

Detection and Partial Characterization of Surface Antigenicity  
in the Cuticle of the Nematode C. elegans

by

Joseph Amatucci

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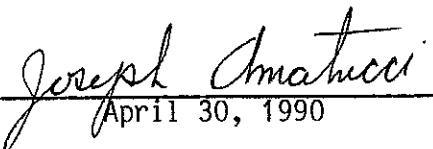
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Dr. Sam Politz,

WPI, Academic Advisor

## INTRODUCTION

The cuticle of the free living soil nematode *C. elegans* is a proteinaceous extracellular structure completely surrounding the animal except for openings at the pharynx, vulva, anus and excretory pore. The synthesis of the cuticle is linked to the animal's molting cycle. Therefore *C. elegans*, especially mutant animals with altered morphology and body shape, has proved a useful model to study developmental genetics (Cox et al 1981; Politz et al 1987).

Because the cuticle is the nematodes' major point of contact with the environment the structure and development of the cuticle can have a significant effect on the animal's survival, especially in the area of host-parasite antigenic response. Although *C. elegans* is not parasitic, many nematodes are, and any insights into the structure and development of the *C. elegans* cuticle could be useful in the study of parasitic nematodes (Philipp 1981; Wakelin 1983).

Previous insight into the structure of the *C. elegans* cuticle and the genetics controlling its synthesis have been acquired studying the antigenic response of different larval and varietal strains to rabbit immune sera directed against the 2-mercaptoethanol (BME) soluble fraction of adult cuticles. This fraction contains the major cuticle collagens characterized by Cox (Cox et al 1981; Politz et al 1986; Politz et al 1987). In the work described in this report, I obtained rabbit antiserum directed against the SDS soluble fraction of adult cuticle. This fraction had become of interest after a monoclonal antibody which was made against general embryo proteins and binds to the surface of live worms was shown to detect an antigen extracted primarily in the SDS fraction. (Donkin 1988). I used this antiserum to detect surface antigenicity in live wild type adults and to detect antigenicity among the proteins of the SDS fraction separated on SDS polyacrylamide gels. These results will hopefully lead to a better understanding of the composition of the SDS fraction and be a foundation for

future studies into the morphology and genetics of the *C. elegans* cuticle using as a tool, the SDS fraction and antisera directed against it.

## MATERIALS and METHODS

### Nematode Strain and Cultivation

The strain of *C. elegans* used was BA1; a *fer-1* mutant which is temperature sensitive, producing defective sperm at 25° and therefore no fertilized eggs (Ward and Miwa 1978). This allowed growth of large population of adults with no contamination from eggs or other larval stages.

Worms were stored as the dauer larva, a long lived nonfeeding developmental alternative to the L3 stage, which becomes an L4 upon resumption of favorable conditions (Cox 1981). Large quantities of dauer larva were raised on 100mm eggwhite plates which were made by boiling one eggwhite in 40ml of distilled/sterile water until well dissolved but not cooked, homogenizing for one minute in a Waring blender and layering 3ml onto a 100mm plate of 1/2 enriched media containing a lawn of *E. coli* OP50 (Cox 1981).

Eggwhite plates were seeded with 12,000 dauer larva each and incubated at 20° for 7-8 days. Dauer larva were washed off the plates with M9 buffer (Brenner 1974), collected by low speed centrifugation, and purified free of bacteria by several washes in M9. Nematode pellets were resuspended in 1% SDS, at 20ml per eggplate for 30 minutes with frequent mixing. Dauers and debris were collected by spinning for 2 min at 2000 rpm in a Beckman GPR centrifuge. Pellets were suspended in 2ml of M9 and spun through a 5ml cushion of ice cold 15% ficoll in 12ml conical tubes for 5 min at 2000 rpm. These pellets were purified dauer larva. Each eggplate yielded approximately  $1 \times 10^5$  dauer larva which were stored in M9 at 16° in wide bottom flasks (1000-25000/ml) allowing a large surface to air contact area (Cox 1981).

### Adult Cuticle Protein Isolation

To obtain a large synchronous adult population for cuticle isolation, thirty 100mm 1/2 enriched media plates of *E. coli* OP50 lawns were seeded with  $1 \times 10^4$  BA1 dauer larva and incubated at 25<sup>o</sup> (to prevent egg production) for 30 hours, which is a few hours after they molt into adults. Approximately 300,000 worms were washed off the plates, separated into 15ml graduated conical plastic tubes and cleansed of bacteria by several washes in ice cold M9. The animals were collected into six tubes and suspended in 7ml of ice cold sonication buffer (SB) (10mM Tris-HCl pH 7.4, 1mM EDTA-diNa, 1mM PMSF). A count was performed to estimate the number of worms per tube (25-50,000).

The nematodes were given three 20 sec bursts from a Branson Sonic Power Co. model W185 sonicator, setting #5, on ice with 40 sec between bursts. Cuticle fragments were collected by spinning for 5 min at 2000 rpm. Some of the first supernatant was saved, frozen in liquid nitrogen and stored at -4<sup>o</sup>. This was called the SB fraction. The pellets were then transferred to microfuge tubes and washed 4x in 500ul SB with 5 min spins in a refrigerated Eppendorf microfuge.

After the fourth wash in SB the cuticle pellets were collected into 1-3 microfuge tubes, suspended in 200ul 1% SDS, 0.125M Tris pH 6.8 per tube, put in a boiling water bath for 2 min and left sitting overnight at room temp. The tubes were microfuged the next day for 5 min and the supernatant was collected, frozen in liquid nitrogen and stored at -4<sup>o</sup>. This was called the SDS fraction. The remaining pellet was washed 5x in SDS buffer, suspended in 0.125M Tris pH 6.8 1% SDS 10% BME, put in a boiling water bath for 2 min and left sitting overnight at room temp. The next day the supernatant was collected and frozen as before. This was the BME fraction (Cox 1981). The cuticle preparation described above to collect the SB, SDS and BME fractions was performed 3x.

