Cell Surface Differentiation of *Chlamydomonas* during Gametogenesis

I. Mating and Concanavalin A Agglutinability

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Accepted September 19, 1973

Sexual agglutination of opposite gametes and agglutination of like gametes by concanavalin A (Con A) were studied in *Chlamydomonas moewusii* and *C. reinhardtii* for the possibility that the surface sites involved in these agglutinating phenomena may be the same. Our data show that the two agglutinating phenomena appeared at different times after the beginning of gametogenesis. Sucrose and mannose block agglutination of the gametes by Con A but do not affect mating ability. Trypsin eliminates mating ability, except in (−) gametes of *C. moewusii*, while Con A agglutinability remains. Monovalent Con A can selectively bind to Con A-binding sites to block agglutination of gametes by multivalent Con A while mating ability is unaffected. The data indicate that the mating agglutinin and the Con A-binding sites are two different flagellar surface agglutinins that occur coincidentally during gametic differentiation of both mating types of *C. moewusii* and *C. reinhardtii*. The function of the Con A-binding site on *Chlamydomonas* gametes is not known.

**INTRODUCTION**

The mating process in *Chlamydomonas*, a unicellular, flagellated green alga, requiring two different mating types in the heterothallic species, has been the subject of numerous investigations (Lewin, 1952, 1959; Forster *et al.*, 1956; Trainor, 1959; Wiese, 1965, 1969; Brown *et al.*, 1968; Wiese and Metz, 1969; Wiese and Shoemaker, 1970; Wiese and Hayward, 1972). The cycle begins when vegetative cells are placed into a minimal nutrient medium after which gametogenesis ensues. Motile, vegetative cells are morphologically indistinguishable from gametes, and the complementary gametes are indistinguishable from each other. When fully differentiated gametes from the two mating types are mixed, a specific agglutination or clumping follows caused by the attachment of the flagellar tips of several complementary gametes (Wiese, 1965). The agglutination phenomenon appears to be as specific as an antigen–antibody reaction (Wiese, 1965).

The clusters begin to dissipate when pairs of complementary gametes emerge, fused by their anterior ends. The flagella are no longer agglutinatable at this time. Eventually, the pair loses motility, and plasmodamy and karyogamy result in a nonmotile, sometimes thick-walled, zygote (Wiese, 1969).

The agglutination reaction of gametes is caused by a glycoprotein (gamone) on the flagellar tips (Forster *et al.*, 1956). An analysis of gamone has shown that of the (+) mating type to be 21% protein while the (−) mating type gamone is 36% protein (Forster *et al.*, 1956). Gamone isolated from one mating type can cause an agglutination of gametes from the opposite mating type (isoagglutination) and this is reciprocal (Wiese, 1965). Isoagglutination of *C. moewusii* and *C. eugametos* gametes can also be caused by the presence of 0.01% concanavalin A (Con A) (Wiese and Shoemaker, 1970; Wiese and Hayward, 1972). Vegetative cells of the respective mating types will not agglutinate with each other,
nor will they react with differentiated gametes (Wiese, 1965) or Con A (Wiese and Shoemaker, 1970).

Wiese and Shoemaker (1970) suggested that Con A forms a complex with the mating-type substances of (+) gametes of *C. eugametos* and *C. moewusii*. However, they found that Con A did not affect the ability of (-) gametes to agglutinate sexually. Wiese and Hayward (1972) studied this further with several species of *Chlamydomonas*. By determining sensitivity to various enzymes, they concluded that the active sites of the (+) gametes of *C. moewusii*, *C. eugametos*, and *C. mexicana* are composed of a carbohydrate while the active sites of the (-) gametes are proteinaceous. Both gamete types of *C. reinhardtii* and *C. chlamydogama* are said to be proteinaceous active sites but were not observed by Wiese and Hayward (1972) to isoagglutinate with Con A.

The difference, if any, between the mating sites and sites that bind Con A on differentiated gametes has not been clarified. This study is intended to distinguish between the mating reaction and agglutination by Con A of *C. reinhardtii* and *C. moewusii* gametes.

