Extraction of Chondroitin Sulfate and Its Current Use

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Abstract:Chondroitin sulfate (CS) is a polyanionic polymer compound. It is an acidic mucopolysaccharide. These compounds are commonly found in the nasal bone, trachea, leg bone, hyoid bone and other parts of mammals, and are the main components of connective tissues such as cartilage, muscle membrane, blood vessel wall and skin. China is the country with the largest amount of livestock slaughtered in the world. However, a large number of animal bones are currently discarded and not fully utilized. Based on this situation, this project used bovine nasal cartilage as the source of chondroitin sulfate. Chondroitin sulfate was extracted by a complex, but simple enzymatic method with high extraction efficiency. In addition, it is well known that chondroitin sulfate also has various pharmacological activities such as anti-inflammatory, antitumor, etc. It is currently used clinically for the prevention and treatment of osteoarthritis, cardiovascular and cerebrovascular diseases. Therefore, this paper also collates and summarizes its clinical and biomedical uses.

Keywords: Bovine Nasal Cartilage; Chondroitin Sulfate; Extract; Uses of Chondroitin Sulfate

1. Introduction

1.1 The Meaning and Purpose of the Topic

China ranks first in the world in terms of the volume of livestock slaughter. However, after animal slaughter, animal bones, which account for a large proportion of the by-products of livestock and poultry, are rarely used in food(1). Chondroitin sulfate (CS) is one of the components of proteoglycan. There are many current production processes. Among them, the quality of the products produced by the dilute alkali-concentrated salt method is higher, but the optimal process conditions have not been determined, and the extraction conditions need to be further optimized.

In recent years, high-end health products made of chondroitin sulfate are also popular. It has the effect of preventing and suppressing cancer. Chondroitin sulfate can also be formulated with other substances into cosmetics for skin and bodybuilding (2).

The purpose of this study is to develop the use of bovine nasal cartilage to extract chondroitin sulfate. Through the experiment, we provide scientific basis for the full utilization of livestock bone resources in China. In addition, this paper will summarize the use of chondroitin sulfate in clinical and biomedical applications.

1.2 Types of Chondroitin Sulfate

Chondroitin sulfate is an acidic mucopolysaccharide, glycosaminoglycan (GAG), it generally has 60 to 80 disaccharide units. It can be divided into five isomers, A, B, C, D and E, which are classified according to the different positions of the sulfate group--see Table 1. Chondroitin 4-sulfate and chondroitin 6-sulfate are the main types (3). Therefore, here in this project, we will use 4-chondroitin sulfate as the main raw material. Also, compared to domestic animals, cartilage of marine organisms is relatively difficult to obtain and less abundant, therefore, we used bovine nasal cartilage as the raw material for extraction experiments.

Table 1. Isomerization of Chondroitin Sulfate

R_1=SO_3^-	R_2=H	R_3=H	Chondroitin sulfate A
R_1=SO_3^-	R_2=H	R_3=H	Chondroitin sulfate B (C_5 differential isomerization)
R_1=H	R_2=SO_3^-	R_3=H	Chondroitin sulfate C
R_1=H	R_2=SO_3^-	R_3= SO_3^-	Chondroitin sulfate D
R_1= SO_3^-	R_2=SO_3^-	R_3=H	Chondroitin sulfate E

1.3 Research Status of Chondroitin Sulfate

1.3.1 Extraction methods of chondroitin sulfate

Degradation and non-degradation methods are the main means of extracting glycosaminoglycans. The non-degradation method is only used to extract proteoglycan

glycosaminoglycans like hyaluronic acid. Chondroitin sulfate can be extracted by the degradation method, with alkaline extraction and protease digestion are the main degradation methods (4). There are many extraction methods for chondroitin sulfate. In summary, there are generally the following categories:

(1) Alkaline extraction method (5): use different concentrations of strong alkaline salt solutions (such as KOH, Na_2CO_3 solution, etc.) to cause the β-position elimination reaction of the O-glycosidic bond (strong alkaline conditions). The sugar-peptide bond is broken, so that the chondroitin sulfate is released from the proteoglycan.

