Purification and Biosynthesis of a Derepressible Periplasmic Arylsulfatase from Chlamydomonas reinhardtii

Eugenio L. de Hostos,* Robert K. Togasaki,‡ and Arthur Grossman§

*Department of Biological Sciences, Stanford University, Stanford, California 94305; ‡Department of Biology, Indiana University, Bloomington, Indiana 47401; and §Department of Plant Biology, Carnegie Institution of Washington, Stanford, California 94305

Abstract. The unicellular green alga Chlamydomonas reinhardtii responds to sulfate deprivation by producing an arylsulfatase (Lien, T., and O. Schreiner. 1975. Biochim. Biophys. Acta. 384:168-179; Schreiner, O., 1975. Biochim. Biophys. Acta. 384:180-193) and by developing the capacity to transport sulfate more rapidly (our unpublished data). The arylsulfatase activity, detectable 3 h after the transfer of the cells to low sulfate medium (<10 μM sulfate), is a periplasmic protein released into the culture medium by cw15, a cell wall–less mutant of C. reinhardtii. We have purified the derepressible arylsulfatase to homogeneity and have raised monospecific antibodies to it. The protein monomer (67.6 kD) associates into a dimer, and the enzyme activity shows an alkaline pH optimum and a Km of 0.3 mM for p-nitrophenylsulfate. Studies focused on arylsulfatase biosynthesis demonstrate that it is glycosylated and synthesized as a higher molecular mass precursor. The mature protein contains complex N-linked oligosaccharides and the primary translation product has an apparent molecular mass ~5 kD larger than the deglycosylated monomer. Since translatable RNA encoding the arylsulfatase can only be detected in cells after sulfate starvation, it is likely that accumulation of the enzyme is regulated at the level of transcription, although posttranscriptional processes may also be involved.

CHLAMYDOMONAS reinhardtii is a unicellular, terrestrial, green alga widely used as a model system for examining physiological and biochemical processes in photosynthetic organisms (Levine, 1969; Rochaix et al., 1983). Like many algae, C. reinhardtii has the capacity to acclimate to various types of nutritional stress. It responds to low CO2 by producing a periplasmic carbonic anhydrase and by elevating the level of inorganic carbon transport (Tsuzuki, 1983; Kimpel et al., 1983; Spalding et al., 1983; Moroney and Tolbert, 1985; Moroney et al., 1987; Coleman and Grossman, 1984; Coleman et al., 1984). In response to phosphate deprivation, C. reinhardtii, like the yeast Saccharomyces cerevisiae (Lemire et al., 1985; Bostian et al., 1983), secretes periplasmic phosphatases (Loppins and Magtang, 1973; Lien and Knutsen, 1973) that may be important for scavenging phosphate from the environment. Under conditions of sulfate deprivation, C. reinhardtii, stops dividing, enlarges, accumulates starch (R. Togasaki, unpublished data), produces an arylsulfatase (Schreiner et al., 1975; Lien and Schreiner, 1975), and develops the capacity to transport sulfate more rapidly (our unpublished data).

Arylsulfatase (EC 3.1.6.1) catalyzes the hydrolysis as follows: 

\[ \text{H}_2\text{O} + \text{Aryl-O-SO}_3^- \rightarrow \text{Aryl-OH} + \text{SO}_4^{2-} + \text{H}^+ \]

Over 40% of the sulfur in soils can be in the form of organic esters and half of these may be arylsulfates (Fitzgerald, 1978). Therefore, arylsulfatases are essential for the mineralization of sulfate by hydrolyzing sulfate esters. The sulfate released is readily available to higher plants and soil microorganisms (Speir and Ross, 1978).

