Ultrasonic-treated fucoidan as a promising therapeutic agent

Fukoidan poddany obróbce ultradźwiękowej jako obiecujący środek terapeutyczny

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Abstract

Fucoidans represent the sulfated heteropolysaccharides that possess a wide range of important pharmacological properties. The properties of a fucoidan depend on several factors, including the molecular weight and the way of extraction. However, the selection of an optimal depolymerization method is necessary to enhance its therapeutic applications. Reducing the molecular weight of fucoidans will make it possible to use them in creating nanoparticles and nanocarriers for, among others, the targeted drug delivery. The molecular mass of the polymer can be changed by means of various methods of depolymerization. In this work, the possibility of application of ultrasonic destruction for decrease in the size of fucoidan molecules for the purpose of expansion of opportunities and spheres of their therapeutic application is considered. This is one of the simple and effective methods of depolymerization of fucoidan, which leads to a decrease in molecular weight without significant structural changes in macromolecules. In addition, methods and potential applications of the ultrasonic extraction of fucoidan from seaweed and the possibilities of their combination are discussed, as well as other physical or chemical methods of extraction.

Key words: antioxidant activity, fucoidan, depolymerization, ultrasonic treatment, ultrasonic extraction

Streszczenie

Fukoidany to siarczanowane heteropolisacharydy o szerokim zakresie farmakologicznie ważnych właściwości. Właściwości fukoidanu zależą od wielu czynników, w tym masy cząsteczkowej i metody ekstrakcji. Jednak w celu poszerzenia możliwości zastosowania terapeutycznego konieczny jest dobór optymalnej metody depolimeryzacji. Zmniejszenie masy cząsteczkowej fukoidanów pozwoli na ich wykorzystanie do tworzenia nanocząstek i nanonośników, w tym do ukierunkowanego dostarczania leków. Masę cząsteczkową polimeru można zmienić przy użyciu różnych metod depolimeryzacji. W niniejszej pracy rozważono możliwość wykorzystania destrukcji ultradźwiękowej do zmniejszenia wielkości cząsteczek fukoidanu. Metoda ta jest jedną z prostych i skutecznych metod depolimeryzacji fukoidanu, która prowadzi do spadku masy cząsteczkowej bez istotnych zmian strukturalnych w makrocząsteczkach. Omówiono również metody i możliwości ekstrakcji ultradźwiękowej fukoidanów z alg, a także możliwość łączenia ich z innymi fizycznymi lub chemicznymi metodami ekstrakcji.

Słowa kluczowe: aktywność przeciwutleniająca, ekstrakcja ultradźwiękowa, fucoidan, depolimeryzacja, obróbka ultradźwiękowa

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Introduction

Fucoidans are of a great interest among biopolymers of marine origin. Fucoidan is a branched sulfated heteropolysaccharide isolated from brown algae and some marine invertebrates.[1] The main monomeric unit of fucoidan is L-fucose. However, the presence in the structure of some amounts of residues of glucose, mannose, xylose[2], galactose[3,4] and glucuronic acid was also established. The structure of fucoidans itself is not uniform and the 2 most common types of backbone can be distinguished. The 1st type is the 1→3-related residues of α-L-fukopyranose, the 2nd type is alternating 1→3- and 1→4-related residues of α-L-fukopyranose.[6] In addition to sulfate groups, which are usually located at the C-2, C-3 and/or C-4 carbon atom of the fucose ring,[7,8] there are acetate groups at the positions C-4 (at the 1→3-related fucose residues) and C-3 (at 1→4-bonds).[8] The properties of the polysaccharide depend on the structural characteristics of the polysaccharide determined by a group of factors (the place of growth, the raw material, the time of its collection, the method of extraction, etc.) and require the selection of an optimal method for obtaining its extraction. The molecular weight of such polysaccharides can vary widely. Low-molecular-weight (3–8 kDa), medium- (from 30 kDa) and high-molecular-weight fucoidans are isolated (may exceed 2000 kDa). Fucoidan has a wide range of biological activity, including immunomodeling,[9] antimicrobial,[10] anti-inflammatory,[11] anticancer,[12] and antiviral activity.[13] It enhances the activity of natural killers, macrophages, dendritic cells and T-cells,[14] and stimulates hemopoies.[15] There is an increasing interest in the possibility of its use as an adjuvant. An adjuvant effect is observed when the vaccine is administered orally.[16] Immunomodulation is observed after absorption of the vaccine in the small intestine. The presence of sulfate ester groups confers a negative charge on the skeleton[17] and in general, the mechanism of action of this polysaccharide in biological interactions with various targets is based on the charge density and chemical properties of the biopolymer itself.[17,18]

