Isolation and characterization of hemagglutinins from the sponge *Dysidea herbacea*

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Summary. The sponge *Dysidea herbacea* (Keller) was found to possess hemagglutinins. The major component, DHA-I, is a protein with a mol.wt of 26,000, which dissociates into subunits of equal size (14,000). It contains large amounts of glutamic acid and aspartic acid residues, but no half-cystine, methionine or histidine residues. DHA-I reacted with rabbit and human AB0 erythrocytes. D-galactose and lactose were effective inhibitors of DHA-I. The sponge also contained a minor component(s) which reacted preferentially with rabbit erythrocytes but not with human AB0 erythrocytes.
Key words. Hemagglutinin; agglutinin; lectin; sponge; *Dysidea herbacea.*

Agglutinins or receptor-specific proteins are widely distributed in nature. Some, particularly those found in the seeds of plants, have been isolated in a homogeneous form and are now being widely used as research tools [2-5]. There is considerable interest in detecting and isolating new hemagglutinins from various sources. Agglutinins seem to be very common in sponges; however, only a few of these hemagglutinins have been isolated and characterized in detail [6-9]. In searching for agglutinins in marine invertebrates collected in the vicinity of Fiji, we have found agglutinating activities in some sponges against various kinds of mammalian erythrocytes. We report here the isolation and characterization of agglutinins from the sponge *Dysidea herbacea* (Keller).

A 0.85% saline extract of *D. herbacea* was found to be highly active against rabbit erythrocytes (titer value 128) and slightly active against human AB0 erythrocytes (titer value 16–32). The activity of *Dysidea* agglutinins was fairly stable over a wide range of pH values and was unchanged between pH 4 and 12. Activity was unaffected by heating at 40–60 °C for 15 min, but was reduced to less than half by heating at 80 °C for 15 min. However, even when the agglutinin was kept in a boiling water bath for 15 min, no complete loss of activity was observed.

D-Galactose and lactose were the most effective inhibitors among the simple sugars tested. Hemagglutinating activity of the crude extract was not lost on the addition of 100 mM EDTA. Agarose gels, either with or without acid-treatment, have been used as an effective affinity adsorbent for D-galactose specific hemagglutinins. Acid-treated Sepharose 4B was found to be effective for the isolation of *Dysidea* agglutinins. The agglutinin adsorbed to the column was eluted with 0.2 M D-galactose. Further separation on Sephadex G-50 gave two active fractions, as shown in the figure. The major component, DHA-I, was obtained from the slow-eluting protein peak in an electrophoretically homogeneous form by separation on DEAE-Toyopearl 650S. Its activity against rabbit erythrocytes and human AB0 erythrocytes was identical with that of the crude extract, indicating that DHA-I represents the activity of *Dysidea* hemagglutinins. The minimum active concentration against rabbit, human B, and human A and 0 erythrocytes was estimated to be 0.06, 0.25, and 0.5 μg protein/ml, respectively. The agglutinating activity of DHA-I was independent of divalent cations, and the titer value was unchanged in the presence of 100 mM EDTA, as was reported for hemagglutinins in the sponges *Aaptos papillata*[8] and *Geodia cydonium*[9]. DHA-I also agglutinated sperms of the starfish *Asterina pectinifera* at a minimum concentration of 5 μg protein/ml. No significant mitogenic activity was observed when this was checked with spleen cells from C3H/He mice at various concentrations ranging from 1.5 to 20 μg protein/ml. Concanavalin A was used as positive reference.