The production of arylsulfatases in response to sulfur deprivation has been described in the eukaryotic organisms Neurospora crassa and Aspergillus nidulans and several different bacteria, including cyanobacteria (for review see Dodgson et al., 1982). In A. nidulans there is a 50-fold increase in arylsulfatase activity during sulfur deprivation (Siddiqui et al., 1966; Apte et al., 1974). On the other hand, in N. crassa, developmental control can supersede physiological control. A 30-fold increase in arylsulfatase activity is observed in germinating conidia irrespective of sulfur availability (Scott and Metzenberg, 1970). We have identified the derepressible arylsulfatase of C. reinhardtii as a periplasmic component, purified it to homogeneity, and elucidated some aspects of its biosynthesis.

Materials and Methods

Strains and Growth Conditions

C. reinhardtii strains 2137, mt+, and cw15, mt+ were grown in Tris, acetate, phosphate (TAP) medium (Gorman and Levine, 1966). In TAP lacking sul-
Assay for Arylsulfatase Activity

The standard assay for arylsulfatase activity was performed essentially as described by Lien and Schreiner (1975): 50 μl of sample was added to 500 μl of 0.1 M glycine-NaOH, pH 9.0, 10 mM imidazole, 4.5 mM p-nitrophenylsulfate (Sigma Chemical Co., St. Louis, MO) and incubated for 10 min at 27°C. The reaction was stopped by the addition of 2 ml of 0.25 M NaOH and the absorbance at 410 nm was measured. Enzymatic activity was derived from a standard absorbance curve of p-nitrophenol (Sigma Chemical Co.) in 0.2 M NaOH. This assay was performed on whole cultures, culture supernatants, and protein fractions. When whole cultures were used the cells were sedimented before measuring the absorbance at 410 nm.

Purification of Arylsulfatase

Log-phase cultures of cw5 (between 2 and 5 × 10^6 cells/ml) were harvested by centrifugation (2000 g, 2 min), washed once, resuspended in TAP-S, and divided into 20-ml subcultures. At regular intervals ^35SO_4^- (New England Nuclear, Boston, MA), neutralized from the carrier-free acid with 1 M Tris-HCl, pH 7.5, was added to individual subcultures to a final concentration of 10 μCi/ml. After 2 h the cells were pelleted by centrifugation (3000 g, 5 min) and the supernatants filtered through glass fiber filters (GF/B; Whatman, Maidstone, England) and mixed with 10 g ammonium sulfate to obtain 70% saturation. After precipitation for 2–5 h at 4°C the suspensions were centrifuged for 10 min at 9500 g. The pellets were redissolved in 1 ml 30 mM Tris-acetate, pH 7.5 (TA) and reprecipitated by the addition of 100% w/v TCA to 10% final. In all experiments protein pellets were prepared and resolved by SDS-PAGE as described previously (Coleman and Grossman, 1984). SDS-PAGE was carried out through 7.5–15% polyacrylamide gradient gels in the buffer system of Laemmli (1970) with low range molecular mass markers (Bio-Rad Laboratories, Richmond, CA) as standards, and were stained with Coomassie Brilliant Blue G-250 (J. T. Baker Chemical Co., Phillipsburg, NJ). The amount of protein loaded per lane varied widely, depending on the complexity of the sample or the specific activity of the labeled proteins, but was generally in the range of 5–30 μg.

Effect of Tunicamycin on Arylsulfatase Synthesis

cw5 cells were grown on TAP to log phase, transferred to TAP-S containing 0, 2, or 4 μg/ml tunicamycin (500 μg/ml stock in methanol; Sigma Chemical Co.), and incubated under standard conditions for 12 h. After the incubation the cultures were made 0.1 M NaCl and gently agitated for 10 min. The cells were pelleted by centrifugation for 5 min at 3,000 g, the supernatants decanted, and pellets sonicated at low power in 20% of the original volume of TAP-S containing 1 mM bezamidine-HCl (Sigma Chemical Co.). The original cultures, sonicated pellets, and supernatants were assayed for arylsulfatase activity. Supernatant proteins were precipitated with ammonium sulfate and prepared for SDS-PAGE as described above.

