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# Extraction of heparin sodium from pig intestinal mucosa by recombinant trypsin mutant

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***ABSTRACT:*** *Heparin sodium is the anticoagulant in clinic, which is mainly produced by enzymatic hydrolysis method. In this paper, the effect of three kinds of trypsin on the extraction of heparin sodium from pig small intestinal mucosa with the assistance of ultrasound-microwave synergy was studied. Heparin sodium extraction process adopts ultrasound-microwave processing, trypsin enzymolysis, D204 resin adsorption, sodium chloride solution elution, and ethanol precipitation. The results showed that the potency of crude heparin sodium by recombinant trypsin mutant Q197W was 97.59 U/mg, more than that of recombinant trypsin wild-type (91.26 U/mg) and standard trypsin (91.05 U/mg).*

***KEYWORDS:*** *Heparin sodium; Recombinant trypsin mutant; Pig intestinal mucosa*

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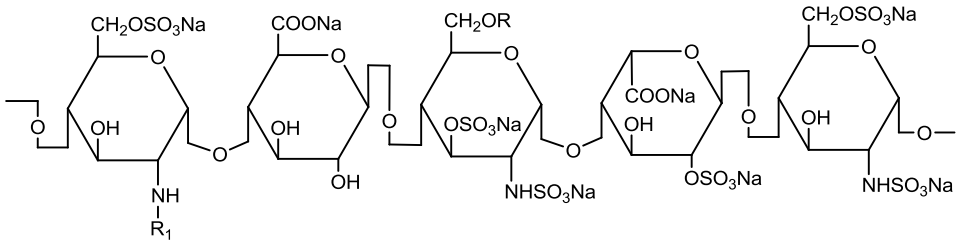
## I. INTRODUCTION

Heparin sodium is the sodium salt of heparin, which is a mucopolysaccharide and anticoagulant with a direct effect that prevents the activation of mammalian blood coagulation (Faham et al., 1996). The structure of heparin sodium is a polysaccharide chain linked by a glycosylation bond between uronic acid and glucosamine (Fig. 1). Combined with heparin, antithrombin exhibits the fast and potent inhibition for coagulant serine esterases: IXa, Xa and thrombin (Buyue et al., 2012). As the main anticoagulant in clinic, heparin sodium also has the functions of lowering blood lipid, anti-cancer, anti-virus, anti-inflammation, reducing blood viscosity and so on (Papa et al., 2000; Dong et al., 2018).




$R_1$= $SO_3Na$ or $COCH_3$ R= H or $SO_3Na$

**Fig. 1** The chemical structure of heparin sodium

Heparin sodium is widely distributed in the intestinal mucosa, lung and liver of mammals. There are two precesses of extracting heparin sodium: salt-hydrolysis and enzymatic hydrolysis. In the 1980s, heparin research group of Si-Chuan University promoted the salt-hydrolysis process to extract heparin sodium from pigs' small intestine (Ma et al., 2016). In the 1990s, enzymatic hydrolysis process of heparin sodium was produced. Enzymatic hydrolysis process is the addition of hydrolase to the intestinal mucosa, causing it to lyse fully, hydrolyzing the protein in the heparin-protein complex and releasing the heparin completely (Tang et al., 2015). With the improvement of the quality and quantity of heparin in the market, the enzymatic hydrolysis technology has been paid more and more attention. The extraction of heparin sodium by enzymatic hydrolysis can greatly reduce the discharge of wastewater, improve the yield, and save cost (Yu et al., 2016).

As a hydrolase for extracting heparin sodium, trypsin can not only hydrolyze the protein in heparin-protein complex, but also remove some oligosaccharides which have less biological activity, reducing the impurity of product, improving the uniformity and purity of heparin sodium (Zhou et al., 1999; Li, 2005). Animal-derived trypsin is limited by raw materials, having long production cycle, high cost and not suitable for large-scale production (Feng et al., 2014). Recombinant trypsin is now widely used in medicine. Compared with the enzyme extracted from animals, the recombinant enzyme has no animal origin, clear background, high purity and no animal virus, and is suitable for the production of drugs. Therefore, in this paper we investigated the application of a highly active trypsin mutant in the extraction of heparin sodium.

