Functional Modification of the 
Chlamydomonas Flagellar Surface

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ABSTRACT Chlamydomonas flagella exhibit force transduction in association with their surface. This flagellar surface motility is probably used both for whole cell gliding movements (flagella-substrate interaction) and for reorientation of flagella during mating (flagella-flagella interaction). The present study seeks to identify flagellar proteins that may function as exposed adhesive sites coupled to a motor responsible for their translocation in the plane of the plasma membrane. The principal components of the flagellar membrane are a pair of glycoproteins (~350,000 mol wt), with similar mobility on SDS polyacrylamide gels. A rabbit IgG preparation has been obtained which is specific for these two glycoproteins; this antibody preparation binds to and agglutinates cells by their flagellar surfaces only. Treatment of cells with 0.1 mg/ml pronase results in a loss of motility-coupled flagellar membrane adhesiveness. This effect is totally reversible, but only in the presence of new protein synthesis. The major flagellar protein modified by this pronase treatment is the faster migrating of the two high molecular weight glycoproteins; the other glycoprotein does not appear to be accessible to external proteolytic digestion. Loss and recovery of flagella surface binding sites for the specific antibody parallels the loss and recovery of the motility-coupled flagellar surface adhesiveness, as measured by the binding and translocation of polystyrene microspheres. These observations suggest, but do not prove, that the faster migrating of the major high molecular weight flagellar membrane glycoproteins may be the component which provides sites for substrate interaction and couples these sites to the cytoskeletal components responsible for force transduction.

Cell surfaces possess many dynamic properties (25). In many cases, cell surface receptors are coupled to intracellular machinery that actively regulates their spatial distribution (24). Many cells are capable of rapidly changing the characteristics of their surfaces through protein turnover and through redistribution of existing components.

The surfaces of both vegetative and gametic flagella of Chlamydomonas reinhardtii exhibit the properties of adhesiveness, motility (receptor redistribution), transmembrane coupling, and rapid protein turnover (6). Further, Chlamydomonas provides certain experimental advantages for the study of cell surface properties because the cells can be easily synchronized, pulse-labeled, and a small subset of the cell surface (that associated with the flagella) possessing a limited number of defined functions can be rapidly separated from the rest of the cell by the act of deflagellation. The flagellar surface consists of an extension of the cell body plasma membrane which is covered by a 20-nm layer of material referred to as the "flagellar sheath" (27) or "fuzzy coat" (2) in addition to mastigosomes ~0.9 μm in length (27, 35). Purified flagellar membranes consist of one predominant class of high molecular weight (HMW) glycoproteins in addition to a number of minor components (2, 19, 22, 23, 29, 35).

Chlamydomonas can be grown vegetatively or induced to differentiate into gametic cells. Vegetative and gametic cells possess certain flagellar surface properties in common although flagella from gametic cells express certain unique properties (6, 11). Both vegetative and gametic flagellar surfaces possess a relatively nonspecific adhesive property that allows them to interact with a solid substrate or artificial markers such as polystyrene microspheres. These flagellar surface "receptors", although not specific in their interactions, are coupled to motile machinery which results in force being applied at the flagellar surface in a direction defined by the long axis of the flagellum (3, 5, 7, 12, 16). Bloodgood et al. (7) have demonstrated that flagellar surface adhesiveness and flagellar surface motility can be independently quantitated. In addition, these two phenomena can be independently and reversibly modified. Hoffman...
and Goodenough (12) reported that chymotrypsin treatment affected flagellar surface adhesiveness whereas trypsin treatment affected flagellar surface translocation of polystyrene microspheres without having any effect on adhesiveness. Bloodgood et al. (7) reported that low temperature (0-4°C) inhibited flagellar surface motility but had no effect on adhesiveness whereas pronase treatment had the opposite effect. One physiological manifestation of these flagellar surface properties is the flagella-dependent gliding of cells in contact with a solid substrate (5, 16).

Mating interactions between gametic cells of Chlamydomonas involve a complex series of flagellar surface events: (a) adhesive interaction of the flagellar surfaces, (b) reorientation of the flagella, (c) flagellar tip activation, (d) "locking in" of the flagellar tips, (e) signaling of these events to the cell bodies, and (f) de-adhesion of the flagellar surfaces (2, 9, 11, 16, 20, 21, 29, 31). It has been separately proposed that flagellar surface motility is involved in the flagellar reorientation (6, 7) and in the signaling (12) events of the mating process. It has also been observed that the characteristics of the gametic flagellar surface can be rapidly altered through protein turnover (30) after specific cell-cell contact during mating.

