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Review Article

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A Review on Polysaccharide Degradation Methods

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Abstract Due to the excellent pharmacological effects of polysaccharides, scholars have attached importance to researches on them. However, the high-value utilization and development of polysaccharides have been greatly limited by the high molecular weight, poor solubility and bioavailability. The degraded polysaccharides have stronger biological activities than those before degradation, such as antioxidant, anti-tumor, immune-regulatory, anti-hyperlipidemia, anti-inflammatory, regulating intestinal microbe and so on. At present, many effective methods have been applied to prepare low molecular weight polysaccharides, like chemical degradation, physical degradation and biodegradation methods.

Keywords Polysaccharides; degradation; biological activity

Introduction

Polysaccharides are an indispensable biopolymer in addition to protein and nucleic acid, which consists of a large number of monosaccharides linked in an almost infinite way by glycosidic bonds [1]. Polysaccharides formed from the same polysaccharide become homopolysaccharides, such as starch and cellulose; while polysaccharides composed of different monosaccharides are called heteropolysaccharides, like arabic gum. The general formula for polysaccharides is $C_x(H_2O_2)_y$ or $(C_6H_{10}O_5)_n$, which is why they are also known as carbohydrates [2].

Polysaccharides are widespread in nature and can be found in bacteria, fungi, plants, algae and animals [2]. Polysaccharides can be components of cell walls of microorganisms or plants, such as peptidoglycan and cellulose. Also they can be energy stores for plants or animals, like glycogen and starch. In addition, there is increasing evidence that polysaccharides have a wide range of special biological activities, such as immunomodulation, anti-tumor, anti-inflammatory, anti-virus, anti-aging, regulation of intestinal flora, etc [3-4]. The polysaccharides extracted from the same species of different origins have different structures and activities. Besides, the polysaccharides obtained from the same species using the different methods also have difference and distinction[5-6]. The molecular weight, monosaccharide composition, acid group content, degree of branching, glycosidic linkage type, main chain conformation and conformation of polysaccharides affect the biological activity of polysaccharides [7]. With the continuous research on polysaccharides, they have attracted significant attention not only in biomedicine, but also in chemicals, cosmetics, food and other fields [8-9].

Most polysaccharides in nature have high molecular weight and poor water solubility, thus making them difficult to be absorbed and utilized by organisms, which limits the application of polysaccharides [10]. Recently, accumulated evidence has demonstrated that lower molecular weight polysaccharides have better biological activity. For instance, it was demonstrated that compared with high molecular weight polysaccharide, low molecular weight polysaccharides have stronger inhibitory effects on Escherichia coli and Staphylococcus aureus [11]. However, if the molecular weight of the polysaccharide is too low, it will not be able to form an active polymeric structure and

its activity will decrease rapidly [6, 12]. In addition, the degradation process not only reduces the molecular weight of the polysaccharide, but increases its radius of gyration. The structure of polysaccharide will change from a compact globule to a randomly coiled structure which exposes more reactive groups [13]. Therefore, modification of polysaccharides by degradation can effectively improve their biological activity. The appropriate degradation methods should be selected according to different needs so that low molecular weight polysaccharides can be prepared for more wide application.

Currently, chemical degradation, physical degradation and biodegradation methods have been widely used for the degradation of polysaccharides [14], and all of them are effective in enhancing their biological activity. This review covers polysaccharide degradation methods that have been widely used in recent years and will provide enlightenment for the study of low molecular weight polysaccharides.

Chemical degradation

The chemical degradation method mainly uses reagents for degradation, while the products need further separation and purification, and the homogeneity is poor. The degradation process may also destroy the reactive groups in the polysaccharide molecules. The current chemical degradation methods mainly use $NaNO_2$, acid or oxidants to degrade polysaccharides.

$NaNO_2$ degradation

$NaNO_2$ in acid solutions has strong oxidizing properties. Using acetic acid as the solvent, $NaNO_2$ causes a diazotization reaction of the polysaccharide, which causes an intramolecular rearrangement to break the polysaccharide chain. Then it is reduced by $NaBH_4$, thus the degradation reaction is finished [15].

Traditionally, $NaNO_2$ is commonly used to degrade chitosan to prepare monosaccharides, and the main influencing factors affecting this reaction are reaction time, reaction temperature and the amount of $NaNO_2$ added [16]. $NaNO_2$ can also be applied to the degradation studies of heparin and its derivatives, the product is low molecular weight heparin sodium [17]. Meanwhile, $NaNO_2$ is commonly used to degrade various glycosaminoglycans and combined with LC-MS analysis for qualitative and quantitative [18].

However, the $NaNO_2$ degradation method has defects, such as the product molecular weight distribution is too wide, the degradation process will destroy the amino group, and it is not friendly to the environment. These defects have limited the use of $NaNO_2$ and prompted the search for better degradation methods.