### **Protein Yield Determination**

To calculate the total amount of protein collected from each cuticle preparation a protein concentration standard curve was made using the Lowry Assay (Lowry 1951). Aliquots of 6-20ul from the SB, SDS and BME fractions were precipitated with 9 volumes of acetone for 3 hrs and dissolved in 400ul of 0.5M NaOH. The samples were compared with the standard curve of bovine serum albumin to determine the total amount of protein.

### **Gel Electrophoresis**

Discontinuous SDS polyacrylamide gel electrophoresis (PAGE) was performed according to the buffer system described by Laemlli and Favre (Laemlli 1973) on a Hoefer Scientific Instruments gel apparatus model SE 400, with a 7.5% separating gel (7.5% 30% acrylamide and 0.8% bis-acrylamide in water, 7.5ml 1.5M Tris-Cl pH 8.8, 3ml 1% SDS, 11.8ml water, 225ul 10% amper, 20ul TEMED) and a 3% stacking gel (1.05ml 30% acrylamide and 0.8% bis-acrylamide in water, 2.63ml 0.5m Tris-Cl pH 6.8, 1.05ml 1%SDS, 7.5ml water, 75ul 10% amper, 15ul TEMED). The running gel was poured leaving enough room for the stacking gel and comb, and then covered with a 1/3 dilution of overlay buffer (0.375M Tris-Cl pH 8.8) until polymerized. The overlay buffer was washed out, the stacking gel poured and the comb inserted. Sample aliquots containing 40ug of protein from each cuticle fraction were boiled for 2 min in sample buffer (0.125M Tris-HCl pH 6.8, 2%SDS, 10% glycerol, 5-10% BME, in a 1:20 dilution of 1% bromophenol blue) and loaded onto the gels under running buffer. Running buffer (6g Tris, 28.8g glycine, and 1g SDS; in 2 liters of water) was loaded onto the gel apparatus which was run at 60 volts until the samples reached the separatory gel, and then at 120 volts for 5 hrs.

The gel was stained for 1 hr in 0.1% coomassie blue, 50% methanol, 10% acetic acid, and destained overnight in 50% methanol, 10% acetic acid. The gel was then

restained for 1 hr in the original stain formula and then destained for several hrs in 10% methanol.

### **Antiserum Preparation**

A young male New Zealand White rabbit (rabbit #80) was immunized intramuscularly and subcutaneously with a total of 1mg of the SDS soluble cuticle proteins (150-300ul depending on which preparation was injected); in 0.125M Tris pH 6.8 and 1% SDS was emulsified in 1 ml of the the RIBI Adjuvant System (RIBI Immunochemical Research inc. Hamilton, Montana) using MPL (monophosphoryl lipid A) + TDM (trehalose dimycolate) + CWS (cell wall skeleton). Total volume injected at each immunization was 1ml (Ribi, Cantrell et al 1986; Azuma, Ribi et al 1974; Ribi 1984).

The rabbit was bled prior to the first immunization to obtain pre-immune serum. 14 days later the rabbit was bled to obtain the first immune serum and given a booster injection identical to the first. 14 days after this the rabbit was bled again to obtain the second immune serum. In all cases the rabbit was bled from the marginal ear vein. As a positive control in the immunofluorescence and immunoblotting experiments I used antiserum #74, supplied by prof. Politz, which was antiserum from a rabbit immunized against the BME soluble cuticle proteins in a previous study.

### **Immunofluorescence**

Strain N2 of *C. elegans* (wild type) was grown on 60mm plates spotted with *E. coli* OP50. Aliquots of about 50 adults were taken from each plate, washed several times by low speed centrifugation in 3ml conical tubes with PBS buffer to remove eggs, larvae and bacteria; and incubated in 2ml glass vials in 30ul of a solution of 54ul PBS/6ul anticuticle antiserum, a 1:10 dilution, for 1.5 hrs at room temp. with shaking (150 rpm). Samples were then washed 3x as before, resuspended in PBS and

incubated in 25ul of FITC (fluorescein isothiocyanate) labeled goat anti-rabbit globulins for 60 min at room temp. with shaking (150 rpm). Worms were then washed 6x as before.

The nematodes were killed on a glass microscope slide by gently heating, covered with a cover slip and photographed on a Zeiss Axioskop with FITC optics at 5x magnification using Kodak Tmax 400 film. Exposures were for 1 and 2 min.

### **Immunoblotting**

Proteins were separated by SDS-PAGE as described above except that 20ug instead of 40ug of protein were loaded onto each lane. Proteins were then electrophoretically transferred to nitrocellulose in a Hoefer Scientific Instruments protein blotting apparatus as described by the manufacturer. The electroblot buffer was 0.025M Tris 0.192M glycine pH 8.3-8.6 , 0.02% of a 20% volume of methanol. The electrotransfer was run at 0.8 amps for 1 hr.

Excess protein binding sites on the nitrocellulose were blocked by incubating for 1 hr in TBS buffer (0.05M Tris pH 7.5, 150mM NaCl) with 1% BSA, at room temp with gentle shaking. The nitrocellulose was then washed in TBS without BSA and incubated overnight at room temp with gentle shaking in 50ml of TBST (TBS, 0.05% Tween-20) and 10ul of anticuticle antiserum; a (1/5000 dilution). The nitrocellulose was next washed 3x in TBST and incubated for 1 hr at room temp with gentle shaking in 50ml TBST with 50ul goat anti-rabbit Ig (A+G+M) conjugated to alkaline phosphatase a (1/1000) dilution.

