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Lectin-Agglutination Properties of Symbiotic and Non-Symbiotic Algae and Hydra Cells

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Abstract

Agglutination properties with six different lectins, of cultured and freshly isolated symbiotic algae (FISA), belonging to *Chlorella* sp. and *Symbiococcum hydrae* (Rahat and Reich, 1989) strains, were investigated, together with cells from symbiotic, aposymbiotic and non-symbiotic strains of *Hydra* from the *Viridissima* and *Vulgaris* groups. FISA and cells from the respective hydra host were agglutinated by at least one common lectin. FISA differed in their agglutination properties from the cultured symbiotic strains, but no clear correlation between the ability of the latter to form symbiosis and the agglutination with lectins was found. Generally, different species of algae and hydra have different, species-specific agglutination properties, which is suggested as an additional tool in their taxonomy. The relevance of these results to the establishment of stable Hydra/algae symbioses is discussed.

Keywords: Chlorellae, freshly isolated algae, Glycoconjugates, *Hydra*, *Hydra*-cells, lectin-agglutination, *Symbiococcum hydrae*, symbiosis

1. Introduction

The first stages in the initiation of symbiotic relationships between *Hydra* and algae involve the cell surface, as suggested by studies using antibodies

(Pool, 1979) or changing the net surface charge of the cells (McNeil et al., 1981). Treatment of algal symbionts with lectins before host infection significantly decreased the number of symbionts taken up by the host (Meints and Pardy, 1980; Reisser et al., 1982; Reich et al., 1990). This indicated a possible involvement of surface glycoconjugates in the establishment of algal symbioses. Our purpose was to find some properties unique to the symbiotic *Hydra* and algae strains that might explain host-symbiont specificity.

We chose the technique of agglutination by lectins because it is a method widely used to differentiate closely related cell populations (Lis and Sharon, 1978, 1986). It has been used successfully in the separation of populations of endoparasites such as *Leishmania* and *Trypanosoma* (Jacobs et al., 1982; Schottelius and Uhlenbruck, 1983; Schnottelius, 1987), and it has been used in distinguishing differences in infectivity of a parasite according to the stages of its life cycle (Rudin et al., 1989).

To detect different sugar residues on the cell-surface, we used six lectins of differing sugar specificity.

In this study we report the comparative lectin-agglutination properties of cultured and freshly isolated *Symbiococcum hydrae* (Rahat and Reich, 1989), and of different cells from *Hydra* sp. and of *Chlorella* strains that were previously tested for their ability to form stable symbiosis with *Hydra viridissima* (Rahat and Reich, 1985a). A wide spectrum of characteristics of these chlorellae has been defined previously (Kessler, 1982; Kessler et al., 1988). The comparison between symbiotic, aposymbiotic and non-symbiotic algae and hydra strains enabled us to follow differences at the cell surface.

2. Materials and Methods

Organisms and growth conditions

Hydra used:

1. Three strains of *H. viridissima*, differing in their geographical origin: Swiss – Ssh, European – Esh, and Jerusalem – Jsh, and the Swiss aposymbiotic strain – Sah (Rahat and Reich, 1986).
2. Twelve hydra strains of the *Vulgaris* group (Campbell, 1983, 1989):
   (a) Eight strains of *H. magnipapillata* (Sugiyama, 1983), of which 5 are symbiotic (Rahat and Reich, 1988), and their respective aposymbiotic strains.
   (b) Three non-symbiotic strains of *H. vulgaris* from Israel (Isr), Europe (Eur) and Australia (Aus) (Rahat and Reich, 1986).

(c) A symbiotic strain of *H. vulgaris* from South Africa (Afro) and its respective aposymbiotic strain (Rahat and Reich, 1986).

3. A non-symbiotic strain of *H. pirardi* (obtained from Prof. Tardent, Dept. of Zoology, University of Zurich).

All hydra strains were grown under continuous illumination of about 2500 Lux (white fluorescent tubes, 30 cm above culture dishes) at $20 \pm 2°$in 'M' solution (Lenhoff and Brown, 1970) and fed three times a week with freshly hatched larvae of *Artemia* sp.

Algae used:

Thirty-seven *Chlorella* strains, 16 of which are symbiotic in *H. viridissima* (Kessler et al., 1988; Rahat et al., 1989), were grown in 5 ml of enriched BBM medium (Rahat and Reich, 1985a) under continuous illumination of 2500 Lux (white fluorescent tubes, 30 cm above cultures), at $20 \pm 2°C$.