The present study examines the role of the HMW glycoproteins of the vegetative Chlamydomonas flagellar membrane in the motile and adhesive properties of the flagellar surface. Use of an antibody prepared against defined flagellar membrane glycoproteins coupled with proteolytic modification of exposed flagellar surface proteins implicates one of the two HMW glycoproteins in the role of motility-coupled flagellar surface receptor.

MATERIALS AND METHODS

Unless otherwise specified, all experiments were performed using vegetatively grown cells of C. reinhardtii, strain pf-18, a nonmotile mutant with a structural defect in the central pair of flagellar microtubules (33). It was necessary to use a strain with paralyzed flagella to obtain quantitative data on flagellar adhesiveness and flagellar surface motility (7). For preparation of antibodies, flagellar membrane components were obtained from a wild type strain, C. reinhardtii, strain 21 gr. Cells were grown synchronously at 22°C in medium I of Sager and Granick (28) using an alternating cycle of 14 h light and 10 h dark. Experiments were performed in fresh growth medium, except as otherwise noted. Cells were labeled with [35S]sulfate by the method of Lefebvre et al. (15). Incorporation of [35S]sulfate into protein was measured using the filter disk assay of Mans and Novelli (18).

Transmission electron microscopy (TEM) was performed as previously described (3) except 2% tannic acid was included in the glutaraldehyde fixative. Flagella were purified by the pH shock (35) or dibucaine (36) procedures. To prepare a membrane vesicle, isolated flagella were resuspended in 10 mM HEPES-KOH, pH 7.4, 5 mM MgSO4, 1 mM dithothreitol (DTT), 0.5 mM EDTA and 25 mM KCl and demembranated by addition of an equal volume of the same buffer containing 0.08% Nonidet P-40 (see Materials and Methods). The material from the HMW glycoprotein band was observed to consist primarily of three components: (a) a HMW glycoprotein band (actually a doublet) migrating with an apparent molecular weight around 350,000, (b) a less prominent, faster migrating component in the molecular weight range of 250-300,000, and (c) a minor component migrating slightly behind α-tubulin (~65,000 mol wt). Silver-staining of the gels does reveal a number of other, very minor components. Little or no detectable tubulin is present in this preparation of flagellar membrane material. For preparation of antibodies, this material was separated on a preparative 5% SDS polyacrylamide gel and the HMW band (indicated by an arrowhead in Fig. 1 b) was excised and injected into rabbits. A purified IgG fraction from these rabbits gave a single, strong precipitin line by double immunodiffusion and crossed immunoelectrophoresis against total flagellar proteins (Fig. 2 a, b); some suggestion of heterogeneity can be seen in Fig. 2a. Incubation of solubilized total flagellar proteins with this antibody preparation followed by immunoadsorption with protein A-Sepharose, SDS PAGE, and autoradiography clearly indicated that the IgG fraction actually contained antibodies specific for two different HMW proteins migrating close together at an apparent molecular weight around 350,000 (Fig. 6 e). Although both of these components are glycoproteins judged by a comparison of acrylamide gels stained with Coomassie Brilliant Blue and periodic acid–Schiff reagents, the faster migrating protein contains more carbohydrate relative to protein. Labeling of an

RESULTS

Characterization of an Antibody Preparation
Specific for the High Molecular Weight Flagellar Glycoproteins