Immuno precipitation of In Vitro Translated Products

Total RNA was extracted from log-phase cells and poly(A)^+ RNA purified by chromatography over PolyU-Sepharose (Pharmacia) using the buffer system of Chomczynski et al. (1978). The RNA was translated in vitro using a reticulocyte lysate system and ^35S)methionine (Amersham Corp., Arlington Heights, IL) as described by the manufacturer. Immuno precipitation of the primary translation products was carried out as described by Schmidt et al. (1979) using protein A-Sepharose (Pharmacia) instead of formalin-
fixed *Staphylococcus aureus* membranes, and the samples were resolved by SDS-PAGE. Fluorograms were prepared using Amplify (Amersham Corp.) as described by the manufacturer.

**Results and Discussion**

**Location of the Arylsulfatase**

The periplasmic location of the arylsulfatase from *C. reinhardtii* was suggested by experiments in which the sediment-ability of the enzyme activity in cultures of wild-type and *cw15* cells was compared. When wild-type sulfate-starved cells are pelleted by centrifugation, the arylsulfatase activity sediments with the cells. However, over 90% of the activity can be recovered in the culture medium of the cell wall-less mutant after sulfate deprivation. Maximal recovery of aryl-sulfatase activity is achieved by making the culture medium 0.1 M NaCl before centrifugation. These results indicate that most of the enzyme is normally periplasmic and may be loosely associated with the plasma membrane or cell wall. In addition, the arylsulfatase activity can be released from wild-type cells by treatment with autolysin (Togasaki, R., unpublished data), the cell wall-degrading enzyme produced by mating gametes of *Chlamydomonas* (Tamaki and Matsuda, 1981). By these criteria the arylsulfatase can be classified as a periplasmic enzyme.

*C. reinhardtii* is known to respond to other types of stress by producing specific periplasmic enzymes. While an aryl-sulfatase is produced by cultures deprived of sulfate, carbonic anhydrase is secreted by cells if cultures are maintained on air levels of CO₂ (Coleman and Grossman, 1983, 1984; Coleman et al., 1984). High levels of CO₂ (e.g., 3% in air) repress the production of the carbonic anhydrase. At least one periplasmic phosphatase is produced when the cells are deprived of phosphate (Lien and Knutsen, 1973; Loppes and Deltour, 1981; Loppes and Matagne, 1973; Ogawa, J., J. Nash, and A. Grossman, unpublished data). Although the phosphatase, the carbonic anhydrase, and the arylsulfatase are all released into the medium by *cw15*, they are released at different rates by wild-type cells during treatment with autolysin (Togasaki, R., unpublished data). These results suggest that proteins exterior to the cell membrane can assume different locations within the cell wall, a complex structure composed of several different layers and containing 70% protein (Goodenough and Heuser, 1985).
While the great majority of the *C. reinhardtii* arylsulfatase is periplasmic or extracellular, the derepressible arylsulfatases produced by *Neurospora* and *Aspergillus* have complex distributions within the fungal tissue (Doddson et al., 1982). In *N. crassa* there are both intracellular and extracellular arylsulfatase activities (Scott and Metzenberg, 1970). The extracellular activities are associated with the cell wall and plasma membrane, while the internal activities are both soluble and particulate.

**Conditions and Time Course of Arylsulfatase Synthesis**

To determine the threshold concentration of sulfate required for the derepression of arylsulfatase synthesis, *cwl5* cells were washed in TAP-S and transferred to medium containing various concentrations of sulfate. The cells were incubated under standard conditions and assayed for activity at regular intervals. Activity is first detected after 3 h in medium containing up to 10 μM sulfate. During the period shown the cell population increases less than twofold. Arylsulfatase activity begins to increase 3 h after the removal of sulfate. An increased lag phase in the appearance of the hydrolytic activity is observed at concentrations of sulfate above 10 μM (not shown), suggesting that depletion of the exogenous sulfate must occur before the initiation of arylsulfatase synthesis. The activity is very stable and is not rapidly lost upon the addition of sulfate to sulfate-depleted cultures.