The industrial production of fucoidan and functional products with its contents is expanding.[19] For these purposes, fractions of low-molecular-weight fucoidan are used more often, since some high-molecular-weight fucoidans have a strong branching of the molecule, which leads to an increase in viscosity[20] and a decrease in the absorption of the polysaccharide due to "limited transport" through the cell membrane".[21]

Furthermore, low-molecular-weight fucoidans exhibit higher biological activity.[22] In such a way, for the use of fucoidan in the pharmaceutical and food industries, it is necessary to develop a quick and easy way to produce low-molecular-weight fucoidan with specified physicochemical properties. Ultrasonic exposure can lead to faster reactions and processes. For polysaccharides, ultrasound is used to extract them from raw materials or through depolymerization process,[23] since the generated acoustic energy is sufficient both to destroy the cell wall of the raw materials[24] and to break bonds in polymer structures.[25]

Ultrasound as a depolymerizing factor

Low-frequency ultrasonic exposure is applicable to the depolymerization of polymeric materials, including naturally occurring ones, and has been used to depolymerize various biopolymers, including polysaccharides, DNA, etc., without altering their chemical structure.[26]

Ultrasonic degradation is characterized by a high decomposition rate of large molecules with a narrow molecular weight distribution,[27] which allows to obtain an aqueous polymer solution without introducing additional purification steps. To reduce the molecular weight of polymers, ultrasonic waves with a frequency of 16 kHz are used.[23] Ultrasonic processing is based on the phenomenon of cavitation. As a result of cavitation, shock waves, intense local heating (about 5000°C) and high pressure (about 1000 atm) are created (Fig. 1).[28] The energy is released to break bonds in any polymeric material,[25] including glycosidic bonds in polysaccharides.[29]

The primary, secondary, and physical sonochemical effects are isolated. The primary effect is associated with all processes occurring in the gas phase inside the bubble, the secondary effect in the solution phase, and the physical effect is caused by the shockwave.[30] It is assumed that polymer chain rupture as a result of sonolysis is not random, but is carried out in the middle of the molecule, with a greater effect observed when exposed to low-frequency ultrasound.[31] Polysaccharides have also been found to depolymerize more rapidly in dilute solutions and with the increased ultrasound time.[32]

