Characterization of the Purified *Chlamydomonas minus* Agglutinin

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ABSTRACT *Chlamydomonas* flagellar sexual agglutinins are responsible for the adhesion of opposite mating-type (*plus* and *minus*) gametes during the first stages of mating. Purification and partial characterization of the *plus* agglutinin was previously reported (Adair, W. S., C. J. Hwang, and U. W. Goodenough, 1983, *Cell*, 33:183–193). Here we characterize the purified *minus* molecule. We show it to be a high molecular weight, hydroxyproline-rich glycoprotein that migrates in the 3% stacking region of an SDS-polyacrylamide gel and is absent from two nonagglutinating *minus* mutants. *Plus* and *minus* agglutinins are remarkably similar, although nonidentical, in amino acid composition, molecular morphology, and reactivity in vivo and in vitro with monoclonal antibodies raised against the *plus* agglutinin. Moreover, the adhesiveness of both *plus* and *minus* agglutinins, when coupled to agarose beads, is abolished by thermolysin, trypsin, periodate, alkaline borohydride, reducing agents, or heat, but unaffected by exo- or endoglycosidases. The *minus* agglutinin, however, migrates just ahead of the *plus* molecule on SDS PAGE, is excluded from an anion-exchange (Mono Q) column, elutes earlier during hydrophobic interaction (Bio-gel TSK Phenyl 5PW) chromatography, and is sensitive to chymotrypsin digestion (unlike the *plus* agglutinin); therefore, it differs from the *plus* agglutinin in apparent molecular weight, net charge, relative hydrophobicity and proteolytic susceptibility. Nevertheless, our results generally demonstrate a high degree of homology between these complementary cell–cell recognition/adhesion molecules, which suggests that they are specified by genes that have a common evolutionary origin.

*Chlamydomonas reinhardi* mating-type *plus* (*mt*+) and mating-type *minus* (*mt−*) gametes recognize and adhere to one another to initiate mating via sexual agglutinins located on their flagellar surfaces (reviewed in references 1 and 2). The processes of gametic recognition and flagellar adhesive interactions are extremely specific for both the mating-type and species of *Chlamydomonas* (3), features attributable to the agglutinin molecules themselves (4, 5). The *plus* and *minus* agglutinins can both be extracted in a biologically active form from *mt*+ and *mt−* gametes by EDTA (4, 6) and quantitated using an in vitro bioassay (4). The *plus* species has been purified by gel filtration chromatography and identified as a high molecular weight, hydroxyproline-containing, fibrous glycoprotein that is present as an extrinsic component of the *mt*+ flagellar surface (4, 5, 7). Recently, Saito and Matsuda (8) have followed similar protocols to extract *minus* agglutinin and have fractionated the activity by hydroxyapatite chromatography. They report that the *minus* agglutinin is also a high molecular weight glycopolypeptide.

To investigate the molecular mechanism that governs agglutinin-mediated intracellular recognition and adhesion in *Chlamydomonas*, it is important that both adhesins be well characterized. This paper describes the characterization of the *minus* agglutinin, purified by Fractogel-75 gel filtration chromatography, and its comparison with the *plus* agglutinin. *Minus* agglutinin is shown to be highly homologous to its *plus* counterpart in amino acid composition, structural morphology, and immunological antigenicity, with both adhesins representing high molecular weight, extrinsic, flagellar glycoproteins, rich in serine and hydroxyproline, that are absent from nonagglutinating mutants. Furthermore, many agents or treatments that perturb the adhesive nature of the *plus* agglutinin are shown to have a similar effect on the *minus* species. Despite these homologies, the two agglutinins differ with respect to net charge, hydrophobicity, electrophoretic mobility, and susceptibility to chymotrypsin digestion.