Isolated flagella from vegetative cells of wild type C. reinhardtii (strain 21 gr) were briefly treated with low concentrations of the nonionic detergent Nonidet P-40 (see Materials and Methods). Much of the material released from the flagella by this treatment pelleted at 100,000 g. Examination of the material in the pellet by negative stain electron microscopy showed it to consist of small membranous vesicles (Fig. 1 a) with an average diameter of 130 ± 40 nm (n = 46); no mastigones were observed in this preparation. This flagellar membrane vesicle preparation, when analyzed by SDS PAGE (Fig. 1 b), was observed to consist primarily of three components: (a) a HMW glycoprotein band (actually a doublet) migrating with an apparent molecular weight around 350,000, (b) a less prominent, faster migrating component in the molecular weight range of 250-300,000, and (c) a minor component migrating slightly behind α-tubulin (~65,000 mol wt). Silver-staining of the gels does reveal a number of other, very minor components. Little or no detectable tubulin is present in this preparation of flagellar membrane material. For preparation of antibodies, this material was separated on a preparative 5% SDS polyacrylamide gel and the HMW band (indicated by an arrowhead in Fig. 1 b) was excised and injected into rabbits. A purified IgG fraction from these rabbits gave a single, strong precipitin line by double immunodiffusion and crossed immunoelectrophoresis against total flagellar proteins (Fig. 2 a, b); some suggestion of heterogeneity can be seen in Fig. 2a. Incubation of solubilized total flagellar proteins with this antibody preparation followed by immunoadsorption with protein A-Sepharose, SDS PAGE, and autoradiography clearly indicated that the IgG fraction actually contained antibodies specific for two different HMW proteins migrating close together at an apparent molecular weight around 350,000 (Fig. 6 e). Although both of these components are glycoproteins judged by a comparison of acrylamide gels stained with Coomassie Brilliant Blue and periodic acid–Schiff reagents, the faster migrating protein contains more carbohydrate relative to protein. Labeling of an...
FIGURE 2  (a) Crossed immunoelectrophoresis of a whole flagella preparation separated in the horizontal dimension reacted against the antiserum produced to the G band shown in Fig. 1 b. One major precipitin band is observed. (b) Double immunodiffusion of the antibody prepared against G band material reacted with whole solubilized flagellar proteins. One major precipitin band is observed.

SDS-acrylamide slab gel with the specific antibody followed by 125I-protein A and autoradiography confirmed the specificity of the antibody preparation for the HMW glycoproteins but was not able to resolve the two components.

The antibody preparation prepared against the HMW glycoproteins strongly agglutinates both vegetative and gametic cells of *Chlamydomonas* strains 21 gr (wild type) and pf-18 (paralyzed flagella) (Fig. 3, also Fig. 11 a) while an IgG fraction purified from preimmune serum did not induce any agglutination. The agglutination always occurred by the flagellar surfaces (Fig. 3); cells in a clump appeared to be held together preferentially by their flagellar tips, reminiscent of the situation in mating aggregates. The resulting clumps routinely contained 50–100 cells or more and rapidly (within 20 min) settled out of solution (Fig. 4). Cells were never observed to be agglutinated by their cell walls suggesting that the antibody preparation was not contaminated with antibodies to any of the HMW cell wall components. Purified flagella also exhibited strong agglutination in the presence of the specific IgG but not the presence of a preimmune IgG preparation. Both vegetative and gametic cells exposed to the antibody have been observed to exhibit thickenings of the distal portion of the flagella (arrows in Fig. 3). This may correspond to the "tipping" response of gametic cells to a whole flagella antiserum reported by Goodenough and Jurivich (10). The observation that the antibody preparation used in the present report agglutinates cells by their flagellar surfaces indicates that portions of one or both of the HMW glycoprotein species with which the antibodies react are exposed at the flagellar surface.

Cells, pretreated with the specific antibody preparation and then extensively washed, bind large numbers of polystyrene microspheres relative to control cells. Although this reaction of polystyrene microspheres with the antibody-treated flagellar surface probably results from a nonspecific affinity of polystyrene microspheres for immunoglobulins, the observation clearly demonstrates that a large amount of specific antibody binds to the flagellar surface. There is no increase in microsphere binding to the flagellar surface of cells exposed to preimmune serum and then washed. Uniform antibody binding to the entire flagellar surface of both vegetative and gametic cells has been directly demonstrated using: (a) FITC-labeled specific antibody followed by fluorescence microscopy, (b) specific antibody followed by FITC-labeled goat anti-rabbit IgG and fluorescence microscopy, (c) specific antibody followed by the peroxidase-antiperoxidase procedure of Sternber-
ger (32), and (d) specific antibody followed by ferritin-labeled goat anti-rabbit IgG and TEM. An example of the results obtained using the peroxidase-antiperoxidase (PAP) procedure is shown in Fig. 5. In addition to a uniform dense staining of the flagellar surface, some reaction product is also consistently observed over the cell body. Two lines of evidence suggest that this reaction product is associated with the general cell plasma membrane and not the cell wall: (a) a cell-wall-less mutant (CW-15) exhibits similar staining over the general cell surface and (b) CW-15, but never wild type, cells are agglutinated by their cell bodies in the presence of the specific antibody. These results strongly suggest that the major membrane glycoprotein exposed at the flagellar surface (the faster migrating of the two components recognized by the specific antibody preparation) is also present in the general cell surface plasma membrane.

