ナミイソカイメンのレクチンの性状

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Short Paper

Purification and Characterization of a Lectin from a Marine Sponge *Halichondria panicea*[*1]

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The unique ability of lectins to combine specifically with saccharides has provided us with valuable research tools for biological and medical investigation.[1)] There is a considerable interest in discovering and isolating new lectins for applications. Lectins have been found in the hemolymph and tissue extracts of marine invertebrates.[2)] However, only a few lectins have been isolated and characterized in detail.[3-5)] In searching for lectins in marine invertebrates, we found that an extract of the sponge *Halichondria panicea* collected in Kohchi Prefecture, Japan showed potent hemagglutinating activity against various mammalian erythrocytes. The present paper deals with the purification and partial characterization of lectins from *H. panicea*.

A saline extract of *H. panicea* agglutinated animal erythrocytes nonspecifically, such as rabbit (titer value 512), sheep (512), and human ABO erythrocytes (256–512). The hemagglutinating activity was sensitive to the heat treatment. The original activity of the extract diluted to titer of 1:64 reduced to 1:4 when kept at 60°C for 15 min. However, the activity was not destroyed even at 80°C for 15 min. The hemagglutinating activity was dependent on the presence of Ca^{++}-ions and the activity of the extract was lost on the addition of 50 mM EDTA. As shown in Table 1, lactose was the most effective inhibitor among the simple sugars tested. D-Galactose, D-galactosamine, and N-acetyl-D-galactosamine also showed inhibitory activity when checked with rabbit erythrocytes. D-Galacturonic acid and D-glucuronic acid also showed a marginal inhibitory effect on hemagglutination. However, there is the possibility that these uronic acids inhibit hemagglutination by chelating divalent cations necessary to the erythrocytes agglutination, since uronic acids possess chelating activity. Other simple sugars tested showed no inhibitory activity at a concentration of 200 mM.

H. panicea lectins were purified by affinity chromatography on HCl-treated Sepharose 4B. The extract was applied to a column (3.2×38 cm) of acid-treated Sepharose 4B previously equilibrated with 50 mM Tris-HCl buffer (pH 8.0) containing 10 mM $CaCl_2$.

Table 1. Hemagglutinating inhibition of the *Halichondria panicea* extract* by simple sugars

Inhibitor	Initial conc.	Maximum inhibiting sugar dilution
D-Galactose	200 mM	1:16
D-Galactosamine	200 mM	1: 8
N-Acetyl-D-galactosamine	200 mM	1: 4
Lactose	200 mM	1:64
D-Galacturonic acid	100 mM	1: 1
D-Glucuronic acid	100 mM	1: 2

* *Halichondria panicea* extract was diluted to titer of 8.

After washing thoroughly with the same buffer to remove unbound proteins, the lectins were eluted with 0.2 M lactose in the Tris-HCl buffer (pH 8.0). This step was effective to remove inactive proteins which were not separable by gel filtration. Further separation was carried out by high speed gel filtration on TSKG-3000SW (0.75×60 cm Toshoh, Tokyo) using 0.25 M phosphate buffer (pH 7.0), which gave three protein peaks; the second and major protein peak showed potent hemagglutinating activity against rabbit, sheep, and human ABO erythrocytes. The other two peaks also showed agglutinating activity, but the activity was less than one-hundredth. The final preparation could agglutinate rabbit erythrocytes at a concentration as low as 140 ng protein/m*l*. In a typical run, 100 g of sponge yielded 0.2 mg protein of the purified lectin.

The purified lectin gave a single band corresponding to a molecular weight of 29,000 in SDS-slab electrophoresis with or without 2-mercaptoethanol. However, in isoelectric focusing the final preparation gave a major band at pH 6.4, and also additional minor three protein bands at pH 6.0, 6.3 and 6.8. The molecular weight of the intact lectins was estimated to be 49,000 by gel filtration on TSK G-3000SW. The ratio of the molecular weight of the subunit to the intact specimen is close to 2.

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