1144

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Preparation of Agglutinin EDTA Extracts: Minus agglutinin was extracted from 3 x 10^7 C. reinhardtii mating-type minus plate gametes (strain CC-621) using buffered EDTA (Sigma Chemical Co., St. Louis, MO) according to the protocol routinely used for plus agglutinin extraction. This procedure has been modified from that previously described (4, 5) in the following ways. Washed gametes are pelleted by brief centrifugation at 8,000 g and resuspended in 15 mM EDTA, 20 mM PIPES (Research Organics, Inc., Cleveland, OH), pH 7.4, at a final cell density of 10^9 cells/ml. Extraction is performed at 25°C until the cells are nonagglutinative with untreated tester gametes of the opposite mating type; unlike Saito and Matsuda (6, 8), we find that our minus gametes require a somewhat longer incubation period (30-45 min) with EDTA than do plus gametes (20-30 min) for full agglutinin extraction. The suspension is then briefly centrifuged at 27,000 g for at least 2 h in a cold cell pellet, and the supernatant is centrifuged at 40,000 g for 30 min. Ammonium sulfate is added to this supernatant to 70% saturation for 30 min on ice. After a 15-min centrifugation at 40,000 g, the pellets are resuspended in 1.5 ml deionized water, dialyzed overnight at 4°C against the same, centrifuged at 100,000 g for 40 min to pellet large cell wall fragments, and the supernatant (crude preparation) is lyophilized and stored at -70°C. The nonagglutinating minus mutants imp-10 (CC-1147) and imp-12 (CC-1148) were treated in an identical fashion to the normal minus strain. All CC numbers denote stock cultures available from the Chlamydomonas Genetics Center, Department of Botany, Duke University (Durham, NC).

In Vitro Iodination of Agglutinin: A lyophilized crude EDTA extract was resuspended in 1.0 ml of column buffer A, which consisted of 20 mM PIPES (pH 7.4), 100 mM KCI (Sigma Chemical Co.), 5 mM EDTA, and 30 mM octyl-β-D-glucopyranoside (Calbiochem-Behring Corp., La Jolla, CA), centrifuged for 10 min at 40,000 g, and the supernatant was divided into 750-μl aliquots. The smaller sample was split into two portions, each of 375 μl, one of which was added to 1.5 ml Bio-Gel P-6-DG (Bio-Rad Laboratories, Richmond, VA) into column buffer A. Another labeled sample was added to the remaining (750 μl) agglutinin aliquot and centrifuged for 10 min at 40,000 g. The supernatant (900 μl), containing 1.5 x 10^8 dpm, was directly loaded onto the gel filtration column for agglutinin purification.

Denaturing PAGE: SDS PAGE, sample preparation, autoradiography, and gel stains (periodic acid-Schiff, [PAS], 1 Coomassie Blue, silver) were performed as described previously (4, 5). Gels containing 3-4 and 4-6% gradients of acrylamide and urea, respectively, were used without stacking regions in some experiments but run at lower voltages than the above gels (40 V for 1 h, then 80 V until the bromophenol blue incorporated in the gradient gel migrated from the end of the gel).

Chromatographic Fractionation of Agglutinin Extracts: Fractionation of minus agglutinin extracts on Fractogel TSK HW-75 (F) (EM Science, Gibbstown, NJ) was performed in a column buffer A (30 mM octylglycoside, 5 mM EDTA, 100 mM KCI, 20 mM PIPES, pH 7.4) as previously described for plus agglutinin (5), without prior chromatography on Sepharose 4B. After equilibration and chromatography, fractions (5 ml each) were collected as void volume. Washes of the smaller sample were pooled into two portions, each of which was adsorbed to a nondenaturing hydrophobic phenyl column (Bio-Gel TSK Phenyl SPW, Bio-Rad Laboratories), in 10 mM potassium phosphate, pH 7.0, containing 1.7 M ammonium sulfate. Bound species were eluted with a decreasing (1.7-0 M ammonium sulfate gradient at a flow rate of 1.0 ml/min (10). Anion-exchange chromatography was performed on a MonoQ HR5/5 column (Pharmacia Fine Chemicals, Piscataway, NJ) in 20 mM HEPES (Sigma Chemical Co.), 5 mM EDTA, pH 7.2. Elution of bound proteins was accomplished with a gradient of 100-500 mM KCI in the same buffer at a flow rate of 1.0 ml/min.