Fig. 1, left, shows a time course for the appearance of arylsulfatase activity in medium with no added sulfate. During the period shown the cell population increases less than twofold. Arylsulfatase activity begins to increase 3 h after the removal of sulfate. An increased lag phase in the appearance of the hydrolytic activity is observed at concentrations of sulfate above 10 μM (not shown), suggesting that depletion of the exogenous sulfate must occur before the initiation of arylsulfatase synthesis. The activity is very stable and is not rapidly lost upon the addition of sulfate to sulfate-depleted cultures.

Fig. 1, right, shows the stained profile of periplasmic proteins before the removal of sulfate (lane 1) and 24 h after the removal of sulfate (lane 8), and the autoradiographic pattern of labeled periplasmic proteins at various times after the removal of sulfate (lanes 2–7). Previous studies (Coleman and Grossman, 1984; Coleman et al., 1984) had shown that 35SO4− could be used effectively to label proteins in vivo in *Chlamydomonas*, whereas labeled amino acids were not effectively taken up by the cells. In these experiments the concentration of labeled sulfate to which the cells were exposed was far below the threshold level that would repress arylsulfatase accumulation. The culture supernatant before the removal of sulfate shows several proteins of high molecular mass (>100 kD) that probably correspond to cell wall components (Iman et al., 1985). There are also several relatively minor bands in the range of 45-100 kD and a very prominent polypeptide (band H) migrating just below the 45-kD molecular mass marker. The autoradiographic pattern observed during the first half hour of labeling (lane 2) in media lacking sulfate is essentially identical (with the exception of band G, which may lack sulfur amino acids, see below) to the pattern observed after labeling sulfate-sufficient cells with [14C]acetate (not shown). The supernatant of starved cultures (lane 8), however, shows a characteristic pattern of seven major bands (A–G) in the range of 45–100 kD. Changes in protein synthesis did not occur immediately after the cells were deprived of sulfate but the synthesis of most of the major bands became evident after 4–6 h of sulfate deprivation, as seen in lane 4 (4–6 h labeling). Other less prominent species with molecular masses >100,000 are also apparent.

Comparison of the stained bands and the in vivo labeled bands indicated changes in the levels of synthesis and/or degradation of various supernatant components during sulfate deprivation. The incorporation of label into band H, the prominent 44-kD polypeptide, for example, continued for several hours after the removal of sulfate (lanes 2 and 3), but decreased dramatically by later time points (e.g., lane 7). Labeling of bands A, B, C, and G (apparent as stainable species in the medium after 24 h of sulfate starvation; G is also present in the medium from sulfate-sufficient cells) during the time course of induction was not clearly detected using 35SO4− as the source of label. If protein was labeled with [14C]acetate during conditions of sulfate deprivation, these polypeptides, especially polypeptides B and C, became heavily labeled (data not shown), indicating that they are deficient in sulfur amino acids. Augmented synthesis of some polypeptides (e.g., F) may occur in response to sulfate deprivation, although without additional data (e.g., immunological) it is impossible to determine whether similarly migrating species from sulfate-sufficient and deficient cultures are identical.

The major extracellular polypeptide in the medium from sulfate-starved cells, band D, has an *M*<sub>r</sub> of 67.6 kD. It is synthesized and accumulates only under starvation conditions and has been shown to be the derepressible arylsulfatase of *C. reinhardtii* (see below).

**Purification**

The derepressible arylsulfatase of *C. reinhardtii* was purified to determine its polypeptide composition, to study its kinetic properties, and to raise antibodies to the homogeneous protein. The capacity for arylsulfatase synthesis by *C. reinhardtii* was first noted by Schreiner et al. (1975) and Lien and Schreiner (1975), who described a purification of the enzyme from a complex mixture of proteins in acetone extracts of sulfate-starved wild-type cells. However, no SDS-PAGE system was used to evaluate the purity of their preparations. Our basic strategy for isolating the enzyme was similar to the strategy followed by Coleman and co-workers (Coleman and Grossman, 1984; Coleman et al., 1984) in the identification of the periplasmic carbonic anhydrase that is released into the culture medium by *cwl5* cells grown under low levels of CO₂.

