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Influence of Carrageenan (E 407) on the Membrane of Enterocytes Investigated by Fluorescent Probes Research Institute of Chemistry (Kharkiv)

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The results presented in this article were obtained in following research «Investigation of long-term effects of regular consumption of foods containing genetically modified organisms in damaged epithelial barrier of the gastrointestinal tract» (state registration number 0110 U000653).

Introduction. Food additive E 407 (carrageenan) is widely used in the modern food industry. It is sulfated polysaccharide, which is produced by extraction from red seaweed. Carrageenan serves as natural thickener in dairy, confectionery and meat products. Moreover, in experimental medicine carrageenan is widely used to model the inflammatory processes. It has been used in the pathophysiology for modeling of peritonitis, pleurisy, arthritis and carrageenan-induced edema of the limbs in rats [6, 13]. Carrageenan-induced inflammation, originally described by Winter, is an acute, non-immune and easily reproducible. Edema and erythema, hyperalgesia develop immediately after subcutaneous injection of carrageenan and they are the result of pro-inflammatory agents such as bradykinin, histamine, tachykinins, components of the complement system, reactive oxygen and nitrogen species [6]. *There is an evidence of oncogenic transformation of cells under the influence of carrageenan, the positive correlation between the use of products with this additive in the diet and an increased risk of breast carcinoma has been found [17]. Also the model of ulcero-necrotizing carrageenan-induced gastroenterocolitis* has been reported *[18]. I*n this connection, the question about the safety of carrageenan as a food additive arises. In modern literature, there are no reliable data about the effect of systematic consumption of carrageenan neither by an adult nor by child or by pregnant women. A model of moderate chronic carrageenan gastroenterocolitis without necrotizing process was elaborated at Kharkiv National Medical University on the basis of the model of necrotizing carrageenan-induced gastroenterocolitis.

At the same time, the mechanism of carrageenan action on enterocyte biomembranes during continious consumption remains unexplored. It is very urgent task, because of the spread of food products containing carrageenan in the diet of the consumers. The investigation of the mechanisms of biomembranes damage will help to develop regulation rules of carrageenan use in food.

The purpose of the research was to study the influence of carrageenan (E 407) on the lipid membrane of enterocytes in rats with fluorescent probes (ortho-hydroxy derivatives of oxazole), which non-covalently bind to cell membranes of experimental animals and have a quick response to changes in their microenvironment [3-5].

Subjects and methods. The female Wistar rats were used for the experiment. Chronic carrageenan-induced gastroenterocolitis was reproduced by the free access of animals to 1% solution of carrageenan in drinking water. Laboratory animals were divided into 2 groups. Group № 1 consisted of intact animals, group №2 consisted of experimental animals with chronic carrageenan-induced gastroenterocolitis**.** A month after the start of the experiment animals were taken from the experiment by decapitation. Manipulations with animals were carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986). The intestine was removed and placed on the cold immediately after decapitation of rats. Intestinal perfusion was performed using cooled physiological solution. Epithelial cells were detached by scraping the inner surface of the intestine by anatomical knife. A suspension of epithelial cells was made in Tris-HCl buffer (pH 7. 4). Fluorescent probes were dissolved in acetonitrile to the initial concentration of 2×10^{-4} mol / l. 10 μl of each corresponding probe was added to the suspension of enterocytes. The final concentration of each probe in the suspensions of investigated membranes is 1×10^{-6} mol/l, thus, the molar ratio of lipid / probe was 1000:1. Then, we measured the fluorescence probes O1O (2 – (2´–OH-phenyl)-5-phenyl-1,3-oxazole) and PH7 (2 – (2´–OH-phenyl)-phenanthrene [10,11] –1,3-oxazole) in physiological solutions containing enterocytes of rats with chronic carrageenan-induced gastroenterocolitis. Measurement of the fluorescence was performed by spectrofluorometer «Hitachi F4010» after 1 hour after the addition of probes to a solution of cells. The fluorescence spectra of probes were measured in the diapason of 340-600 nm with monochromator slit width of excitation and fluorescence 5 and 5 nm, respectively, and the excitation wavelength of 330 nm. The enterocytes of intact healthy animals were used as