The high-pressure liquid chromatographic system employed for hydrophobic interaction and anion-exchange chromatography used a Micromeritics (Micromeritics Instrument Corp., Norcross, GA) solvent delivery system (model 750), gradient programmer (model 752), and variable wavelength ultraviolet detector (model E 54 5 U) on line to an IBM CS 9000 instruments computer. Aliquots (~200 μl) of column fractions were rapidly-desalted through 1 ml of Bio-Gel P-6-DG (9) and analyzed by SDS PAGE/autoradiography and/or PAS staining, microscopic bioassay (4), and radioactivity determinations in a Beckman Gamma 4000 Counting System (Beckman Instruments Inc., Palo Alto, CA). When required, pooled column fractions were concentrated over Aquacide II (Calbiochem-Behring Corp.).

Biochemical and Immunological Analyses: Amino acid analysis for minus agglutinin was conducted as described for plus agglutinin (7).

Abbreviation used in this paper: PAS, periodic acid-Schiff.

Minus agglutinin was coupled to agarose beads for inactivation studies and treated as previously described (11).

Details of antibody production and properties of agglutinin-reactive monoclonal antibodies are presented in separate reports from our laboratory (1, 12). Immunoautoradiography was performed using the spaghetti overlay procedure (13).

Light, Fluorescence, and Electron Microscopy: Agglutinin bioassay by light microscopy was performed according to the method of Adair et al. (4). Quick-frozen, deep-etched samples of purified agglutinins were prepared for electron microscopy according to Heuser (14) and visualized as described previously (5, 7, 15).

Immunofluorescence microscopy of agglutinin-reactive monoclonal antibodies to bound to cells was performed as follows. Cells were washed once with cold buffer of 10 mM HEPES, pH 7.5, 5 mM MgSO_4, 0.5 mM EDTA, 25 mM KCl, and then 2 h in the buffer containing 2% paraformaldehyde (Eastman Laboratory and Specialty Chemicals, Rochester, NY), and washed three times with phosphate-buffered saline (PBS) before incubation for 30 min with monoclonal antibodies (25 μg/ml of a 50% ammonium sulfate cut of ascites fluids). Control samples were incubated with nonimmune mouse serum (Sigma Chemical Co.) or an irrelevant monoclonal antibody (D3, raised against Chlamydomonas dynein, Richard, B.B., and W. S. Adair, unpublished observation). After primary antibody incubations, cells were washed three times with PBS and incubated for 15 min with 5 μg/ml biotinylated horse-anti-mouse Ig (Vector Laboratories, Burlingame, CA). After they were washed twice with PBS and once with 50 mM Na_2CO_3, 0.15 M NaCl, the cells were incubated for 5 min in the latter buffer containing 25 μg/ml fluorescein isothiocyanate-avidin D (Vector Laboratories). The labeled cells were then washed three times with 50 mM Na_2CO_3, 0.15 M NaCl and suspended in mounting buffer (25% glycerol in PBS). A Zeiss Photomicroscope I equipped with an II RS epifluorescence condenser was employed to visualize and photograph the labeled cells through a K500 barrier filter using Kodak Tri-X film, shot at ASA 400 and developed at ASA 800.

RESULTS AND DISCUSSION

Purification of Mating-type Minus Agglutinin

Quantitative release of the minus agglutinin from gametic flagella is readily accomplished by incubation of living gametes with buffered 15 mM EDTA, analogous to the protocol employed for extraction of plus agglutinin (4, 5). Fig. 1 illustrates the purification of an in vitro 125I-labeled minus agglutinin EDTA extract from Fractogel-75 sizing chromatography. Elution of labeled extract components was monitored by radioactive determination and a quantitative bioassay (4) was used to detect agglutinin biological activity. In the first cycle through the column matrix, a major portion of the labeled extract eluted in one broad peak (Fig. 1A). Maximum minus agglutinin activity was predominantly associated with the shoulder of this peak. Autoradiography of column fractions after SDS PAGE revealed a number of labeled bands in the biologically active fractions.