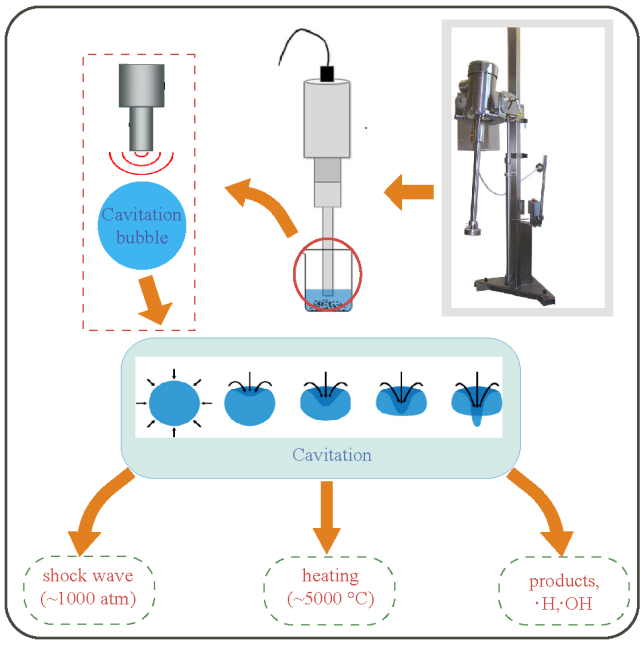

Fig. 1. Types of ultrasonic emission

In addition, it is known that with prolonged and intense exposure to an ultrasonic field, the energy transfer is dampened and fragments of polysaccharides larger than 20 kDa are formed.[33] Therefore, to increase the effectiveness of ultrasonic exposure, a combination of other physical methods is possible, such as radical depolymerization,[34] or introducing additional chemicals into the treatment medium.[35] The ultrasonic treatment itself is possible in a neutral, acidic and alkaline medium. Upon ultrasonic exposure at 300 W to a chitosan solution in acetate buffer with pH 4.4 and a concentration of 0.2%, 0.8%, 1.4% and 2.0%, the polydispersity decreased from 10.10 to 2.11, 3.11, 4.04, and 5.09, respectively.[36] The use of an alkaline medium is possible with low solubility of the depolymerized component or complex, where cleavage occurs from the surface of the swollen particles.[37]

Hydroxyl radicals generated using ultraviolet cavitation also make a great contribution to bond breaking, including glycosidic ones. Therefore, in polysaccharide depolymerization, additional introduction of hydrogen peroxide (H_2O_2) is possible, which catalyzes radical hydrolysis under the action of ultrasonic waves.[38] This ultrasonic treatment leads to the formation of products with a low polydispersity index (PI = 1.38 ±0.001).[38] The efficiency of the depolymerization process itself is improved. High molecular weight (MW) exopolysaccharide produced by a deep-sea hydrothermal bacterium *Alteromonas macleodii* subsp. *fijiensis* biovar deepsane is halved compared to ultrasonic treatment without hydrogen peroxide (MW = 204.5 – 112.7 g/mol).[34] Metal ions can be used as catalysts to increase the amount of hydroxyl radicals in the system. An example of such ions are Fenton systems where Fe^{2+} ions act as a catalyst for the production of such radicals.[33] Ultrasound synergistically increases the effectiveness of the Fenton reaction in decomposing pectin from 448 kDa to 5.5 kDa in just 35 min,[33] and heparin from 14,8 kDa to 4.87 kDa within 20 min.[39] In the process of such depolymerization, no significant chemical changes in the backbone occur, including fucoisylated chondroitin sulfate and loss of sulfate groups.[40] Moreover, when Fenton depolymerization based on the H_2O_2/ascorbic system acid is used, the increase in efficiency of decomposition of fucosylated chondroitin sulfate without loss of fucoidan branches is observed.[41]

Ultrasonic depolymerization of fucoidans

The main property determining the functional value of the polymers is the MW distribution.[42] Unlike acid hydrolysis, ultrasonic depolymerization leads to the production of fucoidan oligomers without changing the monomer composition and quantitative content of sulfo groups.[19] However, the decrease in MW must be controlled because a decrease below the optimal value for a given activity can lead to the loss of this activity.[21] Therefore, it is known that the inhibition of α-amylase activity is possible by fractions of fucoidan with a molecular weight of 637 kDa and 2351 kDa. Fractions with a molecular weight below 43 kDa no longer possess this ability.[21,43]

