

Novel Anticoagulant Compound from Fermented Edible Brown Seaweed, *Laminaria ochotensis*

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Introduction

Heparin, a highly sulfated polysaccharide is used as a blood anticoagulant in laboratories as well as in therapeutics. The disadvantages occurred with heparin give rise to a new area of research for discovering novel substances with blood anticoagulant activity. The anticoagulant activity of marine algae was first reported in 1936. Different techniques such as cold and hot water, mild acids, CaCl₂ (Pericival, 1979) and enzymes are used to extract anticoagulant compounds from brown algae. Fermentation involves the breaking down of complex organic substances into simpler ones and finally forms many end products. The microbial enzymes play an active role in fermentation processes and these end products formed by the action of one or more microorganism, either working together or in a sequence (Atlas, 1995). However, screening of bioactive compounds from seaweed fermentation has not yet been studied. Purpose of the present study was to isolate and purify an anticoagulant compound from fermented edible marine brown seaweed, *Laminaria ochotensis*.

Materials and Methods

L. ochotensis was fermented with 15% sugar (w/v) at 25°C for 10 weeks. Anticoagulant activity was measured from the supernatant of algal mixture at biweekly intervals up to 10th week by activated partial thromboplastin (APTT), prothrombin time (PT) and thrombin time (TT) assay using citrated human plasma. Sample having high APTT activity (6th week) was filtered, ethanol precipitated and freeze-dried. The polysaccharide compound having anticoagulant activity was purified by DEAE ion exchange chromatography followed by Sepharose-4B gel filtration

chromatography. Anticoagulant activity, polysaccharide concentration, and heparin like activity were determined for the collected fractions by APTT, phenol-H₂SO₄, and glycosaminoglycan assay, respectively. Purified polysaccharide was applied to a 0.5% agarose gel to find out the degree of purity of the compound. The molecular mass of the purified polysaccharide was estimated by polyacrylamide gel electrophoresis (PAGE).

Results and Summery

The anticoagulant activity assay showed that the activity was increased up to 6th week, and decreased thereafter. The concentration of our purified compound was 31.0 µg/ml and showed higher APTT activity than commercial heparin. At the same concentration of 31.0 µg/ml, the heparin showed 186.5 sec activity while our purified compound showed an activity of 386 sec. Single spot on agarose gel electrophoresis showed that the compound was purified and polyacrylamide gel electrophoresis (PAGE) results revealed that the molecular mass of the purified polysaccharide compound was between 60 and 500 kD. Therapeutic interest of the algal polysaccharide as an anticoagulant has recently been in highlighted. This purified anticoagulant compound from fermented *L. ochotensis* can be used as a model for anticoagulant agent or could be developed as an anticoagulant agent. This study can be extended to identify the structure and chemical composition of the purified polysaccharide, and to establish a relationship between structure and the function of the identified anticoagulant compounds.

References

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