosan formed a one zone of density and settled in the lower part of the gradient whereas κ -CG was separated into two fractions in the upper and middle parts of the gradient. The coincidence of the maxima of the elution curve for chitosan and κ -CG in their mixture indicated that a κ -CG:chitosan complex formed (Fig. 1b and c).

Two types of complexes with different densities formed irrespective of the initial components ratio. In the case of the polyanion high content in the mixture (κ -CG:chitosan 10:1 w/w (Fig. 1b)), part of the κ -CG remains unbound, otherwise (κ -CG:chitosan 1:10 w/w (Fig. 1c)) part of the chitosan remains free.

We studied the macromolecular structure of the κ -CG:chitosan complexes, obtained at different ratio, compared with the initial polysaccharides by AFM (Fig. 2). Concentrations of the components were the same as in the complexes.

According to the AFM images shown in Fig. 2a, chitosan at a concentration of 0.01 mg/mL produces a homogeneous and very smooth (root-mean-square roughness (RMS) – 0.13 nm) film, which is sensitive to AFM probe influences. At the scanning of chitosan in tapping contact mode, during recording one of the image lines, there was self-excited oscillation of the cantilever. As a result, the probe tip impacts the surface for several times with increased force. The crack on the film surface was observed during rescanning of the same area.

The basis of the chemical structure of the κ -CG is a disaccharide repeating unit, which contains the 3,6-anhydrogalactose. It determines the κ -CG ability of forming a double helical. As shown on the AFM images (Fig. 2b), κ -CG at concentration 0.1 mg/mL forms a three-dimensional network structure with height about 2.5 nm that indicates on intermolecular association of the polysaccharide "side-by-side" (Funami et al., 2007). The network is continuous and composed of branched fibers. The average wall thickness of the network is ($C_{\kappa\text{-CG}} = 0.1 \text{ mg/mL}$) 0.05 μm . Fig. 2d shows a histogram of the meshes area distribution. According to that observed for κ -CG unimodal size meshes area distribution, with the maximum at about 1600 nm².

The macromolecular structure of PEC κ -CG:chitosan 10:1 w/w was different from structure of the initial components with the same concentrations of polysaccharides, that in the complex. As one can see from Fig. 2, the structure of the complex consists of several layers. The bottom layer has a mesh structure similar to the structure of κ -CG, but with considerably smaller wall thickness (0.025 μm). The histogram of the meshes area distribution (Fig. 2e) has a bimodal character with two maxima at about 150 and 300 nm². This is 10 times smaller than the area corresponding to the peak of the histogram for the κ -CG. The second layer is disposed over the reticulated structure and it is composed of long twisted fibrils. In this PEC the amount of the κ -CG was 10 times greater than chitosan, and perhaps the two layers correspond to two types of complexes (heavy and light) which were observed during the centrifugation of PEC in Percoll gradient (Fig. 1b and c).

Table 1
Binding parameters of chitosan with κ-CG.

Binding constant, 10 ⁷			The number of SO ₄ ²⁻ to 1 NH ₃ ⁺	Quantity chitosan associated with 1 mg κ-CG in saturation point, mg
Scatchard's method	Hill's method	Mean value		
2.11 ± 0.31	1.98 ± 0.31	2.04 ± 0.26	1.29 ± 0.13	0.33 ± 0.03

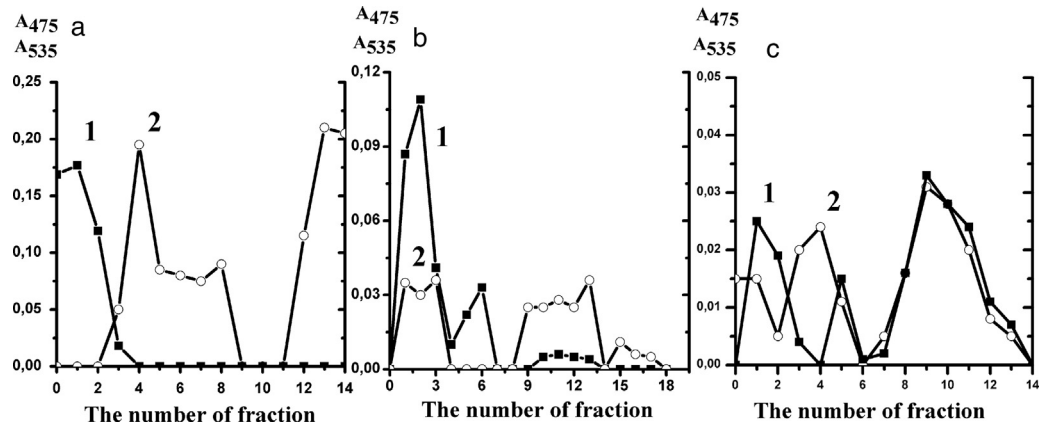


Fig. 1. Centrifugation in Percoll gradient: a – initial polysaccharides; b – κ-CG:chitosan mixtures 1:10 w/w; c – κ-CG:chitosan 10:1 w/w; 1 – content of amino groups chitosan (A_{474} nm); 2 – content of sulfate groups κ-CG (A_{535} nm).