Flagella pretreated with the specific antibody preparation fail to exhibit flagellar surface motility, as visualized by movements of attached polystyrene microspheres. This may be due to the fact that cross-linking of the motility-coupled receptors at the flagellar surface prevents their active redistribution in the plane of the membrane. However, it may also be that extensive cross-linking of the flagellar surface restricts the mobility of many flagellar membrane proteins, including ones not recognized by the antibody. Pretreatment of cells with monovalent Fab fragments of the specific IgG (generated by papain treatment in the presence of cysteine) does not result in an inhibition of surface translocation of polystyrene microspheres. In fact, that is the result that would be predicted if the intact IgG were cross-linking a specific receptor and preventing its lateral movement within the plane of the flagellar membrane. However, it is also the result that would be predicted if the intact IgG were merely restricting lateral mobility of all components through cross-linking of an unrelated membrane component. Hoffman and Goodenough (12) previously reported that pretreatment of C. reinhardii gametic cells with a crude antibody prepared against whole flagella from mating type (+) gametes prevented polystyrene microsphere movement.

The mating of C. reinhardii gametic cells is prevented by a 15-min pretreatment of either of the two mating types with a 1:100 dilution of the antibody preparation specific for the flagellar HMW glycoproteins. The pretreatment with the antibody was performed at a low cell density to minimize agglutination of the cells by the antibody. This antibody-induced inhibition of mating may reflect a genuine involvement of the HMW glycoproteins in the mating process or it may be due to a steric masking of the mating specific sites due to the binding of large amounts of antibody to other sites.

**Effects of Pronase Treatment**

Treatment of *Chlamydomonas* with 0.1 mg/ml pronase in growth medium for 5–6 h at 22°C results in little change in the...
The effect of treatment of cells with 0.1 mg/ml pronase for 4 h on the acrylamide gel electrophoretic pattern of flagellar proteins. Cells were pulse-labeled with [35S]sulfate before treatment. Autoradiograms are shown in A–D and Coomassie Brilliant Blue stained gels are shown in E–F. Lane A—whole flagella from untreated cells; Lane B—whole flagella from pronase-treated cells exhibiting a major new component (G'). The samples shown in lanes E and F result from processing of the flagellar preparations in A and B, respectively, by an immunoadsorption procedure using the antibody preparation specific for the HMW glycoproteins. C and D are the autoradiograms of E and F, respectively. The antibody preparation recognizes two closely migrating flagellar components (arrowheads in E). After pronase treatment, the faster migrating of the components disappears and a new component (G') of much different mobility appears (lane F). This newly generated species is recognized by the antibody prepared to the HMW membrane glycoproteins (D) and hence is presumed to result from proteolytic modification of the faster migrating HMW glycoprotein. HC and LC identify the heavy chains and light chains of the IgG preparation.

level of flagellar surface motility, as measured by the percentage of attached microspheres exhibiting rapid saltatory motility (Fig. 7). However, the same treatment, after a 2-h lag period, results in a drastic reduction in flagellar surface adhesiveness (as judged by adhesion of polystyrene microspheres) between 2 and 4 h after addition of pronase (Fig. 8). The cells recover their control level of flagellar adhesiveness within 2 h after removal from pronase (Fig. 8); the recovery from pronase is inhibited by 10 µg/ml cycloheximide (Fig. 9), a concentration previously shown to completely inhibit protein synthesis in this species and strain of Chlamydomonas (7). It should be stressed that the loss and recovery of motility-coupled adhesiveness are occurring on intact flagella whose lengths do not change during the course of the experiment (Fig. 8). Because every polystyrene microsphere that becomes associated with the flagellar surface exhibits periods of rapid saltatory movement, level of binding is taken as a measure of the level of motility-coupled receptors. Pronase treatment modifies the motility-coupled receptors without affecting the motor itself. Pronase treatment for 6 h has no effect on the swimming or phototactic behavior of wild type cells, even when new protein synthesis is inhibited by the addition of 10 µg/ml cycloheximide.