The nitrocellulose was then washed 3x more in TBS and exposed to the enzyme substrate; 60mg NBT in 2ml 70% DMF, and 30mg BCIP in 2ml 100% DMF, all in 200ml of carbonate buffer (0.1M NaHCO<sub>3</sub>, 1.0mM MgCl<sub>2</sub>, pH 9.8).

### **Collagenase Digestion**



The collagenase used was Form III clostridial collagenase from Advanced Biofactures. The ratio of collagenase:protein was 1 unit of enzyme :1ug acetone precipitated protein. Incubations were performed in collagenase buffer (50mM Tris-HCl pH 7.4, 150mM NaCl, 5mM N-ethylmaleimide, 5mM CaCl<sub>2</sub>) overnight at 37°. Undigested samples were incubated under the same conditions minus the collagenase.

## RESULTS and DISCUSSION

### Partial Characterization of Cuticle Proteins by SDS-PAGE

Three preparations of cuticle proteins were compared. The SDS soluble and BME soluble cuticle protein fractions from the first and second cuticle preparations were separated and analysed on a 7.5% gel (fig.1) and compared to the 6% gel of the similar fractions performed by Cox (Cox et al 1981). My SDS fractions from the different preparations showed similar banding patterns to each other and contained bands with apparent mol. wts. corresponding to those of myosin heavy chain (MHC 210,000 daltons), paramyosin (PM 92,500 daltons) and actin (AC 42,000 daltons), which were seen by Cox. My BME fractions from the different preparations also showed similar banding patterns to each other and displayed bands with the apparent mol. wts. of six of the eight major cuticle collagens seen by Cox, A, B, C, D, E and G (bands corresponding to F and H did not appear on my gel). Additional evidence that the major bands in my BME fractions are the cuticle collagens is the metachromatic staining they display (seen in the original gel but not in the black and white photograph). Studies have shown Coomassie blue to stain collagens pink while staining all other proteins blue (Micko 1978).

The BME fractions from the first, second and third cuticle preparations were later separated on a 7.5% gel after collagenase digestion (fig. 2). This experiment was performed in order to verify the presence of collagen in the BME fraction and in order to test for the presence of contamination of the BME fraction with non-collagen proteins from the SDS fraction. Results from the immunoblotting experiment described below had indicated the possibility of this contamination. A similar experiment by Cox had shown no change in the SDS fraction with collagenase treatment but extensive degradation of proteins in the BME fraction (Cox et al 1981).

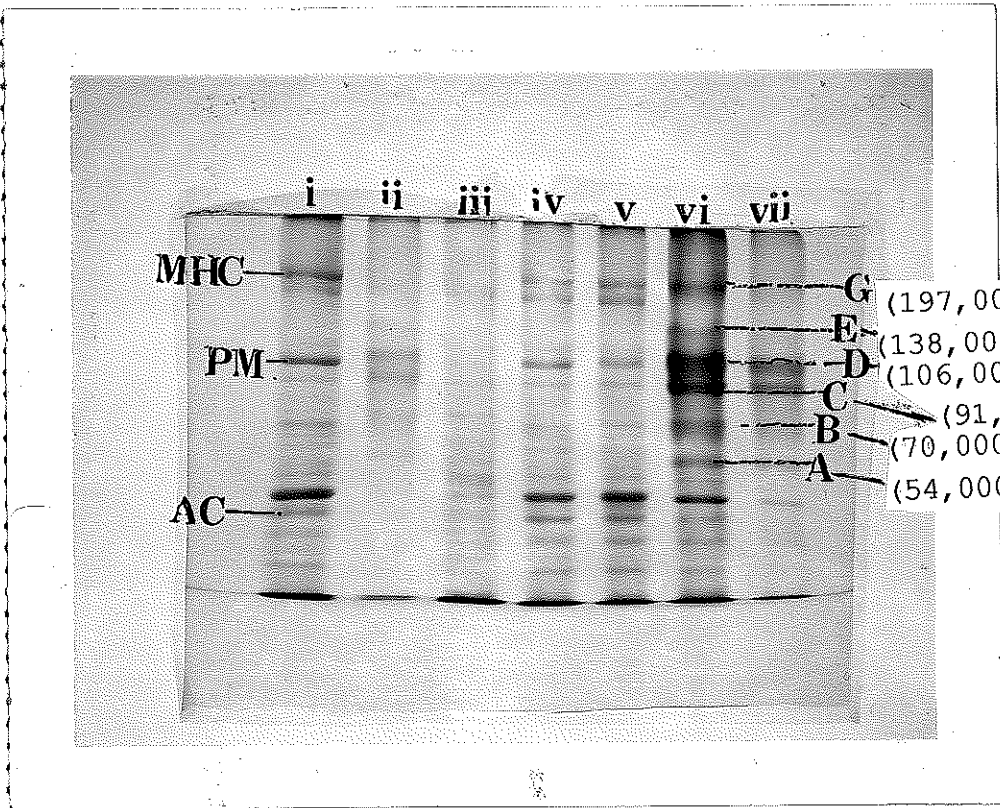
The BME fractions from all three preparations in fig. 2 showed the presence of collagenase sensitive proteins to some degree. The fraction from the first preparation was the least contaminated by non-collagens as only a few faint bands remain after digestion. However, preparations two and three both contained many bands after collagenase treatment. Through comparison with the gel of the SDS soluble fraction performed by Cox, these collagenase resistant bands in the BME fraction appear to be contamination from the SDS fraction and include bands with the apparent mol. wts. of MHC, PM, and AC, which are indicated in fig. 2. Many of the proteins contaminating the second and third preparations may also be in the first, but not in sufficient quantity to stain. A possible indication of this is the band at mol. wt. 48,000 (7.0cm lane ii fig. 2) in the digested BME lane from the first preparation. Of the bands contaminating the second and third preparations, this one is the most prominent; the fact that this band is so faint in the first preparation may indicate that many other bands are also contaminating it but are too weak to stain.