## II. MATERIALS AND METHODS

### 2.1 Materials and Reagents

Recombinant trypsin mutant Q197W and wild-type (Our laboratory preparation); Standard trypsin (Sigma Co., Ltd.); N-benzoyl-L-arginine ethyl ester and azure A (Shanghai Macklin Biochemical Technology Co., Ltd.); sodium chloride (Sinopharm Chemical Reagent Co., Ltd.); absolute alcohol (Tianjin Fuyu Fine Chemical Co., Ltd.); D204 resin (The Dow Chemical Co. ); Pig intestinal mucosa (market in Yichang).

### 2.2 Instrument and Equipment

Ultraviolet photometer (Shimadzu Co., Ltd.); Ultrasonic Cleaner (Zhengzhou Borui Ultrasonic Equipment Co., Ltd.); Microwave oven (Midea Co. , Ltd.); Vacuum drying box (Shanghai Kuntian Laboratory Instrument Co., Ltd. ).

### 2.3 Method

#### 2.3.1 Heparin Sodium Extraction Steps

(1) Resin pretreatment

New resin, with 5% NaOH solution for 3 ~ 5 hours, often stirring, washing to neutral. Soak in 7% hydrochloric acid solution for 3 ~ 5 hours, stirring frequently, washing in water until neutral, then soak in 18% saline, set aside.

(2) Cleanse the pig small intestine

The pig small intestine is rinsed with clear water inside and outside.

(3) Scraping and grinding of intestinal mucosa

Cut the intestine with scissors, scrape the mucosa inside the small intestine, and put 50g of the intestinal mucosa in the grinder with a small amount of distilled water to grind it into a minced shape.

(4) microwave processing

Add 750 mL distilled water in a 1000 mL beaker, add the ground mucosa and mix it, put it in the microwave oven (power 100W) for 10 minutes, then adjust the pH to 8.5 with dilute NaOH solution.

(5) Ultrasonic wave assistance

The material was put into the ultrasonic cleaner and sealed with the plastic film, and treated with ultrasonic wave (power 360W) for 40 min.

(6) Adjusting pH

After ultrasonic wave process, the pH of the feed solution was adjusted to 9 by dilute NaOH solution at 37 ℃, and then put into a constant temperature water bath at 37 ℃.

(7) Enzymolysis

Add 150mL 2% trypsin into the solution, stir for 10 minutes, then add 1.5 g NaCl, stir for 10 minutes and place in a 50 ℃ incubator for 3 hours.

(8) Adsorption

The solution was heated to 76 ℃ for 30 minutes, the impurities were removed by nynon filter, then cooled to 55 ℃, 20 g pre-treated resin was added, and stirred at 55 ℃ for 12 hours.

(9) Elution

The resin was obtained by filtrating the adsorbed solution, rinsing the resin with hot water at 60 ℃, rinsing it with water, then loading it into the ion chromatography column, and eluting the resin with 200 mL saturated NaCl solution at the rate of 1.1 mL/min under the action of an intelligent constant flow pump, elution with the same saturated NaCl solution was repeated 3 times. Then 100 mL saturated NaCl solution was used for the second elution, repeated elution 3 times.

(10) Precipitation by ethanol

The two batches of eluent were added 95% ethanol. There are white particles precipitated, then filtered to collect precipitation.

(11) Vacuum drying

The precipitate is placed in a vacuum drying box at 50 ℃ until constant weight.

**2.3.2 Activity Determination of Crude Heparin Sodium**

(1) Barbiturate 0.1104 g is dissolved in the 0.5 mol/L NaOH solution, and diluted with distilled water to 10mL.

(2) Azure 0.1 g is dissolved completely with a small amount of distilled water, diluted to 100 mL, filter it and store the filtrate in refrigerator. When in use, 5 mL of storage filtrate is taken and 25 mL of distilled water is added, and mixed evenly.

(3) The sample of 5mg is accurately weighed and diluted to 50mL with water to prepare 0.1 mg/mL solution. 1 ~ 5 mL of the assay solution are taken respectively and added distilled water to 5 mL. The absorbance value is determined at 505 nm after full shaking, and the average value of the absorbance of 3 parallel samples is obtained. Activity of crude heparin sodium is determined by standard curve.