Ultrasonic degradation has been found to not lead to significant structural changes in fucoidan macromolecules.[44] When sonochemically treated in an aqueous medium, fucoidan isolated from sea cucumber at an intensity of 508 W/cm^2 and a frequency of 21–25 kHz retained repeated linear tetrasaccharide blocks only with partial destruction of unsulfated fucose units.[45] After 220 min of such treatment, the average molecular weight of fucoidan decreased from 338 to 91 kDa. Depolymerization of fucoidan from *Sargassum fulvellum* by high-intensity low-frequency treatment (25 kHz, 200 W) in the presence of H_2O_2 leads to the acceleration of the decomposition of fucoidan. The resulting product retained the structural features of the original biopolymer without altering the functional groups, such as sulfate and monosaccharide units.[46] The infrared (IR) spectra of the treated fucoidan were identical to the spectrum of the native polysaccharide, with the exception of the peak of the bond stretch absorption band C=O (1730 cm^{-1}) and the peak of asymmetric bond stretch COO^- (1630 cm^{-1}).

At an ultrasonic depolymerization of a fucoidan from *Sargassum muticum*, the shift of peaks of molecular weight from 80 kDa and 40 kDa up to 65 kDa and 25 kDa, accompanied with the increase in antioxidant properties of polysaccharide, with the maximum value of indicators of samples processed at 80 kHz within 120 min is observed. These samples have also shown the inhibiting action on growth of cells of carcinoma of a neck of the womb (HeLa 229).[47]

The further use of depolymerized fucoidan is possible in various fields, including the creation of nanoparticles and nanocarriers. At the same time, the use of high-molecular-weight fucoidan leads to the production of only large particles. For example, when high-molecular-weight fucoidan reacts with chitosan, large aggregates are formed.[48] With a decrease in the size of fucoidan, the size of particles formed with chitosan also decreases. Thus, with a decrease in molecular weight from 340 kDa to 123 kDa in a ratio of fucoidan to chitosan 1:1, there is a drop in the size of the formed particles by 50–70 nm. Furthermore, an increase in the quantitative content of fucoidan in the system at some stages led to an increase in the difference of nanoparticles size.[48]

Ultrasonic extraction of fucoidan

The main method for extracting fucoidans from raw materials is the use of acid solutions at a temperature of 70–100°C with the separation of alginates using Ca^{2+}.[49] The H_2O_2 can be used to remove polyphenolic compounds. However, it is now increasingly popular to use physical

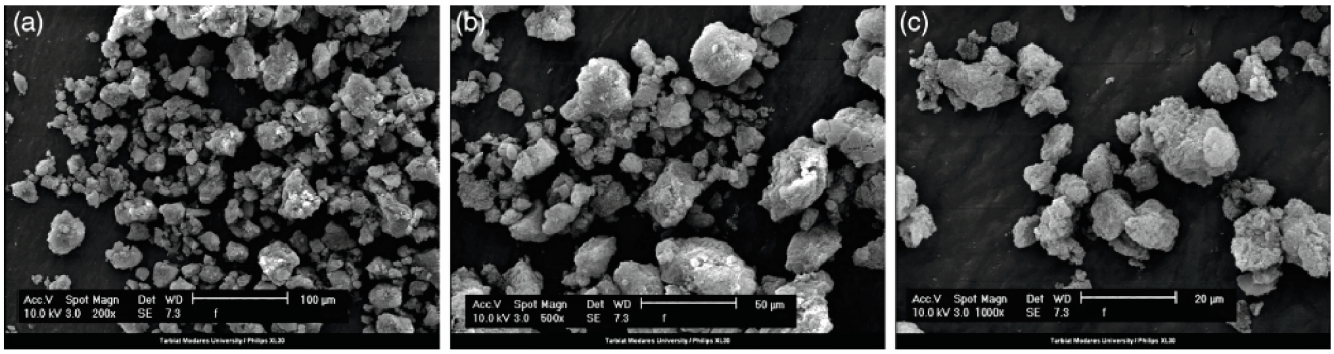

Fig. 2. Scanning electron microscope (SEM) picture (×200, ×500 and ×1000) of fucoidan extracted with ultrasonics[54]

extraction methods to reduce or eliminate the complete use of toxic solvents with an increased extraction efficiency.[21] However, it is necessary to control and optimize the ultrasonic processing time of the raw material to avoid damage to the target compound.[50]