The initial results from fig. 1 indicated that the separation of the SDS soluble and BME soluble proteins was satisfactory and successful in that results similar to those arrived at by Cox were achieved. However the subsequent collagenase digestion of the BME fractions from the three preparations showed evident contamination by SDS soluble proteins with preparations two and three being the most contaminated.

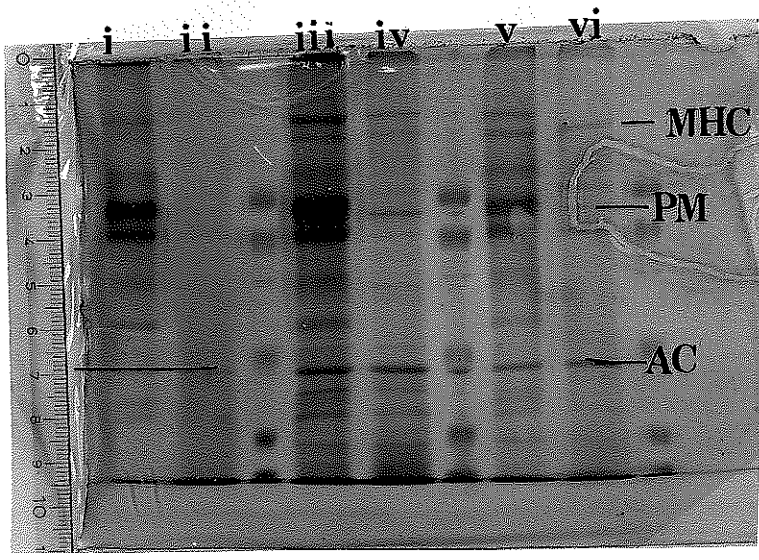
The most probable reason for the lesser amount of contamination in the first BME preparation was that a greater number of washes of the cuticle pellet in 1% SDS (5x), before BME extraction, were carried out than in the second and third preparations (3x). This does not however rule out the possibility that the first preparation is also contaminated. Although the possible contamination may not be sufficient to be seen on a stained gel it may be sufficient to elicit an immune response from antiserum made against the SDS fraction. The results of this collagenase digestion experiment call into question whether additional washes in 1% SDS before BME extraction can ever

reduce the level of contaminating non-collagen proteins to immunologically insignificant levels.

**FIGURE 1** 7.5% SDS Polyacrylamide gel electrophoresis of BA1 cuticle proteins released by sonication and treatment with SDS and BME. Proteins were stained with Coomassie blue. Lane i; SDS soluble proteins from first cuticle prep. Lane ii; BME soluble proteins from first cuticle prep. Lane iii; proteins released during sonication from first cuticle prep. Lanes iv and v; SDS soluble proteins from second cuticle prep. Lanes vi and vii; BME soluble proteins from second cuticle prep. In lane i, the muscle proteins, myosin heavychain (MHC 210,000 mol. wt.), paramyosin (PM 92,500 mol. wt.), and actin (AC 42,000 mol. wt.) are indicated. In lane vi the major cuticle collagens are labeled and their mol. wts. indicated. Each lane contains 40ug of protein.



**FIGURE 2** 7.5% SDS polyacrylamide gel electrophoresis of BME soluble proteins from BA1 cuticles, with and without collagenase digestion. Proteins were stained with Coomassie blue. Lanes i and ii; proteins from first cuticle prep. without and with collagenase digestion respectively. Lanes iii and iv; proteins from second cuticle prep. without and with collagenase digestion respectively. Lanes v and vi; proteins from the third cuticle prep. without and with collagenase digestion respectively. All other lanes are mol. wt. markers. In lane iv the bands suspected of being the large muscle proteins from the SDS fraction (MHC, PM, and AC) are indicated. Each lane contains 40ug of protein.