Acid degradation

Acid degradation is attributed to the protonation of the oxygen atom in the glycosidic bond, resulting in the breakage of the carbon-oxygen bond, thereby producing the low molecular weight polysaccharide. In general, when polysaccharides are hydrolyzed by acid, branched glycosidic bonds are more easily hydrolyzed than main chain glycosidic bonds, furanosides are more easily hydrolyzed than pyranosides, ketoses are more easily hydrolyzed than aldoses, neutral sugars at non-reducing ends are easily hydrolyzed, and glycosidic bonds linked to glyoxylates are difficult to hydrolyze.

Polysaccharides are commonly degraded by heating in some inorganic acids such as phosphoric acid, hydrochloric acid and sulfuric acid. Experimentally, it was proved that six representative sulfated polysaccharides could be prepared by microwave-assisted hydrochloric acid degradation [19]. However, the recovery efficiency of some polysaccharides degraded in solution is low and the product quality is not uniform. Therefore, solid-state degradation methods have been used, wherein solid flake chitosan is degraded directly with hydrogen chloride in the presence of small amounts of water, resulting in products with narrow molecular weight distributions [20].

In recent years, a number of scholars have used organic acids such as trifluoroacetic acid or acetic acid to degrade polysaccharides. For example, using trifluoroacetic acid to degrade Cordyceps sinensis fungal exopolysaccharides and konjac glucomannan, and the results showed that the acid degradation products were more suitable for the growth of bacteria [21]. Furthermore, the degradation rate of chitosan in oxalic, citric, succinic, malic, and tartaric acids has also been studied with the results that oxalic acid was the fastest and malic acid the slowest [22].

The acid degradation method is simple to operate, but the molecular weight of the degraded polysaccharide is widely distributed, which is difficult to purify, and also has certain pollution. In addition, higher acidity may reduce the sulfate content of polysaccharide chains, which is an important group for the biological activity of polysaccharides [23]. Depending on the needs, the appropriate method should be chosen.

Oxidative degradation

Polysaccharides can be degraded by oxidation in the presence of strong oxidizing agents. The most commonly used oxidizing agent are ascorbic acid and H_2O_2. However, there are also a few examples of the use of $NaBO_3$, ClO_2 and Cl_2, which are still in the research stages [15]. This paper focuses on the mechanism and methodological study of ascorbic acid degradation and H_2O_2 degradation.

Ascorbic acid addition to polysaccharide solutions induces a series of changes in polysaccharide properties, such as lower molecular weight and smoother apparent morphology, while the functional group structure and monosaccharide composition of the polysaccharide will stay largely constant [24]. Ascorbic acid degradation of polysaccharides is based on the Fenton reaction resulting in hydroxyl radicals attacking the polysaccharide backbone and causing degradation [25-26]. If ascorbic acid is paired with hydrogen peroxide, metal ions or ultrasound, more powerful active hydroxyl radicals are generated. Today, scholars generally agree that the oxidative degradation of polysaccharides by ascorbic acid is non-specific and stochastic. Its study has to be further.

H_2O_2 degrades large polysaccharides because it can generate reactive oxygen species such as HOO^-, $HO^\bullet$ and $^\bullet O_2^-$. Free radicals can abstract hydrogen atoms from all cyclic C-H bonds to form carbon-centered radicals and break polysaccharide chains via β-scission [7, 27]. In the degradation of polysaccharides with H_2O_2, the production of free radicals can be activated by the introduction of metal ions, ascorbic acid or peroxidase, or by radiation treatment [28]. H_2O_2 degradation may have an effect on the physicochemical and structural properties of polysaccharides. In the case of H_2O_2 degradation can expose more hydrophilic groups of polysaccharides, thus increasing their water solubility [29]. The thermal stability also increases because the degradation forms a more stable intermediate product with a lower content of bound water [30]. In addition, H_2O_2 degradation can change the molar ratio and concentration of monosaccharides, but left out the monosaccharide types [7, 31]. With the advantage of non-toxic and no by-products, H_2O_2 oxidative degradation of polysaccharides is an ideal chemical degradation method. However the reaction of H_2O_2 degradation for the preparation of low molecular weight polysaccharides was mainly influenced by the H_2O_2 concentration, reaction time and reaction temperature [23]. Therefore, in this reaction, the operating conditions are difficult to control, and if the reaction time is long, it will affect the appearance quality of the product.

Physical degradation

Physical degradation method is a simple to operate, easy to control, non-polluting method with high degradation efficiency. The commonly used methods are radiation method and ultrasonic method.

Radiation method

Radiation degradation method mainly adopts ionizing radiation such as electron beam, γ-ray, ultraviolet light, X-ray and microwave to break the glycosidic bonds in polysaccharides and induce the degradation of polysaccharides, thus improving the biological activity of polysaccharides. This method is less expensive and the product is of high quality.

There are experiments comparing the degradation of hyaluronic acid by electron beam irradiation, γ-ray irradiation, and microwave irradiation and found that the radiation not only reduced the molecular weight of the polymer, but also changed its structure. In particular, hyaluronic acid degraded by microwave radiation was found to have increased UV absorption at 265 nm and changed its color, which may lead to an increase in its antioxidant activity. In contrast, hyaluronic acid after degradation by electron beam and γ-rays had lower polydispersity and less change in UV spectrum [32].