Symbiococcum hydrae (*S. hydrae*) (Rahat and Reich, 1989) was cultured under the same conditions.

For each cultured alga strain tested, a two-week "old" culture was used, from which the respective "young" culture was inoculated 24 hr before the agglutination test.

Freshly isolated *S. hydrae* and native symbiotic chlorellae (Zoochlorellae) were prepared by homogenizing the respective symbiotic hydra strains before each experiment.

Chemicals

Lectins:

The lectins used, their abbreviations and sugar specificities are listed in Table 1. Lectins were purchased from Makor Chemicals Ltd., Jerusalem, with the exception of Con-A, which was purchased from Sigma Chemical Company. Lectin solutions were prepared at final concentration of 500 μg/ml in 300 mOsmol NaCl for *Hydra*-cells experiments, or in BBM pH 7.0 for algal-cells experiments. In the agglutination experiments of the cultured algae, the concentration of RCA-120 was 120 μg/ml. These specific concentrations were based on results obtained from preliminary dilution tests conducted for each lectin.

Table 1. List of lectins, their abbreviations and sugar specificity

Lectin	Initials	Specificity sugar
Concanavalin-A	Con-A	α-D(+)-Mannose
Phytohemagglutinin	PHA-L	oligosaccharides
Tetragonolobus	TET	α-L-Fucose
Wheat germ	WGA	Nac-D-Glucosamine
Soybean	SBA	NAc-D-Galactosamine
Ricinus agglutinin	RCA-120	D(+)-Galactose

Sugars:

NAc-Galactosamine, NAc-Glucosamine and α-L(−)-Fucose were purchased from Sigma (Israel), D(+)-Maltose and D(+)-Galactose were purchased from E. Merck (Germany), and D(+)-Mannose was purchased from NBCO (Cleveland, Ohio).

For inhibition tests, all sugar solutions were prepared in 30 mOsmol NaCl or in BBM pH 7.0 at 0.25 M final concentration.

Agglutination experiments

Agglutination experiments were carried out in 96-well culture-dishes with flat bottom wells (Costar, USA) in 50 μl final volume. Cells were incubated in the experimental solution for 1 hr at room temperature, in a water-bath shaker, at 100 strokes/min. The results were graded from −, no agglutination to ++++, depending on the degree of agglutination, as shown in Fig. 1 and according to Sela et al. (1970) and Dwyer (1974).

Preparation of algal cells

Algal cultures were centrifuged, transferred to Eppendorf micro test tubes and washed ×4 with 1 ml BBM solution, pH 7.0. FISA were prepared before each experiment as described by Cantor and Rahat (1982): Symbiotic hydra were homogenized, the homogenate washed several times with M solution by centrifugation, discarding the supernatants containing the animal tissue debris. From the last washing, 0.25 μl of cell suspension was transferred into the "well" that contained 0.25 μl appropriate lectin or control solution. Zoochlorellae do not survive outside hydra cells for more than a few hours, thus "old" and "young" cultures of these algae could not be examined.