To more fully resolve components eluting in this region, the most active fractions (fractions 20-26) were pooled, concentrated, and rechromatographed. The major agglutinin activity was now found within the peak of remaining 125I-labeled extract EDTA-extracted material (Fig. 1B). Peak minus agglutinin activity co-fractionated with two high molecular weight polypeptides, and these fractions otherwise contained only relatively minor amounts of other labeled components. When these two high molecular weight molecules were separated by high-performance liquid chromatography hydrophobic interaction chromatography, minus agglutinin activity was invariably associated with the slower-migrating, higher molecular weight gel band (as described below); this molecule is therefore named polypeptide A~. By analogy with the similarly migrating gel band of active plus agglutinin (5), which we henceforth designate polypeptide A. 1
**Minus Agglutination Mutants**

*Chlamydomonas* mutants unable to agglutinate sexually with gametes of the opposite mating-type have been isolated in both *plus* and *minus* strains and genetically characterized (22, 23). Adair et al. (5) have shown that EDTA extracts from nonagglutinating *plus* mutants lack the agglutinin polypeptide $A^-$. To learn whether *minus* nonagglutinating mutants lack polypeptide $A^-$, EDTA extracts (inactive) were prepared from *imp-10* and *imp-12* strains, and subjected to Fractogel-75 purification in a manner analogous to that employed for active *minus* extracts, and the fractions were analyzed by SDS PAGE. Fig. 2 (lanes 2 and 3) demonstrates that both mutants lack a polypeptide $A^-$ component, which supports the identification of this high molecular weight glycoprotein as the active *minus* agglutinin molecule. In addition, molecules with the morphology of $A^-$ are present on the surface of gametic, but not vegetative, *minus* cells when observed in situ (15). The mutants do, however, produce glycopolypeptides that migrate further into the stacker region of the gel (Fig. 2). These species, which are also produced by wild-type gametes, derive from the flagellum but are not involved in adhesion; they are described in detail elsewhere (15).

**Visualization of Purified Minus Agglutinin**

Fig. 3 compares representative isolated molecules of *plus* and *minus* agglutinins as visualized by the quick-freeze, deep-etch technique. Clearly, the *plus* and *minus* agglutinins are homologous. Both have four morphologically distinct regions: a terminal head, a straight portion, a flexible domain, and a terminal hook. The *minus* agglutinin however, displays a "shepherd's crook" conformation near the head region, and its head is somewhat larger. A detailed structural comparison of these proteins is provided in a separate report (15).

**Amino Acid Composition of the Minus Agglutinin**

Table I presents the amino acid composition of the *minus* agglutinin. Included for comparison purposes are profiles previously published for the *plus* agglutinin (7) and for 2BI, one of the major *C. reinhardtii* cell wall glycoproteins (24). The three *Chlamydomonas* high molecular weight glycoproteins are remarkably similar in overall composition. Furthermore, values for the *C. reinhardtii* sexual agglutinins closely resemble those reported for the *minus* agglutinin of *C. eugamatos* (25).

As expected, the *plus* and *minus* agglutinins are more similar to one another than to the cell wall glycoprotein, presumably reflecting their more similar evolutionary histories. Note that the *minus* agglutinin, like its *plus* homologue and 2BI, contains high levels of hydroxyproline and serine residues. As discussed previously, these two amino acids serve as points
for oligosaccharide attachment in a variety of plant glycoproteins, including *Chlamydomonas* cell wall glycoproteins, and the hydroxyproline residues are thought to confer on the protein its fibrous properties (7).

**Differential Electrophoretic Mobility of Plus and Minus Agglutinins**

Both *plus* and *minus* agglutinins migrate slowly in SDS PAGE gels (Fig. 4, lanes 1 and 4). As described in detail elsewhere (15), one or more faster-migrating glycoproteptides, collectively designated *B* (Fig. 4, lanes 2–4), often contaminate peak agglutinin fractions; since the *B* material alone has no adhesive activity, however, it is not relevant to the present study. Agglutinin fractions enriched for *A*+ (Fig. 4, lane 1) consistently display maximal activity, whereas fractions containing abundant band *B* (lane 2) are much less active. Of interest here is that the polypeptide *A*– band reproducibly migrates farther into the 3% stacking region of SDS PAGE gels than does polypeptide *A*+, regardless of the purification method. This difference is most easily observed with longer electrophoretic runs on urea/polyacrylamide gels containing gradients of 3–4% acrylamide and 4–6% urea, conditions that provide improved separation as well as increased migration (without loss of resolution) of both bands *A* and *B* (Fig. 4, lanes 3 and 4). The increased migration of active *minus* agglutinin relative to active *plus* agglutinin presumably reflects a structural disparity under denaturing conditions between the *plus* and *minus* sexual adhesins.