Increasing of the chitosan concentrations by 10 times results in supramolecular structure changes. By the AFM data (Fig. 3a), chitosan composed of densely packed spherical particles. The RMS value is 14.3 nm, corresponding to at least a level surface, unlike chitosan at a lower concentration (Fig. 2a).

κ-CG at low concentrations ($C=0.01$ mg/ml) forms long thread-like structures. Fibers cross over one another, which lead to the increase of the height at the crossover points. That may correspond to the formation of the double-structure characteristic of κ-CG (Sokolova et al., 2013).

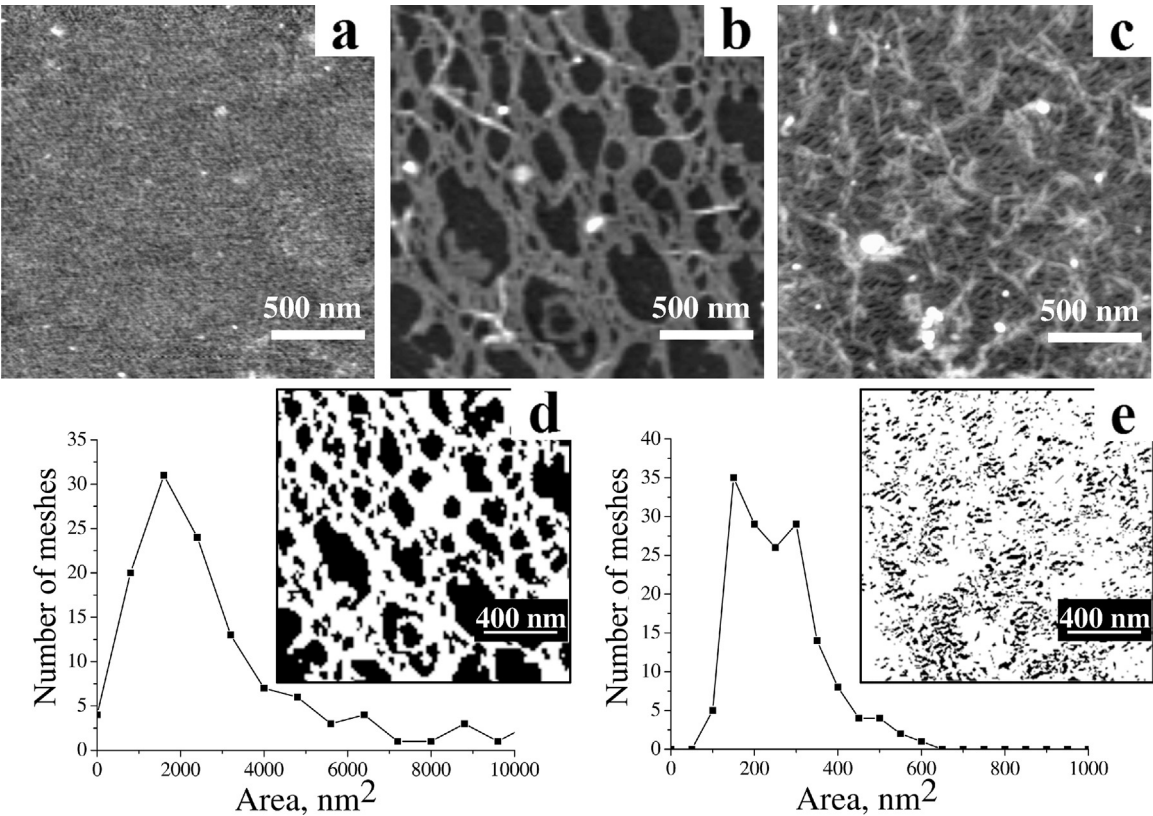


Fig. 2. AFM topography images: a – chitosan, $C=0.01$ mg/ml, b – κ-CG, $C=0.1$ mg/ml, c – PEC κ-CG:chitosan 10:1 w/w; and a histogram of the meshes area distribution: d – κ-CG, $C=0.1$ mg/ml; e – PEC κ-CG:chitosan 10:1 w/w; on the inserts one can see the calculation masks.