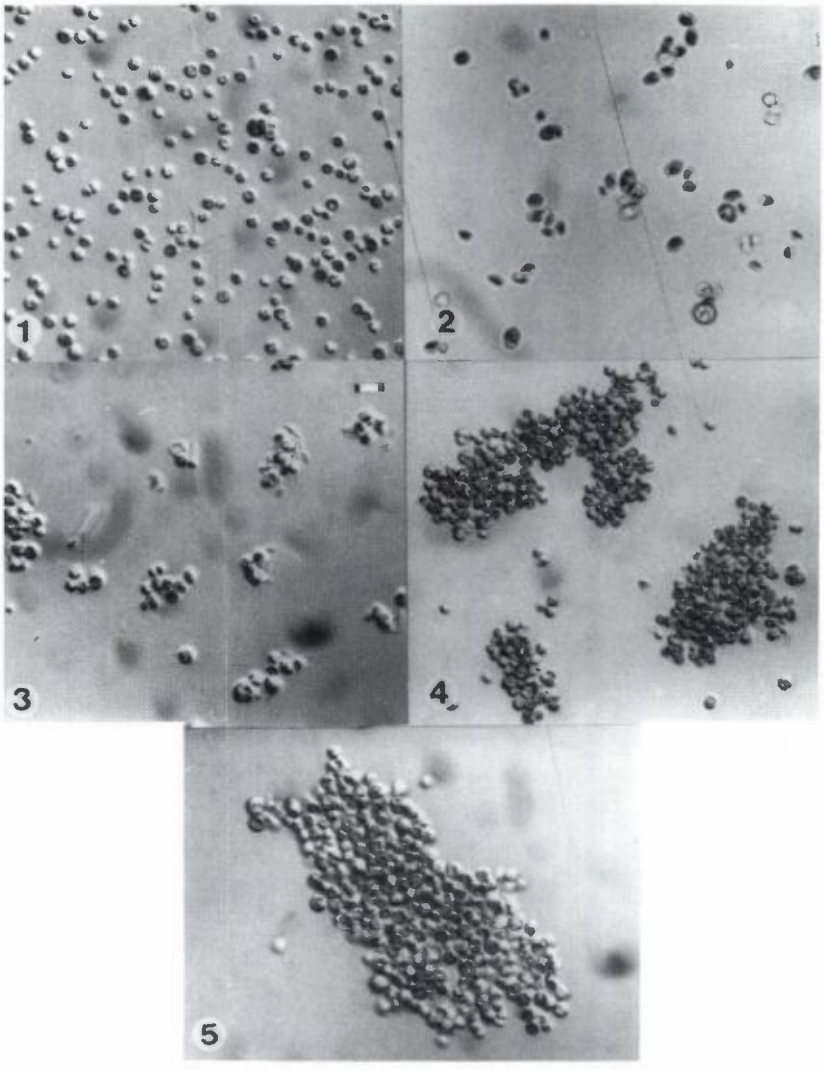

Figure 1. Degrees of agglutination of different *Chlorella* strains with Con-A at 500 μg/ml final concentration.

1. *C. kessleri* strain 211–11g control, – no agglutination.
2. *C. sacc. var. saccharophila* strain 211–11d, + reaction.
3. *C. kessleri* strain NC(64)P, ++ reaction.
4. *C. kessleri* strain 211–11g, from 14 days old culture, +++ reaction.
5. *C. kessleri* strain 211–11g, from 1 day old culture, ++++ reaction. Scale: bar = 10 μm.

Preparation of hydra cells

Hydra cells were obtained by cutting 24 hr starved polyps into small pieces, incubating them for 90 min in a hyperosmotic solution of 30 mOsmol NaCl, followed by vigorous pipetation. Twenty-five μl of the obtained cell suspension was transferred into the "well".

Controls

For each sample the following controls were performed:

1. Cells were incubated without lectin.
2. The lectin was incubated with the specific inhibitory sugar for 15 min before the cells were added. (No appropriate inhibitory sugar for PHA-L was found).
3. Lectins were incubated with a non-specific sugar, maltose, for 15 min before adding the cells.

Only lectin-specific agglutination was recorded as a positive result

3. Results

The lectin-agglutination properties of the different cells tested are summarized in Tables 2–5.

Agglutination of algal cells

Cultured Chlorella

Of the 37 strains of cultured chlorellas we studied, the 18 symbiotic strains showed almost no agglutination with any of the lectins applied. Con-A was the only lectin that caused a weak reaction of + or ± in 9 of the strains (Table 3). Some of the 19 non symbiotic strains showed a stronger reaction than the symbiotic ones, especially with Con-A. None of these 19 strains agglutinated with RCA and SBA (Table 4).

Cells from young cultures usually agglutinated stronger than cells from old cultures and in some cases cells from young cultures agglutinated also in the absence of lectins.

Table 2. Lectin-agglutination properties of cells from the different *Hydra* strains. 1. For the full name and detailed origin see Materials and Methods. 2. Symbiotic strain. 3. Aposymbiotic strain. 4. Swiss strain of *H. viridissima* infected with the native symbiont of Jsh. 5. Forms stable symbiosis with *Chlorella*. 6. Forms stable symbiosis with *Symbiococcum hydrae*. 7. For full names and sugar specificity see Table 1. 8. Levels of agglutination as described in Fig. 1.

