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Purification and characterization of a lectin from hard roe of skipjack tuna, *Katsuwonus pelamis*

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Introduction

Lectins or carbohydrate-binding proteins are proteins of non-immune origin, which is able to agglutinate cells, and precipitate polysaccharides and glycoconjugates (Goldstein et al., 1980). Lectins with diverse physiological roles are widely distributed to the various plant, microorganisms, invertebrates and vertebrates. The ubiquitous occurrence of lectins in nature and their ability to recognize complementary sugars provided a stimulus for the continuous research for their biochemical properties and physiological functions (Raz and Lotan, 1987). Recently, lectins of fish eggs can be classified into three groups according to their saccharide binding specificity. Lectins of the first group are noted for their pronounced binding affinity for L-rhamnose and D-galactose. The second group has affinity to L-fucose and D-glucose, and finally, the third group exhibits elevated binding affinity to sialoglycoconjugates. In the present paper, we report the purification and some properties of a lectin from hard roe extracts of skipjack tuna, *Katsuwonus pelamis*, including structural properties with respect to recognition between sugar and the lectin.

Materials and Methods

Hemagglutination and hemagglutination inhibitory assay. Hemagglutination activity was assayed by the two-fold serial dilutions of a lectin solution (50 μl) in 96-well microtiter plate using 3% (v/v) suspension of human blood erythrocytes washed with PBS-Ca, phosphate buffered saline containing 10 mM $CaCl_2$ (pH 7.4). The protein solution (50 μl) was mixed with 50 μl

erythrocytes and incubated at room temperature for 1 h. The activity was expressed as titer, i.e. the reciprocal of the highest dilution showing complete agglutination. Specific activity was expressed as titer of lectin per mg of protein. The hemagglutination activity was also tested using 3% (v/v) suspensions of rat and mouse blood erythrocytes. Hemagglutination inhibitory test was conducted using the same way as hemagglutination activity determination. The lectin solution (25 μl) was allowed to react with various concentrations of the test sugar solutions (25 μl) after preincubation (room temperature, 15 min) for 1 h. The 3% (v/v) suspension of 50 μl of blood erythrocyte suspension 3% was then added to the mixture and the activity was measured after standing at room temperature for 1 h. The results were expressed by the minimum concentration of the inhibitors.

Results and Discussion

A lectin (KPL) from the hard roe extract of skipjack tuna, *Katsuwonus pelamis* was purified by gel filtration on Sephadex G-100 and affinity chromatography on asialofetuin-Sepharose 4B. The molecular mass of the purified lectin was estimated to be approximately 150 kDa as determined by gel filtration, and 37.5 kDa by SDS-PAGE, under non-denaturing and denaturing conditions. The lectin specifically agglutinated human blood type B erythrocytes, but not other human blood types. Hemagglutination inhibitory test indicated that D-galactose, lactose and asialofetuin among the materials tested were potent inhibitors of KPL-induced hemagglutination. The test exhibited an optical pH 6.0-8.5 and temperature, 40℃ in the present of Ca^{2+}. In additon, its activity was inhibited by EDTA and restored by addition of the divalent metal cation. The amino-terminal amino acid sequence of the lectin was revealed as Pro-Val-Gln-Leu-Cys-Asp-Ala-Lys-Cys-Thr.

References

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