

**Separating and purifying polysaccharide,
polypeptide from sea cucumber and their
functions**

(해삼으로부터 다당류와 폴리펩타이드의
분리, 정제 및 기능성)

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Separating and purifying polysaccharide, polypeptide from sea cucumber and their functions

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Abstract

For the studying and utilizing the thorn sea cucumber synthetically, the method preparing polysaccharide and polypeptide from sea cucumber was studied. The comparatively rational craft for preparing crude polysaccharide is fresh raw materials deal with oar form, 60% of the ethanol precipitate after hydrolyzed by protease and vibrated with ultrasonic wave auxiliary. The purification of polysaccharide and removal of protein from crude polysaccharide were made through precipitation method using the acetate. The polypeptide is obtained by concentrating in vacuum, freeze-drying after mixing precipitations of two times. Polysaccharide acute poisoning experiment indicate there is no bad reaction when $LD_{50} > 5000g/kg$, there strain rate of liver tumor H11 approach present generally acknowledged tumor treat medicine cyclophosphamide, and there is no side effect. The fatigue resistance function of polypeptide experiment also indicates that the fatigue resistance ability of mouse which fed on added sea cucumber polypeptide has a great improvement.

Key words: Sea cucumber Polysaccharide Polypeptide Protease ; Cyclophosphamide

Preface

Sea cucumber, *Ludwigothurea agrisea*, (Echinodermata, Holothurioide, and Aspidochirotida) survives almost 50 million years long. Nearly there are 1100

species of sea cucumbers in the whole world; more than 40 species[1]

'are edible sea cucumbers. The thorn sea cucumber of yellow and Bohai Sea district is the famous and is the most precious and the highest in nutritive value of the world. Since ancient times, the sea cucumber is the health and nutritive food. <Food Herbal> of Ming Dynasty pointed out that sea cucumber has the host to make up the vitality, to incite benefits of keeping in good health function which vital organs of the human body and dispels the deficient health.[2] "Compendium of Material Medical collection" the traditional Chinese medicine ancient book classified the sea cucumber as the help medicine in Qing Dynasty[3]. It recorded that the sea cucumber is warm, function as ginseng, so called sea ginseng and tastes sweet and salty, invigorate the kidney, benefit the marrow , dispel the phlegm, benefit the urinate, strengthen body activity and treat the impotence, kill the sore worm" In recent years the Chinese medicine proposed treating tumor[4] , recycled obstacle anemia [5]and diabetes [6]with sea cucumber's using separately or group side has made good result, at the same time in assisting various speciesof herbal cuisine or the food therapy group side , the sea cucumber has got extensive approval and application [7].

In recent years, with deeply researching on assortment determining and classification , physiology and biochemistry, separation, qualification and biomedical function of active material and biomedical function of sea cucumber by using the modern science and technology both at home and abroad, people find the sea cucumber contains many kinds of materials with important biological activation, such as toxin, polysaccharide, soap glycoside, polypeptide etc. These bioactive materials have functions such as antitumor, blood coagulation , resisting the thrombus , easing pain , raising the immunity , resisting tired , antiviral , lowering the fat of blood , preventing and curing the rheumatoid and arthritis ,etc. Looking for and developing the new medicine and processing the functional food from sea cucumber has already become the most important content[8]. This text makes preliminary research on Separating and purifying polysaccharide, polypeptide from sea cucumber and its function

1. Material and Method

1.1 Material

The sea cucumber purchases in Changdao Island of Yantai and they are fresh. The reagents are all analytically pure. The mice are standard mice of Kunming, heavy 18-21g per mouse.

1.2 Main equipment

Ultrasonic wave, Freeze-drying equipment, Reaction pot, low temperature hypervelocity centrifuge, etc.