The ultrasonic extraction of fucoidan from the raw material is carried out under the influence of low-frequency ultrasound with a short duration in time, possibly in an acidified medium. In addition, both aqueous and alcoholic systems may be used as the treatment medium. Treatment of *Sargassum muticum* in water with ultrasound at a frequency of 40 kHz, with a power of 150 W for 5–30 min at 25°C led to the production of fucoidan with a high yield (147.6 ±8.0 g/kg of raw materials).[51] The combination of such extraction with ion exchange chromatography allowed the total fucoidan fraction from the *Fucus evanescens* brown algae to be divided into 2 fractions at a ratio of 1:0.2.[52] The fractions differ in structural characteristics, namely the presence and location of the acetate group, the content of galactose and xylose residues.[52] With respect to the structural features, fucoidan isolated by ultrasound has a lower fucose content.[53] The fucoidan obtained by ultrasonic extraction from *Nizamuddinia zanardinii* spp. at a ratio of water to raw materials of 80:1 (power 196 W, extraction temperature 70°C for 58 min) showed a noticeable inhibition of the growth of cancer cells HeLa (62.36%) and HepG2 (56.83%) (Fig. 2).[54]

In the work of Okolie et al., the extraction was carried out by ultrasound at a frequency of 20 kHz for 35 min in an aqueous medium containing 0.01 M HCl, followed by the treatment of the extract with 2% (w/v) $CaCl_2$ and 4 volumes of 95% ethanol.[55] The fucoidan obtained from *Ascophyllum nodosum* spp. showed a high prebiotic activity similar to that of the standard prebiotic inulin. The addition of fucoidan extracts to MRS (de Man, Rogosa and Sharpe) broth, with a final concentration of 0.1 and 0.5%, significantly ($p < 0.05$), but not dose-dependently, improved the growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* strain.[55] When alcohol was used as a solvent in ultrasonic extraction, the fucoidan yield increased by 16.8%, compared to the extraction with hot water. In this way, fucoidan was obtained from *Sargassum mcclurei* at a solvent to algae ratio of 24:1, the extraction time of 49 min at 54°C, with the ultrasound power being 360 W.[56]

Ultrasonic exposure is also known to lead to an increase in enzyme activity when used together. The enzymatic ultrasonic extraction method allows for obtaining a lower molecular weight polysaccharide with a higher fucoidan yield compared to the ultrasonic method (from 3.6% to 7.87%).[57] The average molecular weight of fucoidan isolated by the ultrasonic method was 1020.85 kDa and enzymatic ultrasonic was 443.70 kDa.[57]

In addition to chemical agents, ultrasonic exposure during extraction can be combined with other mechanical effects. For example, the combination of ultrasonic exposure (200 W, 20 kHz, 55°C) and microwave exposure (700 W, 90°C) resulted in an increase in the fucoidan sulfate content of 27.16%.[58]

Conclusions

Due to the biological activity of fucoidans, interest in them remains high. However, the selection of an optimal depolymerization method is necessary to enhance their therapeutic applications. Reducing the molecular weight of fucoidans will make it possible to use them in creating nanoparticles and nanocarriers for, among others, the targeted drug delivery. Ultrasound destruction is applicable for these purposes. This is one of the simple and effective methods of fucoidan depolymerization, which leads to the decrease in molecular weight without significant structural changes in macromolecules. This approach is simple and can be used on an industrial scale. The ultrasonic extraction method is useful for extracting fucoidans from algae. Yet, the use of the ultrasonic method in combination with chemical (for example, acidic, enzymatic) and physical (for example, microwave radiation) methods allows to increase the yield of the desired product.

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