The Chlamydomonas Cell Wall Degrading Enzyme, Lysin, Acts on Two Substrates within the Framework of the Wall

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Abstract. The Chlamydomonas cell wall is a multilayered, extracellular matrix containing 20–25 proteins and glycoproteins, many of which are highly enriched in hydroxyproline. 80–90% of the wall protein is located in a crystalline portion of the wall that is soluble in sarkosyl-urea solutions as well as in chaotrope solutions. Although the wall has no cellulose, it contains a noncrystalline, highly insoluble framework portion that is responsible for the integrity and overall shape of the wall. In the present report we show that the framework of the wall is composed of two components that are acted upon by lysin, a wall degrading enzyme released by mating gametes. One, which makes up the major portion of the framework, is insoluble upon boiling in SDS-PAGE sample buffer. Lysin treatment of this portion leads to its physical degradation and the concomitant appearance of several SDS-dithiothreitol-soluble polypeptides ranging in relative molecular mass from >400,000 to <60,000. The second component is the flagellar collar. This hollow cylinder composed of striated fibers aligned in parallel array serves as the tunnel in the wall through which the flagella protrude. Our evidence indicates that the primary collar polypeptide is a 225,000-Mr molecule that itself has at least two functional domains. One domain, contained in a 185,000-Mr fragment, permits the self-association of the molecules to form the main body of the collar. The second part of the molecule anchors the collar to the wall framework via sarkosyl-urea-insensitive, SDS-dithiothreitol-sensitive linkages.

The cell wall of the biflagellated alga, Chlamydomonas reinhardtii, is a complex, multilayered structure that contains 20–25 proteins and glycoproteins, many of which are highly enriched in hydroxyproline (6, 8, 11, 14, 22, 23, 25, 26–28, 30). Unlike the cell walls of higher plants, this wall has little if any cellulose (12, 22), but derives its form and shape from an insoluble framework (10, 14, 28). A substantial amount of information has accumulated about the ultrastructure and the biochemistry of this extracellular matrix. In the first ultrastructural study of the wall, Roberts et al. showed that it is a seven-layered structure, with the outer layers arranged in a crystalline pattern (28). The inner layer, or inner wall, appeared to be amorphous (28). More recently, Goodenough and Heuser have used rapid-freeze deep-etch methods to provide additional information about the architecture of the wall. They found that there are actually only five layers and that each layer is constructed from a distinct set of components (6, 8).

In conjunction with these ultrastructural methods, biochemical studies on the wall have indicated that a portion of the wall, which retains the overall shape of the wall, is insoluble in perchlorate, lithium chloride, and other chaotrope agents, as well as in sarkosyl-urea solutions (8–10, 14). This portion of the wall, which we have called the framework, and which is operationally defined by its solubility properties, corresponds to the inner wall or W-2 layer seen in the electron microscope (6, 8, 18, 20, 26, 28). The outer portions of the wall, which are solubilized by these solutions, are capable of self-assembly and form wall crystals when the salts are removed (8–10). If the insoluble portion of the wall is provided as a template, these peripheral molecules will reassemble onto it to form what appears to be an intact wall (1, 9, 10).

There is less information available about the framework, yet this structure appears to be responsible for the overall shape and integrity of the wall. Moreover, the framework must be covalently modified during the mating reaction in order for the entire wall to be released before fusion of mt+ and mt− gametes (reviewed in 7, 32). The cell wall degrading enzyme, lysin, whose release is signalled by flagellar adhesion, has been studied by several groups (1, 2, 13, 18–20, 29, 30, 33). There is evidence that it is a metalloprotease (18, 20) and our laboratory and others have shown that the framework of the wall is a major site of action of lysin (6, 14, 18). Detailed information about the framework molecules that are acted upon by lysin has been lacking. In studies on walls isolated from nonmating cells, Matsuda et al. (18) showed changes in the SDS-PAGE profile of frameworks. Working with intact and lysin-released and degraded walls, Monk et...
al. (24) also found lysin-dependent changes in the SDS-PAGE profile of the walls. In particular, both groups reported that a polypeptide of slightly \( \geq 200,000\ M_r \) disappeared after lysin treatment. Goodenough and Heuser found that lysin treatment of the salt-insoluble portion of walls led to the appearance of molecules shaped like fishbones (6).