**MATERIALS AND METHODS**

The (+) and (-) gametes of *Chlamydomonas reinhardtii* (Nos. 89 and 90, respectively, from the Culture Collection of Algae, Indiana University) and *C. moewusii* (Nos. 96 and 97, respectively) were grown on the following medium (pH 6.8):

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl₂ 2H₂O</td>
<td>0.1 gm/l</td>
</tr>
<tr>
<td>MgSO₄ 7H₂O</td>
<td>0.6</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>0.4</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>2.15</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.09</td>
</tr>
<tr>
<td>Na citrate</td>
<td>0.5</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>0.001</td>
</tr>
<tr>
<td>Agar</td>
<td>15 gm/l</td>
</tr>
<tr>
<td>H₂BO₃</td>
<td>618.40 µg/l</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>880.88</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>109.03</td>
</tr>
<tr>
<td>CoCl₂</td>
<td>2.60</td>
</tr>
</tbody>
</table>

Culture conditions were 22 C ± 1 at 350 ft-c in a 12-12 hr light-dark cycle.

In order to induce gametogenesis, the surface of agar culture plates 3-4 weeks old were scraped and the cells suspended in induction medium which contained the growth medium nutrients reduced to one-tenth the concentration except for the phosphate buffer which was reduced to one-third and the NH₄Cl which was eliminated. The pH was adjusted to 7.6. After being washed 3 times in induction medium, cell suspensions of *C. reinhardtii* were allowed to stand in light at room temperature for 1-2 hr or until ready for use during that day. *C. moewusii* required a dark period of about 5 hr after induction before good production of gametes was possible.

Mating ability was determined by mixing equal volumes of suspended gametes in a watchglass and observing the agglutination or clumping of gametes, which was usually immediate, under a dissecting microscope. Concanavalin A (2X crystallized, Nutritional Biochemicals) was applied directly to individual gamete suspensions resulting in a final concentration of 0.01%. Isoagglutination was detected almost immediately or within a minute or two.

Monovalent Con A was prepared according to the procedure of Burger and Noonan (1970). One milligram crystalline Con A (Miles-Yeda) was dissolved in 1 ml of sterile induction medium. The Con A solution was incubated with 0.1% trypsin (Nutritional Biochemicals) at 37°C for 5 hr. The action of trypsin was stopped by the addition of soybean trypsin inhibitor (Miles-Seravac, Ltd.) to a final concentration of 0.1%. The solution was then filter-sterilized, diluted, and applied to gamete suspensions in a final concentration of 0.001% Con A.
RESULTS

Cells of *C. reinhardtii* from agar plates were suspended in induction medium in light and checked every half-hour from time 0 of the induction period for the appearance of mating ability and Con A agglutinability. *C. moewusii* was suspended similarly but maintained in the dark during this time. Mating ability of each mating type was determined by mixing it with fully differentiated gametes of the opposite mating type that were started the night before and by observing agglutination, which usually occurred immediately. The mating reaction in both mating types of *C. reinhardtii* appeared after 1–1.5 hr in induction medium in light and was maintained during the rest of the experimental period. *C. moewusii* developed sexual compatibility after 3–3.5 hr in darkness. Light seemed to be inhibitory to gamete development in *C. moewusii* if cells were exposed from time 0. In separate experiments, agglutinability by Con A occurred 0.5–1 hr before, 0.5–1 hr after, or at the same time as sexual agglutinability depending on the previous culture conditions of the cells. In any event, Con A agglutinability was independent of the appearance of sexual agglutinability.

If the sexual contact sites and the Con A-binding sites are separate, then the highly specific nature of these binding sites might possibly enable us to selectively block or affect one site without affecting the other. Since trypsin is known to eliminate the mating reaction (Wiese and Metz, 1969), gametes were incubated with 0.1% trypsin in induction medium at pH 7.6 for 1 hr at room temperature. They were washed and checked for mating ability with control gametes. No sexual agglutination was observed for both mating types of *C. reinhardtii* and for the (+) gametes of *C. moewusii* (Table 1). In the same experiment, mating ability of *C. moewusii* (−) gametes (No. 97) was not eliminated by trypsin. Trypsinized gametes of all mating types showed isoagglutination in the presence of Con A (Table 1). Trypsin had eliminated the mating sites of 3 gamete types without eliminating the Con A-binding sites. Forty-five minutes after washing, the mating reaction of the previously trypsinized gametes returned to full strength. This would indicate that there is a continual production or replacement of the mating sites.