### Detection of Surface Antigenicity by Indirect Immunofluorescence

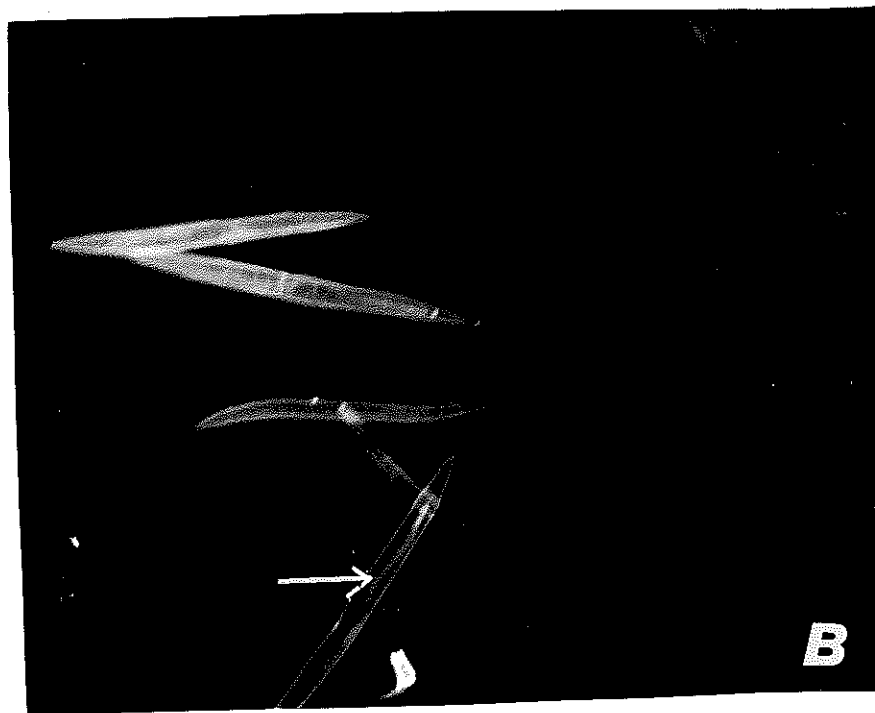
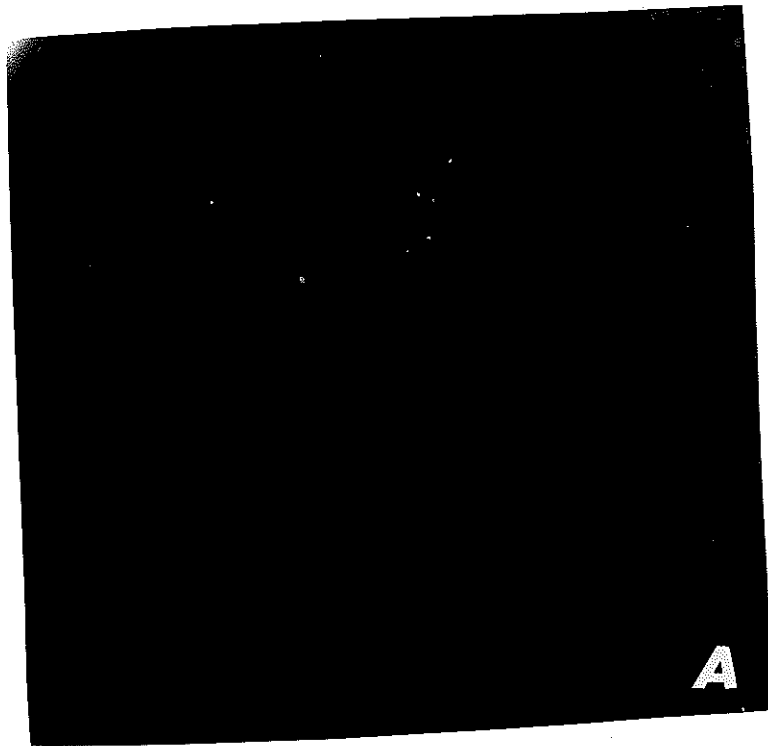
In order to detect whether the SDS soluble fraction of the *C. elegans* cuticle contains any surface antigens, antiserum from a New Zealand White rabbit (rabbit #80) immunized with the SDS fraction was tested for antibody binding to live adult nematodes by indirect immunofluorescence (fig. 3). As a negative control the live worms were incubated with pre-immune serum from rabbit #80. As a positive control the nematodes were incubated with immune serum from rabbit #74 (a rabbit immunized against the BME fraction from a previous study and provided by professor Politz for this project) which is known to bind to the surface of live adult nematodes (Politz et al 1990).

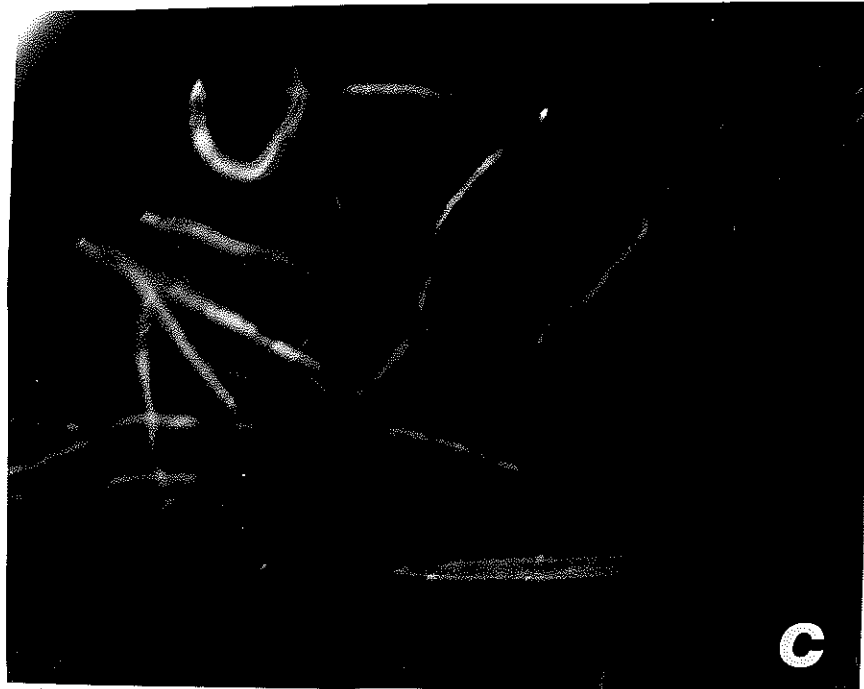
Pre-immune serum from rabbit #80 did show a very slight reaction with what seems to be the gut of the worms (fig. 3A), however there was no binding detected to the cuticle surface. Serum from the first post immune bleeding of rabbit #80 bound to the cuticle surface with some animals (shown by arrow) revealing a slight binding to the gut (fig. 3B). Although the serum from the second post immune bleeding of rabbit #80 also showed a positive reaction to the cuticle surface, there was a greater reaction with the gut (fig. 3C). The immune serum from rabbit #74 reacted very strongly with the cuticle surface (fig. 3D).

Because the nematodes in this experiment were never incubated with the secondary antibody (the FITC labeled goat anti-rabbit globulins) without having first been incubated with one of the rabbit antisera, the background fluorescence is unknown. Therefore I do not know if the slight reaction seen with the gut in fig. 3A is the result of rabbit #80 having possibly been exposed to a prior nematode infection or a background reaction.

These results do indicate the presence of a surface antigen of the *C. elegans* cuticle being extracted in the SDS soluble fraction.