DHA-I is a protein which has a mol. wt of 26,000, as determined by high-speed gel filtration on Toyo Soda TSK G-3000 SW. The isoelectric point was estimated to be 4.5. On polyacrylamide disc gel electrophoresis in sodium dodecyl sulfate with and without 2-mercaptoethanol, it showed a single band having an apparent molecular weight of 14,000 when stained with coomassie brilliant blue. No significant amount of carbohydrate was detected. Agglutinins generally show a high content of acidic and of hydroxy amino acids and a low content of half-cystine and of

Table 1. Amino acid composition of *Dysidea herbacea* agglutinin (DHA-I)

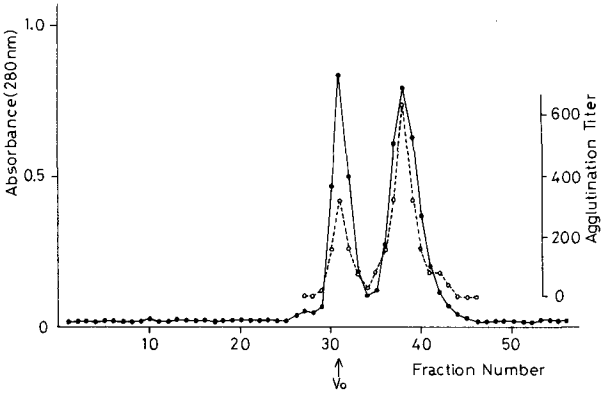
Amino acid	mol%	Amino acid	mol%
Asp	9.6	Met	0
Glu	11.2	Ile	8.7
Ser	4.5	Leu	8.3
Thr	3.9	Tyr	6.1
Gly	9.6	Phe	7.9
Ala	5.5	Trp	ND*
Pro	5.6	Lys	8.1
Val	7.1	His	0
Cys (half)	0	Arg	3.9

*Not determined.

Table 2. Hemagglutinating inhibition of *Dysidea herbacea* agglutinin (DHA-I) by simple sugars

Sugars	mM*	Sugars	mM*
D-Galactose	1.6	N-Acetylneuraminic acid	–**
D-Glucose	–**	N-Acetyl-D-galactosamine	6.3
D-Mannose	–**	D-Galactosamine	6.3
D-Fucose	–**	N-Acetyl-D-glucosamine	–**
D-Arabinose	–**	D-Glucosamine	–**
D-Xylose	–**	D-Galacturonic acid	6.3
Lactose	1.6	D-Glucuronic acid	25

*A minimum concentration of sugars required to inhibit the eight units agglutinin solution. **No inhibition at 100 mM.



Chromatography of *Dysidea herbacea* hemagglutinin on Sephadex G-50. Partially purified *Dysidea* hemagglutinin was applied to a column of Sephadex G-50. The column was eluted with 0.05 M Tris-HCl buffer, pH 8.0 containing 0.2 M NaCl. Fractions were analyzed for UV absorption at 280 nm (●—●) and for hemagglutinating activity against rabbit erythrocytes (○—○). Vo: void volume.

methionine. As shown in table 1, glutamic and aspartic acids were high in abundance in DHA-I and comprised more than 20% of the total residues. A high content of aromatic acids, tyrosine and phenylalanine, was also observed. No half-cystine, methionine or histidine was detected. Lack of half-cystine indicates that the subunits of DHA-I are held together by a non-covalent bond, as has been shown in many hemagglutinins from various sources[5].

Table 2 shows the results of an inhibition test for hemagglutionation against rabbit erythrocytes by simple sugars. D-Galactose and lactose among the sugars tested showed strong inhibitory activity. The activity was also inhibited by D-galactosamine, N-acetyl-D-galactosamine, N-acetyl-D-galactosamine, and D-galacturonic acid. D-Glucuronic acid, a well-known inhibitor for the aggregation factor of sponge cells, showed only weak inhibitory activity. None of other sugars tested showed any effect at a concentration of 100 mM.