To determine if pronase is exercising its effect as a proteolytic enzyme, the loss of protein-bound radioactivity from the flagella was monitored. Cells were pulse-labeled for 1 h under conditions where the flagella remained intact but incorporated considerable radioactivity, preferentially into membrane components (4, 6). The specific activity of flagella isolated from control cells was compared with that of cells treated with pronase under conditions that severely reduced flagellar adhesiveness (4 h at 0.1 mg/ml) (Table I). Residual pronase was inactivated using phenylmethylsulfonyl fluoride (PMSF) before deflagellation and flagellar integrity during purification was monitored by phase contrast and TEM. Regardless of

Figure 7 Effect of pronase (0.1 mg/ml) treatment on flagellar surface motility. The open and closed circles represent data from two independent experiments.

Figure 8 Effect of pronase (0.1 mg/ml) treatment on flagellar surface adhesiveness (circles) and flagellar length (triangles). Filled circles and open triangles: pronase treated cells; open circles and closed triangles: control cells. Cells were removed from pronase at 4 h; recovery of flagellar adhesiveness was almost complete 2 h later.

Figure 9 Effect of cycloheximide on the recovery of flagellar adhesiveness after pronase treatment. Cells were treated with 0.1 mg/ml pronase for 4 h, at which time all cells were removed from pronase and placed in fresh medium alone (squares) or medium containing 10 µg/ml cycloheximide (circles). Inhibition of protein synthesis with cycloheximide prevented the recovery from pronase treatment.
whether total or TCA precipitable counts were measured, 60% of the radioactivity that was incorporated into the flagella during the pulse was lost to the medium through the action of pronase (Table I). The observations support the interpretation that pronase treatment is resulting in a proteolytic modification of the flagellar surface coat. The difference in counting efficiency between aqueous samples of whole flagella counted in Aquasol (*) and processed filter disks counted in Liquifluor ($).

The difference in the number of cpms between lines 2 and 3 reflects the difference in counting efficiency between aqueous samples of whole flagella counted in Aquasol (*) and processed filter disks counted in Liquifluor ($).

Does the pronase treatment that results in loss of flagellar adhesiveness (Fig. 8) and loss of protein bound label from the flagellum (Table I) also result in any alteration in the ultrastructure of the flagellar surface? TEM of control and pronase treated cells, fixed in the presence of 2% tannic acid in order to enhance the appearance of the flagellar surface coat, shows no significant difference in ultrastructure (Fig. 10a–d). The appearance of the flagellar membrane and the surface coat in both cross section and longitudinal section is essentially normal after 4 h of treatment with 0.1 mg/ml pronase. It is somewhat surprising that the distinct functional alteration of the flagellar surface resulting from pronase treatment is not accompanied by any obvious morphological correlate. This suggests that a good deal of the material comprising the flagellar surface coat may consist of nonprotein associated carbohydrate, probably in the form of glycolipid. Acrylamide gel electrophoresis of whole flagella, when stained for carbohydrate by the periodic acid-Schiff procedure, reveals a large band of carbohydrate staining that does not correlate with any Coomassie Blue staining material (6). This material is not seen on gels of surf.ace resulting from pronase treatment is not accompanied by any obvious morphological correlate. This suggests that a good deal of the material comprising the flagellar surface coat may consist of nonprotein associated carbohydrate, probably in the form of glycolipid. Acrylamide gel electrophoresis of whole flagella, when stained for carbohydrate by the periodic acid-Schiff procedure, reveals a large band of carbohydrate staining that does not correlate with any Coomassie Blue staining material (6). This material is not seen on gels of surf.ace resulting from pronase treatment is not accompanied by any obvious morphological correlate. This suggests that a good deal of the material comprising the flagellar surface coat may consist of nonprotein associated carbohydrate, probably in the form of glycolipid. Acrylamide gel electrophoresis of whole flagella, when stained for carbohydrate by the periodic acid-Schiff procedure, reveals a large band of carbohydrate staining that does not correlate with any Coomassie Blue staining material (6). This material is not seen on gels of surf.ace resulting from pronase treatment is not accompanied by any obvious morphological correlate. This suggests that a good deal of the material comprising the flagellar surface coat may consist of nonprotein associated carbohydrate, probably in the form of glycolipid. Acrylamide gel electrophoresis of whole flagella, when stained for carbohydrate by the periodic acid-Schiff procedure, reveals a large band of carbohydrate staining that does not correlate with any Coomassie Blue staining material (6). This material is not seen on gels of