## III. RESUITS AND DISCUSSION

As shown in Fig. 2, the heparin sodium product by trypsin mutant process is white powder. The yield and potency of crude heparin sodium by recombinant trypsin mutant is 11.74% (using 50g intestinal mucosa) and 97.59 U/mg, more than the standard trypsin and recombinant trypsin wild-type. So recombinant trypsin mutant could be used in the production of heparin sodium to improve product yield and quality.

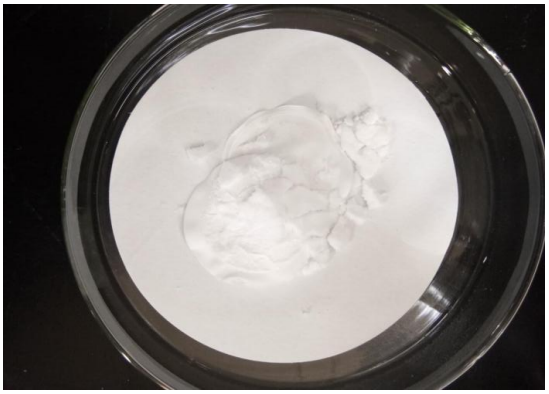


**Fig. 2** Appearance of heparin sodium by trypsin mutant process

**Table 1** The weight and potency of crude heparin sodium by different enzymes

| Enzyme | Weight of crude heparin sodium (g) | Potency (U/mg) |
|---|---|---|
| Standard trypsin | 4.38 | 91.05 |
| Recombinant trypsin wild-type | 5.62 | 91.26 |
| Recombinant trypsin mutant Q197W | 5.87 | 97.59 |

## IV. CONCLNSIONS

In this experiment, different types of trypsin were used to extract heparin sodium from pig small intestinal mucosa through ultrasound-microwave assisting enzymolysis process. The results showed that the extraction effect of crude heparin sodium by recombinant trypsin mutant Q197W was better than recombinant trypsin wild-type and standard trypsin, which could be used in the production of heparin sodium to improve product yield and quality.

**Conflict of interest**

The authors declare that they have no competing interests.

## ACKNOWLEDGEMENTS

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## REFERENCES

[1]. Faham S, Hileman RE, Fromm JR, Linhardt RJ, Rees DC. (1996) Heparin Structure and Interactions with Basic Fibroblast Growth Factor. Science 271: 1116–1120

[2]. Buyue Y, Misenemer TM, Sheehan JP. (2012) Low molecular weight heparin inhibits plasma thrombin generation via direct targeting of factor IXa: Contribution of the serpin-independent mechanism. Journal of Thrombosis and Haemostasis10: 2086-2098

[3]. Papa A, Danese S, Gasbarrini A. (2000) Potential therapeutic applications and mechanisms of action of heparin in inflammatory bowel disease. Alimentary Pharmacology & Therapeutics 14(11): 1403-1409

[4]. Dong B, Zhu Y, Wu D. (2018) Clinical application and troubleshooting of heparin pump in hemodialysis. China Medical Equipment 2: 132-134
[5]. Ma ZK, Ma L. (2016) Research progress in extraction methods of heparin sodium from animal organs. Advances in Veterinary Medicine 37(5): 106-108
[6]. Tang BC, Tong YG, Pan CW. (2015) Study on the process of enzymatic hydrolysis of heparin sodium with complex enzymes. Modern Chemical Industry 35(003): 98-100
[7]. Yu HN, Zhang P, Tao YH, Shen SR, Shan WG. (2016) Study on the process of preparing heparin by immobilized enzymatic hydrolysis of porcine small intestinal mucosa. Research and Development of Natural Products 28(2): 300-306
[8]. Zhou XW, He W. (1999) Purification of heparin sodium by enzymatic degradation. Chinese Journal of Biochemistry and Molecular Biology 3: 488-490
[9]. Li HG. (2005) The purification and potency analysis of heparin sodium. Journal of Shanxi University (Natural Science Edition) 4: 429-431
[10]. Feng XT, Liu SL, Huang X. (2014) Construction,expression and activity analysis of recombinant porcine trypsinogen. Progress in Pharmaceutical Sciences 38(12): 916-921

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