Short Paper

A Hemagglutinin in the Sponge *Phyllospongia foliascens*: Isolation and Characterization*¹

Hisao Kamiya,*² Koji Muramoto,*²
Takaharu Hoshino,*³ Masatoshi Yamazaki,*⁴
and Uday Raj*⁵

(Accepted February 6, 1986)

Recently, we have found high agglutinating activity against various kinds of animal erythrocytes in some sponges collected in the vicinity of Fiji and have isolated the D-galactose-specific hemagglutinin from *Dysidea herbacea*.¹⁾ Another species of the sponge *Phyllospongia foliascens* was found to contain nonspecific hemagglutinins against human ABO erythrocytes. We report here the isolation and characterization of a hemagglutinin in *P. foliascens*.

The extract of *P. foliascens* was prepared by squeezing sponge tissues with an equal volume of 50 mM Tris-HCl buffer, pH 8.0 containing 145 mM NaCl. It was centrifuged at 10,000 rpm for 30 min at -4°C. *Phyllospongia* hemagglutinin was purified by affinity chromatography on acid-treated Sepharose 4B. As shown in Fig. 1, it was adsorbed on acid-treated agarose gels and was eluted with 200 mM D-galactose in the buffer. Hemolysin(s), which interfered hemagglutination assay of the extract, was separated effectively in this step. The main active fractions (indicated by horizontal bar in Fig. 1) were combined and ultrafiltered using UK-10 membrane (molecular cut 10,000). The retentate was dialyzed against 25 mM Tris-HCl buffer and loaded onto a column of DEAE-Toyopearl 650S (1.2×12 cm) previously equilibrated with the starting buffer (25 mM Tris-HCl buffer, pH 8.0). The column was first eluted with the starting buffer and subsequently with a linear gradient of 0 to 500 mM NaCl in the buffer. The active fractions were pooled, ultrafiltered, and dialyzed against distilled water. In a typical run, a 100 g of frozen *Phyllospongia* yielded 1.2 mg of the final preparation.

Purified *Phyllospongia* hemagglutinin agglutinated human ABO erythrocytes and rabbit erythrocytes. The minimum active concentration against rabbit blood cells was estimated to be 1.1 µg protein/ml. The activity was independent of divalent cations, and the titer value was unchanged in the presence of 100 mM EDTA, as was reported for agglutinins from various kinds of sponges.¹⁻³⁾ In hemagglutination inhibition tests with 14 available simple sugars, lactose, N-acetyl-D-galactosamine, D-galacturonic acid, and N-acetylneuraminic acid inhibited hemagglutination of the test solution with eight units at the concentration as high as 100 mM. Although *Phyllospongia* hemagglutinin was adsorbed on acid-treated agarose gels and was eluted by D-galactose, D-galactose itself was inactive at the concentrations tested. Similar observation was also reported for *Geodia* hemagglutinin.³⁾ No signi-

Fig. 1. Affinity chromatography of *Phyllospongia* hemagglutinin.

The extract (1.6 g protein) was applied to a column of acid-treated Sepharose 4B (3.2×38 cm). The column was eluted with 50 mM Tris-HCl buffer containing 145 mM NaCl and subsequently with 200 mM D-galactose. Fractions (6 ml) of eluate were analyzed for absorption at 280 nm (○) and for activity (●). The active fractions (horizontal bar) were combined.

Table 1. The amino acid composition of *Phyllospongia* hemagglutinin

Amino acid	Mol %	Amino acid	Mol %
Asx	14.0	Met	1.5
Glx	10.5	Ile	7.1
Ser	6.4	Leu	6.6
Thr	5.9	Phe	4.5
Gly	6.9	Trp	ND*
Ala	4.2	Tyr	5.3
Cys (half)	0	Arg	3.8
Pro	6.0	Lys	7.2
Val	6.4	Hir	3.9

* Not determined.

ficant mitogenic activity was observed when checked with spleen cells from C3H/He mice at various concentrations ranging from 1.5 to 100 µg protein/ml.

The molecular weight of *Phyllospongia* hemagglutinin was estimated to be 38,000–40,000 by Fast Protein Liquid Chromatography on Superose 12 (1.0×30 cm, Pharmacia NJ). In SDS-polyacrylamide disc gel electrophoresis with or without 2-mercaptoethanol, it showed a single band corresponding to a molecular weight of 15,000. The purified specimen, however, gave multiple protein bands in isoelectric focusing. It showed the major band at pI 6.4 and two faint bands at pI 6.2 and 6.6, suggesting that *Phyllospongia* hemagglutinin is composed of at least three isolectins. Neutral sugar content was determined to be 4.7% by phenol-sulfuric acid method using glucose as a standard. The amino acid composition is listed in Table 1. The lack of half-cystine indicated that disulfide bonds were not involved in the association of subunits, as have been shown in many hemagglutinins from various sources.⁴⁾

References

- 1) H. Kamiya, K. Muramoto, T. Hoshino, and U. Raj: *Experientia*, **41**, 1201–1202 (1985).
- 2) H. Bretting, E. A. Kabat, and M. E. A. Pereira: *Biochemistry*, **15**, 5029–5038 (1976).
- 3) P. Vaith, G. Uhlenbruck, W. E. G. Muller, and G. Holz: *Develop. Comp. Immunol.*, **3**, 399–416 (1979).
- 4) E. R. Gold and P. Balding: *Receptor-specific Proteins, Plant and Animal Lectins*. Excerpta Medica, Amsterdam, 1975, pp. 405–424.

*¹ Studies on the Bioactive Marine Metabolites—V.

*² School of Fisheries Sciences, Kitasato University, Iwate 022-01, Japan (神谷久男, 村本光二: 北里大学水産学部).

*³ Mukaishima Marine Biological Station, Hiroshima University, Mukaishima, Hiroshima 722, Japan (星野孝治: 広島大学理学部向島臨海実験所).

*⁴ Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01, Japan (山崎正利: 帝京大学薬学部).

*⁵ Institute of Marine Resources, The University of the South Pacific, Suva, Fiji (ユディ・ラジ: 南太平洋海洋資源研究所).