1.3 Methods

1.3.1 Sea cucumber polysaccharide separation

1.3.1.1 Raw polysaccharide separation

Technological process:

Fresh sea cucumber → -70°C quick-freeze → Shearing machine cutting into slices → 8 times of water → Rub and grind colloiddally → Ultrasonic wave and proteinase → 95% to the system of 60% ethanol → Static for 12 hours → Centrifuge Sediment(polysaccharide) → Clear liquid of upper Strata (Polypeptide mixture)

1.3.1.2 Polysaccharide purification

Technological process:

Crud polysaccharide → 5% watery solution → Hydrogen peroxide solution decolorization → Potassium acetate → Spend the night in 4 → of refrigeration → Centrifuge → Sediment(have clear liquid upper amalgamate to the first circulation clear liquid upper) → Biuret method measures recessiveness → multimolecular polysaccharide mixture → Function determination

1.3.2 Polypeptide separation preparation

Technological process:

Two times centrifuge clear liquid upper →Dialysis →Ultra filter diaphragm graduation separation →Molecular confirmation → Function experiment → Product development

1.3.3 Polysaccharide preliminary function experiment

1.3.3.1 Acute toxic experiment[9]

Using the Horn law, obtains thorn sea cucumber polysaccharide LD50 is >5000mg/kg to divide three groups to fill the stomach, then observe phenomenon.

1.3.3.2 Transplanting suppressing rate of H22liver cancer[9]

Every group of mouse 15, sea cucumber extract give medicine group animal separately according to 50 mg/kg on inoculate abdominal cavity inject to medicines from the next day such as tumor, once a day, it takes 11 days in succession . The cyclophosphamide group is singly injects to medicines at the tumor of inoculating abdominal cavity next day (only give medicines 1 time). Physiological saline group offer with medicine group equal physiological saline of capacity (0.2 ml/10g)

Drug efficacy appraisal: Stop medicines and put mice's cervical vertebra dislocation to death next day. First measures the body weight, latter dissects the hypodermic lump block, weigh it.

Tumor suppression rate % = $(1 - \text{medicine group lump weight} / \text{physiological saline comparison group lump weight}) \times 100\%$.

1.3.4 The fatigue resistance function experiment of mixed polypeptide[10]

According to 0.10g/kg, 0.20g/kg, 0.30g/kg of every kg weight compared with ordinary feeding for 30 days. Make little mouse shoulder a heavy task 5% of weight, swim in swimming pool, 30cm of depth of water, determine drowned death time.

2. Result and discussion

2.1 Optimizing on polysaccharide preparation

2.1.1 Optimizing on raw polysaccharide preparation

Take the water volume, the neutral protease, the hydrolysis time as three major effects factors, set up three factors, three levels orthogonal experiment

Table 1. Sea cucumber raw polysaccharide and Hydrolysis rate craft factor table

Factor	Water (A)	Enzyme (B) %	Hydrolysis time (C) h
1	6 time	1 . 0	5
2	8 time	1 . 5	7
3	1 0 time	2 . 0	9

*The amount of water added and enzyme amount are subject to fresh sea cucumber's weight after the district viscera

Table 2. Result of crude polysaccharide rate and total nitrogen quantity orthogona

NO	A	B	C	polysaccharide rate (%)	total nitrogen(%)
1	1	1	1	0.35	2.39
2	1	2	2	0.64	2.61
3	1	3	3	0.84	3.03
4	2	1	3	0.62	2.58
5	2	2	2	0.95	3.13
6	2	3	1	0.52	2.56
7	3	1	2	0.77	2.90
8	3	2	3	0.44	2.44
9	3	3	1	0.72	2.66

From the table 2, the direct-viewing analysis and the variance analysis result indicated that the enzyme hydrolysis time has the remarkable difference ($P < 0.05$) while Other factors donot have remarkable differences. Total nitrogen rateis close from direct-viewing to the variance result.