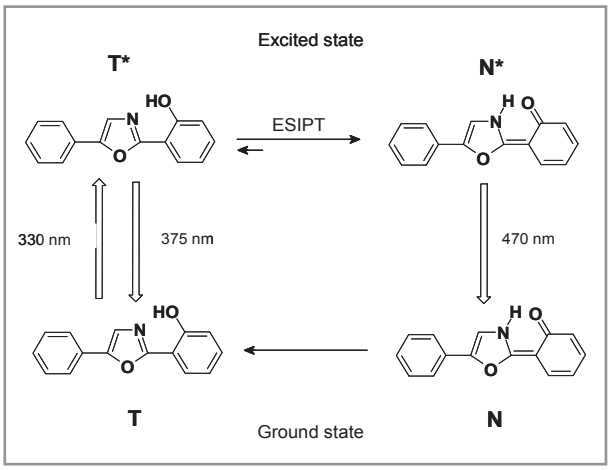

Fig. 1. Scheme of excited state proton transfer (ESIPT) reaction in 2 – (2′-OH-phenyl)-5-phenyl-1,3-oxazole (probe O1O). Upwards arrow shows the electronic excitation and downwards arrow represents the light emission (fluorescence). Corresponding maxima of fluorescence are measured in nanometers.

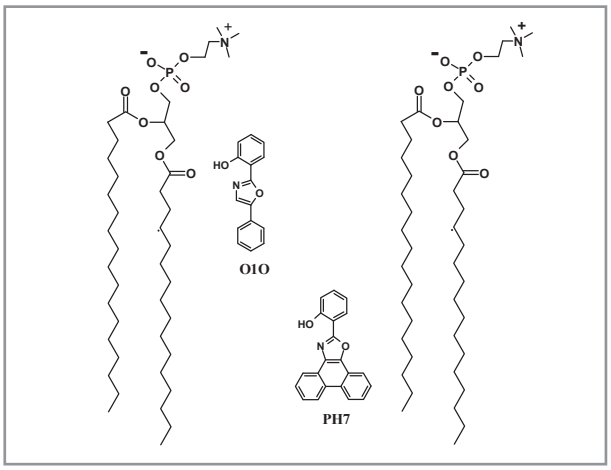

Fig. 2. Expected location and orientation of fluorescent probes O1O and PH7 based on their fluorescence properties in lipid membranes [3-5] and on the basis of their structural similarity with fluorescent probes with known localization in lipid membranes [15]. Two molecules of phosphatidylcholine from the outer monolayer are showed to denote the localization of the probes.

a control sample. The fluorescent probes, successfully used in the past for studies of biological membranes [3-5]: 2 – (2′-OH-phenyl)-5-phenyl-1,3-oxazole (probe O1O) and 2 – (2′-OH-phenyl)-phenanthrene [10,11] –1,3-oxazole (probe PH7), were used for investigation of carrageenan influence on the membrane of enterocytes. The choice of fluorescent probes O1O, PH7 (ortho-hydroxy 2,5-diaryl-1,3-oxazole) for investigation of carrageenan influence on physico-chemical properties of biological membranes was due to the fact that the fluorescent characteristics of the probes depend on the physico-chemical properties of their microenvironment: *hydrogen bonding ability* (i. e., the ability to form hydrogen bonds), the polarity and viscosity of the microenvironment [2-5, 8-10].

Results and discussion. It has been known that in the excited state the ortho-hydroxy 2,5-diaryl-1,3-oxazole is characterized by excited state proton transfer (ESIPT) reaction (**Fig. 1**): hydroxyl group in the ortho-position of the lateral benzene ring acts as protonodonor and the nitrogen atom of oxazole ring acts as proton acceptor [2, 8-10]. The result of the ESIPT reaction is the formation of phototautomeric form (T*), fluorescent in significantly longer wavelengths in comparison with the initial form (N*) [2, 8-10].