**FIGURE 3** Indirect immunofluorescent staining of live wild type adult nematodes. Animals were incubated with various rabbit anticuticle antisera and an FITC labeled goat anti-rabbit secondary antibody as described in materials and methods. A; worms incubated with pre-immune serum from rabbit #80. B; worms incubated with the first immune serum from rabbit #80. C; worms incubated with the second immune serum from rabbit #80. D; worms incubated with immune serum from rabbit #74. All samples were stained with 3ul of the appropriate serum and photographed under 5x magnification for a 1 min exposure.





### Characterization of Cuticle Antigenicity by Immunoblotting

The SB, SDS, and BME soluble cuticle fractions of *C. elegans* were separated by SDS-PAGE, transferred to nitrocellulose and incubated with anti-cuticle antiserum from either rabbit #80 or #74 (fig. 4).

Among the SDS fraction lanes incubated with pre-immune, first immune and second immune serum from rabbit #80 (lanes i fig. 4A, 4B, 4C respectively) the first immune serum reacted more strongly than the pre-immune and the second immune serum reacted more strongly than the first. Paramyosin, indicated in fig. 4A, 4B and 4C is the only known SDS soluble protein which could be identified. Similarly the BME fraction lanes incubated with pre-immune, first immune or second immune serum from rabbit #80 (lanes ii fig. 4A, 4B, 4C respectively) also showed the latter sera reacting more strongly.

Between adjacent lanes of the SDS and BME fractions incubated with the same antiserum from rabbit #80, either pre-immune, first immune or second immune (lanes i and ii from fig. 4A, 4B, and 4C respectively), there are numerous bands, most smaller than mol. wt. 90,000 (about 4.0 cm) that appear in both lanes. Paramyosin however is the only protein with a mol. wt. corresponding to the apparent mol. wt. of one of the bands in this blot.

The reaction of immune serum from rabbit #74 with both the SDS and BME fractions was so strong that no separate bands could be discerned (lanes i and ii fig. 4D). The SB fraction (lanes iii) did show separate bands but the pattern did not match with that of the SB fraction incubated with serum from rabbit #80.

The reaction of pre-immune serum #80 to the SB, SDS and BME fractions could indicate that the rabbit was exposed to a nematode infection prior to immunization. However because this immunoblot was never performed using the secondary enzyme linked antibody, without first using the primary antiserum, it is not known if any of the

binding seen in fig. 4A is a genuine or background reaction.

Several bands recognized by the first and second immune sera from rabbit #80 in the BME fraction are also present in the SDS fraction. These bands in the BME fraction could be contamination from the SDS fraction against which rabbit #80 was immunized. The difficulty in correlating these bands is two fold. 1; other than MHC, PM, and AC there is no definition of what proteins constitute the SDS fraction, unlike the the BME fraction which contains the eight major cuticle collagens, and 2; it is possible that a number of proteins in the SDS fraction elicit a strong immunogenic response while existing in such minute quantities that they do not stain on the SDS gel with Coomassie blue and are thus difficult to identify.

Immune serum from rabbit #74, which was immunized against the BME fraction, reacted equally well with the SDS and BME fractions in fig. 4D. Although the level of contamination by SDS soluble proteins in the particular BME fraction used to immunize rabbit #74 is unknown, the cross reactivity of this serum appears similar to that of serum #80.

The results from this immunoblot along with those from the collagenase digestion in fig. 2 put to question the purity of the BME fractions used in this study and raise uncertainties about the specificity of the immune serum from rabbit #74 which was immunized with a BME fraction of unknown content.

Collagenase digested and undigested samples of the SDS soluble and BME soluble cuticle fractions were separated by SDS-PAGE, transferred to nitrocellulose and incubated with either the first immune serum from rabbit #80 or the immune serum from rabbit #74 (fig. 5)

The lanes containing the SDS fraction incubated with the first immune serum #80 displayed no reduction or elimination of bands after collagenase digestion (lane i fig. 5A, 5B). Bands with the apparent mol. wts. of PM and AC are seen and indicated in