2.1.2 Optimization of the refined polysaccharide preparation

2.1.2.1 Decolorization

Table 3. Comparison decolorization effect on crude polysaccharide

Method	No	Product Rate(%)	Average Product Rate(%)	Product Appearance Appraisal
Hydrogen peroxide solution metho	1	88.5	88.5	Nature White
	2	89.0		Nature White
	3	87.9		Nature White
Active charcoal metho	1	76.2	74.8	Nature White
	2	74.5		Nature White
	3	73.8		Nature White

The composite index of decolourization (50→, 15% of the 15% H_2O_2 ,6h) of the obvious hydrogen peroxide solution is higher than the active charcoal from the table3 and the post-processing and attaches in the product recycling use the hydrogen peroxide solution law to be more advantageous.

2.1.2.2 Remove the protein

Comparing Sevag method[11-12], the trichloroacetic acid method[13]and the acetate method[14]to remove protein effect, the results in table 4. The results indicated that the acetate method had a great advantage. On one hand the

efficiency is higher, on the other hand may reduce the ethyl alcohol precipitation working procedure, reduces the cost, and is more advantageous to removed protein recycling use. Sea cucumber's protein (polypeptide) is a matter with extremely high application value.

Table 4. The comparison of several removed protein craft effects

Method	OD280nm	OD260nm	protein (%)	polysaccharide Recycling(%)	Sugar (%)
Polysaccharide (unremoved protein)	0.243	0.215	3.52	-	35.6
Sevag (5times)	0.011	0.012	-	68.5	49.1
Trichloroacetic acid method(3 times)			-	59.8	47.5
Acetate method			-	72.1	60.2

2.2 Mixed polypeptide preparation craft optimization

Have clear liquid upper retrieve ethanol amalgamate with second time. Distilled water dialyze more than 24 hours to concentrate for the first time. Mixed the polypeptide products through two kinds of crafts. The method of freeze-drying and spraying method. Compare two kinds of method: Products appearance, freeze drying method such as being water soluble is it sprays dry law to superior to, but it helps industrialization to produce in succession to spray dryly.

2.3 Polysaccharide preliminary function experiment

2.3.1 Acute toxic experiment

Use the Horn method, obtain thorn sea cucumber polysaccharide LD50

>5000mg/kg. Fills in the stomach process, discovered mouse's fur color, spirit, appetite has not changed, the mouse is not dead, explained should belong trying the non-toxic matter, see table 5.

Table 5. Toxic experiment with thorn sea cucumber

Dosage (mg/kg .d)	5000	2500	1250	1000
Male	0/5	0/5	0/5	0/5
Female	0/5	0/5	0/5	0/5

2.3.2 Transplant nature H22 liver cancer suppression rate

Table 6. Polysaccharide influence to mice transplanting H22 liver cancer growth

Team	Dosage(mg/kg)	tumor weight(g)	tumor restrain rate(%)
Physiological saline	-	1.99±1.03	0
polysaccharide 40%	100	0.02±0.01**	49.2
cyclophosphamide	100	0.01±0.005 ^{##}	49.7

##: N=10, Compares with the physiological saline group, #P<0.05;##P<0.01; compares with the Phosphorus cyclophosphamide, *P<0.05, **P<0.01

Statistics indicates, compared with physiological saline group, 40% of sea cucumber's extract is suppressed the little mouse transplanting H22liver cancer to have high significance difference (P<0.05) to grow by the product of trying. And positive medicine surround cyclophosphamidecontrast group (100 mg/kg) compared with physiological saline group, H22 liver cancer grow inhibition have high significance difference (P<0.01) to mice transplant.

2.4 Mixed polypeptide fatigue resistance functionexperiment

Table 7. The swimming pool test of artificial mic

Sample		Animal Number	Average Time (min)	Difference with contrast group (min)	Compare with contrast group (%)
Contrast group	Ordinary feed (day)	20	18.02±1.91	-	100
Mixed polypeptide	0.10g/kg	20	20.78±2.12	+2.76	115.3
	0.20g/kg	20	22.11±1.64	+4.09	122.7
	0.30g/kg	20	27.13±1.95*	+9.11	150.8