The presence of two-band fluorescence allows to perform ratiometric measurement, i. e. to use the ratio of phototautomeric form and the initial form fluorescence intensities (I_{T^*}/I_{N^*}) as a parameter for evaluation of the physical and chemical properties of the microenvironment. The use of ratiometric fluorescent probes allows to exclude the measurement error associated with the deviation of the fluorescent probe concentration (e. g., uneven distribution of the fluorescent probe in various membranes) and the measurement error associated with a deviation of fluorescence technique settings (deviation of the intensity of the exciting source, a change in focus, changes in the sensitivity of the photodetector, etc.) [10].

Compounds that differ in their lipophilicity [3-5] were selected for the present study. It is expected that the regions of localization of selected probes in the membrane are different and correspond to the lipophilicity of the probes (**Fig. 2**) [1, 3-5]. Expected location and orientation of O1O and PH7 in lipid membranes is based on their fluorescence properties in lipid membranes [3-5] and on the basis of their structural similarity with fluorescent probes with known localization in lipid membranes [1]. The location of the probes in lipid membrabes: probe O1O is located in the region of glycerol residues of phospholipids and in the region of carbonyl groups of phospholipids; probe PH7 is located in the region of methylene chains of phospholipids and in the center of the bilayer (**Fig. 2**).

A noticeable increase in the intensity and hypsochromic (i. e. short-wavelength) shift of the lonwavelength fluorescence band of probe O1O tautomeric

Table

Fluorescence intensity of probes O1O and PH7 in enterocyte membranes of animals with carrageenan-induced gastroenterocolitis

Sample	Fluorescence intensity,a. u.					
	Probe O1O			Probe PH7		
	375 nm	470 nm	I_{470}/I_{375}	425 nm	485 nm	I_{485}/I_{425}
Control	5,0	23,7	4,7	67,4	124,1	1,8
Gastroenterocolitis	5,3	79,4	15,0	64,6	117,9	1,8

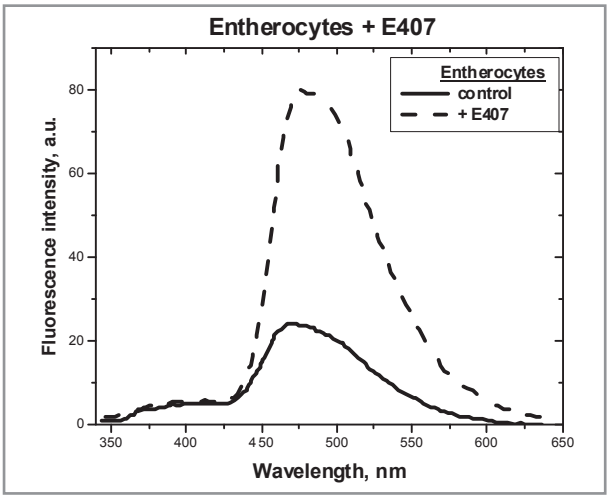

Fig. 3. The fluorescence spectra of the probe O1O in solutions containing enterocytes: (a) control (solid line), (b) the animals with carrageenan-induced gastroenterocolitis (dashed line).

form (I_{T^*}) was observed as a result of carrageenan action on enterocytes, at the same time, the intensity of the fluorescence band of the probe O1O normal form (I_{N^*}) was almost unchanged (**Table, Fig. 3**). Thus, the intensity ratio of the tautomeric and the initial forms I_{T^*}/I_{N^*} of probe O1O increases under the influence of carrageenan (E 407) (**Fig. 4**).