Hydra strain strain **1**	Hydra species	Symb. **5** with Chlor	Symb. **6** with Sc.	Con-A μg/ml 500	PHA-L μg/ml 500	TET **7** μg/ml 500	WGA μg/ml 500	SBA μg/ml 500	RCA120 μg/ml 500
75-19-8 Sym **2**	*H. magnipapillata*	– **8**	+	+++	–	++++	+++	+++	++
75-19-8 Apo **3**	"	–	+	+++	–	++++	+++	++	++
Mh-1 Sym	"	–	+	++++	–	++++	+++	+++	++
Mh-1 Apo	"	–	+	+++	–	++++	++++	+	++
Maxi Sym	"	–	+	++	–	+++	++	–	–
Mini4-Apo	"	–	+	++	–	+++	++++	–	++
Nem-3 Sym	"	–	+	+++	–	++	+++	–	+++
Nem-3 Apo	"	–	+	++	–	++	++	+	++
Reg-16	"	–	+	+++	+	++++	++++	++	+
MS-1	"	–	–	++	–	++++	++++	+	+
L – 4	"	–	–	++	–	+	+++	+++	++
Afro Sym	*H. vulgaris*	–	+	–	–	–	++++	+++	++++
Afro Apo	"	–	+	–	–	–	++	++	+++
Eur.	*H. vulgaris*	–	–	+	–	+++	–	+	++++
Isr.	"	–	–	++	+	+++	++	++	++++
Aus.	"	–	±	++	–	+++	++	–	+
Pirardi	*H. pirardi*	–	–	+	–	+	++	–	–
Ssh	*H. viridissima*	+	–	++	–	++++	+	–	+++
Sah	"	+	–	++	–	++++	±	–	+++
Sah/Ja **4**	"	+	–	±	–	++++	±	–	+++
Jsh	"	+	–	–	–	++++	–	–	+
Esh	"	+	–	–	–	++++	–	–	+

Table 3. Lectin agglutination properties of free-living symbiotic *Chlorellae*.

1. For full names and sugar specificity see Table 1.
2. Levels of agglutination as described in Fig. 1.
3. Cells from young cultures agglutinated stronger.
4. Cells from young cultures agglutinated less.

Alga strain	Alga species	Con-A μg/ml 500	PHA-L μg/ml 500	TET 1 μg/ml 500	WGA μg/ml 500	SBA μg/ml 500	RCA120 μg/ml 120
211-1d	*C. sacch.* var. *saccharophila*	+ 2	–	–	–	–	–
211-9b	"	+	–	–	–	–	–
211-9a	"	++ **4**	+ **3**	–	±**3**	±	±
211/11n	*C. fusca* var. *vacuolata*	+	– **3**	– **3**	– **3**	– **3**	–
211-11n	"	+	–	–	±	–	–
211/8p	"	+	–	–	–	–	–
211/8b	"	–	–	–	–	–	–
211-8b	"	+	–	–	–	–	–
211-11g	*C. kessleri*	+++ **3**	–	–	–	–	–
NC64A(P)	"	++ **3**	–	–	–	–	–
211-2a	*C. luteoviridis*	+	–	–	–	–	+
FS	*C. protothecoides*	–	±	–	–	±	–
211/6	"	–	–	–	–	–	–
211/11a	"	–	–	–	–	–	–
211/7a	"	–	–	–	–	–	–
211-7a	"	–	–	–	–	–	–

Freshly isolated Symbiotic Algae (FISA)

The three strains of *Chlorella*, freshly isolated from *H. viridissima* had common qualitative agglutination properties (Table 5). All agglutinated with Con-A, TET, and RCA, at different levels (FISA from Jsh agglutinated also with SBA), and did not react with the other lectins. There was no clear similarity between their agglutination properties and those of the cultured symbiotic strains.

Table 4. Lectin-agglutination properties of free-living non-symbiotic *Chlorellae*.

1. For full names and sugar specificity see Table 1.
2. Levels of agglutination as described in Fig. 1.
3. Cells from young cultures agglutinated stronger.
4. Cells from young cultures agglutinated less.