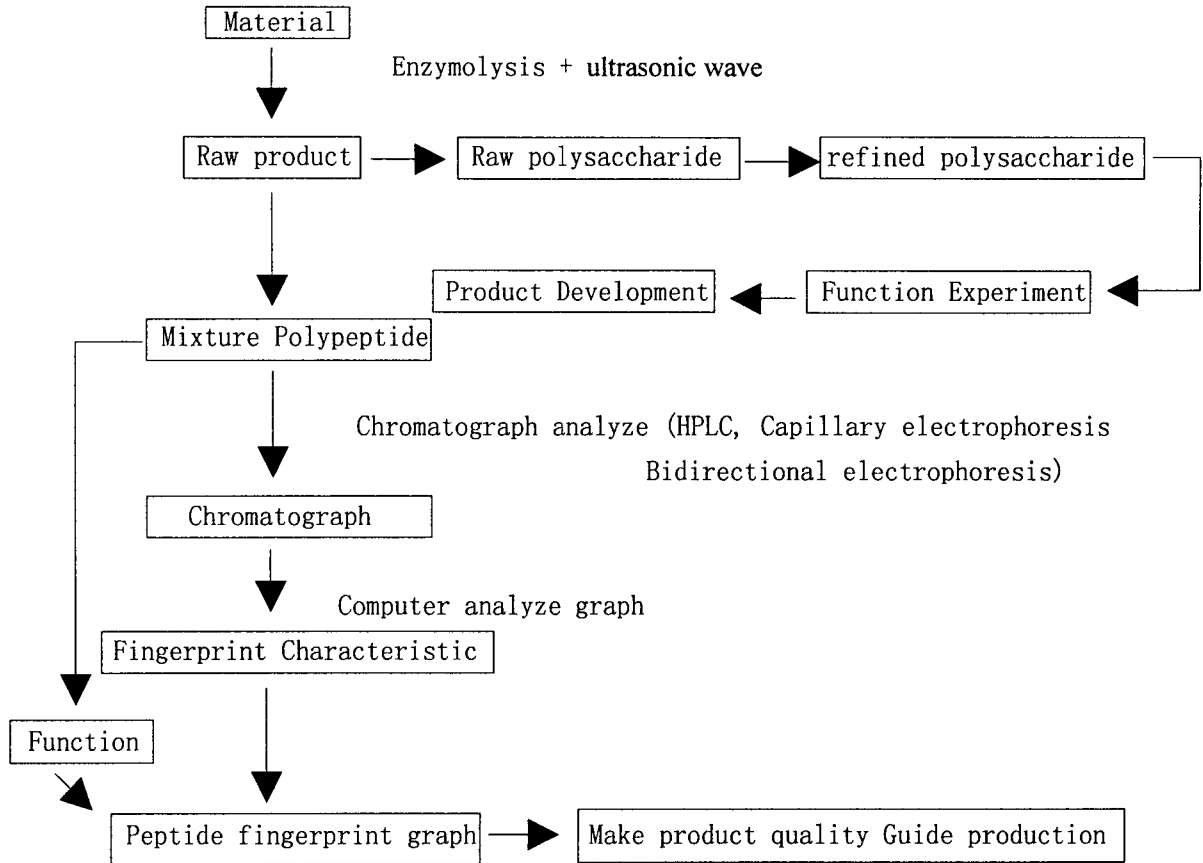
* Significantly different from the contrast group ($p < 0.05$)

It is obviously seen from the Tab.7 that adding sea cucumber's peptide in the fodder, has a remarkable result to mice fatigue resistance ability. And its mechanism waits for a further discussion from both molecule and physiology biochemical study.

3. Conclusion and prospect

The nutritive value of the sea cucumber has been praised highly by the human since ancient times. Research indicate that sea cucumber for medicinal purposes, health care value is quite high. However, because its price is expensive and due to people's traditional diet habit, the research to the sea cucumber has remained in low repeatedly studying level all the time. At the same time the research of the active material of sea cucumber's physiology has been confined to a certain material all the time, mutually divides active material increased the research cost and prevented deepening research step. Sea cucumber is treasure all over, so it is great helpful to discuss its application and development.

General Idea:



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