Hemagglutinins in Marine algae

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We surveyed the Japanese marine algae for hemagglutinins with rabbit erythrocytes. Algae used in this screening were 53 species; 6 species of green algae, 42 of red algae and 5 of brown algae. The results showed the presence of the active components with strong activities in 14 species; 4 species of the green algae, 9 of the red algae and 1 of the brown algae. The extracts of the algae which gave positive results were further examined for hemagglutinating activity against other erythrocytes of animals and human A, B, O and AB groups. Of the 14 species positive to hemagglutination with rabbit erythrocytes, 5 species showed no hemagglutinating activity with human erythrocytes. The results also indicated that the extract of a green alga *Ulva arasaki* reacted preferentially with erythrocytes of human A and O groups, and that those of both a green alga *Bryopsis hypnoides* and a red alga *Laurencia undulata* showed more specific activities with human B erythrocytes than with the others. There were no marked features in the distribution of the hemagglutinins among marine algae tested.

Hemagglutinins have been known to be proteins or glycoproteins which recognize some carbohydrate structures specifically and bind the carbohydrates¹⁻⁴. It may have been considered that binding specifically to some carbohydrates provokes to produce many unique biological activities such as cell agglutination, precipitation of polysaccharides, mitogenic activity, antitumour activity and toxicity¹⁻⁴. For these unusual biological activities, hemagglutinins have provided useful tools in immunology, cell biology, cancer research and other areas of research activities. Various reports¹⁻⁴ revealed to date that hemagglutinins were widely distributed in nature, though the studies have been mainly made in higher plants and invertebrates. Recently, two groups of investigators have screened for hemagglutinins in tropical marine algae⁵ and British marine algae⁶⁻⁸, respectively with only human erythrocytes, and reported that only some species gave positive results with very weak and nonspecific activities. One exception was a red alga *Ptilota plumosa* which possessed a hemagglutinin with the specificity to human B erythrocytes^{8,9}.

In our preliminary test using algal extracts, we found that rabbit erythrocytes rather than human erythrocytes were much more susceptible to hemagglutination. We therefore surveyed the Japanese marine algae for hemagglutinins with rabbit erythrocytes. The extracts of the algae which

showed positive results were further examined for hemagglutinating activity against other erythrocytes of animals and human A, B, O and AB groups. This paper reports the results.

Materials and Methods

Materials

The algae used in this survey are listed with localities and dates of collection in Table 1. The taxonomic classification used for the Japanese algal species in the Table follows that of Chihara¹⁰. Their algal specimens, after collection, were frozen as soon as possible and stored at -20°C , or air-dried and kept at room temperature, until used.

Preparation of test solution

Twenty g of each specimens of the algae in freezing were cut to small pieces and to them 40 ml of 0.85% sodium chloride solution were added. The mixture was immediately homogenized with a handy-ultrahomogenizer and filtered with a gauze after 1 h. The filtrate was centrifuged in cold at 10000 rpm for 25 min and the supernatant was further filtered with a Toyo No. 2 filter paper. The solution thus obtained was made up to a 40 ml of volume with 0.85% sodium chloride solution and examined for hemagglutinating activity. On the other hand, 2 g of each specimens of the air-dried samples were extracted with 20 ml of 0.85% sodium chloride solution in the similar way. The

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Table 1—continued

Species	Collection		Treatment before used	Titer of extract with erythrocytes									
	Locality	Date		Rabbit	Horse	Sheep	Duck	Chicken	Human	A	B	O	AB
<i>Chondria crassicaulis</i>	Eh	April 16, 1980	f	—									
	Eh	May 15, 1980	f	—									
<i>Laurencia intermedia</i>	Es	May 12, 1979	d	—									
<i>L. undulata</i>	Ki	May 27, 1979	d	—									
	Wk	June 13, 1980	f	2 ⁸	2 ²	—	—	—	2 ³	2 ¹⁰	2 ³	2 ⁸	
	Ho	December 20, 1980	f	2 ¹²					2 ¹	2 ⁷	2 ¹	2 ¹	
<i>L. okamurai</i>	Hu	March 15, 1980	f	—									
PHAEOPHYCOPHYTA													
DICTYOTALES													
Dictyotaceae													
<i>Dictyopteris prolifera</i>	Wk	June 13, 1980	f	—									
<i>D. undulata</i>	Wk	June 13, 1980	f	—									
CHORDARIALES													
Ishigeaceae													
<i>Ishige okamurai</i>	Es	May 12, 1979	d	2 ⁸	2 ⁸	2 ⁴	2 ⁷	2 ⁸	2 ⁸	2 ⁴	2 ⁸	2 ⁸	
SCYTOSIPHONALES													
Scytosiphonaceae													
<i>Hydroclathrus clathratus</i>	Eh	March 18, 1980	f	—									
FUCALES													
Sargassaceae													
<i>Sargassum thunbergii</i>	Eh	March 18, 1980	f	—									

Hu, Ujina, Hiroshima Prefecture; Ho, Onomichi, Hiroshima Prefecture; Eh, Hojo, Ehime Prefecture; Es, Satonisaki, Ehime Prefecture; Ki, Ibusuki, Kagoshima Prefecture; Wk, Kada, Wakayama Prefecture. f, sample frozen and stored at -20°C; d, sample air-dried and kept at room temperature. (—), no hemagglutination.

resultant solution was made up to a 20 ml of volume and used as a test solution.