(2) Enzyme extraction method (6): This method is based on the specificity of the protease in the protein. Enzymes such as trypsin, papain, streptomycin, etc. are used to digest the protein into amino acids, and then the difference in solubility of chondroitin sulfate and the amino acid is used to extract the chrondroitin sulfate.

(3) The method of mixed extraction with alkali and salt (7). The chondroitin sulfate is not degraded under strong alkaline conditions. The principle of this method is as follows: chondroitin sulfate is released through the breaking of the O-glycosidic bond. Then, the protein can be precipitated under conditions of differing ionic strength.

Among the above extraction methods, the alkali extraction method has high extraction efficiency and simple operation, but the extraction conditions are harsh. Therefore, relatively high yield, high cost but short cycle time, non-polluting complex enzymatic method has more advantages, and it is now a more ideal preparation method.

1.3.2 Separation and purification of chondroitin sulfate

The chemical composition of chondroitin sulfate is relatively complicated, and the extract contains impurities such as protein, amino acid, and inorganic salt. Therefore, it is necessary to separate and purify the chrondroitin sulfate from these “contaminants”. The main methods for the separation and purification of chondroitin sulfate are:

1.3.2.1 Solvent separation and purification method

The solvent method can separate chondroitin sulfate and impurities from each other (8). The main principle is to use the different solubility of various chemical components in cartilage to separate and purify the chrondroitin sulfate. Hydrophilic groups such as sulfuric acid groups in chondroitin sulfate make it highly soluble in water and minimally soluble in organic solvents. Commonly used organic reagents include ethanol and methanol. Higher purity chondroitin sulfate can be obtained by ethanol precipitation. By dissolving the lower purity chondroitin sulfate with 2% (w/v) NaCl solution, then adding a certain amount of ethanol, centrifugation (10 min, 10000r/min), and incubation at low temperature (about 4^0C), approximately 70% to 78% of chondroitin sulfate can be purified.

1.3.2.2 Quaternary ammonium salt precipitation separation and purification method

The formation of the quaternary ammonium salt complex is accomplished by adding a polyanionic quaternary ammonium compound (such as cetylpyridinium chloride, etc.) to the chondroitin sulfate (9). The solubility of the complex in water is low. This complex has very low solubility in aqueous solutions with low ionic strength, but very high solubility in high ionic strength solutions. This property can make the separation and purification of chondroitin sulfate convenient and efficient. Usually, 0.1%-1.0% (w/v) is the concentration of chondroitin sulfate, and the ratio of 1:3 is usually the mass ratio of the reaction of chondroitin sulfate and quaternary ammonium salt.

2. Chondroitin Sulfate Extraction

2.1 Raw Materials, Reagents and Equipment

Raw material: fresh beef nose cartilage, purchased from the slaughter market in Lianyungang, Jiangsu.

Reagents: Chondroitin sulfate standards, papain, and trypsin were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China), chondroitin sulfate samples, monosaccharides and dextran standards were purchased from Fluka Co., (Shanghai, China), chicken albumin was purchased from Sigma Co., Ltd. (Jiangsu, China), absolute ethanol, sodium acetate, L-cysteine hydrochloride, oxalic acid tetraacetic acid-disodium, cetylpyridinium chloride (CPC), sodium

chloride, Coomassie brilliant blue G250, carbazole, pyridine, methanol, potassium sulfate, barium chloride, gelatin, concentrated sulfuric acid, phosphoric acid, potassium bromide, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium sulfate, sodium hydroxide, etc. were all made in the laboratory.

2.2 Experimental Method

2.2.1 Extraction of Chondroitin Sulfate

First, remove the adhering meat and fat on the surface of beef nose cartilage, soak in absolute ethanol for 4 h, dry at 60℃, and crush. Then weigh 10 g of bovine cartilage powder, add 0.01 mol/L EDTA and 0.01 mol/L cysteine buffer at a ratio of 1:10, and add 1.5% complex enzyme (papain:trypsin=1:2), 65 ℃ water bath reaction for 24 hours.