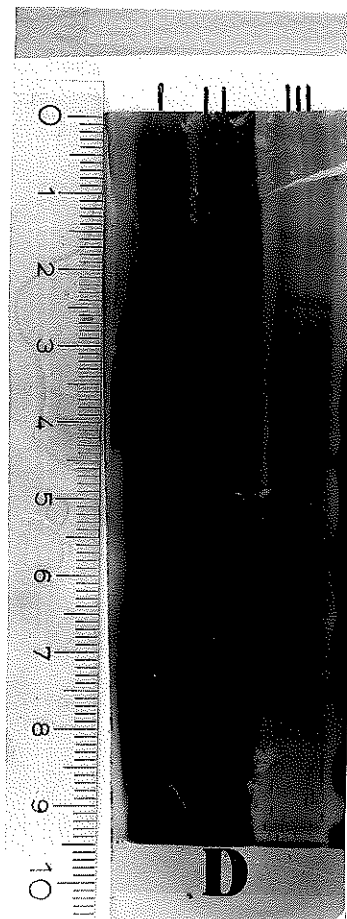
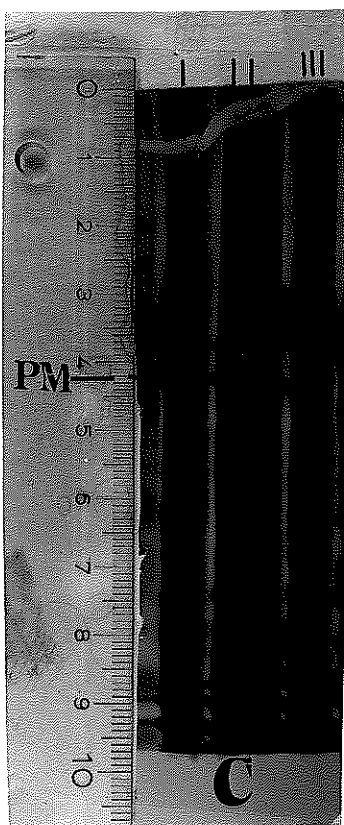
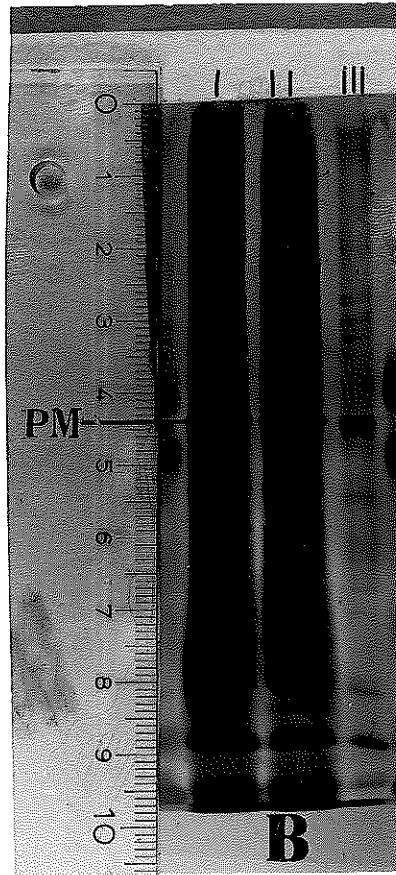
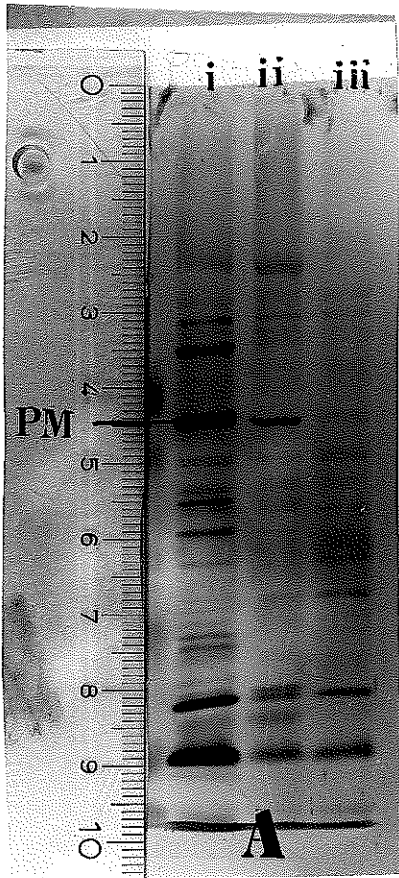
the figure. Many other bands in these two lanes also appear in the immunoblot of the SDS fraction mentioned above which was previously incubated with immune serum #80 (fig. 4B lane i). These results give evidence of an immunological response by rabbit #80 to certain non-collagens in the SDS fraction.

The lanes of the BME fraction incubated with the first immune serum #80 displayed a reduction or elimination of a relatively few bands after collagenase digestion, all larger than mol. wt. 100,000 (3.0 cm) (lane ii fig. 5A 5B). When comparing lane i with lane ii in figure 5A and 5B, many of the bands seen in the SDS fraction are also seen in the BME fraction although fainter, including the band suspected of being PM, both before and after collagenase digestion. This is further evidence that SDS soluble proteins are contaminating the BME fraction.

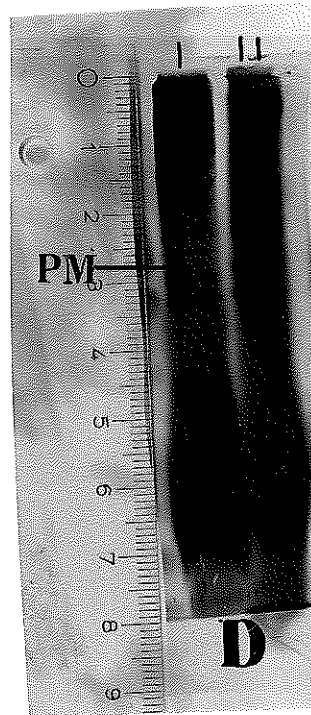
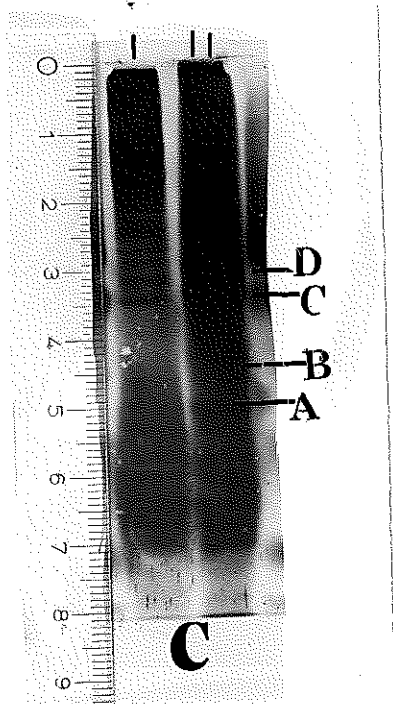
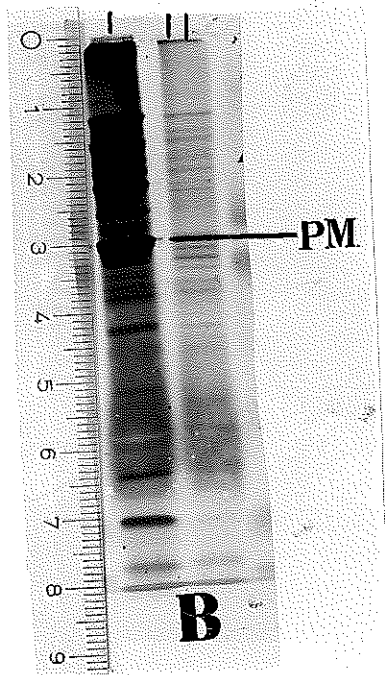
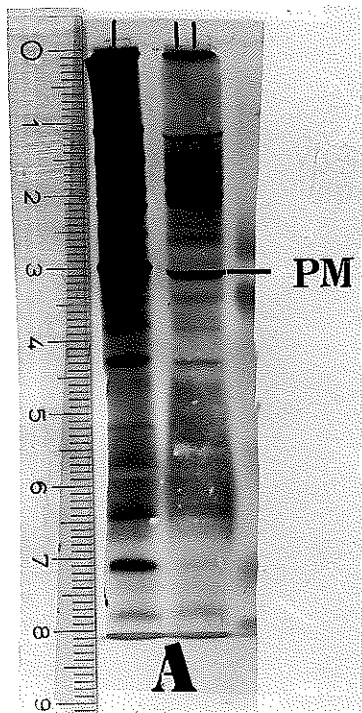
The SDS fraction incubated with immune serum from rabbit #74 revealed no reduction or elimination of bands after collagenase digestion (lane i fig. 5C 5D) and its banding pattern did not match with that of the SDS fraction incubated with immune serum #80 except for the band with the apparent mol. wt. of PM (indicated in the fig.). The BME fraction incubated with immune serum #74 showed bands with the mol. wts. of the major cuticle collagens A, B, C, and D (indicated in lane ii fig 5C) which were collagenase sensitive and did not appear in the lane with the digested fraction (lane ii fig. 5D). Several bands in this digested BME fraction are also seen in the digested and undigested SDS fraction incubated with serum #74, including one with the apparent mol. wt. of PM. This is more evidence still that SDS soluble proteins are contaminating the BME fraction.

