

A novel preparation method for a proteoglycan in a matrix with collagen from salmon (*Oncorhynchus keta*) nasal cartilage and its affinity to L-selectin

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Abstract

An intact proteoglycan was extracted from salmon (*Oncorhynchus keta*) nasal cartilage containing type II collagen and prepared using a novel extraction procedure in water containing a sugar fatty acid ester as an edible detergent. This isolation step suppressed the degradation of the proteoglycan and simultaneously afforded a proteoglycan-type II collagen matrix. The extracted proteoglycan was purified, and its properties were compared with those prepared via different extraction procedures using gel permeation chromatography and polyacrylamide gel electrophoresis. Furthermore, the interaction between the purified proteoglycan and human L-selectin was analyzed using a bio-layer interferometry biosensor assay; the proteoglycan demonstrated strong binding to L-selectin.

Keywords : Salmon nasal cartilage, proteoglycan, sugar fatty acid ester, L-selectin binding

I Introduction

The extracellular matrix of cartilage is a complex of proteoglycans, hyaluronan, collagens, and other proteins.¹⁻³⁾ Proteoglycans are composed of glycosaminoglycan (GAG) chains and a core protein in which the hydroxyl group of serine/threonine residues are attached to a linkage tetrasaccharide. Aggrecan, a brush-type, large chondroitin sulfate proteoglycan is one of the major components of salmon nasal cartilage.⁴⁾ Several reports on the physiological activities of salmon (*Oncorhynchus keta*) nasal proteoglycan indicate stimulatory activity in the immune system in mice and curative properties for acute colitis induced by dextran sulfate sodium in rats.⁵⁾ The anti-aging effects of proteoglycans in hairless mice have also been investigated.^{6, 7)} Based on these observations, it is potentially very important to isolate proteoglycan samples from the tissue of salmon nasal cartilage.

Several reports on the isolation and preparation of proteoglycan molecules from tissue materials demonstrate that homogenization and extraction steps are important for obtaining large amounts of intact proteoglycans.⁸⁾ However, either the quality or recovery of proteoglycans reported in previous reports seems to be inappropriate for nutraceutical

and cosmeceutical materials.⁹⁻¹¹⁾ The current study describes a new approach for the preparation of a proteoglycan from salmon nasal cartilage, which is a source of nutraceutical and/or cosmeceutical materials. The relationship between the anti-inflammatory effects of the proteoglycan and L-selectin^{12, 13)} was also investigated.

II Materials and Methods

1. Abbreviations

CS: chondroitin sulfate; Δ Di-0S: 2-acetamide-2-deoxy-3-*O*-(β -D-*gluco*-4-ene-pyranosyluronic acid)-D-galactose; Δ Di-4S: 2-acetamide-2-deoxy-3-*O*-(β -D-*gluco*-4-ene-pyranosyluronic acid)-4-*O*-sulfo-D-galactose; Δ Di-6S: 2-acetamide-2-deoxy-3-*O*-(β -D-*gluco*-4-ene-pyranosyluronic acid)-6-*O*-sulfo-D-galactose; Δ Di-diS_B: 2-acetamide-2-deoxy-3-*O*-(2-*O*-sulfo- β -D-*gluco*-4-ene-pyranosyluronic acid)-4-*O*-sulfo-D-galactose; Δ Di-diS_D: 2-acetamide-2-deoxy-3-*O*-(2-*O*-sulfo- β -D-*gluco*-4-ene-pyranosyluronic acid)-6-*O*-sulfo-D-galactose; Δ Di-diS_E: 2-acetamide-2-deoxy-3-*O*-(β -D-*gluco*-4-ene-pyranosyluronic acid)-4, 6-di-*O*-sulfo-D-galactose; Δ Di-triS: 2-acetamide-2-deoxy-3-*O*-(2-*O*-sulfo- β -D-*gluco*-4-ene-pyranosyluronic acid)-