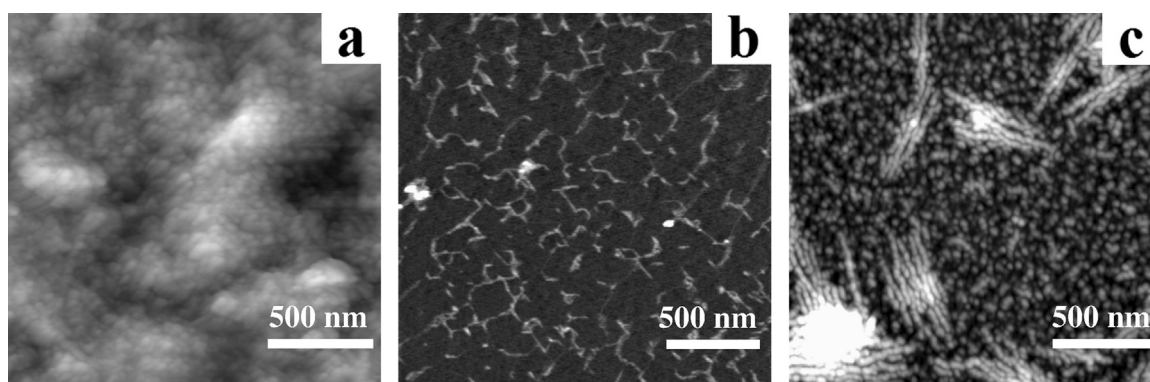


Fig. 3. AFM topography images: a – chitosan, C=0.1 mg/ml, b – κ -CG, C=0.01 mg/ml, c – PEC κ -CG:chitosan 1:10 w/w.

Table 2

Comparative gastroprotective activity of PECs κ -CG:chitosan.

Sample	The average number of ulcers per 1 rat	The total number of ulcers in the group (6 rats)	Pauls index	Antiulcer activity, units
Control	5.8 ± 1.1	35	5.8	–
κ -CG:chitosan 10:1 w/w	2.8 ± 1.0 ($p=0.07$)	17	2.3	2.5
κ -CG:chitosan 1:10 w/w	1.8 ± 1.0 ($p=0.03$)	11	0.9	6.4

$p < 0.05$ —differences with control are significant.

Table 3

Gastroprotective activity κ -CG, chitosan and their complexes in comparison with Phosphalugel.

Sample	The average number of ulcers per 1 rat	The total number of ulcers in the group (6 rats)	Pauls index	Antiulcer activity, units
Control	9.2 ± 1.6	55	9.2	–
κ -CG	2.8 ± 1.5 ($p=0.01$)	17	1.9	4.8
Chitosan	6.2 ± 1.9 ($p=0.24$)	37	6.2	1.5
κ -CG:chitosan 10:1 w/w	4.2 ± 1.3 ($p=0.03$)	25	4.2	2.2
κ -CG:chitosan 1:10 w/w	1.3 ± 1.0 ($p=0.002$)	8	0.4	23.0
Phosphalugel	1.0 ± 0.4 ($p=0.0004$)	6	0.7	13.1

$p < 0.05$ —differences with control are significant.

The AFM image of PEC κ -CG:chitosan (1:10 w/w) shows the formation of morphologically heterogeneous structure, which is different from those of PEC κ -CG:chitosan (10:1 w/w) and from initial polysaccharides. As one can see from Fig. 3, sub-layer consists of spherical segments that possibly correspond to free chitosan. The heterogeneity observed in the structure is in line with the data obtained by centrifugation, whereby PEC divided in the Percoll gradient into two fractions (Fig. 1c). Also tightly packed formations of complex κ -CG:chitosan 1:10 was observed which probably represent fibrils of κ -CG (average length of $0.5 \mu\text{m}$) consisting of segments chitosan.

The observed features of the molecular organization of the PECs κ -CG:chitosan reveal that the dependence of the ratio of the initial polysaccharides can have a significant impact on their biological effects in the body. We investigated gastroprotective and anti-ulcerogenic effect of these complexes (10:1 and 1:10, w/w) to test this assumption.