Initial concentration and fractionation of the protein in the culture medium from sulfate-starved *cwl5* cells was achieved by precipitation with ammonium sulfate (Fig. 2 and Table I). Comparison of Fig. 2, lanes 2 and 3, shows that ammonium sulfate fractionation (40–70% saturation cut) resulted in a small enrichment of the major supernatant components (bands A–G) with respect to total protein. Losses of arylsulfatase activity in the discarded 0–40% ammonium sulfate fraction were variable and generally ranged from 20–40%. This initial precipitation, however, eliminated low molecular mass polypeptides, and polysaccharides and cell wall debris that were secreted into the medium by *cwl5* and which could have been problematic to subsequent steps of the purification. Passage of the 40–70% ammonium sulfate fraction over blue agarose, followed by a stepwise elution with NaCl, achieved a sixfold purification (see Table I for specific activities). Lanes 4 and 5 of Fig. 2 (column flow-through) show that most of the periplasmic proteins do not bind to the gel
Table 1. Purification of Arylsulfatase from the Extracellular Medium of cw15

| Sample                        | Total activity | Specific activity |
|-------------------------------|----------------|------------------|
| % U/mg                        |                |                  |
| Ammonium sulfate fractionation |                |                  |
| 0-70%, Cells with sulfate     | 0              | 0                |
| 0-70%, Cells without sulfate  | 100            | 11               |
| 40-70%, Cells without sulfate | 57             | 12               |
| Blue agarose chromatography   |                |                  |
| Flow-through                  | 0.4            | 1                |
| Wash with TA                  | 1              | 2                |
| Wash with 0.1 M NaCl          | 0.08           | 2                |
| Elution with 0.2 M NaCl       | 55             | 72               |

Upon overloading samples eluted from the blue agarose column that exhibit arylsulfatase activity onto polyacrylamide gels, a minor polypeptide contaminant was sometimes observed. However, active enzyme eluted from the blue agarose column could be purified to homogeneity by gel filtration. The enzyme chromatographed through Sephacryl S-200 or S-300 as a single broad peak with a mobility corresponding to a dimer of 137 kD (data not shown). Lien and Schreiner (1975) have reported a size of 150 kD for the *C. reinhardtii* arylsulfatase. After gel filtration only a single polypeptide species, co-migrating with band D, was observed after denaturing PAGE (Fig. 2, lane 9).

Blue agarose has been used previously in the separation of mammalian arylsulfatases (Ahmad et al., 1977). The interaction of the enzymes and the dye ligand Cibachrom F3GA is not well characterized. The possibility that some arylsulfatases may bind arylsulfonates (Aril-SO3−) on the dye ligand has not been substantiated, and Ahmad and co-workers (1977) have concluded that the affinity of one of the mammalian arylsulfatases is due to nonspecific ionic interactions in which the dye ligand serves as a complex ion exchanger. Since the *C. reinhardtii* arylsulfatase remained active and bound to the matrix in the presence of the substrate p-nitrophenylsulfate, the substrate analog p-phenolsulfonate, and sulfate (data not shown), nonspecific interactions may explain the binding we observed. The striking fractionation observed correlates with the difference in the isoelectric point of the arylsulfatase from other periplasmic proteins. When periplasmic proteins were subjected to electrophoresis cathodically in an acid buffer system (Reisfield et al., 1962), the only protein that migrated into the gel was the arylsulfatase (our unpublished data). Lien and Schreiner (1975) have reported its isoelectric point as ~pH 9.0.

Arylsulfatase activity eluted from the blue agarose column was subjected to electrophoresis on preparative SDS polyacrylamide gels and the 67.6-kD polypeptide was excised and electroeluted from the gel matrix, combined with Freund's adjuvant, and injected into rabbits. Antibodies prepared to this polypeptide exhibited a single precipitin line when reacted with total extracellular protein from sulfate-deprived *cw15* cultures in double immunodiffusion tests (Ouchterlony and Nilsson, 1973), indicating that the antibody is monospecific (data not shown).