The increase of the intensity of the tautomeric form fluorescence band (I_{T^*}), hypsochromic (i. e. short-wavelength) longwavelength shift of the tautomeric form fluorescence band and increased ratio of I_{T^*}/I_{N^*} for O1O probe indicates a decrease in polarity and hydrogen-bonding ability of the microenvironment of probe O1O in the enterocyte membranes of rats with gastroenterocolitis. Such decrease in polarity and hydrogen-bonding ability of the microenvironment of probe O1O indicates

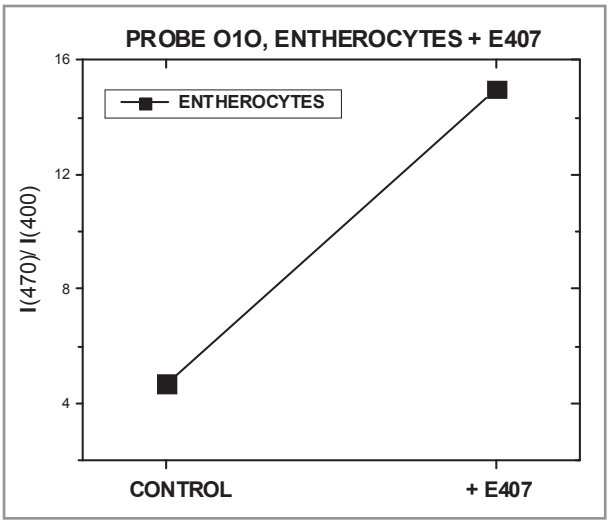

Fig. 4. The ratio of the tautomeric and the initial form intensities I_{T^*}/I_{N^*} for O1O probe in the membranes of enterocytes.

a decrease in hydration of the probe microenvironment in the erythrocyte membranes of the experimental group.

Taking into consideration the emulsifying properties of carrageenan [14, 16], the discussed reduction of hydration might be caused by the possible accumulation of carrageenan (E 407) in the regions of probe O1O localization may increase the microviscosity of the lipid bilayer and, therefore, may lead to increase of orderliness of the membrane phospholipid molecules (i. e. may lead to the increase in membrane lipid order), which in turn, may contribute in dehydration of the lipid bilayer [7, 11, 12]. According to literature [7, 12], the similar dehydration of the lipid membrane due to the increase in orderliness of the phospholipid molecules (i. e. due to the increase in membrane lipid order) is observed in case of cholesterol and its esters accumulation in the lipid bilayer. At the same time, in case of the probe pH 7 no significant change was found in its fluorescence parameters under the influence of carrageenan (E 407) on enterocytes (**Table**). The absence of changes in the regions of the probe pH7 localization (i. e., in the rather hydrophobic regions of the lipid bilayer) may be explained by the fact that, carrageenan, the structure of which have polar hydroxyl and charged sulfonyl groups, is localized in more polar regions of the lipid bilayer.

Thus, it was shown that fluorescent probe O1O (2 – (2´-OH-phenyl)-5-phenyl-1,3-oxazole) might be used as an indicator for estimation of the nature and types of changes in the structure of enterocyte membranes of rats under the influence of carrageenan (E 407). It was found that the changes in the membranes of enterocytes under the influence of carrageenan occurred in the regions of probe O1O localization, i. e. in quite polar regions of the membrane: presumably, in the region of glycerol residues of phospholipids and in the region of the carbonyl groups of phospholipids. The influence of carrageenan (E 407) didn't lead to changes in the regions of probe pH 7 membrane localization, i. e. in a more hydrophobic region of the enterocyte membranes: presumably, in the in the region of methylene chains of the phospholipids and in the bilayer center.

It has been known that the absorption of essential nutritional factors such as amino acids, glucose, vitamins takes place in the small intestine. Disturbance of enterocyte membrane, which occurs under the influence of carrageenan, reduces the absorption of nutrients in the gastrointestinal tract, which may significantly aggravate the course of gastroenterocolitis. In addition, carrageenan-induced disorder of enterocyte transport function may be explained by its possible effect on the structure of protein transporters localized at the apical surface of enterocytes, which indicates the impact of a multi-vector influence of carrageenan on the morpho-functional state of gastrointestinal tarct in experimental animals.