Second, after the reaction is over, centrifuge (10 min, 10000 r/min), the supernatant is precipitated with 1.25 times ethanol at 4°C for 12h, the precipitate is re-dissolved in water, and then precipitated with 2 times volume of ethanol containing 0.5% sodium acetate, centrifuged (10 min, 10000 r /min) to obtain the crude chondroitin sulfate extract.

2.2.2 Purification of Chondroitin Sulfate

First, dissolve the centrifuged chondroitin sulfate extract in 200 ml of 0.5 mol sodium chloride containing 5% CPC and place at 30°C overnight.

Then, centrifuge (10 min, 10000 r/min), dissolve the precipitate in 100 ml of 2 mol sodium chloride:ethanol (100:15, v/v), and then precipitate with 3 times the volume of ethanol.

Next, centrifuge (10 min, 10000 r/min), re-dissolve in 100 ml of 2mol sodium chloride: ethanol (100:15, v/v), and then precipitate with 3 times the volume of ethanol.

Finally, centrifuge again and dissolve the precipitate in water, dialyzed with cellophane, then put the dialyzed mixture into a rotary evaporator to concentrate, and then freeze-dry to obtain the purified chondroitin sulfate.

2.3 Experimental Results

Using bovine nasal cartilage as the material, the compound enzymatic method (papain: trypsin=1:2) was used to degrade the bovine nasal cartilage by ethanol precipitation and quaternary ammonium salt precipitation. The purified yield was 22.1%.

3. The current Uses of Chrondrotin Sulfate

Acid and N-acetyl-D-galactosamine of chondroitin sulfate (CS) are connected by 1,3 glycosidic bonds to form disaccharides, and disaccharides are connected by β-1,4 glycosidic bonds. The unique structure of chondroitin sulfate also determines that it will have a wide range of uses in clinical, biomedicine and other aspects.

3.1 Clinical Application

CS can effectively remove or reduce lipids and other lipids deposited on the walls of arteries and veins (10), significantly reduce plasma cholesterol, thereby preventing the formation of atherosclerosis, preventing coronary heart disease, angina pectoris, myocardial infarction, coronary atherosclerosis, and myocardial ischemia.

3.2 Application in Biomedicine

Scaffolding made of gelatin, chondroitin sulfate C and hyaluronic acid trimer can accelerate the regeneration of human NP cells and can be used as a human active scaffold (9). The type I collagen scaffold containing CS inoculated with chondrocytes significantly accelerates cell proliferation and can be used as tissue engineering articular cartilage. The collagen scaffold is prepared by freeze-drying, and the surface of the CS cross-linked scaffold can improve the biocompatibility and mechanical properties of collagen.

4. Summary

This thesis studied the extraction of chondroitin sulfate with bovine nasal cartilage as raw material, and we obtained the following conclusions.

(1) Compound enzymatic hydrolysis is an ideal method for extracting chondroitin sulfate in the laboratory. After continuous parallel comparison experiments, the optimal extraction conditions are: the compound enzyme (papain: trypsin=1:2) is added at 1.5%, the material-to-liquid ratio is 1:10, and the extraction is performed at a temperature of 65°C for 24 hours. Ethanol precipitation method and the quaternary ammonium salt precipitation method to degrade and purify CS can be used with a material-liquid ratio is 1:10, and an enzyme concentration of 1.5% using these methods, the purification yield is 22.1%.

(2) The research on the extraction of chondroitin sulfate in this paper has described data, and results that can provide a theoretical reference for the preparation and utilization of chondroitin sulfate. However, there are still many imperfections in the thesis, especially for many experimental results that are difficult to explain clearly in theory. These all need to be further improved and perfected in future work.

This thesis also summarizes several major uses of chondroitin sulfate in biomedicine and clinical application. CS has a variety of pharmacological activities. Long-term use has little toxic and side effects, which is important for biochemical drugs. In recent years, with the continuous deepening of research, it has also been discovered that CS has potential applications and development prospects in the fields of anti-parasitic and viral infection, tissue repair, anti-tumor, and as a drug carrier.

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