| Table 1 |
| Loss of Label from Pronase-treated, Pulse-labeled Flagella |
| Flagellar adhesiveness (microspheres/flagellum) | Control | Pronase | Control |
| | 0.27 | 0.04 | 15 |
| Total flagellar label, cpm/mg protein | 47.79 x 10^6 | 19.34 x 10^6 | 40 |
| Flagellar TCA PPT label, cpm/mg protein | 35.46 x 10^6 | 13.46 x 10^6 | 38 |

The difference in the number of cpms between lines 2 and 3 reflects the difference in counting efficiency between aqueous samples of whole flagella counted in Aquasol (*) and processed filter disks counted in Liquifluor ($).
Figure 11 (a) Chlamydomonas reinhardtii strain pf-18 vegetative cells massively agglutinated in the presence of the antibody prepared against the major glycoprotein (G) of the flagellum. (b) Pretreatment of cells with pronase under conditions that result in loss of the motility-coupled receptors on the flagellar surface abolishes the ability of the antibody to agglutinate the cells.

The antibody preparation specific for the two major membrane glycoprotein bands fails to agglutinate pronase-treated cells (Fig. 11b), presumably because that portion of those components normally exposed at the flagellar surface and accessible to interaction with the antibody is removed during pronase treatment. Because the antibody preparation recognizes both glycoprotein components but the ability of this preparation to agglutinate flagellar surfaces is lost concomitant with modification of only the faster migrating of the two components, it is concluded that the slower migrating of the two HMW glycoprotein species is not exposed at the flagellar surface. During recovery of cells from pronase treatment, antibody agglutinability reappears suggesting that new, unmodified copies of the faster migrating of the two glycoprotein species are being synthesized and inserted into the flagellar membrane. This presumption is supported by the observation that pronase effects are not reversed in the absence of new protein synthesis (Fig. 9). The specific antibody agglutinability of the flagellar surface returns concomitant with recovery of the motility-coupled flagellar surface receptors (Fig. 8) suggesting that the faster migrating of the two closely migrating HMW glycoproteins may be functioning as the motility-coupled cell surface receptor.

DISCUSSION

The Chlamydomonas flagellar surface exhibits many dynamic properties (6). One such property has been visualized as the rapid, saltatory movements of exogenous markers (polystyrene microspheres) occurring on both vegetative and gametic flagellar surfaces (3, 7, 12). This system of force transduction occurring at the flagellar surface finds its physiological expression in the processes of flagella-dependent gliding motility (5, 16) and flagellar orientation during mating (6, 12, 16, 20). Since all polystyrene microspheres that become mechanically coupled to the flagellar surface exhibit periods of movement, we define those surface exposed components with which the markers associate as motility-coupled receptors. The process of flagellar surface adhesion (be it to a polystyrene microsphere, to another flagellar surface or to a solid planar substrate during gliding) appears to be a process distinct from the flagellar surface motility. The two phenomena can be independently quantitated (7) and can be individually altered (7, 12).

The present study makes use of proteolytic modification of the flagellar surface, along with an antibody specific for the HMW class of flagellar membrane glycoproteins to correlate changes in particular flagellar components with loss of the motility-coupled flagellar surface receptors. Two major lines of evidence resulting from this study point to one of the HMW flagellar membrane glycoproteins (the faster migrating of this closely migrating pair of components) as a reasonable candidate for the motility-coupled flagellar surface receptor; (a) this component is radically modified in apparent molecular weight concomitant with a loss of motility-coupled flagellar surface adhesiveness, and (b) the agglutination of cells by their flagellar surfaces in the presence of an antibody specific for this component is lost concomitant with loss of flagellar surface adhesiveness and reappears concomitant with the return of the motility-coupled flagellar adhesiveness. It is presumed that, during recovery from pronase treatment, the cell synthesizes new, unmodified copies of the HMW flagellar glycoproteins and inserts these into the surface of the intact flagellum. Turnover of proteins in the intact Chlamydomonas flagellum has been demonstrated and it was found that the flagellar membrane-associated proteins, especially the HMW glycoproteins, turn over much more rapidly than the axonomal components (4, 6, 30).