Conclusions.

1. The consumption of carrageenan leads to the following structural changes in the enterocyte membranes of experimental animals: to increase in microviscosity of the lipid bilayer, to increase in the orderliness of

phospholipid molecules in the membrane (i. e. due to the increase in membrane lipid order) and to dehydration of membranes.

2. The use of fluorescent probes (ortho-hydroxy oxazole derivatives) allows to estimate the type and nature of the damaging effect of carrageenan on the structural components of membranes. Thus, the mentioned method, using ortho-hydroxy oxazole derivatives as fluorescent probes, may be suggested for application in the practical medicine for the study of the negative effects on the human body similar to carrageenan food supplements.

Prospects for further research. Our data substantiate the importance of research aimed at understanding the mechanisms of action of food additives and to evaluate their safety for human health.

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ДОСЛІДЖЕННЯ ВПЛИВУ КАРАГЕНІНУ (Е 407) НА МЕМБРАНИ ЕНТЕРОЦИТІВ МЕТОДОМ ФЛУОРЕСЦЕНТНИХ ЗОНДІВ

Посохов Є. О., Ткаченко А. С., Корніенко Є. М.

Резюме. За допомогою флуоресцентних зондів – орто-гідроксипохідних оксазолу в експерименті на щурах вивчено вплив на структури мембран ентероцитів тварин доданого до їх раціону каррагеніну (Е 407). Встановлено, що каррагенін змінює гідратацію мембран ентероцитів експериментальних тварин. Показано, що під впливом каррагеніну (Е 407) відбуваються зміни у полярних областях біомембран, в той же час, вплив цієї харчової добавки не призводить до змін у гідрофобних ділянках мембран ентероцитів. Характер виявлених змін дозволяє зробити висновок про збільшення мікров'язкості мембран ентероцитів щурів з хронічним каррагенін-індуцированнм гастроентероколітом.

Ключові слова: ентероцит, біомембрана, каррагенін, флуоресцентні зонди.

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ИССЛЕДОВАНИЕ ВЛИЯНИЯ КАРРАГЕНИНА (Е 407) НА МЕМБРАНЫ ЭНТЕРОЦИТОВ МЕТОДОМ ФЛУОРЕСЦЕНТНЫХ ЗОНДОВ

Посохов Е. А., Ткаченко А. С., Корниенко Е. М.

Резюме. При помощи флуоресцентных зондов – орто-гидроксипроизводных оксазола в эксперименте на крысах изучено влияние на структуры мембран энтероцитов животных добавленного в их рацион каррагенина (Е 407). Установлено, что каррагенин изменяет гидратацию мембран энтероцитов экспериментальных животных. Показано, что под влиянием каррагенина (Е 407) происходят изменения в полярных областях биомембран, в то же время, воздействие этой пищевой добавки не приводит к изменениям в гидрофобных участках мембран энтероцитов. Характер выявленных изменений позволяет сделать вывод об увеличении микровязкости мембран энтероцитов крыс с хроническим каррагенин-индуцированнм гастроэнтероколитом.

Ключевые слова: энтероциты, биомембрана, каррагенин, флуоресцентные зонды.

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Influence of Carrageenan (E 407) on the Membrane of Enterocytes Investigated by Fluorescent Probes

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Summary. The influence on enterocytes membrane structure of added to the diet of rats food-additive carrageenan (E 407) was studied using fluorescent probes (derivatives of ortho-hydroxy oxazole). It was found that carrageenan changed hydration membrane of enterocytes in experimental animals. It was shown that carrageenan (E 407) caused changes in the polar regions of biological membranes and at the same time, the consumption of this food-additive didn't lead to any changes in the hydrophobic parts of the membrane of enterocytes. Nature of detected changes allows to make a conclusion that enterocyte membranes in rats with chronic carrageenan-induced gastroenterocolitis have increased microviscosity.

Key words: enterocytes, biomembrane, carrageenan, fluorescent probes.

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