**Chromatographic Behavior of Plus and Minus Agglutinins**

Comparable elution profiles are obtained for *plus* or *minus* agglutinin preparations fractionated on a variety of gel-filtration sizing columns, including Fractogel-75. In contrast, the adhesins behave quite differently on anion-exchange (MonoQ) and hydrophobic interaction (Bio-Gel TSK Phenyl SPW) columns. Whereas *plus* activity is retained on a MonoQ column (20 mM HEPES, 5 mM EDTA, pH 7.2) and subsequently eluted with a 100–500 mM KCl gradient (Fig. 5), *minus* activity is excluded from the anion-exchange resin. Therefore, it appears that the agglutinins differ in net charge, a feature that might facilitate their interaction in vivo. Note that this column is also useful for separating bands *A* and *B* of *plus* agglutinin.

Although both *plus* and *minus* agglutinins are efficiently adsorbed to the hydrophobic phenyl matrix (1.7 M ammonium sulfate, 100 mM potassium phosphate, pH 7.0), each elutes (with decreasing ammonium sulfate concentration) at an independent region of the gradient (Fig. 6), a characteristic retained when *plus* and *minus* extracts are combined and co-chromatographed (not shown). Because *minus* activity elutes before the major extract contaminants (Fig. 6A), and *plus* activity is retained until after most of the wall material has been released (Fig. 6B), both agglutinins can be effectively purified to near homogeneity on the nondenaturing hydrophobic phenyl column. In addition, the high molecular weight gel band that co-elutes during F-75 purification with the *minus* agglutinin can be separated from the active adhesin by such hydrophobic interaction chromatography, as it elutes at a region intermediate to the elution positions of the *minus* agglutinin. However, the band that elutes at a region intermediate to the elution positions of the *minus* agglutinin.

### Table 1. Amino Acid Compositions of the *mt*+ and *mt*– Sexual Agglutinins

| Amino acid | (+) Agglutinin | (–) Agglutinin | Wall protein |
|------------|----------------|----------------|--------------|
|            | (No. of residues per 1,000) |            |
| Lys        | 22 ± 8.5       | 41             | 39           |
| His        | 31 ± 9.2       | 20             | 6            |
| Arg        | 36 ± 3.8       | 22             | 35           |
| Asx        | 95 ± 4.0       | 104            | 112          |
| Thr        | 59 ± 5.7       | 67             | 75           |
| Ser        | 103 ± 13.3     | 113            | 78           |
| Cys        | 88 ± 17.5      | 83             | 64           |
| Pro        | 43 ± 9.3       | 56             | 67           |
| Gly        | 88 ± 11.2      | 88             | 71           |
| Ala        | 77 ± 7.8       | 78             | 86           |
| Val        | 46 ± 8.0       | 62             | 68           |
| Cys        | 50.6           | ND             | ND           |
| Met        | 10 ± 4.0       | 8              | 12           |
| Ile        | 27 ± 3.8       | 35             | 38           |
| Leu        | 51 ± 9.9       | 63             | 69           |
| Trp        | ND             | ND             | ND           |
| Tyr        | 15 ± 1.2       | 11             | 31           |
| Phe        | 28 ± 8.3       | 31             | 38           |
| Hyp        | 123 ± 13.6     | 120            | 112          |

ND, not determined

* Values expressed are mean ± SD from three separate analyses (except 1/2 cysteine).