One interpretation of these studies is that the polypeptides observed when frameworks were run on SDS gels were responsible for the integrity of the framework. Recently, however, we reported that boiling in SDS-PAGE sample buffer did not solubilize the frameworks of intact walls (14). Light microscopy showed that the frameworks were still visible. Thus, the polypeptides seen by SDS-PAGE of frameworks, although they may be associated with the framework and some degraded by lysin, are not essential for the basic integrity of the framework.

In the present report we show that there are two morphologically and biochemically distinct lysin substrates within the framework. One is the SDS-dithiothreitol (DTT)-insoluble portion of the framework. Lysin action on it leads to its becoming soluble in SDS-PAGE sample buffer and to its morphological disintegration. These changes are accompanied by the appearance on SDS-PAGE gels of several polypeptides, ranging in relative molecule mass from \( > 400,000 \) to \( < 60,000 \). The SDS-DTT-soluble portion of the framework also contains a lysin substrate. Our evidence indicates that this is the flagellar collar, the structure within the wall through which the flagella protrude. Lysin action on collars leads to their release from the wall concomitant with conversion of a 225,000-\( M_r \) polypeptide to a 185,000-\( M_r \) polypeptide that is the major polypeptide in isolated collars.

**Materials and Methods**

**Cultures**

Methods for maintaining stock cultures, cell growth in suspension, cell harvesting, and induction of gametes were essentially the same as reported previously (14, 30).

**Isolation of Walls from Mechanically Disrupted Gametes**

Walls were released from ethanol-extracted mit gametes by agitation with glass beads, and then harvested by differential centrifugation. The details of this method were described previously (14). Walls prepared by this method were resuspended in 10 mM Hepes, 1 mM Ca\(^{2+}\), pH 7.2 (HC buffer), at \( \sim 2 \) mg protein/ml with 0.02% sodium azide and stored at 4°C.

**Preparation of Frameworks**

Mechanically isolated walls were incubated for 1 h at 23°C in sarkosyl-urea extraction buffer that contained 0.7% sarkosyl, 6 M urea, 0.1 M KCl, 5 mM EDTA, 1 mM DTT, and 10 mM Tris, pH 7.2. The insoluble portion (frameworks) were harvested by centrifugation at 26,000 g for 30 min at 4°C in a RC-5B refrigerated centrifuge, with a rotor SA-600 (Sorvall Instruments, Norwalk, CT). The sample was washed at least three times in HC buffer and resuspended in the same buffer. The frameworks were stored at a concentration of \( \sim 0.2 \) mg protein/ml in HC buffer with 0.02% sodium azide at 4°C.

**Isolation of Shed Walls**

Walls shed into the medium by mating mit\(^+\) and mit\(^−\) gametes were prepared as described previously (14, 30).

### Preparation of Lysin

Crude lysin was prepared from the medium of mating gametes as previously described (14, 30). Lysin was partially purified using carboxymethyl cellulose ion exchange chromatography and stored at \( -20°C \) in HC buffer. Lysin obtained by this method contained \( \sim 20 \) µg protein/ml. For prolonged incubations of lysin with wall fractions, the incubation mix contained 0.02% sodium azide.

**SDS-PAGE**

Linear 4-12% acrylamide slab gels with a gradient of 1-8 M urea were prepared according to the Jarvik and Rosenbaum (15) modification of the Laemmli procedure (16). Samples were mixed with concentrated sample buffer to yield a final concentration of 2% SDS, 1 mM EDTA, 400 mM DTT, 10 mM Tris, pH 8.0, then heated in a boiling water bath for 3 min, loaded onto the gels, and subjected to electrophoresis overnight at 5-7 mA. Gels were fixed, treated with periodate (4), and stained with silver using the procedure of Merril et al. (21).

**Electron Microscopy**

Samples were negatively stained with 1% uranyl acetate as previously described (31) and examined in a Philips 301 electron microscope.