It was found that the PECs κ -CG:chitosan had various gastroprotective effects depending on its composition. PEC κ -CG:chitosan 10:1 w/w reduced ulcers in the stomach in two times, and PEC κ -CG:chitosan 1:10 w/w in three times in comparison with the control (Table 2). Thus when the samples are injected 1 h before the ulcerogenic impact, the complex with a higher content of κ -CG exhibits a moderate activity, while the complex with excess of chitosan has significant gastroprotective activity.

The further study κ -CG, chitosan and their PEC in comparison with drug Phosphalugel showed that the activity of the complex with a high content of polycation (κ -CG:chitosan 1:10 w/w) exceeded the activity of the reference drug, while the PEC with a high content of polyanion (κ -CG:chitosan 10:1 w/w) demonstrated a low gastroprotective effect (Table 3). It is interesting to note the low activity of initial polysaccharides (4.55 mg/kg, which corresponds to the maximum content of the complexes), as compared

Table 4

Anti-ulcerogenic activity κ -CG, chitosan and their PEC at the prophylactic injection to rats with indomethacin-induced stomach ulcer.

Sample	The average number of ulcers per 1 rat	The total number of ulcers in the group (6 rats)	Pauls index	Antiulcer activity, units
Control	7.0 ± 1.2	42	0.7	–
κ -CG	7.3 ± 1.4	44	0.7	1.0
Chitosan	7.5 ± 1.8	45	0.7	1.0
κ -CG:chitosan 10:1 w/w	9.2 ± 1.7	55	0.9	0.8
κ -CG:chitosan 1:10 w/w	6.7 ± 1.8	40	0.7	1.0
Phosphalugel	7.2 ± 2.0	43	0.7	1.0

$p > 0.05$ at all groups—differences with control are insignificant.

with Phosphalugel. This data shows that the increase of gastroprotective effect of PEC κ -CG:chitosan 1:10 w/w may not be directly related with gastroprotective properties of chitosan.

As indicated above, chitosan and κ -CG have various biological activities, including antiinflammatory, immunomodulatory and reparative. Such activities may have a modifying effect on the stomach mucosa, increasing its resistance to the effects of pathological factors. An experiment was conducted with a three-day prophylactic injection of κ -CG, chitosan and their PEC to evaluate the possible contribution of non-specific activity of polysaccharides in antiulcer effect. The last injection of the samples was done a day before the reproduction of ulcerative lesions, which eliminated the direct contact of the tested samples with indomethacin on the mucosal surface of the stomach. The experiment shows that the introduction of polysaccharides in this mode has not reduced the amount of gastric ulcers induced versus control. There is only reducing the excitability of the gastric mucosa in animals treated with complexes, especially complex κ -CG:chitosan 10:1 w/w, resulting in a reduction of mucosal hyperemia (Table 4). Phosphalugel did not exhibit antiulcer activity in these conditions as well.

4. Conclusion

The soluble PEC κ -CG:chitosan was obtained. Binding constant value showed high affinity of κ -CG to chitosan. The complex formation of κ -CG:chitosan 1:10 and 10:1 w/w was proved by centrifugation in a Percoll gradient. Summarizing the results of biological studies, it may be noted that aqueous solutions of PEC κ -CG:chitosan ($C=0.5$ mg/ml) have gastroprotective activity which depend on their composition. Complex κ -CG:chitosan 1:10 w/w possesses higher gastroprotective activity than the complex κ -CG:chitosan 10:1 w/w. The test polysaccharides and their complexes at preliminary three-days injection do not increase own protective properties of gastric mucosal.

The differences in the effectiveness of PECs κ -CG:chitosan is likely to be associated with features of the supramolecular structure fixed by AFM. We showed that the supramolecular structure of the complexes is different from each other and from the macromolecular structure of the initial polysaccharides. According to this data, κ -CG ($C=0.01$ mg/ml) forms long threadlike structures, whereas a tenfold increase in the concentration produced mainly coarse three-dimensional networks with a large amount of meshes. Considering that the concentration of κ -CG in aqueous solution of PEC κ -CG:chitosan 1:10 w/w was 0.045 mg/ml, it can be assumed that its high gastroprotective activity is provided by a regular formation of the protective layer which formed due to the optimum conditions of complexation for surface fibrils. These results suggest that the gastroprotective effect of complexes can be associated with the formation of the protective layer on the surface of the stomach mucosa, which hinders direct contact with the ulcerogenic agent (indomethacin).

Acknowledgments

The work was supported by the Programs for Fundamental Research of the Presidium of the Russian Academy of Science "Molecular-cell biology" and by the Presidium of Far-East Branch of the Russian Academy of Science and a grant no. 12-II SD-05-006.

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