FIGURE 4 Immunoblot of *C. elegans* cuticle fractions incubated with A, pre-immune serum from rabbit #80; B, first immune serum from rabbit #80; C, second immune serum from rabbit #80; D, immune serum from rabbit #74. In A,B,C and D lanes i, ii and iii are the SDS, BME and SB fractions respectively. The band with the apparent mol. wt. of PM in each lane is indicated. Each lane contains 20ug of protein.





**FIGURE 5** Immunoblot of *C. elegans* cuticle fractions with and without collagenase digestion. A and B, cuticle proteins incubated with the first immune serum from rabbit #80 without and with collagenase digestion respectively; C and D, cuticle proteins incubated with immune serum from rabbit #74 without and with collagenase digestion respectively. In A, B, C and D lane i is the SDS fraction and lane ii is the BME fraction. Bands with the apparent mol. wts. of PM and the the major cuticle collagens are indicated. Each lane contains 20ug of protein. Digested proteins were incubated with collagenase overnight at 37<sup>0</sup> with 1 unit enzyme/1ug acetone precipitated protein. Non-digested samples were incubated under the same conditions minus the collagenase.



## CONCLUSION

The main goal of this project was achieved when the SDS soluble fraction of the *C. elegans* cuticle elicited an antiserum containing antibodies that bind to the surface of live nematodes. Although previously a monoclonal antibody was shown to recognize a surface antigen detected in the SDS soluble fraction (Donkin 1988), this study demonstrates for the first time surface antigenicity in the SDS fraction from adult cuticles.

In the course of this project it was discovered that it is apparently difficult to isolate the separate cuticle fractions without contamination from the proteins of other fractions. This cross fraction contamination seems most prevalent in the case of SDS soluble proteins finding their way into the BME fraction. Evidence for this particular contamination was revealed by the presence of collagenase resistant proteins in the SDS-PAGE of the BME fractions in fig. 2, and in the cross reactivity of immune serum from rabbit #80 with collagenase resistant proteins in the immunoblot of the BME fractions in fig. 5. In both cases the bands contaminating the BME fraction seem to match with bands in the SDS fraction and include the muscle proteins PM and AC, known to be in the SDS fraction (Cox et al 1981). The cross reactivity of immune serum from rabbit #74 with proteins in the SDS fraction in fig. 4 and fig. 5 is evidence that this SDS protein contamination occurred in the particular BME fraction used to immunize rabbit #74. This questions the specificity of immune sera directed against any BME fraction derived in the way the one in this project and the one used to immunize rabbit #74 were.

One of the difficulties in determining whether a cuticle preparation was clean enough to eliminate any immunologically significant contamination is that these significant amounts may be too minute to stain on an SDS polyacrylamide gel. Future cuticle preparations could be tested with a more sensitive staining method such as the

PVDF immobilon-P transfer membrane which can detect as little as 50ng of protein/ band, about 20x more sensitive than staining a gel with Coomassie blue (Matsudira 1989).

In several experiments in this study the results indicated the possibility that rabbit #80 had been exposed to a prior nematode infection. It is not known if pre-immune serum #80's slight reaction with the nematodes' gut in the immunofluorescence experiment and lighting up of bands in the SDS and BME fractions of the immunoblot are genuine immune reactions, because tests for background reaction were done in neither case. The use of specific pathogen free rabbits to produce antisera in any future studies should resolve these ambiguities.

By demonstrating surface antigenicity in the SDS fraction and revealing some of the difficulties in extracting pure cuticle fractions, especially the BME fraction, this project is a foundation for work in characterizing the components of the SDS fraction, with particular attention to the surface antigens. Possibly through the production of monoclonal antibodies specific for surface antigens on the adult cuticle, the SDS fraction may become a useful tool for studying the genetics of *C. elegans*.

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