### Results

**Lysin Treatment of Mechanically Isolated Walls**

Mechanically isolated walls were prepared as previously described (14). Fig. 1 A shows that the walls were sac-shaped structures, \( \sim 5-10 \) µm long. Relatively few were completely intact, having been torn into large fragments by the isolation procedure. Fig. 1 B shows that the basic shape of the wall was retained even after boiling in SDS-PAGE sample buffer. If, however, these walls were treated with lysin first they were rendered soluble in SDS-PAGE sample buffer, as shown in Fig. 1 C.

Analysis by SDS-PAGE indicated that nonlysin treated, mechanically isolated walls (Fig. 2, \( - \)) contained 20-25 polypeptides, including three major polypeptides of \( M_r \) 300,000, 150,000, and 140,000 (corresponding to GP2, GP3A, and GP3B according to the nomenclature of Goodenough, et al. [6, 8]), which have been shown to be major constituents of the peripheral or crystalline region of the wall (6, 8, 14, 26). In addition a polypeptide of 225,000 \( M_r \) (indicated by the arrowhead in Fig. 2) was a noticeable constituent of these preparations. Analysis of lysin-treated walls by SDS-PAGE (Fig. 2, \( + \)) showed that most of the polypeptides were unaffected by the lysin treatment. The 225,000-\( M_r \) polypeptide was an exception, however, because it disappeared with lysin treatment.

**The 225,000-M r, Framework Polypeptide Has Solubility Properties Similar to Flagellar Collars**

Fig. 3 A shows a light micrograph of wall frameworks that lost ion exchange chromatography and stored at \(-20°C\) in HC buffer. Lysin obtained by this method contained \( \sim 20 \) µg protein/ml. For prolonged incubations of lysin with wall fractions, the incubation mix contained 0.02% sodium azide.

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**The 225,000-M r, Framework Polypeptide Has Solubility Properties Similar to Flagellar Collars**

Fig. 3 A shows a light micrograph of wall frameworks that resulted from the sarkosyl-urea extraction, which removed \( \sim 90\% \) of total wall protein. These structures were less phase-dense than intact walls, but otherwise were similar in appearance. Frameworks were also examined by electron microscopy. Fig. 3 B shows that the frameworks were relatively
intact and that flagellar collars were still present in most. Collars are the specialized portion of the wall through which the flagella protrude. Fig. 3 C shows a higher magnification view of the pair of collars circled in Fig. 3 B. The striated nature of these structures is apparent at this magnification.

The frameworks were also analyzed by SDS-PAGE. Fig. 4 A, lane 1 shows the intact walls, and lane 2 shows the frameworks. The major constituent of the SDS-DTT-soluble portion of the frameworks was the 225,000-Mr polypeptide. A weakly staining band could also be seen at 100,000 Mr, and some preparations were contaminated with small amounts of GP2 (see below). To investigate the relationship between the 225,000-Mr polypeptide and collars, frameworks that had been boiled in SDS-PAGE sample buffer for 3 min were centrifuged at 200,000 g (TL-100 table top ultracentrifuge, TLA 100.2 rotor, 75,000 rpm; Beckman Instruments, Inc., Palo Alto, CA) for 15 min at 4°C and examined in the electron microscope. Initial examination at low magnification (Fig. 4 B) suggested that the collars were still present, because the frameworks contained two collar-like structures. Most of these structures (Fig. 4 B, arrows) looked like empty holes, though, rather than typical collars. Examination at higher magnification revealed that what appeared to be a receptacle for the collars remained, but the striated structure itself was gone.

To learn more about the stability of collars, frameworks were incubated for 3 min in SDS-PAGE sample buffer at room temperature and then harvested as above. SDS-PAGE analysis (Fig. 5 A) showed that the 225,000-Mr polypeptide was not solubilized by this treatment. The supernatant (Fig. 5 A, left lane) contained some contaminating GP2 that was present in this particular preparation of frameworks, but none of the 225,000-Mr polypeptide was present. Rather, the 225,000-Mr species remained in the pellet (Fig. 5 A, right lane). And electron microscopy showed that the striated col-
Degradation of the Sarkosyl-Urea-insoluble Portion of Walls Was Accompanied by Significant Changes in Polypeptide Composition

