Extraction, Purification and Application Prospect of Ricin

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Abstract: In this paper, the extraction methods and application of ricin were studied according to its toxicity characteristics. Ricin is a highly toxic plant protein with two polypeptide chains, including ricin, ricinine, ricin allergen and hemagglutinin. Ricin is mainly found in castor beans, and the ricin is the most toxic. At the same time, with the exploration of biomedicine in recent years, ricin, a kind of biological insecticide with “no residue” and “no pollution” , has been attached great importance. The use of ricin as a targeting agent has also received considerable attention. Therefore, the prospect of high purity extraction and utilization of ricin in biomedicine is very broad.

Key words: Ricin; Extraction; Application prospect; Botanical pesticides

DOI: 10.12184/taposatWSP2633-456911.20200101

Introduction: Ricin has very good scientific use value, and in medicine, pesticides and other aspects, it is widely used. Especially in medical field, it can be used as targeted anti-tumor drugs. Ricin is toxic to humans and mammals and is toxic to almost all eukaryotic cells. When a single toxin molecule enters a cell, it is enough to stop the entire cell's protein production completely. Castor-oil is the mature fruit of castor-oil plant. Its oil can be used as important chemical raw material to produce high-grade lubricating oil, paint and surface agent. The defatted cake contains a lot of protein, but there is a little ricin in it, which limits the utilization of this protein. If ricin can be removed completely, it can be an important source of protein in feed. In terms of toxicity, the use of ricin is harmful, but it can be used to develop potent killer directed drugs for cancer cells, while ricin can also be used as a natural insecticide for biological pesticides. Therefore, effective extraction and purification of ricin will play an important role in the future biomedical market.

1. Process of Extraction and Purification of Ricin

It is necessary to find a method of high purity and high extraction rate because of ricin’s high purity. Based on the synthesis of other experimental methods and discussion of this research

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Funded project: 2019 Innovation and Entrepreneurship Training Program for college students of Huazhong University of Science and Technology, No. 19YA-14.

group, the following extraction method is obtained.

(1) Major reagents and apparatus

Dehulled castor seeds (from Inner Mongolia Weiyu Biotechnology Co., Ltd.), disodium phosphate (analytical purity), sodium dihydrogenphosphate (analytical purity), phosphoric acid solution (analytical purity), PEG6000, d-galactose, sodium chloride, dialysis bag, chromatography column, ultracentrifuge (KDC-160HR), electronic balance (BS210S).

(2) Extraction of crude ricin

0.2 MOL / L Sodium dihydrogenphosphate and Disodium phosphate were mixed evenly according to the volume ratio of 77:23. Ph was adjusted by 0.1 mol / L phosphoric acid to reach about 6.0-7.5. The required PBS solution was prepared. The Ph of PBS solution should be controlled at 6.0. A proper amount of shelled castor seeds and anhydrous ethanol were added into a mortar and ground to homogenate. The slurry was immersed in PBS solution at Ph 6.0 for 12 hours and supernatant was extracted. The liquid was soaked overnight at 4 °C with 60% saturated ammonium sulfate solution, then centrifuged and precipitated. The precipitate was then dialyzed for 24 hours, followed by dehydration with PEG6000 to obtain the crude extract of ricin.

(3) Purification of crude extract of ricin

A 50 ml acid burette containing sepharose 4B was purified from the crude extract of ricin, balanced with PBS at Ph 6.5, and the crude protein solution was sampled. The column was eluted by Ph 6.5 PBS, and the column was rebalanced by Ph 5.0 Acetic acid-sodium acetate buffer solution, it was then eluted with a buffer solution containing 0.2 mol/L d-galactose and Ph 5.0 acetic acid-sodium acetate. One test tube was collected at intervals of 5 ml, and the absorbance was measured by UV spectrometer immediately after collection. The absorbance was used to draw the elution curve of toxic protein in volume, which was convenient for later comparison. When the toxic protein is completely sucked out, sodium chloride can be added into the elution solution to the concentration of 0.14 mmol/ L, which can wash off the Hemagglutinin.