A third, but weaker, argument stems from a combination of the pronase observations along with the immunocytochemical localization studies. Based on the results shown in Fig. 5, the faster migrating of the two HMW flagellar glycoproteins is the only major flagellar membrane component exposed at the flagellar surface, although other, minor surface-exposed components can be revealed by iodination procedures and other, very minor flagellar components are affected by pronase treatment (Fig. 6). The immunocytochemical data indicate that this component is localized over the entire flagellar surface, a property necessary for a component associated with flagellar surface motility. It is questionable whether any other surface-exposed component is present in large enough amount to be associated with a property of the entire flagellar surface.

Hoffman and Goodenough (12) reported that another proteolytic enzyme, chymotrypsin, also affected the adhesiveness of the flagellar surface for polystyrene microspheres. In contrast to the present study, those authors did not provide (a) any data on the effects of chymotrypsin on surface motility, (b) any data on the ultrastructure of the enzyme-treated flagella, (c) any evidence that the enzyme was exhibiting proteolytic activity during exposure to the cells or that new protein synthesis was necessary for recovery, (d) any information on alterations of flagellar proteins induced by the enzyme treatment, or (e) provide any evidence for loss of surface-exposed antigenic sites from the flagellar surface during enzymatic treatment or their recovery after removal of the enzyme. The observations on the
effects of chymotrypsin reported by Hoffman and Goodenough (12) have been confirmed in this laboratory. Further, it has been observed by TEM that chymotrypsin treatment, as with pronase treatment, does not significantly alter the ultrastructural appearance of the flagellar surface coat under conditions where >90% of the control level of flagellar adhesiveness has been lost.

Pronase treatment has been reported to prevent mating-specific flagellar agglutination in C. reinhardtii (34). In the present work, it has been observed that an antibody preparation that reacts with the principal pronase-sensitive protein exposed at the vegetative flagellar surface inhibits mating-specific flagellar agglutination of gametic cells. This observation suggests that the HMW glycoprotein common to vegetative and gametic flagellar membranes may be involved in mating-specific agglutination, although it must be modified during gametogenesis in such a manner as to acquire gametic and mating-type specific properties. Goodenough and Adair (9) have reported that the sexual agglutinin of the C. reinhardtii gametic flagellar membrane has a similar mobility on SDS-acrylamide gels to that of the HMW glycoproteins of the vegetative flagellar membrane.

Ramanathan et al. (26) have shown that pronase treatment of Paramecium removes the fuzzy coat from the ciliary surface. At the same time, this treatment preferentially hydrolyzes the principal ciliary membrane protein, the Immobilization Antigen (250,000 mol wt). In the present work, it has been observed that pronase treatment has little effect on the ultrastructure of the fuzzy coat of the Chlamydomonas flagellar membrane (Fig. 11). However, this same treatment radically (but reversibly) alters the functional properties of the flagellar surface (Figs. 8, 9, and 11a) concomitant with modification of one of the principal HMW glycoproteins of the flagellar membrane. The insensitivity of the surface coat to pronase, a broad spectrum protease, suggests that much of the ultrastructural appearance of this layer is due to carbohydrate, possibly associated with lipid. This is supported by the observation that whole flagella and detergent extracts of flagella (but not axonemes) exhibit a prominent carbohydrate staining region on acrylamide gels which is not associated with any detectable protein staining.

Careful inspection of Fig. 6a, and b reveals certain minor alterations of the electrophoretic pattern of pronase-treated flagella in addition to the major alteration occurring in the HMW glycoprotein region. Therefore, the possibility cannot be totally excluded that a rather minor component of the electrophoretic pattern (not visible in Fig. 1b, a gel of the membrane vesicle preparation) is indeed the actual motility-coupled cell surface receptor and is being lost from and added back to the flagellar surface with kinetics similar to those observed for the major HMW component reported in this paper. Definitive assignment of flagellar surface-exposed proteins as being involved in dynamic flagellar surface properties awaits the isolation of mutant strains of Chlamydomonas defective in these membrane proteins. The ability of the antibody preparation described in this report to agglutinate cells by their flagellar surfaces (Figs. 3, and 4) is currently being exploited as a means of selecting for mutant strains defective in the principal surface-exposed glycoprotein of the Chlamydomonas flagellum.

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