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Effects of Se-enriched polysaccharides produced by *Enterobacter cloacae* Z0206 on alloxan-induced diabetic mice

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ABSTRACT

In this study, the water-soluble selenium-enriched exopolysaccharides (Se-ECZ-EPS) were isolated from submerged culture broth of *Enterobacter cloacae* Z0206 through fermentation, ethanol precipitation and deproteinization. The protective effects of Se-ECZ-EPS on alloxan-induced diabetic mice were investigated. Diabetes was induced in ICR (Institute of Cancer Research) mice by administration of single doses of alloxan intraperitoneally (190 mg/kg body weight). Se-ECZ-EPS at a dose of 200 mg/kg body weight were administered per os (p.o.) as single dose per day to diabetes-induced mice for a period of 42 days. The decrease in body weight, serum insulin level, and the increase in blood glucose level, glycosylated serum protein (GSP), total cholesterol (TC) and triglycerides (TG) in liver were observed in diabetic mice. On the other hand, oral administration of Se-ECZ-EPS resulted in a significant reduction in fasting blood glucose levels, GSP, TC and TG contents in liver coupled with improvement of body weight and serum insulin level in comparison with diabetic control group. These results suggest that Se-ECZ-EPS possess significant protective and anti-diabetic effects in alloxan-induced diabetic mice.

1. Introduction

Diabetes mellitus (DM) is a serious chronic metabolic disease which now afflicts 3% of population world-wide, and it can be divided into two major categories (type-1, or insulin-dependent diabetes mellitus, and type-2, or non-insulin-dependent diabetes mellitus) based on individual etiologies, with a great majority (95%) of type-2 [1]. The major feature of type-2 diabetes is hyperglycemia and dyslipidemia resulting from defects in both insulin secretion and insulin resistance [2], and the key strategy in treating patients with type-2 diabetes is maintenance of blood glucose level [3]. There are many oral hypoglycemic agents for the treatment of type-2 diabetes currently, such as biguanides and sulfonylureas, however, these synthetic agents are associated with certain drawbacks, such as adverse side effects, decreased efficacy over time, ineffectiveness against some long-term diabetic complications and low cost-effectiveness [4]. Therefore, it is necessary to explore and discover novel safer and more effective drug for diabetes.

Selenium (Se) is an essential micronutrient, which is actively involved in animal physiology via a variety of selenoproteins [5]. These selenoproteins play preventive role in some degenerative conditions including cancer, inflammatory diseases, neurological diseases, aging, infertility and infections through specific cellular pathways [6]. It is reported that the Se supplementation decreased plasma glucose levels in diabetic rats [7] and human beings [8]. Se has also been found to induce a sustained improvement of glucose homeostasis in diabetic individuals by an insulin-like action, and to regulate vital metabolic processes such as glycolysis and gluconeogenesis [9]. Due to higher biological activities, lower toxicity compared with inorganic selenium, organic selenium can be absorbed and utilized rapidly. Lots of studies indicated that organification of selenium through microorganism fermentation technique provides a feasible and economic approach for production of organic selenium compounds and it becomes the focus in recent years [10].

Enterobacter cloacae Z0206, a bacterial strain, can produce large amounts of exopolysaccharides. In our previous studies [11], we found that *E. cloacae* Z0206 could accumulate Se in the form of Se-enriched exopolysaccharides (Se-ECZ-EPS) efficiently during cultivation with selenium. The major component of Se-ECZ-EPS with the average molecular weight of 29,300 Da was composed of glucose, galactose and mannose with a molar ratio of 8.530:0.061:0.706, and administration of this major component to cyclophosphamide-exposed mice resulted in improvement of cellular and humoral immune responses [11]. It has also been reported that glycoproteins from *E. cloacae* showed antitumor effects on mice with S180 tumors (real), and F3, one of the glycoprotein components, could distinctly inhibit the growth of QGY7703 (liver cancer), A549 (glandular cancer of the lungs), Kato III (gastric carcinoma) and Sw1116 (intestinal cancer) cell strains [12].

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However, little is known about the anti-diabetic effects of Se-ECZ-EPS produced by *E. cloacae* Z0206, which would allow a better understanding of the functional effects about those macromolecules, and be beneficial to explore new more bioresources. The objective of the current study was to prepare and extract Se-ECZ-EPS produced by *E. cloacae* Z0206 through fermentation, ethanol precipitation and deproteinization. Based on the experiments above, the possible anti-diabetic effects of Se-ECZ-EPS in alloxan-induced diabetic mice were further investigated.

2. Materials and methods

2.1. Materials

E. cloacae Z0206, the Se-ECZ-EPS producing bacterial strain, was identified and kept in our laboratory, and it has been collected by China General Microbiological Culture Collection Center. Alloxan was purchased from Sigma Chemical Co. (St. Louis, MO, USA). OneTouch Ultra blood glucosimeter was obtained from Johnson & Johnson Co. (New Brunswick, NJ, USA). All other reagents were of the highest purity and commercially available.

2.2. Microorganism cultivation

E. cloacae Z0206 was initially grown on PDA medium (fresh potato, 20% (w/v); dextrose, 2.0%; agar, 2.0%) at 30 °C for 1 day, and then transferred to 250 ml flasks containing 80 ml of seed culture medium (fresh potato, 20% (w/v); dextrose, 2.0%; peptone, 0.2%; yeast extract, 0.3%) and incubated on a rotary shaker at 250 rpm for 18 h at 30 °C.