![Figure 4 Electrophoretic mobilities of polypeptides A+ and A–. Purified *mt*+ and *mt*– agglutinins were analyzed by urea/polyacrylamide SDS PAGE (4–6 and 3–6%, respectively). 1.0-ml fractions, dialyzed extensively against dH2O and lyophilized to dryness, were resuspended in 20 μl SDS sample buffer (4). 6 μl of each sample was loaded per lane. Quantitative agglutinin bioassays (4) of the purified fractions were performed before lyophilization. Lane 1, peak Fractogel-75 purified *mt*+ agglutinin (1.024 U activity); lane 2, Fractogel-75 fraction enriched for “band” B; lane 3, peak MonoQ/Fractogel-75 purified *mt*+ agglutinin; lane 4, peak Fractogel-75 purified *mt*– agglutinin. Arrows indicate the *mt*+ agglutinin (A+), *mt*– agglutinin (A–), and heterogeneous “band” B (B) region. Note that the slowest-migrating PAS-reactive band in lane 4 does not correspond to polypeptide A+; it is frequently found in both *mt*+ and *mt*– extracts, and when isolated by hydrophobic interaction chromatography it lacks agglutinin activity.**
FIGURE 5 Purification of mt+ agglutinin by anion-exchange chromatography. A crude mt+ agglutinin extract (1.5 x 10¹¹ cells), resuspended in 1.0 ml 20 mM HEPES, pH 7.2, buffer containing 5 mM EDTA, was adsorbed to a MonoQ column, and eluted with a 100-500 mM KCl gradient (see Materials and Methods). 150-µl aliquots of each fraction (1.0 ml total) were rapid-desalted (9) and analyzed by quantitative bioassay (4). For SDS PAGE (3.5-15%), the desalted aliquots were lyophilized and resuspended in 15 µl SDS sample buffer; 6 µl was loaded per lane. Fixed gels were PAS stained (see Materials and Methods). Note that polypeptides A+ and B are resolved and that agglutinin activity is associated with A+ alone. The arrowhead denotes the stacking gel interface.

and plus agglutinins (not shown). Since selective desorption depends on the strength of the hydrophobic interaction between the nonpolar amino acid groups of the protein and the phenyl substituents of the matrix (10), the lengthy retention time of plus agglutinin on this column is suggestive of its being relatively more hydrophobic than the minus agglutinin.

Inactivation of Minus Agglutinin Conjugated to Agarose Beads

Purified agglutinin, when covalently attached to an inert support such as agarose beads, retains potent biological activity in assays using living gametes of the complementary mating type (11). Such immobilized preparations provide a sensitive and rapid in vitro assay system for assessing the effects of various agents (chemical, enzymatic, etc.) on agglutinin activity (11).

Table II compares data obtained for immobilized minus agglutinin subjected to various treatments with results previously published (11) for the effects of these treatments on beads coupled with plus agglutinin. Most of the treatments have a similar effect on both agglutinins. Thus, both species are sensitive to digestion by thermolysin or trypsin, alkaline borohydride reductive modification of carbohydrate, heating above 65°C (although minus may be slightly more heat labile than plus), periodate oxidation (see also reference 6), and thiol reduction. Unlike C. eugamatos (16-18), minus agglutinin is not inactivated by a-galactosidase, nor is plus activity destroyed by a-mannosidase. An interesting difference is seen with a-chymotrypsin: minus agglutinin activity demonstrates a partial loss of activity at 0.1 mg/ml and a complete loss of activity at 1 mg/ml, whereas plus activity is unaffected by even 10 mg/ml of this protease. Since the overall amino acid composition of minus agglutinin, as compared with plus agglutinin, is not enriched for aromatic amino acids (see Table I), it is unlikely that the chymotryptic-induced loss of minus biological activity results from a generalized degradation of minus agglutinin by this protease. If the selective inactivation of minus agglutinin results from chymotryptic cleavage at or near the minus adhesive site, this enzyme should be useful for probing the differences in the plus and minus mating-type-specific adhesion sites.