To study the effects of lysin on frameworks, samples of the sarkosyl-urea-insoluble portion of walls were incubated with lysin for varying amounts of time up to 15 h and then examined in the electron microscope and analyzed by SDS-PAGE. The effects of lysin treatment on the morphology of the frameworks was evaluated by electron microscopy. Fig. 6 shows samples negatively stained after 0, 1, 2, 3, 5, and 15 h of lysin treatment. Two features of the samples were apparent. First, the collars (Fig. 6, arrowheads) remained visible throughout the course of the experiment. In the 15-h sample, many loose collars were observed, often in clumps of three to five collars. Second, there was a progressive diminution of the staining intensity of the entire framework. The frameworks appeared to become thinner and less well defined, but even at 15 h remnants were still observed.

The results of the SDS-PAGE (Fig. 7) indicated that lysin treatment had dramatic effects on the gel profile of the frameworks. Whereas the profile of the starting material was dominated by the 225,000-Mr polypeptide (Fig. 7, lane O'), with time it began to disappear at the same time that a new polypeptide of 185,000 Mr began to appear. By 15 h there was little if any of the 225,000-Mr species remaining. (The contaminating GP2 peripheral wall protein showed some variability and also was present in the lysin preparation used for these experiments [Fig. 7, lane L]. The significance of this...
Figure 4. Boiling in SDS-PAGE sample buffer solubilized the 225,000-M_r polypeptide and collars but not the remainder of the framework. (A) SDS-PAGE analysis (4-12% gels) of intact walls (lane 1) and frameworks (lane 2). The 225,000-M_r polypeptide is indicated on the right. Frameworks were boiled in SDS-PAGE sample buffer for 3 min, harvested by centrifugation, resuspended in a volume of HC buffer equal to the original sample volume and examined by electron microscopy. (B) Low magnification view of boiled frameworks. Collarlike structures that appeared to be empty (arrows) were still present. (C) Higher magnification view of the boiled frameworks showed that the striated collars were no longer present. Bars: (B) 1 μm; (C) 0.25 μm.

Figure 5. The relationship between collars and the 225,000-M_r polypeptide. Frameworks were incubated at room temperature for 3 min in SDS-PAGE sample buffer, centrifuged at 200,000 g for 15 min and the pellet was resuspended in a volume of HC buffer equal to the original sample volume. (A) Both samples were then boiled in SDS-PAGE sample buffer and analyzed by SDS-PAGE on 4-12% gels. The lane on the left is the supernatant and the lane on the right is the pellet. The 225,000-M_r polypeptide was not solubilized by incubation in SDS-PAGE sample buffer at room temperature. (B) Electron micrograph of the frameworks incubated in SDS-PAGE sample buffer at room temperature. The striated collars were present in the frameworks. Bar, 0.25 μm.
Figure 6. The effects of lysin on the morphology of frameworks. Frameworks incubated with partially purified lysin for 0, 1, 2, 3, 5, and 15 h were negatively stained and examined by electron microscopy. Collars (arrowheads) remained visible throughout the incubation, although the rest of the framework became progressively less distinct. Bar, 1 µm.

Figure 7. SDS-PAGE analysis of frameworks incubated with lysin. Frameworks (15 µl in HC buffer) were incubated at room temperature with partially purified lysin (2.5 µl in HC buffer) for 0', 15', 1, 2, 3, 5, and 15 h. EDTA (2.5 µl) was added and the samples were boiled in sample buffer and analyzed by SDS-PAGE on 4-16% gels. Lane L is the lysin used for the experiment. Relative molecular mass markers are shown at the left. The 225,000- and 185,000-M_r polypeptides are indicated on the right. The unlabeled arrows on the right indicate polypeptides that appeared after lysin treatment.
variability is unclear.) Several other new polypeptides appeared including molecules of 195,000, 160,000, 130,000, 120,000, 100,000, and 80,000 Mr (Fig. 7, arrows). In addition to these changes in the lower relative molecular mass portion of the gel, there were also dramatic changes in material in the stacking gel and at the stacking gel-running gel interface. In the starting sample there was a small amount of material at the interface (Fig. 7, lane 0'). But with time this material became much more prominent and in the 15-h sample (Fig. 7, lane 15h) it extended from the top of the stacker into the upper portion of the running gel.