(4) Determination of Hemagglutinin activity

The fresh sheep blood was centrifuged for 10 minutes. The sheep red blood cells were precipitated and washed 2-4 times with normal saline, and the suspension of A5201.93 was prepared. At this point, the eluted Hemagglutinin was taken and its agglutinating activity was tested with the control experiment. 1 ML distilled water was used as the blank control group and 1 ML PBS (0.1 mol / L, Ph 6.0) as the control group. 1 Ml of Hemagglutinin solution and 1 ml of suspension of sheep red blood cells were added to each group and left overnight at room temperature for observation. The above concentration of the three components was 0.020 mg / Ml.

2. The Future of Application of Ricin

(1) Mechanism of ricin

Ricin is a kind of Heterodimer phytotoxic protein formed by the disulfide bond of RTA and RTB. The RTB binds to glycoproteins or glycolipid receptors on the cell surface using lactose binding sites on its amino acid residues, and the cell transports RTA to the cell via endocytosis. When the toxin molecules are transported to rough endoplasmic reticulum (rer), they are dissociated by disulfide isomerase. The dissociated RTA is active. By ERAD pathway, RTA can remove Adenine, inhibit the synthesis of intracellular protein, and result in cell death.

(2) Preparation of biological reagents using the characteristics of RTA and RTB and the mechanism of ricin

Biological reagents are prepared by using the characteristics of RTA and RTB and the mechanism of ricin. According to the mechanism of action of ricin, RTA is mainly used to exert the effects of toxic proteins, based on the different functional characteristics of RTA and RTB, it is often combined with proteins that have specific binding properties and is used in the preparation of anti tumor monoclonal antibody. RTB can bind non-specific receptor cells, so it is often combined with functional proteins for the preparation of related reagents. For example, RTB binds to nicotinic acetylcholine receptor proteins that act on the striated muscle to produce monoclonal antibody, a fusion protein used to treat strabismus and boost immune function. Based on the ability of RTB to bind non-specifically to receptor cells, it is possible to study both the mechanism of cell death by ricin and the ability of RTB to act as a vector for transporting functional proteins, to study the mechanism of action of functional proteins. In addition, ricin can be used in the preparation of antagonistic agents and vaccines. The preparation of neutralizing antibody and small molecule antagonistic peptide reagent are the main research of antagonists, and the preparation of recombinant vaccine, microsphere vaccine and super vaccine are the main research of vaccines.

(3) The role of ricin in botanical pesticides

The use of castor-oil plants in plant-based pesticides are also numerous. When different organic solvents were used to extract ricin, the purified products were tested as insecticides to small insects such as Plutella Xylostella and canopy caterpillars. It was found that the inactivated substances used to kill insects were mainly ricin and ricinine, and the purpose of the ricin is to contact. Therefore, the use of castor beans and ricin as plant-based pesticides, in the process of no residual pest control of crops will be able to achieve a great degree of effect. But the relative molecular weight of ricin is so large that it is difficult for RTA to work in insect cells with the help of RTB. Therefore, if certain methods, such as enzymatic degradation or chemical methods, can be used to reduce the molecular weight of ricin while maintaining its biological activity and normal function, is expected to be even more effective as a ricin.

3. Conclusion

With the deepening of environmental protection and health awareness, people's demand for green environmental protection food and natural bio-medicine is also gradually rising, so accelerating the trend of bio-medicine development is inevitable. Ricin is a natural extract that is easily broken down by the body and does not accumulate in the body. It can be used to solve the problem of toxic residues and secondary food chain re-poisoning. It can also solve the problem of resistance of pests to chemical pesticides. At the same time, it can be used as a tumor-oriented drug in the treatment of cancer. High purity extraction and utilization of ricin is bound to be greatly developed in the future.

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