A minor component(s), appearing in the fast-eluting protein peak from a Sephadex G-50 column, was found to contain few contaminants in analytical disc gel electrophoresis. Differing from DHA-I, it reacted preferably with rabbit erythrocytes showing a minimum active concentration of 1.2 µg protein/ml but not with human AB0 erythrocytes even at a concentration of 150 µg protein/ml. Although hemagglutinating activity of the crude extract was not affected by the addition of EDTA, as mentioned above, the activity of the minor component(s), comprising about 30% of the total activity, was lost in the presence of EDTA at a concentration as low as 5 mM. This discrepancy may be mainly due to the unreliability of the microtiter plate method used determination of hemagglutinating activity.

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Carbohydrase and esterase activity in the gut of larval *Callosobruchus maculatus*

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Summary. Carbohydrase activity has been demonstrated in homogenates of the alimentary tracts of late instar larvae of *C. maculatus*: β-D galactosidase, α-D glucosidase and N-acetyl β-D glucosaminidase activities were comparable and significantly greater than α-D galactosidase, β-D glucosidase and α-D mannosidase. The effects of pH and substrate concentration are reported. The presence and changes in pattern of non-specific esterase activity in larval and adult gut homogenates is also described.
Key words. Callosobruchus maculatus; digestive enzymes; glycosidases; esterases.

Callosobruchus maculatus is a major storage pest of the cowpea, *Vigna unguiculata*[1]. To date relatively little information is available concerning the physiology and biochemistry of digestion in these important pest insects. Podoler and Applebaum[2] isolated and partially characterized an α-amylase from a total larval extract of *C. chinensis* and more recently Gatehouse and Anstee[3] have reported the presence, and partially characterised, four carbohydrase enzymes in homogenates of the alimentary tract of adult male and female *C. maculatus*. The present study was carried out to extend further our knowledge of the digestive enzymes present in the adult insect and to provide information concerning the presence and nature of various carbohydrase and esterase activities in late larval guts of *C. maculatus*, as a basis for future physiological investigations on digestion.

Materials and methods. A culture of *Callosobruchus maculatus* originating from Campinas, Brazil, was reared and maintained on seeds of *Vigna unguiculata* at 28 ± 0.5 °C and 60% relative humidity. The photoperiod was arranged to give 12 h light and 12 h dark.

Preparation of gut homogenates: The alimentary tracts were removed by dissection from late instar larvae and sexually mature animals of both sexes which had previously been killed by decapitation. Homogenization was carried out in cold (ca. 4 °C) distilled water using a Potter-Elvehjem homogenizer with a teflon pestle (clearance 0.1–0.15 mm) with 20 passes of the plunger at 1000 rev/min; the homogenization tube was surrounded by ice throughout this procedure. The resulting homogenate was then centrifuged at 9000 × g for 5 min at 4 °C in a Haematocrit centrifuge and the supernatant retained for enzyme assay. All homogenates were freshly prepared.

The activities of α-D and β-D glucosidase, α-D and β-D galactosidase, α-D-mannosidase and N-acetyl β-D-glucosaminidase were determined by the estimation of p-nitrophenol liberated by hydrolysis of the corresponding p-nitrophenyl glycoside as described previously[3,4]. The composition of the reaction mixtures was as follows:

Aryl α-D glucosidase: 200 µl 0.1 M McIlvane's citrate-phosphate buffer[5], pH 5.2, 50 µl 51.2 mM p-nitrophenyl α-D-glucopyranoside (to give a final concentration of 9.5 mM) and 20 µl homogenate were incubated at 37 °C for 15 min in sealed Eppendorf tubes, unless otherwise stated in the text. The reaction was terminated by the addition of 1 ml 50 mM NaOH and the absorbance read at 405 nm.

Aryl β-D glucosidase: as for aryl α-D glucosidase except for the use of the substrate p-nitrophenyl β-D glucopyranoside.

Aryl α-D galactosidase: as for aryl α-D glucosidase except for the use of the substrate p-nitrophenyl α-D galactopyranoside (35 mM: to give a final concentration of 6.5 mM).

Aryl β-D galactosidase: as for aryl α-D galactosidase except for the use of the substrate p-nitrophenyl β-D galactopyranoside.