Alga strain	Alga species	Con-A µg/ml 500	PHA-L µg/ml 500	TET 1 µg/ml 500	WGA µg/ml 500	SBA µg/ml 500	RCA120 µg/ml 120
211/1e	*C. vulgaris*	+++ **2**	–	–	–	–	–
211/21	"	+++ **4**	–	+	–	–	–
CE-76	"	+	–	–	–	–	–
NC64A(M)	"	–	–	–	–	–	–
211/8k	*C. sorokiniana*	++ **4**	++ **4**	++ **4**	–	–	–
211-8k	"	–	–	–	–	–	–
211-11k	"	+++ **4**	–	+	–	–	–
211-32	"	+++	–	–	–	–	–
211-40a	"	–	±	+	–	–	–
211-40b	"	–	–	±	–	–	–
211-40c	"	– **3**	–	±**3**	–	– **3**	–
C-1.1.8	"	++	+	+++	+	–	–
211/11c	"	–	–	–	–	–	–
Prag A14	"	++ **3**	–	–	–	–	–
211-34	"	+	–	–	++	–	–
211-14a	*C. zofingiensis*	++++	–	–	–	–	+
211/8e	*C. homosphaera*	++	+	+	–	–	–
232/1	*C. fusca* var. *rubescens*	+ **4**	–	–	–	–	–
C-1.1.9	*C. minutissima*	++ **4**	+	–	+	–	–

Table 5. Lectin agglutination properties of FISA from different hosts.

1. For full names and detailed origin see Materials and Methods.
2. For full names and sugar specificity see Table 1.
3. Levels of agglutination as described in Fig. 1.

Alga strain	Alga species	Con-A μg/ml 500	PHA-L μg/ml 500	TET 2 μg/ml 500	WGA μg/ml 500	SBA μg/ml 500	RCA120 μg/ml 500
Ssh	*Chlorella*	±3	±	++	–	–	+++
Esh	"	+	–	++	–	–	±
Jsh	"	+	–	++	–	+	+++
75-19-8	*Symbiococcum hydrae*	++++	–	–	–	–	+++
Mh-1	"	++	–	–	–	–	–
Nem-3	"	++	–	–	–	–	–
Afro	"	++	–	–	–	–	–
Free living	"	++++	–	–	–	–	++++

S. hydrae, either cultured or freshly isolated from the native host *H. magnipapillata* (strain 75-19-8), showed common agglutination properties. *S. hydrae* isolated from other hydra hosts lost their ability to agglutinate with RCA, and had a weaker reaction with Con-A (++ instead of ++++) (Table 5). This may indicate that these hosts have changed some surface properties of their endosymbionts.

Agglutination of Hydra-cells

Cells from the three strains of *H. viridissima* showed common lectin-agglutination properties with only minor differences (Table 2). All cells agglutinated strongly with TET and RCA-120, less with Con-A and WGA and none agglutinated with PHA-L and SBA.

The native symbiotic algae affect the agglutination properties of their host cells with Con-A or WGA, according to the strain. Cells from all eight strains of *H. magnipapillata* differed quantitatively in their agglutination with Con-A, TET, WGA and qualitatively with the other lectins applied (Table 2).

Cells from the symbiotic and the respective aposymbiotic 'Afro' (*H. vulgaris*) strain, agglutinated with WGA, SBA and RCA. In all these cases, cells from

the symbiotic strain agglutinated stronger. Cells from three non symbiotic *H. vulgaris* strains and *H. pirardi*, differed in their agglutination patterns, as shown in Table 2. The only similarity between them was their agglutination with Con-A and TET.

4. Discussion

Our study was aimed to find correlations between the presence of specific surface glycoconjugates on interacting hydra and algae cells and their ability to form symbioses.

We used six lectins, differing in their sugar specificity, each separately applied. Cell agglutination indicated that their surface possessed the carbohydrate structures for which the tested lectin was specific. Negative agglutination results did not mean the total absence of these sugar molecules, as considerably amounts of lectins can bind to cells without causing agglutination (Lis and Sharon, 1977, 1986).

Cultured chlorellae

Con-A was, with some exceptions, the only lectin that caused agglutination at different degrees, indicating the presence of mannose (and possibly glucose) residues on the cells' surfaces, distributed in patterns causing agglutination.

With all the other lectins, this group of algae showed almost no agglutination (Tables 3 and 4). There was no correlation between the agglutination pattern with any of the lectins and the ability of these algae to live as endosymbionts in *H. viridissima*. Their agglutination pattern was species-specific like most of their other physiological and biochemical characteristics (Kessler, 1982; Kessler et al., 198; Rahat et al., 1989). This species-specificity is concomitant with the results reported for other algal species tested for their lectin binding properties (Sengbusch and Muller, 1983). We propose that in the study of the various *Chlorella* species and strains, agglutination with lectins can be used as an additional taxonomic characteristic.