Fig. 3 shows a Western blot of extracellular proteins from matrix, with the notable exception of band D. The last traces of band A are eluted with 0.1 M NaCl (lane 6), while protein D and arylsulfatase activity are eluted with 0.2 M NaCl (lane 7). The identity of the arylsulfatase with band D was further confirmed by electroeluting the activity-stained band obtained after nondenaturing PAGE of periplasmic proteins and by subjecting this material to SDS-PAGE. The only polypeptide detected in the region of the gel exhibiting arylsulfatase activity co-migrates with band D (lane 8).

**Figure 2.** SDS-PAGE of polypeptides from different stages in the purification of the arylsulfatase. Lanes 1-7 correspond to samples 1-7 in Table I. Lane 8 shows the re-electrophoresis of activity-stained protein resolved by nondenaturing PAGE (see Materials and Methods). Lane 9 shows the protein present in the fraction eluted from a Sephacryl S-300 column that exhibited the highest level of arylsulfatase activity.
cw15 screened with these antibodies. The antibodies did not react with proteins in the periplasmic fraction of sulfate-sufficient cells (lanes 1 and 4), however, they did react strongly with purified arylsulfatase (lanes 3 and 6) and with the same polypeptide in preparations of extracellular proteins from sulfate-deprived cells (lanes 2 and 5). Furthermore, while the antibodies did not inhibit arylsulfatase activity, they did precipitate the enzyme activity in combination with protein A-Sepharose (data not shown).

**Properties**

Using purified arylsulfatase we have investigated several properties of the enzyme. Fig. 4, left, shows that the enzyme had a broad activity optimum at a pH of ~10; our values are higher than those reported by Lien and Schreiner (1975). The derepressible periplasmic alkaline phosphatase synthesized by cw15 also had a very alkaline pH optimum (~12) (Ogawa, J., and A. Grossman, unpublished data). The stimulation of enzyme activity by imidazole described previously (Lien and Schreiner, 1975) is most pronounced at low pH but does not seem to shift the pH optimum.

Fig. 4, right, shows a double reciprocal plot from which a $K_m$ (p-nitrophenylsulfate) of 0.3 mM was derived. This value is comparable with the value of 0.2 mM reported by Lien and Schreiner (1975) when activity was determined at 38°C in 0.1 M veronal-HCl, 10 mM imidazole, pH 7.8.

Microorganisms produce a wide variety of sulfatases with different substrate specificities. The classification of the *C. reinhardtii* enzyme as an arylsulfatase is based on its activity towards the chromogenic substrates p-nitrophenylsulfate and nitrocatecholsulfate. Since there are numerous potential substrates available in the soil (Fitzgerald, 1978) and on cell wall polymers, we considered the possibility that this enzyme may not be limited to arylsulfates as substrates. The cell wall of *C. reinhardtii*, composed primarily of glycoprotein and sulfated glycoprotein (Goodenough and Heuser, 1985), provides a hydrophilic shell that protects the cells from desiccation and acts as a barrier confining extracellular proteins but giving access to small molecules. The sulfated oligosaccharides linked to the protein components may play an important role in hydration of the cell wall (Roberts et al., 1980). Most of the sulfur in the cell wall is in the form of sulfate esterified to sugars, which accounts for 1–2% of the cell wall by weight (Roberts et al., 1980) and could be an ideal, although limited source of sulfate during periods of deprivation. In *Bacillus subtilis* teichoic acid in the cell walls may serve as a source of phosphate under stress conditions (Grant, 1979). We have tested the hypothesis that the cell wall glycoproteins are an endogenous substrate for the extracellular arylsulfatase using cell wall labeled with $^{35}$S by the method of Roberts et al. (1980) and prepared by the method of Imam et al. (1985). In these experiments, crude and purified arylsulfatase activities were unable to release radioactive sulfate into solution from cell wall material even after extended incubation periods (data not shown).