Sucrose and mannose (inhibitors of Con A agglutination reaction) were tested separately for their effect on either the mating or Con A reaction. Fully differentiated gametes of both mating types which demonstrated agglutinability with opposite gametes and with Con A, were suspended separately in induction medium containing 0.02 M of either sucrose or mannose. Addition of Con A did not cause isoagglutination, but mixing the sugar-treated gametes with untreated opposite gametes did elicit the mating reaction (Table 1). Sucrose and mannose blocked the action of Con A but had no effect on sexual agglutinability.

### TABLE 1

**EFFECT OF SUCROSE, MANNOSE, AND TRYPsin TREATMENT ON Mating Ability AND Con A AGGLUTINABILITY OF GAMetes OF Chlamydomonas reinhardtii AND C. moewusii**

<table>
<thead>
<tr>
<th>Species and mating type</th>
<th>Control</th>
<th>0.02 M Sucrose</th>
<th>0.02 M Mannose</th>
<th>0.1% Trypsin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Opposite gamete</td>
<td>Con A</td>
<td>Opposite gamete</td>
<td>Con A</td>
</tr>
<tr>
<td>No. 89 <em>C. reinhardtii</em> (+)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>No. 90 <em>C. reinhardtii</em> (−)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>No. 96 <em>C. moewusii</em> (+)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>No. 97 <em>C. moewusii</em> (−)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>

* + = agglutination; 0 = no agglutination.*
In an attempt to determine whether trypsin could uncover or reveal the mating factor which may actually be on the flagellar surface of vegetative cells but masked (Burger, 1969) or distributed differently (Nicolson, 1972), vegetative cells of C. reinhardtii were treated with 0.1% trypsin in growth medium (pH 6.8) for 1 hr at room temperature. Induction medium was not used since it alone would elicit appearance of the binding sites. The cells were then washed with growth medium. The trypsin-treated vegetative cells did not agglutinate with fully differentiated opposite gametes nor with Con A.

Burger and Noonan (1970) were able to enzymatically cleave Con A, which is multivalent, and use the monovalent molecule to bind to the Con A sites of tumor cells allowing the cells to return to a normal, nontumor type of cell growth. In Chlamydomonas monovalent Con A selectively bound and covered the Con A sites without affecting sexual agglutination (Table 2). Monovalent Con A was prepared according to the procedure of Burger and Noonan (1970) and suspensions of differentiated gametes were treated with the monovalent molecule. Multivalent Con A was then added to determine if the Con A-binding sites were successfully covered. No agglutination occurred in the presence of multivalent Con A after monovalent Con A treatment (Table 2). Opposite gametes readily agglutinated with monovalent Con A-treated gametes. The Con A-binding sites were successfully covered without affecting the mating reaction (Table 2). This was true of both mating types of each species.

**DISCUSSION**

It is apparent from the data presented here that the mating sites and Con A-binding sites are two different flagellar surface agglutinins that appear during gametogenesis of Chlamydomonas reinhardtii and C. moewusii. The two types of sites were not revealed on trypsinized vegetative cells suggesting that appearance was due to de novo synthesis during gametogenesis or to storage of the active sites inside the cell. Gametes affected by trypsin renewed the mating sites within 45 min after trypsin is removed indicating a continual and rapid production of the mating agglutinin. Con A agglutination of C. reinhardtii was observed in this investigation but not by others (Wiese and Hayward, 1972). The role of Con A-binding sites on the flagellar surface of Chlamydomonas gametes is unknown.

The work of Wiese and Hayward (1972) suggested that the mating and Con A-bind-

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<table>
<thead>
<tr>
<th>Species and mating type</th>
<th>Controls</th>
<th>Monovalent Con A treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agglutination with opposite gamete</td>
<td>Isoagglutination with whole Con A</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. reinhardtii (+)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. reinhardtii (-)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. moewusii (+)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. moewusii (-)</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* + = agglutination; 0 = no agglutination.
ing sites of (+) gametes of C. moewusii are one and the same. Our data show that the two types of sites are separate. Monovalent Con A successfully blocked the Con A sites as shown by the lack of agglutination in the presence of multivalent Con A, and yet (+) gametes treated with monovalent Con A were able to sexually agglutinate with control (−) gametes and vice versa. Additionally, sucrose and mannose, Con A inhibitors, did not block sexual agglutination of C. moewusii as they might if the (±) mating sites could bind Con A, since the (−) mating site would have to be specific for α-D-glucopyranosyl residues (Goldstein et al., 1965). The reason that Wiese and Hayward (1972) did not observe mating by (+) gametes treated with low concentrations of multivalent Con A may be due to steric hindrance by the larger multivalent molecule which may have bound very close to the mating site.