Certain algae change their surface molecular composition during their life cycle (Kurn and Sela, 1979). In some of our strains, old cells agglutinated differently compared to the young cells, depending on the lectin applied. This suggests changes of specific surface receptors during the algal life cycle.

Freshly isolated zoochlorellae

The three strains of native zoochlorellae, freshly isolated from the European, Swiss and Jerusalem strains of *H. viridissima*, showed common agglutination properties. This correlates with successful cross-infection between these three host strains (unreported results). This indicates that the algal specific surface-molecules combination might be host-affected. Another possibility is that these native symbiotic strains, *a priori* possessed a favorable glycomolecules makeup as a preadaptation, and little or no changes in their surface combination occurred, upon entering the host cell.

All three strains of native symbiotic chlorellae agglutinated with Con-A, RCA-120, and TET, in contrast to the cultured chlorellae. These differences may result from their symbiotic way of life, indicating some common characteristics of native symbiotic algae. Algae cells have surface plasticity which confer a makeup that permits permanent residency inside *Hydra* cells. The capability of the algae to respond to the host-cell stimuli by changing their surface glycoconjugates, might be a useful preadaptation for a symbiotic alga (or for any endosymbiont). Such cell wall modifications in Con-A receptors, occurring as a consequence of symbiosis, were reported for freshly isolated phycobionts from several lichens (Bubrick and Galun, 1980).

There was a correlation between the agglutination patterns of FISA and their respective host cells. This can also be an adaptation to endosymbiotic life, i.e. sharing with the host common cell-surface molecules (markers?). Such results were obtained by us with FISA from the 75-19-8 strain of *H. magnipapillata* and their hydra host cells (see below), and by Marx and Preveling (1983) with lichens, where the mycobionts and the freshly isolated phycobionts bound at least one common lectin.

Cells of Hydra viridissima

Cells from polyps of the three strains of *H. viridissima* had common agglutination properties. All possess on their cell-surface different amounts of Mannose, NAc-Glucosamine, Fucose and Galactose residues, while NAc-Galactosamine might be absent. The quantitative differences in the cells' reaction with the lectins might be due to differences in the number and/or distribution of these sugar residues on the cells' surfaces, which also seem to be species-specific.

One of our purposes was to determine whether the presence of symbiotic algae alter the agglutination properties of their host cells. It seems that there is a limited influence. The results presented in Table 2 show that the Swiss

symbiotic algae do not affect the Con-A receptors on their host cell surface, but they do affect the WGA receptors. The Jerusalem symbiotic algae had an opposite effect, as they affected the Con-A receptors only. These variations between the three strains of native symbiotic algae supplement their differences in morphology, cell-wall resistance to SDS and DNA base composition, previously reported by Huss et al. (1989).

Some symbiotic algae cause changes to their surrounding perialgal membrane (Meier et al., 1980, 1984). These changes are believed to prevent phagosome-lysosome fusion, enabling the algae to survive inside the host cell (Reisser, 1988). In *Dictyostelium discoideum*, phagocytosis of yeasts by ameboid cells, led to the disappearance of WGA receptors from the membrane of the phagosome, and this phenomenon seemed specific for yeast phagocytosis and WGA receptors only (Ryter and Hellio, 1980). Such phenomena together with membrane recycling, can cause the differences in the agglutination patterns of the host cells, as detected with lectins.

Cultured and freshly isolated S. hydrae

The only known naturally occurring symbiotic *H. magnipapillata* is the 75-19-8 strain (Rahat and Reich, 1985b). All the other *H. magnipapillata* strains were artificially infected (Rahat and Reich, 1988) with the symbiotic alga isolated from the 75-19-8 strain (Rahat and Reich, 1989). We tested whether the changes in the cell surface of the algal endosymbiont are host-specific, by using different hydra strains hosting *S. hydrae*. The variations in the agglutination patterns between FISA from these "new symbiotic" strains were hydra strain-specific, and were thus apparently caused by the different hydra host.