Comparison of the starting sample (Fig. 7, lane 0') with the 15-h sample (lane 15h) indicated that the lysin treatment was associated with the appearance of much more protein in the gel than could be accounted for by degradation of any of the polypeptides apparent in the gel of the starting sample. Therefore, most of the polypeptides probably were derived from the SDS-DTT-insoluble portion of the framework. On the other hand, the increase in the 185,000-Mr polypeptide with the concomitant decrease in the 225,000-Mr species was consistent with the idea that the 185,000-Mr molecule was a degradation product of the 225,000-Mr molecule.

Effects of Lysin on the SDS-DTT-insoluble Portion of Frameworks

To determine which of the polypeptides that appeared during lysin degradation were derived from the SDS-DTT-insoluble portion of the frameworks, frameworks were boiled for 3 min in SDS-PAGE sample buffer, harvested by centrifugation, and washed several times in HC buffer. These extracted frameworks were then treated with lysin for 2, 6, and 16 h (Fig. 8, lanes 2, 6, and 16 h). A control sample (Fig. lane 16C) was incubated for 16 h with 5 mM EDTA, an inhibitor of lysin. Fig. 8, lane L shows the lysin used in the experiment. As was observed with the intact frameworks, there was a large amount of very high relative molecular mass material that was produced upon lysin treatment. In addition, several lower molecular mass polypeptides appeared that were not present in the starting material. Included among these were molecules similar to those produced by lysin treatment of intact frameworks of ~150,000, 120,000, 100,000, and 60,000 Mr. Interestingly, all of these polypeptides, as well as the material in the stacking gel, were most prominent in the 2 and 6 h samples and by 16 h it was apparent that degradation was almost complete. We found that rate of degradation varied somewhat from experiment to experiment depending on the particular sample of frameworks and lysin that was used. In the experiment shown here, even though the rate of degradation was more rapid than with the nonlabeled frameworks, the pattern of new polypeptides that appeared after lysin treatment was similar to that obtained with intact frameworks (Fig. 7).

Of particular interest was the result that the 185,000-Mr species did not appear. This result was consistent with the notion that it was derived from degradation of the 225,000-Mr molecule, which we tentatively identified (above) as the primary polypeptide in flagellar collars. In this regard, wall molecules of this relative molecular mass had been observed in our previous experiments with walls shed into the medium by mating gametes (14). As we reported, shed wall frameworks had variable amounts of the 185,000- and 225,000-Mr species. In addition, in separate studies we had found that collars were shed by gametes early in the mating reaction and were observed to slip off of the flagella of the mating cells at the same time that the remainder of the wall was being shed (34). With this in mind we harvested wall preparations from the medium of mating gametes to determine if they contained free collars.

Figure 8. SDS-PAGE analysis of the effects of lysin on the sarkosyl-urea-insoluble portion of mechanically isolated walls that was also insoluble after boiling in SDS-DTT. 700 µl (~1.5 mg protein/ml) of the sarkosyl-urea-insoluble portion of mechanically isolated walls were mixed with a concentrated stock solution of SDS-PAGE sample buffer to give a final concentration of 2% SDS and 400 mM DTT, and boiled for 3 min. The sample was then centrifuged at 200,000 g for 15 min at 4°C in a TL-100 centrifuge (Beckman Instruments, Inc.) and the resulting pellet was washed three times in HC buffer. The final pellet was resuspended in 150 µl of HC buffer. 25 µl portions of this preparation were mixed with 3 µl of lysin and incubated for 16 (L), 6 (6), and 2 h (2). A control sample was incubated for 16 h with HC buffer alone (16C), and lysin (3 µl) to which 25 µl of HC buffer was added also was incubated for 16 h (L). At the end of the incubation the samples were analyzed by SDS-PAGE on 4-12% gradient gels. The asterisk on the top left indicates very high relative molecular mass material that appeared after lysin treatment and the arrows indicate lower relative molecular mass polypeptides that also appeared during the lysin treatment. The arrowhead on the left shows where the 185,000-Mr polypeptide ran in these gels.
Isolation of Flagellar Collars from the Medium of Mating Gametes

