Preparation of collagen and collagen peptides from bluefin tuna skin (bone and scale) and their action on stressed HepG2 cell

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Collagens were extracted from bluefin tuna (*Thunnus Orientails*) abdominal skin and the reagent grade of salmon skin collagen was used for standard sample. The peptides of the bluefin tuna abdominal skin collagen and the salmon skin collagen were prepared by reacting with trypsin (pH7.8) at 37°C for 5 min. The yield of collagen from frozen bluefin tuna abdominal skin was 4.1%. The solubility of the bluefin tuna abdominal skin collagen was 93.8±2.2% and the salmon skin collagen was 100.0±3.9% at the concentration of 100 µg in 1 mL of distilled water. The degree of hydrolysis (DH) of peptides from bluefin abdominal tuna skin collagen was 96.2±4.1% and that of peptides from salmon skin collagen was 53.1±2.5%. The main protein band of the bluefin tuna abdominal skin collagen and the salmon skin collagen in SDS-PAGE were different in two α1 chains and α2 chain, respectively. The bluefin tuna abdominal skin collagen reduced (22%) of cell growth of HepG2, relatively.

These days consumption of fish was increased worldwide and the Japanese especially, consume a wide range of fish species daily. Particularly, Bluefin tuna (*Thunnus Orientails*) is one of the famous tunas in Japan and its consumption and culture has been increasing. However, great quantities of these wastes like skin, bone, and fin are produced in many fish shop and fish-processing factories. These wastes are dumped in which they caused pollutions emit with an offensive odor. However, fish bone, skin, scale and fins are good source of collagen. Collagen is a common protein that makes up a significant part of the living body, whether human and animal. As a structural protein, collagen is essential to build the body's physical structure, and as an extracellular matrix it acts as a supporting framework over which our cells are arranged. Also, collagen is widely and diversely used in food, medicine, and cosmetics; the consumption of collagen has increased with the development of new industrial application.

Aquatic animals have been increasing attention as a backup collagen resource since bovine spongiform encephalopathy (BSE). But the study about collagen of bluefin tuna and their action on cancer cell is not sufficient from the tuna wastes.

In this study, we report preparation of type I collagen and collagen peptides and that of action on HepG2 cell.

Measurements

- Collagen preparations

- Collagen recovery rate and yield

- Solubility determination of type I collagen in distilled water

- Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting

- Preparation of collagen peptides

- Degree of collagen hydrolysis

- HepG2 cell culture and MTT assay

Results and Discussions

The protein recovery rate was 1.8 g/100 g and the yield of type I collagen from freezing bluefin tuna abdominal skin was 4.1%.

Solubility of type I collagen from bluefin tuna abdominal skin and salmon skin in distilled water decreased as concentration of collagen increased. Two collagen samples showed high solubility under a concentration of 0.1 mg/mL but the solubility reduced markedly after 5 mg/mL concentration. The bluefin tuna abdominal skin collagen was high solubility than the salmon skin collagen within all range of concentration in distilled water. This seems to the results from different imino acid contents.

SDS-PAGE pattern showed that bluefin tuna abdominal skin collagen and salmon skin collagen were composed of two different α chains (α1 and α2) and β chain. The density of α1 is higher than that of α2 of bluefin tuna abdominal skin collagen and salmon skin collagen and this result were similar pattern with the previous report of other fish species and is typical of type I collagen. The estimated molecular weight for α1and α2 chains, using marker standard were approximately 120 kD and 112 kD. The β chain is a dimmer and molecular weight was approximately 205 kD of samples. The prepared antisera against fish collagen were reacted with bluefin tuna abdominal skin collagen and salmon skin collagen. The reactivity of salmon collagen seemed to be high but the reactivity of bluefin tuna abdominal skin

collagen was not relatively high.

Degree of hydrolysis of salmon skin collagen peptide was 53.1% whereas degree of hydrolysis of bluefin tuna abdominal skin collagen peptide was 96.2%. The degree of hydrolysis of the prepared salmon skin collagen peptide was lower than that of collagen peptides from bluefin tuna abdominal skin by 43%. This results maybe seems to be difference of amino acid content and imino acid content between the bluefin tuna abdominal skin collagen and the salmon skin collagen.

The bluefin tuna skin collagen and their peptides and the salmon skin collagen were added to HepG2 cell by 20 μg/1.0×10^4 cells. The bluefin tuna skin collagen had 22% reduction of the cell growth regardless sample adding timing but salmon skin collagen did not reduce the cell growth, completely. The action of both preparation collagen peptides did not reduce on the cells growth.

This interesting result suggested that utilization value of collagen from bluefin tuna abdominal skin waste as a functional component on cancer cell. Moreover, studies of the mechanism for reduction effect of cell proliferation and the effect of bluefin tuna abdominal collagen on other cancer cells will be needed.

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