**Studies of Arylsulfatase Biosynthesis**

Arylsulfatase appears to be a glycoprotein that is synthesized as a higher molecular mass precursor before export into the periplasmic space. Table II shows that tunicamycin suppressed the appearance of arylsulfatase activity in the medium of cw15 cultures as well as in the cell pellet. At the
Table I. Effects of Tunicamycin on the Appearance of Arylsulfatase Activity

| Activity          | Tunicamycin |
|-------------------|-------------|
|                   | 0 µg/ml     | 2 µg/ml | 4 µg/ml |
| In supernatant    | 10          | 0.24    | 0       |
| In cell pellet    | 1.2         | 0.13    | 0.11    |

cw15 cells were transferred to medium lacking sulfate and containing varying amounts of tunicamycin. Cell pellets and supernatants were assayed for arylsulfatase activity after 12 h.

levels used tunicamycin does not inhibit protein synthesis based both on total incorporation of $^{35}$SO$_4$ and on the profile of radiolabeled cytoplasmic proteins in the cells (Coleman and Grossman, 1983). Fig. 5 (cf. lanes 2 and 3) shows that the group of periplasmic proteins characteristic of sulfate stress were not present in the supernatant of tunicamycin-treated cells. This is particularly apparent for bands D (arylsulfatase) and F. In double immunodiffusion tests no arylsulfatase antigen could be detected in either cellular or extracellular protein preparations from cultures treated with 4 µg/ml tunicamycin (data not shown).

Suppression of synthesis by tunicamycin is a good indication that the arylsulfatase has N-linked oligosaccharides. To investigate the extent and nature of its glycosylation, the protein was subjected to digestion with Endo H and Endo F (Kobata, 1979). Endo H and F will cleave between the N-acetylglucosamine bonds of N-linked oligosaccharides. Endo H, however, can only function if the distal oligosaccharide is of the unmodified, high mannose type. Fig. 6 shows that digestion with Endo F generates four discrete bands of lower molecular mass than the mature protein (lane 3) while incubation with Endo H (lane 4) has no effect on the glycoprotein. Denaturation by boiling in digestion buffer before incubation was necessary to render the arylsulfatase sensitive to Endo F. The appearance of four bands during the incubation with the endoglycosidase suggests that the arylsulfatase monomer has at least four N-linked glycosylation sites. The deglycosylated polypeptide (Fig. 6, lane 2) has an apparent molecular mass of 63.1 kD. Since only Endo F is effective in the digestion, the oligosaccharide must be complex and have a low mannose content. We have not investigated the possible occurrence of O-linked sugars, which are very prevalent in cell wall glycoprotein (Roberts et al., 1980).

To determine whether the arylsulfatase is synthesized as a precursor, antibodies were used to identify the primary translation product after in vitro translation of poly(A)$^+$ RNA from cw15 cells grown in the presence and absence of sulfate. There are few differences between the products of in vitro translation reactions primed with mRNA from sulfate-sufficient and sulfate-deprived cells (Fig. 6, cf. lanes 5 and 6). (One clear change is the disappearance of a major polypeptide with an $M_r$ of ~20 kD in translation products from sulfate-starved cells.) When these primary translation products were incubated with antiaryl sulfatase antibodies, a labeled polypeptide was precipitated only from the reactions primed with mRNA obtained from sulfate-deprived cells (Fig. 6, cf. lanes 7 and 8). This primary translation product has an apparent molecular mass of 68.2 kD, ~5 kD larger than the smallest product generated after Endo F digestion of the mature protein. These results suggest that the arylsulfatase is synthesized with a presequence, which may be involved in targeting the protein to its final subcellular location. A similar difference in the molecular mass of the C. reinhardtii periplasmic carbonic anhydrase and its primary translation product has been observed (Coleman and Grossman, 1984).

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