Cultured and freshly isolated *S. hydrae* from all the hosts, agglutinated with Con-A, but the cultured algae and those isolated from the native host strain (75-19-8) agglutinated stronger, i.e. ++++ compared to ++. With RCA there was a marked host influence: The cultured algae and those isolated from the 75-19-8 strain, agglutinated at a level of +++, while *S. hydrae* isolated from Mh-1, Nem-3 and Afro symbiotic hydra-strains did not agglutinate with RCA at all. The similarity between the cultured strain and the algae isolated from the native hydra host indicates that this specific *Hydra* strain did not affect the surface-glycoconjugates of the symbiont, while the other artificially infected host strains did. These differences may also be related to other already reported differences between *in vivo* and *in vitro* cultured *S. hydrae*, concerning their cell wall morphology (Rahat and Reich, 1989), and lectin binding properties (Reich et al., 1990). Differences in lectin-binding properties between

freshly isolated and cultured symbionts were also reported for lichens (Bubrick and Galun, 1980) and *P. bursaria* (Reisser, 1988).

S. hydrae isolated from the symbiotic *H. vulgaris* strain agglutinated with Con-A (the lectin causing agglutination in all strains of *H. magnipapillata* tested), while their host cells did not. It appears that in brown hydra the host cell has limited influence on the cell-surface glycoconjugates composition of its symbionts, while in *H. viridissima* the influence is stronger.

Agglutination properties common to host and symbiont cells were found with some lectins only, and they are open for further study as to their possible role in establishing stable symbiosis.

Cells of H. magnipapillata

All *H. magnipapillata* strains originate from Japan. Some were collected there at different localities and some were isolated by sexual inbreeding in Prof. Sugiyama's laboratory in Mishima, Japan (Sugiyama, 1983). The different origin may account for some of the differences in their lectin-agglutination patterns, e.g. Mini-4 and Maxi-i strains share a common parent, the Sapporo strain (Sugiyama and Fujisawa, 1977a) and both did not agglutinate with SBA. The strains used here were reported to differ in many other morphological and physiological characteristics (Sugiyama and Fujisawa, 1977a,b); Schaller et al., 1977; Sugiyama, 1982; Takano and Sugiyama, 1983), and in their symbiotic capacity (Rahat and Reich, 1988).

Agglutination at different levels with Con-A, TET, and WGA was a common property of cells from all *H. magnipapillata* strains tested.

The different *H. magnipapillata* strains infected with the same symbiotic alga – *S. hydrae*, were used for testing the mutual influence of the host and the symbiont on their cells surface-glycoconjugates. In some strains the presence of the symbiotic algae increased the level of agglutination of their host cells with certain lectins (Table 2). On the other hand, *S. hydrae* isolated from the three "new symbiotic" host strains; Maxi-1, Nem-3, and Afro, lost their ability to agglutinate with RCA-120, indicating specific changes in their galactose surface residues, as a result of symbiosis.

We conclude that both host and symbiont may affect the surface-glycoconjugate configuration of each other.

Cells of H. vulgaris

The Afro strain of *H. vulgaris* forms stable symbiosis with *S. hydrae* (Rahat and Reich, 1986), although its cells did not agglutinate with Con-A or TET like the cells from the symbiotic strains of *H. magnipapillata* and *H. viridissima*. This shows that the presence of surface mannose or glucose residues, as revealed by agglutination with Con-A, is not necessary in establishing symbiosis in *Hydra*. Each of the three non-symbiotic *H. vulgaris* strains has specific agglutination patterns resembling those of the *H. magnipapillata* strains.

Cells from *H. pirardi* had a distinct lectin-agglutination pattern different from that of the non-symbiotic *H. vulgaris* or the *H. viridissima* strains.

5. Conclusions

There are significant differences between the agglutination properties of the native symbiotic algae and the symbiotic and non-symbiotic cultured strains.

FISA and cells from the respective hydra host agglutinated with at least one common lectin, but we could not find correlations between specific surface-glycoconjugates on the interacting hydra and algae cells, and the capacity to form symbioses.

There is a limited affect of the host on the agglutination properties of its symbionts, and in some cases, the endosymbionts also affected the agglutination patterns of their hosting cells.

Surface-sugars probably are not a major factor determining symbiotic compatibility. However they might be part of the complex set of phenomena (Smith, 1988), resulting in the specificity present in hydra/algae symbioses.

Lectin-agglutination properties are species-specific characteristics in cells of both *Hydra* and alga species, and can thus be used as an additional systematic tool, complementary to the already existing taxonomic criteria.

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