Erythrocytes

Rabbit blood was obtained from the animal kept in our laboratory. Horse, sheep, duck and chicken blood were obtained from Toyo Seorogical Inc., and human A, B, O and AB blood from Clinical Examination Center in Fukuyama Medical Association. A 2% erythrocyte suspension in saline was prepared from each type of blood after washings three times with saline.

Assay of hemagglutinating activity

Rabbit erythrocytes were first used in this screening. A serial twofold dilution of the test solution was made in a final volume of 25 μ l saline in microtiter V-plate wells using a Takasly microdiluter. To each well an equal volume of one of the 2% rabbit erythrocyte suspension was added. The plate was gently agitated for ca. 30 sec to mix the suspension and kept at 37°C for 30 min. After the plate was further left to stand at room temperature for 1.5 h, agglutination was observed. The activity was expressed as titer, the reciprocal of the

highest twofold dilution exhibiting positive hemagglutination. The extracts of the algae which gave positive results were further examined for hemagglutinating activities against erythrocytes of horse, sheep, duck, chicken and human A, B, O and AB groups in the similar way.

Results and Discussion

Of 53 species tested, 14 species; 4 species in 6 of the green algae, 9 in 42 of the red algae and 1 in 5 of the brown algae showed positive results as shown in Table 1. The red algae *Carpopeltis flabellata*, *Gracilaria verrucosa* and *Laurencia undulata* always contained the hemagglutinins with relatively strong activities in any different collections with each exception of one collection. Both extracts from two different collections of the red alga *Grateloupia turuturu* possessed hemagglutinins, but with weak activities. The red algae *Hypnea japonica*, *Gymnogongrus flabelliformis*, *Ceramium kondoi* and *Campylaephora hypnaeoides* gave positive results respectively in only one of each two or three collections. Other active species, the green

algae *Ulva perutusa*, *U. arasaki*, *Bryopsis hypnoides*, *Codium fragile*, the red alga *Pachymeniopsis elliptica* and the brown alga *Ishige okamura* were examined in only one collection, respectively, but the extracts of all the species except *U. perutusa* gave the relatively strong activities. The higher titer of the extracts for hemagglutination with rabbit erythrocytes were obtained with the green algae *B. hypnoides*, *C. fragile*, the red algae *P. elliptica*, *Carpodellia flabellata*, *Gracilaria verrucosa* and *L. undulata*. The extract of *I. okamura* gave a pseudohemagglutination with all erythrocytes tested as did tannic acid. This pseudohemagglutinin could be also extracted with a hot 70% ethanol and transferred into the outer fraction during dialysis.

Of the 14 species which gave positive results against rabbit erythrocytes, 5 species, *Grateloupia turuturu*, *P. elliptica*, *Carpodellia flabellata*, *Ceramium kondo* and *Campylaeophora hypnaeoides* showed no hemagglutinating activity against human erythrocytes. The extracts of other 9 species active with both erythrocytes were also more active against rabbit erythrocytes than against human erythrocytes. These data revealed that hemagglutinins in marine algae are more sensitive to rabbit erythrocytes than to human erythrocytes. BLUNDEN *et al.*⁶⁾ have reported the presence of hemagglutinins with very weak activities against human erythrocytes in only 19 of 105 species of the British marine algae. Other two reports^{5,7,8)} on the surveys for marine algal hemagglutinins using human erythrocytes have also indicated the similar degrees of the presence and activities of the hemagglutinins. In the present survey with rabbit erythrocytes for hemagglutination, we could detect the presence of hemagglutinins with relatively strong activities in 14 of 53 species tested. We therefore proposed the use of rabbit erythrocytes rather than human erythrocytes in the screening for marine algal hemagglutinins.

Also in this study, the extracts of some species reacted preferentially with erythrocytes of human blood ABO groups. The extract of *U. arasaki* agglutinated only human A and O erythrocytes, though with weak activities, suggesting the possibility of the presence of an anti-A + H hemagglutinin. That of *B. hypnoides* was much more active (titer, 2¹⁰) against human B erythrocytes than each titer, 2⁸ against the others. The extract of *L. undulata* also showed the higher titer, 2¹⁰ against human B erythrocytes than each titer, 2⁸ against the others, though the anti-B activity decreased

to the level of the titer, 2⁸ after the extract was left at 4°C overnight. In the previous studies on marine algal hemagglutinins, it has been reported⁹⁾ that only a red alga *Ptilota plumosa* hemagglutinin showed the specificity to human B erythrocytes. The anti-B hemagglutinin gave the titer value of 2⁸, 2⁷ and 2⁸ against human A, B and O erythrocytes, respectively. The degrees of the specificity to human B erythrocytes are the clearly higher with both hemagglutinins of *B. hypnoides* and *L. undulata* than with that of *P. plumosa*. Therefore, the former two species may provide new sources of anti-B hemagglutinin, a relatively little number of which have been found in nature.

Both extracts of *B. hypnoides* and *P. elliptica* also showed hemolytic activities. It is interesting that the extract of *B. hypnoides* produced the lysis preferentially with human A, B, O and AB erythrocytes with the hemolytic titer values of 2, 2, 2⁸ and 2⁸, respectively.

Isolations and characterizations of the algal hemagglutinins are now under progress.

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