mt+ and mt− gametes were mixed together for 3 min during which time they specifically adhered to each other via their flagella, released their cell walls due to the action of lysin, and began to fuse to form zygotes. Further wall degradation was inhibited by the addition of EDTA (14, 29, 30, 32) and the cells were immediately harvested from the medium by centrifugation. The remaining supernatant was centrifuged again at higher speed to harvest wall constituents. Examination of this sample by electron microscopy (Fig. 9) showed that during wall shedding the collars had been released from the walls and were observed in small collections on the grid (arrowheads). These results made it possible to separate the collars from the remaining part of the walls.

To purify collars, the sample was extracted with sarkosyl-urea, and the sedimented material was centrifuged in 2.8 M CsCl at 200,000 g (TL-100 ultracentrifuge, rotor No. TLS-55; Beckman Instruments, Inc., Palo Alto, CA) for 17 h at 4°C as previously described (31). The collars, which formed a band near the bottom of the gradient, were collected and examined by electron microscopy. The only cellular structures visible were the square-shaped collars (Fig. 10 A). Some collars had one side folded back, reflecting the fact that the collars are indeed hollow cylinders. The sample was also analyzed by SDS-PAGE. Fig. 10 B shows that the major polypeptide present was one of 185,000 M\(_r\), with a small amount of material near the top of the gel. In experiments not shown we found that these collars were similar to the collars present in the lysin treated frameworks shown in Fig. 6 in that they were unaffected by further (overnight) incubation with lysin or by incubation in SDS-PAGE sample buffer at room temperature; but, boiling in this buffer did render them soluble. These results were consistent with the idea presented above that the 185,000-M\(_r\) polypeptide that appeared during lysin treatment of frameworks was a primary constituent of collars.

Discussion

Chlamydomonas Wall Contains Several Biochemically and Ultrastructurally Unique Domains

The results presented here are consistent with the following model for the structure of the Chlamydomonas cell wall. The wall is comprised of three biochemically distinct regions: (a) a wall-shaped framework that is insoluble both in sarkosyl-urea solutions and after boiling in SDS-DTT solutions; (b) flagellar collars, which are insoluble in sarkosyl-urea, and that are linked to the framework via bonds that are sensitive to boiling in SDS-DTT solutions; and (c) a peripheral region that is loosely associated with the framework via bonds that can be disrupted by chaotropic salts or sarkosyl-urea solutions and that make up the crystalline portion of the wall. Our evidence, which is consistent with results of Matsuda et al. (18), indicates that only the two domains that are insoluble in sarkosyl-urea are acted upon by the wall-degrading enzyme, lysin. SDS-PAGE analysis showed no detectable, lysin-dependent changes in any of the peripheral wall polypeptides (Fig. 2).
The domain of the wall that appears to be responsible for its overall shape and integrity is the highly insoluble framework, which is resistant to boiling in SDS-DTT solutions. Even after this harsh treatment structures with the basic shape of the wall remained visible by light microscopy. In these properties it is similar to the peptidoglycan component of the bacterial cell wall (5).

Treatment of frameworks with the wall-degrading enzyme, lysin, lead to their disintegration and was accompanied by the appearance of SDS-DTT-soluble polypeptides. The most prominent of these breakdown products was a very high molecular mass material that remained in the stacking gel or just entered the running gel. In addition several other polypeptides ranging from 195,000 to 80,000 Mr, appeared as a consequence of lysin treatment. These lysin-dependent changes in the integral part of the wall were obvious only after the other wall proteins were removed because this framework portion represented such a small fraction of the total wall protein.