Our results with trypsinized gametes of C. moewusii were opposite to what Wiese and his co-workers observed (Wiese and Metz, 1969; Wiese and Hayward, 1972). They reported the (−) gametes (No. 97) to be sensitive to trypsin in that the mating ability of this mating type could be eliminated with such treatment while the (+) mating type was not affected. When we observed in our experiments that the (+) gametes of C. moewusii were sensitive to trypsin rather than the (−) mating type, we reordered cultures from the Culture Collection of Algae, Indiana University to discount the possibility that we had mislabeled our cultures. When the experiment was repeated, our results were still the same as before. Additionally, we stained No. 96 with phenol red and combined it with No. 97 to observe pairing. Our observations showed that No. 96 was the swimming partner of the pair, therefore, the (+) gamete (Lewin, 1952). This is contrary to their designation by the Culture Collection (Starr, 1964) but consistent with the designation used by Wiese and co-workers (Wiese, 1965; Wiese and Metz, 1969; Wiese and Shoemaker, 1970; Wiese and Hayward, 1972).

The distinction between the two different agglutinins observed in the present study raises the question of whether other agglutinins remain undetected on the flagellar surface. It also presents problems when attempting to relate flagellar surface structure with either agglutinating function. The flagellar surface architecture of C. moewusii gametes has been shown to have parallel rows of particles that spiral slightly down the longitudinal axis of the flagellum (Brown et al., unpublished data). C. reimitzdii has mastigonemes on both vegetative and gametic cells (Lau rendi and McLean, unpublished data; Ringo, 1967; Witman et al., 1972). A direct relationship between either particles or mastigonemes and the agglutinating sites is yet to be demonstrated.

Normal mammalian cells treated with trypsin were found to behave temporarily like tumor cells and were easily agglutinated by Con A (Burger, 1970). Early evidence indicated that the Con A-binding sites were unmasked on normal cells treated with the enzyme (Burger, 1969). Later evidence suggests that the sites are always present (Mallucci, 1971) but become rearranged in clusters on the cell surface during trypsin treatment thereby making the cells more easily agglutinated by Con A (Nicolson, 1972). Trypsin treatment of vegetative cells of Chlamydomonas did not cause them to gain sexual or Con A agglutinability. Such results suggest de novo synthesis of the flagellar surface agglutinins. At least two possibilities exist for the expression of agglutinability during gametogenesis: one, these agglutinating factors are produced in the cell and transported to the outside (Jamieson and Palade, 1967; Neutra and Leblond, 1966); or two, there is an on-site synthesis (Petit et al., 1968; Roberts et al., 1968). These possibilities are currently being investigated in our laboratories.

The role of Con A-binding sites on the
flagellar surface of *Chlamydomonas* gametes remains a mystery. Our evidence shows that these sites apparently have no role in sexual agglutination but occur coincidentally on gametes that have been induced by nutrient deficient conditions. Holley (1972) has postulated that cell surface changes of tumor cells, of which Con A agglutination is one characteristic, result in an uptake of critical nutrients which stimulate growth. The possibility exists that Con A sites are a characteristic of any cell attempting to increase its nutrient uptake as *Chlamydomonas* vegetative cells might do after transfer to the nutrient-deficient induction medium. Under these conditions, certain nutrients, probably nitrogen (Trainor, 1959), could reach a critically low level within the cell and trigger both gametogenesis and membrane modifications of which the latter would try to restore the balance of nutrients within the cell. On the other hand, although the active terminal group on Con A-binding site of both *Chlamydomonas* gametes and mammalian tumor cells may be the same, the rest of the molecule and its function may be quite different.

The authors would like to thank Drs. T. Bonner and L. Kline and Mr. C. Laurende for their many helpful discussions and reading of the manuscript. The technical aid of Mrs. Linda Kranick is greatly appreciated.

Supported by grants from the Monroe County Cancer and Leukemia Association, the Research Foundation of the State University of New York, and NSF.

REFERENCES


BROWN, R. M., McLEAN, R. J., and INO, A., Unpublished data.