Immunologic Relatedness of Plus and Minus Agglutinins

Monoclonal antibodies directed against determinants found on the plus agglutinin have been generated in this laboratory and are described in detail elsewhere (1, 12). Three classes of agglutinin-reactive monoclonal antibodies have been identified. Two are partially (class III) or extensively (class I) cross-reactive with other Chlamydomonas flagellar or wall components; the third (class II) reacts specifically with polypeptide A and the polypeptide B family (1, 12, 15).

All three monoclonal antibody classes, although initially selected for their ability to isoagglutinate plus gametes by their flagella (1), prove to be equally effective at isoagglutinating minus gametes. Fig. 7 illustrates this cross-reactivity for two of these antibodies using immunofluorescence microscopy. Like the patterns observed for plus (1, 12), the minus gametes display highly labeled flagella and cell walls with class I antibody (Fig. 7A), and punctate flagellar staining and unlabeled cell walls with class II antibody (Fig. 7B). Similar mating-type cross-reactivity is also observed with Class III
monoclonal antibodies (not shown).

To learn whether the antibodies were recognizing the same surface molecules on both plus and minus gametes, class I and II monoclonal antibodies were used to probe a partially purified minus agglutinin preparation, after SDS PAGE, by spaghetti overlay immunautoradiography (25). Antigen–antibody complexes were detected using 125I-sheep anti-mouse Ig, F(ab)_2, followed by autoradiography. Fig. 8 shows that both class I (lane 1) and class II (lane 2) monoclonal antibodies recognize the minus agglutinin. Since recent immunotopo­graphical mapping studies have demonstrated that the class II monoclonal antibodies bind to a repeating determinant along the length of the plus agglutinin rod (1, 12), the plus and minus agglutinins may have common features in their polypeptide backbones, consistent with their highly similar amino acid compositions and morphologies.

In conclusion the minus sexual agglutinin has been extracted from C. reinhardtii minus gametes with 15 mM EDTA, and purified by Fractogel-75 sizing chromatography, and the purified adhesin was characterized. These studies have demonstrated that, overall, minus agglutinin is remarkably similar to the plus sexual adhesin in amino acid composition, immunological antigenicity, structural morphology, and sensitivity or resistance to various agents or treatments.

Such homology between the C. reinhardtii sexual agglutinins contrasts with the sexual agglutination factors purified from three genera of ascomycetous yeast. The latter differ markedly in the two yeast mating types: one factor is a large, heavily-glycosylated, heat-stable component that is inactivated by reducing agents (19, 20).

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A second difference between the agglutinins of Chlamydo­monas and yeast lies in their in vitro reactivity. The purified yeast factors can interact in vitro to form stable complexes. Although the molecular mechanism for this recognition/adhesion process is not yet clear, the factors clearly have enough molecular information to effect these biological functions. By contrast, purified (or other nonliving) agglutinin preparations
Figure 7 Immunofluorescence staining of mt" gametes. Paraformaldehyde-fixed gametes were stained with Class I monoclonal A15 (row A) and Class II monoclonal A12 (row B) as described (references 1 and 12, and in Materials and Methods). Cell body fluorescence in row B derives from chlorophyll (most clearly seen in color photographs, in which chlorophyll-related fluorescence appears red), whereas cells in row A display additional antibody-associated fluorescence (green in color photographs) due to cross-reaction of Class I antibodies with cell wall glycoproteins (1, 19). Control cells, stained with preimmune or irrelevant antisera, reproducibly showed no antibody-associated fluorescence (1, 19). × 1,200.

Figure 8 Immunolabeling of mt" agglutinin. Minus agglutinin, partially purified by Fractogel-75 chromatography, was subjected to SDS PAGE (4-8% gel) and probed with Class I monoclonal A15 (lane 1) and Class II monoclonal A12 (lane 2) by the spaghetti overlay procedure (13). Antigen–antibody complexes were detected with 125I-sheep anti-mouse Ig, F(ab)\textsubscript{2}, 24 h exposure.

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from Chlamydomonas show no evidence of interacting with each other, but instead require the participation of one living partner (26). Thus, it appears that the Chlamydomonas cell-cell recognition/adhesion event involves more than the simple presentation of the appropriate mating-type-specific agglutinins on the flagellar surfaces.
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