SDS-DTT-sensitive Linkages among Framework Molecules Are Sufficient to Maintain Framework Integrity

Although the framework of the wall is responsible for the integrity of the wall and contains lysin-sensitive sites, wall structure can be maintained after some of these lysin-sensitive sites have been cleaved. Thus, shed walls or mechanically isolated walls that have been briefly treated with lysin have the normal wall appearance by phase-contrast microscopy (14); but, they are no longer stable to boiling in SDS-DTT. It is framework molecules that are still responsible for the shape of the shed walls, however, because they remain insoluble in sarkosyl-urea (14). Therefore, there are sarkosyl-urea resistant, SDS-DTT-sensitive linkages in the framework of the wall that contribute to wall structure. Possibly during wall assembly these bonds serve as temporary linkages until covalent cross-links are formed.

Primary Polypeptide of the Flagellar Collar Is a 225,000-Mr Polypeptide that Is Cleaved by Lysin to a 185,000-Mr Species

The data are consistent with the idea that the flagellar collars in intact walls are composed of a 225,000-Mr polypeptide and can be summarized as follows. (a) The only polypeptide solubilized from intact wall frameworks by boiling in SDS-PAGE sample buffer was the 225,000-Mr polypeptide; SDS-PAGE sample buffer at room temperature was insufficient for solubilization of the 225,000-Mr species and both of these properties were also characteristic of isolated collars and collars present in intact frameworks. (b) The collars that remained after extensive lysin treatment of frameworks or that were shed into the medium during mating contained the 185,000-Mr polypeptide. (c) This polypeptide was generated during lysin treatment of frameworks that contained collars, but not during lysin treatment of frameworks whose collars had been removed by boiling in SDS-PAGE sample buffer. (d) During lysin treatment of frameworks, as the 225,000-Mr polypeptide disappeared, the 185,000-Mr species showed a concomitant increase.

In the course of these experiments Goodenough and Heuser reported in abstract form that flagellar collars were one of the few structures visible after extensive lysin treatment of Chlamydomonas cell walls (6a). They also found that the sedimentable material remaining after this extensive lysin treatment was composed of a polypeptide of ~185,000-Mr, and that a molecule of ~225,000-Mr, had disappeared from the gel.

According to the ideas presented here, the 225,000-Mr polypeptide must have at least two functional domains. One of these domains, contained in the 185,000-Mr polypeptide
is capable of self-association to form the striated portion of the collar. The other domain, contained in the remaining 40,000-Mr portion of the molecule, links the collar to the framework of the wall. Lysin cleaves this smaller portion of the polypeptide, releasing the collar from the framework. This lower relative molecular mass domain may be cleaved into even smaller fragments by lysin, because we did not routinely observe a peptide of this size in lysin-treated frameworks.

**Relevance to Wall Biogenesis**

The results presented here lead to some predictions about wall biogenesis. The fact that several polypeptides of a variety of relative molecular masses are generated from the SDS-DTT-insoluble framework of the wall suggests that it was originally assembled by the cross-linking of polypeptides synthesized and secreted from the cell. Of course it is unlikely that the polypeptides generated by lysin treatment are the same as those originally assembled into the framework. This would be true only if the original cross-linking sites were identical to the lysin cleavage sites and there is no reason to postulate this. Since we and others (14, 27) have shown that wall frameworks also contain a substantial amount of carbohydrate, the data do not permit us to predict if the polypeptides were cross-linked via their amino acids or via carbohydrate linkages. In this regard Voigt (34) reported that the perchorlate-insoluble portion of the wall was sensitive to α-amylase prepared from Bacillus licheniformis. We recently found, however, that the wall-degrading activity in such amylose preparations could be accounted for by contaminating proteases (13).

Consideration of the structure of the wall raises the question as to the role played by the different components. The most likely explanation for the collar is that it permits flagellar function in this walled cell. On the other hand it is not clear why the major portion of the wall should have a relatively soluble portion (the crystalline or peripheral region) and a highly insoluble portion (the framework). Because it has the properties of a highly cross-linked, macroscopic polymer the framework could serve as the key structural element for the wall. The crystalline portion may serve as a permeability barrier as well as a barrier to pathogens, especially since it is the outermost layer of the wall. Alternatively, or in addition, because it is one of the first portions of the wall to form during wall biogenesis, it may serve, as Goodenough et al. have suggested, as a delicate "mold" within which the polymer the framework could serve as the key structural element for the wall.

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