Ultrasonic method

Ultrasonic method is more commonly used than radiation method. Under ultrasound with appropriate frequency and power, the glycosidic bonds of macromolecular polysaccharides can be interrupted. Currently, there are two main mechanisms of ultrasonic degradation, which are mechanical bond breaking and oxidation-reduction reaction of free radicals [33]. One is that cavitation bubble ruptures with a shear force that can break the polysaccharide chain [34], while the other is that cavitation bubble collapse generates local high pressure and high temperature to provide energy for free radical production [35]. The ultrasonic degradation of polysaccharides is based on the second-order kinetic model and the midpoint chain scission model [36]. The monosaccharide composition and proportion of degraded polysaccharides may change, but can exhibit more favorable physicochemical properties and biological activities.

Ultrasound is often applied in connection with other methods as an auxiliary tool, for example, the degradation effect of ultrasound in combination with H_2O_2 is significantly higher than that of ultrasound or H_2O_2 alone [37].

Ultrasonic degradation is not only efficient and environmentally friendly, but can also affect the biological activity of polysaccharides by changing their physicochemical properties. However, the equipment and technology are not yet ready and the production cost is high, so further research is needed to achieve industrialization.

Other method

In addition to radiation and ultrasonic methods, it has been reported that high temperature and high pressure methods can also be applied to degrade polysaccharides, and the degraded products exhibit more effective biological activities [38]. Similarly, polysaccharide degradation was found in black garlic obtained by heat treatment of fresh garlic, and this reaction was not due to the presence of enzymes [39]. High temperature degradation has a huge potential for industrial purposes.

Although the physical degradation method is not as widely used as the chemical degradation method, it still has certain application value.

Biodegradation

The biodegradation method primarily uses enzymes to degrade polysaccharides and obtain low molecular weight polysaccharides or oligosaccharides. In the natural environment polysaccharide degradation processes occurring enzymes play an important role, such as polygalacturonase and pectin methyl esterase may affect the cell wall and thus lead to the softening of bananas [40]. Enzymes can specifically break the glycosidic bonds and reduce the molecular weight of polysaccharides, thus enabling polysaccharide easier absorbed and utilized by the organisms. The effective functional groups and the main structure of polysaccharides are not destroyed during the enzymatic degradation process, so the products have high activity. Furthermore, enzymatic degradation reaction conditions are mild and do not introduce impurities. It is an ideal degradation method with fast degradation speed and low pollution, and overcomes the disadvantages of wide molecular weight distribution and poor homogeneity of chemical degradation products [41]. However, the conditions of the enzymatic reaction are demanding, with temperature, pH, enzyme concentration and substrate concentration all having a significant effect on the enzyme-catalyzed reaction. In addition, the relatively high price and cost of enzymes is one of the disadvantages of enzymatic degradation.

In a previous study, it was pointed out that the chemical degradation method could cause loss of sulfate content of fucoidan [42]. However, if fucosidase produced by Flavobacterium RC2-3 is uesd to degrad high sulfate content fucoidan, fucoidan with molecular weight between 5-10 kDa will have the best tyrosinase inhibitory activity, antioxidant activity and antimelanogensis ability [43]. In addition, a new technology, that is high pressure pretreatment combined with a complex enzyme for degradation of seaweed polysaccharides was established. The product plays an vital role in weight loss by improving the intestinal microbiota and increasing the synthesis of SCFAs [44]. Similarly, using rhamnoside endonuclease to degrade the Hericium erinaceus polysaccharide showed that the product could enhance the immune effect of macrophages, which indicated that the immunoregulatory fuction of polysaccharide was greatly enhanced after enzymatic degradation [45]. To solve the problems of poor

stability and continuous use of enzymes, enzyme immobilization can be used to overcome these problems and thus make them economical for industrial use. For example, pectinase can be immobilized in calcium alginate beads and showed that the immobilization optimized the reaction conditions for pectin degradation and improved the thermal and operational stability of pectin [46-47].

By now, various specific and non-specific hydrolases have been found for the degradation of polysaccharides. Enzymatic degradation is an efficient and practical method to enhance the biological activity of polysaccharides, which has potential applications in the areas of food and medicine.

Conclusion

Polysaccharides found in nature have high utilization value, but most of them are difficult to be applied directly, which must be modified in order to show better biological activity. There are many ways to modify polysaccharides, not only to reduce their molecular weight, but also to graft different substituents, such as sulfation, carboxymethylation, acetylation, and so on. These are important ways to develop and utilize polysaccharide drugs. In the future, research on low molecular polysaccharides could focus on industrial production and elucidation of the structure-effect relationships.

This article summarizes the method for preparing low molecular weight polysaccharides. There are many degradation methods of polysaccharides, each of them has its own advantages and disadvantages, and an appropriate method should be chosen to prepare low molecular weight polysaccharides according to different requirements. Practical and efficient degradation methods will bring considerable economic and social benefits to the people.

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