Cultivation medium contained: sucrose, 2.5%; peptone, 0.5%; yeast extract, 0.5%, K_2HPO_4, 0.2%; KH_2PO_4, 0.1% and $MgSO_4{\cdot}7H_2O$, 0.05%. Se-ECZ-EPS production was performed in a 10 dm^3 bioreactor (Shanghai Biotech Ltd., China) with 7 dm^3 cultivation medium with stirring rate of 200 rpm at 30 °C for 2 days. The initial pH was 7.5 and the inoculation volume was 5.0% (v/v). The concentration (20 μg/ml) and time of adding selenium into the culture (6th h) were chosen according to our previous report [11]. Aeration rate (1.0 vvm), growth temperature, foam level, dissolved oxygen tension and pH were measured and/or controlled by the bioreactor control unit.

2.3. Preparation of Se-ECZ-EPS

The fermentation liquid was centrifuged at 4500 × *g* for 20 min to remove the mycelia after cultivation. The supernatant was evaporated under reduced pressure at 50 °C, then precipitated upon addition of 4 volumes of cold 95% EtOH and kept at −20 °C overnight. The resulting precipitate was collected by centrifugation at 7600 × *g* for 15 min at 4 °C. The precipitates were dissolved in distilled water and deproteinized by a combination of trypsin and papain enzymolysis and the Sevag method [13], then precipitated with 4 volumes of cold 95% EtOH at 4 °C overnight. After centrifugation at 7600 × *g* for 15 min at 4 °C, successive washes with anhydrous ethanol, acetone and ether, and drying under vacuum at 40 °C, Se-ECZ-EPS were obtained.

2.4. Analysis of Se-ECZ-EPS

The content of polysaccharides in Se-ECZ-EPS was quantified by phenol–sulfuric acid method [14]. Selenium contents were determined spectrophotometrically using a modified method of Kessi et al. [15] with 850 fluorimeter (HITACHI).

For primary structural analysis, the major component of polysaccharide in Se-ECZ-EPS (Se-ECZ-EPS-1) was purified using the methods described by Xu et al. [11]. Briefly, Se-ECZ-EPS was subjected to a DEAE-52 anion-exchange chromatography column (2.6 cm × 50 cm) eluting with distilled water at a flow rate of 30 ml/h. The major peak collected was concentrated and fractionated over a Sephadex G-100 column (1.6 cm × 50 cm) eluting with distilled water at a flow rate of 12 ml/h. Then the main peak fractions were dialyzed and lyophilized to give a white powder, Se-ECZ-EPS-1. The infrared spectra of Se-ECZ-EPS-1 (KBr disc) were recorded in frequency range of 4000–400 cm^{-1} using a Fourier transform infrared (FT-IR) spectrophotometer (Nicolet Nexus 670, Thermo Electron, USA).

2.5. Animals

Female ICR (Institute of Cancer Research) mice, weighting 26 ± 2 g, were supplied by Experimental Animal Center of Zhejiang Province. The mice were housed in plastic cages at room temperature (22 ± 1 °C) under a 12 h light-dark cycle, and provided with commercial pellet diet and water ad libitum. The mice were adapted to diet and general conditions of vivarium for 1 week before the experiment. All experiments were performed in accordance with the institutional ethical guideline.

2.6. Induction of diabetes

Diabetes was induced in 16-h fasting mice by a single intraperitoneal injection of freshly prepared alloxan (190 mg/kg body weight, dissolved in 0.9% normal saline). Whole blood samples were obtained from the tail vein of the overnight fasted mice 72 h after alloxan injection, and blood glucose levels were measured with OneTouch Ultra (Johnson & Johnson, New Brunswick, NJ, USA) blood glucosimeter. The mice with blood glucose levels at 10–28 mmol/l were considered diabetic and used for the study.

2.7. Experimental design

In the experiment, a total of 30 mice (20 diabetic surviving mice, 10 normal mice) were used and divided into three groups of 10 each. Group I (CON): normal control mice received physiological saline by oral administration; Group II (DM): diabetic control mice received physiological saline by oral administration; Group III (DM + Se-ECZ-EPS): diabetic mice received Se-ECZ-EPS (200 mg/kg body weight) in aqueous suspension daily by oral administration. The experiment lasted for 42 days. Body weights of mice were recorded every two weeks.

At the end of the experiment, the animals were fasted overnight and sacrificed by cervical dislocation. Blood samples were collected for blood glucose level analysis and then centrifuged at 960 × *g* for 15 min at 4 °C to separate the serum. Livers of mice were immediately excised, washed with ice-cold saline to remove the blood, blotted dry and stored at −70 °C.

2.8. Biochemical measurements

Blood glucose levels were measured with OneTouch Ultra (Johnson & Johnson, New Brunswick, NJ, USA) blood glucosimeter. Serum insulin was assayed by ELISA with a microplate reader (Denley Dragon Wellscan MK 3, Thermo, Finland) and glycosylated serum protein was determined by fructosamine assay with Architect CI8200 chemistry analyzer (Abbott Laboratories, Abbott Park, IL, USA) using corresponding commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

About 0.5 g of liver was minced and homogenized (10%, w/v) in normal saline solution, centrifuged at 660 × *g* for 10 min at 4 °C, and the resulting supernatant was collected. Concentration of total protein, total cholesterol (TC) and triglycerides (TG) were determined